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Hypolipidemic and hepatoprotective effects of *Ficus bengalensis* aerial roots in STZ-induced diabetic animals

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ABSTRACT

Ficus bengalensis Linn. (Moraceae) root used in the Indian traditional medicine for the treatment of diabetes. The present study deals with the scientific exploration of antidiabetic potential of *Ficus bengalensis* (*F. bengalensis*) aerial roots in severely-diabetic animals in addition to its effect on diabetes induced disturbed lipid profile and hepatic injury. The antidiabetic activity of aqueous extract of *F. bengalensis* aerial root was evaluated by using normal and streptozotocin (STZ)-induced diabetic rats. The acute effect of aqueous extract was evaluated by administering 300 mg/kg to normal and STZ-induced severely-diabetic animals (FBG>250 mg/dl) were treated once a day for 21 days. Blood glucose levels, serum insulin, serum lipids, body weights and different biochemical parameters were also carried out. After 21 days of treatment with extract the maximum reduction in Fasting blood glucose (FBG) (33.8%) was observed in severely-diabetic rats treated with aqueous extract 300 mg/kg dose. Serum lipid levels were reversed towards neat normal and a control in the loss of body weight was observed in treated rats as compared to diabetic control. Levels of serum lipids were also observed in treated rats as compared to diabetic control. These results suggest that aqueous extract of aerial roots of *F. bengalensis* possess significant hypolipidemic and hepatoprotective effects.

1. Introduction

In India, the art of herbal healing has very deep roots in tribal culture and folklore[1]. A multitude of herbs spices and other plant materials have been described for the treatment of diabetes throughout the world[2-5]. The available synthetic drugs for Type-2 diabetes have certain limitations, such as adverse effects and high rates of secondary failure. In recent decades, a number of new antidiabetic remedies from medicinal herbs, relatively free from side effects, have been sought.

'Indian Banyan Tree', the holy tree of India is known as 'Bargad' in Hindi. It is a very large tree distributed throughout the country and in botany, it is known as *Ficus bengalensis*. Information based on ethnomedicinal survey reveals that the herbal preparations of different parts of F. bengalensis have been considered as effective, economical and safe for curing various diseases in Indian traditional system of medicine.

F. bengalensis fruit extract has been reported for its anti-tumor and anti-bacterial activities[6]. The aerial hanging roots of *F. bengalensis* have been reported as anti-diarrhoeal agents[7]. The plant is used in folk medicine for respiratory disorders and certain skin diseases[8]. Its bark was also reported for anti-stress and anti-allergic potential in treating asthma[9]. The bark of *F. bengalensis* has been traditionally used for the management of diabetes mellitus. Oral administration of bark extract showed lowering of blood glucose level in STZ diabetic rats and enhancement of serum insulin levels in normoglycemic as well as diabetic rats[10].

It is interesting to note that dimethoxy ethers of leucocyanidin 3-O-beta-D-galactosyl cellobioside[11] and leucopelargonidin-3-O-alpha-L-rhamnoside[12], isolated from *F. bengalensis*, have been found to possess significant blood glucose lowering and serum insulin raising action whereas methyl ether of the same flavonoids have significant antioxidant action[13]. Anti-oxidant effect of aqueous extract of the bark of F. bengalensis has also been evaluated in hypercholesterolemic rabbits[14]. Since its aerial roots have characteristic property of growing downwards from the branches and finally back to soil and had already been explored by our research group[15] for diabetes management in normal as well as sub- and mild-diabetic animals. Therefore, the present study was carried out on severely-diabetic models for evaluating the antidiabetic, antilipidemic and hepatoprotective effects on long term treatment of aqueous extract of aerial roots of *F. bengalensis*.. Improvements in haemoglobin (Hb), total protein (TP) and body weight (bw) and urine sugar were also studied during the treatment period.

2. Experimental

2.1. Chemicals

Streptozotocin (STZ) was purchased from Sigma-Aldrich Co., USA. Serum glucose, total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C), alkaline phosphates (ALKP), Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT) and serum creatinine (CREAT) were estimated using

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commercial kits purchased from Baeyer Diagnostics India Ltd. Uristix was purchased from Baeyer Diagnostics India Ltd.

2.2. Plant material

Fresh aerial roots of *Ficus bengalensis* were collected from the Garden of Allahabad University in the month of July and were identified by Prof. Anupam Dixit, Taxonomist, Department of Botany, University of Allahabad, Allahabad, India. A voucher specimen AB/425/06 has been submitted in our department for further reference.

2.3. Preparation of plant extract

The roots were air-dried and cut into small pieces, the pieces were mechanically crushed. 4 kg of crushed aerial roots were continuously extracted with distilled water using soxhlet up to 48h. The extract was filtered and concentrated in rotary evaporator at 35-40ºC under reduced pressure to obtain a semisolid material, which was then lyophilized to get a powder (12.32%, w/w).

2.4. Experimental animals

Female albino Wistar rats of approximately the same age group and having body weight 180-230 g were obtained from Central Drug Research Institute (CDRI) Lucknow, which were used for all the experiments. Animals were kept in our animal house at an ambient temperature of 27 ± 3ºC and 50 ± 5% relative humidity with a 12 h each of dark and light cycle. Animals were fed with pellet diet (Pashu Aahar Kendra, Varanasi) and distilled water ad libitum. The Institutional Ethical Committee approved the study.

2.5. Induction of diabetes in rats

Diabetes was induced by a single intraperitoneal injection of freshly prepared solution of STZ at the dose of 55 mg/kg in 0.1 M citrate buffer (pH 4.5) to the overnight fasted rats[16]. Fasting blood glucose (FBG) level was estimated at the time of induction of diabetes and post parandial glucose (PPG) was checked regularly up to stable hyperglycemia after about one week of induction. Severely-diabetic rats with FBG > 250 mg/dl were selected for the study.

2.6. Preliminary phytochemical screening

Preliminary phytochemical screening[17,18] revealed the presence of phenolic compounds, protein, carbohydrate and tannins.

2.7. Analysis of total phenolic content

Total phenolic contents in the extracts were determined by the modified Folin-Ciocalteu method as described earlier[19]. An aliquot (100 µl) of the extracts was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (75 g/l) of sodium carbonate. The tubes were vortexed for 15 s and allowed to stand for 30 min at 40°C for color development. Absorbance was recorded against reagent blank at 765 nm using the Simadzu UV-VIS spectrophotometer. Samples of extract were evaluated at a final concentration of 0.1 mg/ml. Total phenolic contents were expressed as mg/g gallic acid equivalent.

2.8. Estimations

Estimations for various biological parameters were carried out using standard procedures like glucose, TC, HDL-cholesterol and TG levels in blood serum, measured spectrophotometrically[20,21]. LDL-cholesterol was calculated from the above measurement using Friedwald formula [TC- HDL+VLDL][22] and VLDL cholesterol from the formula TG/5. Hepatic enzymes viz. ALKP, SGOT, SGPT[23,24] were estimated in blood serum in addition to CREAT[25], total protein (TP)[26] and haemoglobin[27]. Urine sugar was detected by reagent-based uristrix from Bayer Diagonostics India Ltd.

2.9. Experimental design

From our previous findings, effect of variable doses of 100, 200, 300 and 400 mg/kg bw of aqueous extract of *F. bengalensis* aerial roots (FBAR) on blood glucose level (BGL) of normal, sub- and mild-diabetic models have been studied and the results were compared with a reference drug, Glipizide; 300 mg/kg bw was found to be the most effective dose in case of all the models[28], therefore, this dose (300 mg/kg) was taken for severe studies. The dose of 300 mg kg-1 was taken for evaluation in case of severely-diabetic models, since it was identified as the most effective dose in case of normal, sub- and mild-diabetic models in our previous study.

Four groups of six rats each as were fasted overnight.

Group I : Normal control
Group II : Diabetic control
Group III : Diabetic + Glipizide (2.5 mg/kg)
Group IV : Diabetic + FBAR aqueous extract of (300 mg/kg)

Group I (Normal control) and Group II (Diabetic control), received vehicle (distilled water only), whereas, group III was treated with the dose of 2.5 mg/kg body weight of standard drug glipizide orally. Group IV was treated with the dose of 300 mg/kg body weight of aqueous extract aerial roots of F. bengalensis. A number of biochemical parameters viz. TC, TG, LDL, VLDL, HDL, TP, Hb, were studied initially in the beginning and then weekly up to twenty one days, in addition to FBG in order to get a complete bioactive profile of *F. bengalensis* aerial roots. Blood samples (approx 1.5 ml) were collected from tail vein weekly for conducting all the experiments.

Antidiabetic activity was assessed with the most effective dose (300 mg/kg) in severely- diabetic rats by conducting fasting blood glucose (FBG).

2.9.1. Acute oral toxicity study

Acute oral toxicity of aqueous extract of *F. bengalensis* aerial root was performed on female albino wistar rats, according to OECD Guidelines-425. Four groups of rats of either sex (six animals per group, three females and three males) were orally administered with a single dose of 10 and 15 times of the most effective dose 300 mg/kg of aqueous extract of *F. bengalensis* aerial roots. The rats were observed continuously for 2 h under the following profiles[29].

(i) Behavioral profile. Alertness, restlessness and fearfulness.
(ii) Neurological profile. Touch response, pain response and gait.
(iii) Autonomic profile. Urination.

After a period of 24 h, 72 h and 21 days they were observed for any lethality or death.

2.9.2. Statistical analysis

Data were statistically evaluated using one-way ANOVA, followed by a post hoc Scheffe's test using the SPSS computer software, version 7.5. The values were considered significant when $P < 0.05$ as compared to control.

3. Results and discussion

3.1. Serum glucose levels

Table.1 reveals the results of 21 days treatment of the extract on fasting blood glucose levels. Significant reduction was observed in the extract treated rats.

3.2. Serum lipid profile

Fig.1 deals with the effect of similar treatment, as mentioned above in 3.1, on lipid profile of severely-diabetic rats. A decrease in the serum triglyceride, total cholesterol, LDL and VLDL levels, and an increase in the HDL cholesterol levels were observed.

3.3. Serum enzymatic, Hb and TP assays

Table 2 shows the effect of similar long term treatment on enzymatic as well as Hb and TP assays of normal and severely-diabetic rats. A fall of 35.5% and 29.0% in SGOT and SGPT levels was observed in case of treated diabetic rats. Moreover, ALKP and CRTN of the extract-fed diabetic rats were reduced by 35.6% and 32.3% respectively. However, in the animals treated with the extract for 1 month, there was an increase in haemoglobin and total protein in diabetic animals by 11.4% and 21.8% respectively.

Total phenolic content

The total phenolic content *F. bengalensis* aerial root aqueous extract was found to be 70 mg/g gallic acid equivalent[30].

3.4. Body weight and urine sugar level

Table 3 gives an idea of the changes occurred in body weight and urine sugar during treatment. There was a gradual increase of body weight in the normal control while the diabetic control continued to lose weight. However, treated diabetic group gained 8.1% weight as compared to diabetic control and the body weights of diabetic treated rats were towards normal range. In the normal untreated (control) and extract-fed animals, there was no evidence of urine sugar. In the diabetic control animals, the urine sugar remains at +4 level throughout the experiment whereas, it decreased from +4 to +1 in case of treated diabetic rats.

Table 1: Effect of aqueous extract of *Ficus bengalensis* aerial roots on serum glucose level

Groups	Treatment	Fasting plasma glucose concentrations (mg/dl)			
		1st day	7th day	14th day	21st day
I	Normal control (DW)	78.6 ± 4.2	87.3 ± 2.6	93.4 ± 1.6	88. 8 ± 3.2
II	Diabetic control (DW)	278.8 ± 7.8	291.6 ± 16.1	312.6 ± 12.0	326.0 ± 7.2
III	Diabetic + Glipizide (2.5 mg/kg)	266.4 ± 3.2	236.5 ± 1.1	227.2 ± 1.2	197.6 ± 1.14
IV	Diabetic + aqueous Extract (300 mg/kg)	273.4 ± 3.6	246.7 ± 2.2	239.7 ± 2.6	216.3 ± 2.4

$p<0.05$ as compared to diabetic control
$p<0.01$ as compared to diabetic control

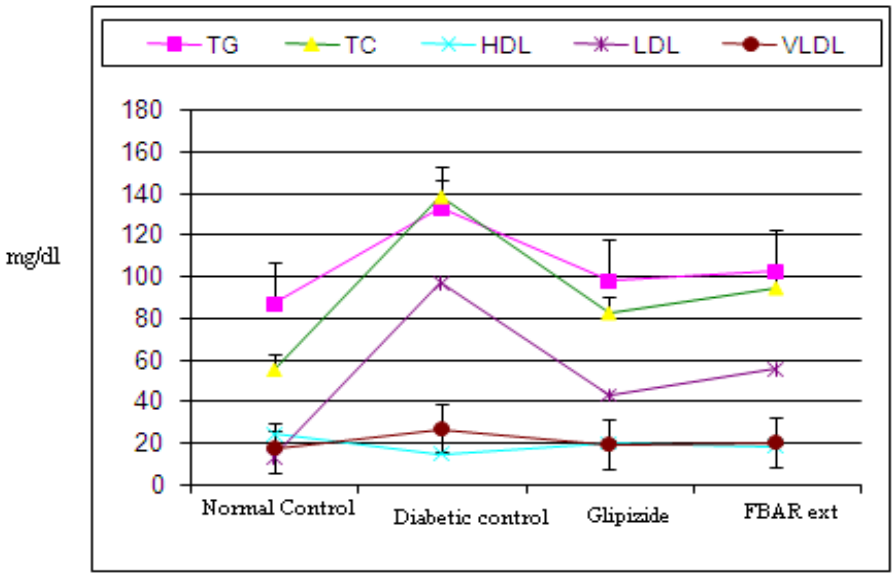
Table 2: Effect of 21 days long term treatment of aqueous extract of *Ficus bengalensis* aerial roots on SGOT, SGPT and ALKP, CREAT levels of normal and diabetic rats

Groups	Treatment	**SGOT (U/L)**		**SGPT(U/L)**	
		Before treatment	After 21 days treatment	Before treatment	After 21 days treatment
I	DW	66.7 ± 2.8	66.2 ± 3.6	28.4 ± 1.2	28.6 ± 2.2
II	DW	102.8 ± 2.4	120.5± 3.4	52.8 ± 6.4	64.5 ± 5.2
III	Glipizide 2.5 mg/kg	114.6 ± 3.4	77.4 ± 2.2*	58.8 ± 3.5	54.4 ± 4.6*
IV	300 mg/kg	117.6 ± 3.6	79.2 ± 3.5*	57.6 ±2.6	56.2 ± 2.8*
Groups	Treatment	ALKP **(U/L)**		CREAT **(mg/dl)**	
		Before treatment	After 21 days treatment	Before treatment	After 21 days treatment
I	DW	77.4 ± 4.6	78.2 ± 4.6	0. 96 ± .14	1.10 ± 0.12
II	DW	148.6 ± 3.2	156.4 ± 6.4	1.76 ± 3.8	2.14 ± 3.2
III	Glipizide 2.5 mg/kg	151.6 ± 4.6	98.6 ± 6.8*	1.2 ± 2.6	1.10 ± 3.8*
IV	300 mg/kg	153.2 ± 5.2	104.6 ± 4.6*	1.81 ± 3.4	1.15 ± 3.5*

Table 3: Effect of 21 days long term treatment of aqueous extract of *Ficus bengalensis* aerial roots on urine sugar and body weight of normal and diabetic rats

Experimental Animals	**Treatment**	**Pre-treatment levels**	**Post-treatment levels**		
			7 days	**14 days**	**21 days**
		Urine sugar (g/lt)			
Normal control	D W	Nil	Nil	Nil	Nil
Diabetic control	D W	+++	+++	++++	++++
Diabetic + Glipizide	2.5 mg/kg	++++	+++	+++	+
Diabetic + aqueous Extract	300 mg /kg	++++	++++	+++	+
		Body weight (g)			
Normal control	D W	185	185	188	188
Diabetic control	D W	195	196	196	210
Diabetic + Glipizide	2.5 mg/kg	180	180*	175*	182*
Diabetic + aqueous Extract	300 mg /kg	185	185*	195*	202*

Fig 1: Effect of 21 days long term treatment of aqueous extract of *Ficus bengalensis* aerial roots on TG, TC, HDL, LDL and VLDL of normal and diabetic rats



3.5. Acute Toxicity Studies

Experiment was carried out on normal healthy rats. The behavior of treated rats appeared normal. There was no lethality or any toxic effects found at any of the doses selected until the end of the study period.

4. Discussion

F. bengalensis is a national tree of India called as "Bargad tree". The present study was undertaken to identify the biomedical profile of aqueous extract of aerial roots of F. bengalensis in vivo. Though, *F. bengalensis* is reported to possess varied medicinal properties such as anti-stress, anti-allergic and antidiabetic but the study of its effect on diabetes induced hepatoprotective and lipid profile has been reported for the first time in the present study. The hypoglycemic and antidiabetic effects of aqueous extract of *F. bengalensis* aerial roots had already been reported and the dose of 300 mg kg-1 had been identified as the most effective dose, by our research group, in case of normal, sub- and mild-diabetic rats. Hence, in the present study the severely- diabetic animals were treated once a day for thirty days only with this identified dose.

It is well known that type 2 diabetes mellitus leads to an increase in TC, TG, LDL and VLDL cholesterol levels with decrease in HDL cholesterol contributing thereby to coronary artery diseases[31-33]. Thus, lowering the serum lipid concentration through dietary or drug therapy seems to be associated with decreased risk in cardio vascular diseases[34]. However, high level of HDL, being good cholesterol, is associated with low risk of coronary heart diseases, while its decreased level is associated with an increased risk of heart diseases.

The present study of severely-diabetic rats showed enhanced HDL cholesterol level and significantly declined levels of TC, TG, LDL and VLDL after 30 days treatment of *F. bengalensis* aqueous extract, once a day indicating thereby a significant improvement in diabetes induced disturbed lipid profile.

In addition to lipid profile other important biochemical parameters such as blood serum AST, ALT, ALKP and CREAT have also been taken into consideration in order to evaluate the improve diabetes induced in liver and kidney function impairment. It is interesting to note that the diabetes induced enhanced levels of these parameters were also found to decrease significantly. The long term treatment of aqueous extract of *F. bengalensis* aerial roots reduces AST, ALT and ALKP levels indicating thereby its hepatoprotective effect against liver injury. The significantly decreased CREAT level was the clear indication of its protective effect against kidney damage.

Generally haemoglobin is much below the normal level in diabetic subjects[35] and the present study reveals that such decreased levels of haemoglobin can be improved by thirty days treatment of *F. bengalensis* aerial roots aqueous extract. Moreover, total protein and body weight were also found to increase during treatment period. Another interesting finding was the significant decrease in urine sugar level after thirty days treatment.

5. Conclusion

In conclusion, it can be stated that *F. bengalensis* aerial roots have high hypolipidemic as well as hepatoprotective potential in addition to antidiabetic efficacy. Hence its aqueous extract can be used for the treatment of severe-diabetics.

Abbreviations:

ALKP	:	Alkaline Phosphatase
BGL	:	Blood Glucose Level
CREAT	:	Creatinine
FBG	:	Fasting Blood Glucose
GTT	:	Glucose Tolerance Test
HDL	:	High Density Lipoprotein
LDL	:	Low Density Lipoprotein
TG	:	Triglyceride
TC	:	Total Cholesterol
SGOT	:	Serum Glutamate Oxaloacetate Transaminases
SGPT	:	Serum Glutamate Pyruvate Transaminases

References

1. K. N. Singh and B. Lal, *Journal of Ethnopharmacology*, **2008**, 115, 147-159.
2. A. N. Kesari, R. K. Gupta and G. Watal, *Journal of Ethnopharmacology*, **2005**, 97, 247-251.
3. R. K. Gupta, A. N. Kesari, P. S. Murthy, R. Chandra, V. Tandon and G. Watal, *Journal of Ethnopharmacology*, **2005**, 99, 75-81.
4. M. D. Ivorra, M. Paya, and A. Villar, *Journal of Ethnopharmacology*, **1989**, 27, 243-275.
5. R. J. Marles,and N. R. Fransworth, *Phytomedicine*, **1995**, 2, 137-189.
6. O. Mousa, P. Vuorela, J. Kiviranta,S. A. Wahab, R. Hiltunen and H. Vourela, *Journal of Ethnopharmacology*, **1994**, 41, 71-76.
7. P. K. Mukherjee, K. Saha, T. Murugesan, S. C. Mandal, M. Pal, and B. P. Saha, *Journal of Ethnopharmacology*, **1998**, 60, 85-89.
8. K. R. Kirtikar and B. D. Basu, Indian medicinal plants, Lalit Mohan Publication, Calcutta, **1935**, 499.
9. D. J. Taur, S. A. Nirmal, R. Y. Patil and M. D. Kharya, *Natural Product Research*, **2007**, 21, 1266-1270.
10. S. Achrekar, G. S. Kaklij, M. S. Pote and S. M. Kelkar, Hypoglycemic activity of Eugenia jambolana and Ficus bengalensis: Mechanism of Action. In Vivo. **1991**, 5, 143-147.
11. R. V. Kumar,and K. T. Augusti, *Indian Journal of Biochemistry and Biophysics*, **1989**, 26, 400-404.
12. K. T. Augusti, R. S. Daniel, S. Cherian, C. G. Sheela and C. R. Nair, *Indian Journal of Medical Research*, **1994**, 99, 82-86.
13. R. S. Daniel, B. C. Mathew,K. S. Devi and K. T. Augusti, Indian *Journal of Experimental Biology*, **1998**, 36, 902-906.
14. R. Shukla, S. Gupta, J. K. Gambhir, K. M. Prabhu and P. S. Murthy, *Journal of Ethnopharmacology*, **2004**, 92, 47-51.
15. R. K. Singh, S. Mehta, D. Jaiswal, P. K. Rai and G. Watal, *Journal of Ethnopharmacology*, **2009**, 123, 110-114.
16. G. Brosky, and J. Logothelopoulos, *Diabetes*, **1969**, 18, 606-609.
17. C. K. Kokate, Practical Pharmacognosy. Vallabh Prakashan, New Delhi, **1994**, 105–107.
18. J. B. Harborne, Phytochemical Methods. Chapman & Hall, London, **1998**, 60.
19. K. Wolfe, X. Wu and R. H. Liu, *Journal of Agriculture and Food Chemistry*, **2003**, 5, 1609- 614.
20. C. C. Allain, *Clinical Chemistry*, **1974**, 20, 470.
21. G. Buccolo and M. David, *Clinical Chemistry*, **1973**, 19, 476-482.
22. J. Friedwald, Y. R. Levy and S. D. Friedrickson, *Clinical Chemistry*, **1972**, 18, 499-502.
23. Z. Klin, *Biochem*., **1970**, 8, 658.
24. T. U. Hansman, *Clinical Chemical Acta*, **1976**, 70, 19.
25. R. J. Henry, D. C. Canon and J. W. Winkelman, Clinical Chemistry Principles and Technics, second ed. Harper and Row, **1974**.
26. R. D. Strickald, M. L. Freeman and F. F. Gurule, *Analytical Chemistry*, **1961**, 33, 125-128.
27. K. E. Van and W. G. Zijlstra, *Clinical Chemiatry Acta*, **1961**, 6, 538-544.
28. R. K. Sharma, S. Chatterji, D. K. Rai, S. Mehta, P. K. Rai, R. K. Singh, G. Watal and B. Sharma, *Journal of Medicinal Plants Research*, **2009**, 3, 944-948.
29. R. Barik, S. Jain, D. Qwatra, A. Joshi, G. S. Tripathi and R. Goyal, *Indian Journal of Pharmacology*, **2008**, 40, 19-22.
30. C. S. Sharma, R. K. Nema and V. K. Sharma, *S. J. Pharm. Sci*., **2009**, 2(2): 42-47.
31. D. Bainton,N. E. Miller, C. H. Botton, J. W. G. Yarnell, P. M. Suretman, I. A. Baker, B. Lewis and P. C. Elwood, *Britsh Heart Journal*, **1992**, 68, 60-66.
32. K. Arvind, R. Pradeep, R. Deepa and V. Mohan, *Indian Journal of Medical Research*, **2002**, 116, 163-176.
33. E. H. Temme, H. P. G. Van, E. G. Schouten and H. Kesteloot, *Acta Cardiology*, **2002**, 57, 111-115.
34. G. G. Rhoads, C. L. Gulbrandse and A. Kagen, *New England Journal of Medicine*, **1976**, 294, 293-298.
35. H. B. Chandalia and P. R. Krishnaswamy, *Current Science*, **2002**, 83, 1522-1615.