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Hypoglycemic and hypolipidemic activities of *Terminalia arjuna* stem bark in alloxan induced diabetic rats

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Abstract

Objective: To study the effect of 50% ethanolic extract of *Terminalia arjuna* bark on glucose and lipid profile in serum, kidney, liver and adipose tissue in alloxan induced diabetic rats. Methods: Oral administration of the *Terminalia arjuna* stem bark extract daily at a dose of 250 and 500 mg/kg body weight to alloxan induced diabetic rats for a period of 30 days. Results: Significant reduction in glucose and lipid profile in serum and tissues of alloxan induced diabetic rats was noticed, the extract even under high concentration (500 mg/kg body weight) showed no toxic effect in control rats. Conclusion: The study indicates that *Terminalia arjuna* 50 % ethanolic stem bark extract at both doses, 250 and 500 mg/kg body weight, restored all the lipids and glucose parameters to near normal values and the dose of 500 mg/kg body weight showed more effect.

Keywords: Lipid profile, alloxan, diabetes, hypolipidimic, *Terminalia arjuna*

1. Introduction

Diabetes mellitus is a chronic disease characterized by high blood glucose levels due to absolute or relative deficiency of circulating insulin levels [1]. Diabetes mellitus is a world wide health problem afflicting millions in both developed and developing countries. It is the prime cause of chronic kidney failure, blindness, high blood pressure and premature coronary artery disease [2]. In recent years emphasis is on the development of drugs from plants for the treatment of various diseases including diabetes mellitus, the incidence of which is very high all over the world especially in India. The reason is that plant drugs could be effective and at the same time have less or no side effects [3]. Alloxan diabetic model resembles type 1 diabetes (insulin dependent diabetes mellitus) without significant insulin resistance [4]. *Terminalia arjuna* is an important cardiotonic plant described in ayurveda, belonging to the

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family *Combretaceae*, a tropical woody tree, occurring throughout India [5]. In traditional literature *T. arjuna* has been recommended as a remedy for heart disease and diabetes. So it was considered worthwhile to investigate the *T. arjuna* stem bark on glucose and lipid profiles in both serum and tissues of different organs in normal and alloxan-induced diabetic rats. It has been found that only meagre work has been so for done on alloxan-induced diabetics rats using *T. arjuna* bark extract.

2. Materials and methods

2.1 Plant material

The wet *Terminalia arjuna* bark was collected from Siruvani coastal of Agali in Kerala. The specimen was identified and certified by Botanical Survey of India (BSI) Coimbatore.

2.2 Preparation of 50 % ethanolic extract:

Terminalia arjuna was used in the form of crude 50% ethanol extract and this extract was prepared according to the traditional system of medicine. The shade dried and coarsely powdered stem bark (1kg) was extracted with 50% alcohol in cold for 72 h. The extract was filtered and distilled on water bath, a reddish brown syrupy mass was obtained and it was finally dried at low temperature under reduced pressure in a rotary evaporator. A crude residue (75g) was obtained giving a yield of 7.5%. The hypoglycemic and hypolipidemic effects were evaluated by oral administration of the extract on the alloxan induced diabetic rats.

2.3 Animals

Male albino rats of Wistar strain weighing about 150 – 200 g, obtained from the Medical College of Trichur (Kerala) were used for the study. They were fed a standard rat pellet diet (Sai Durga feeds, Bangalore) and water was provided *ad libitum* and maintained under standard laboratory conditions. (Temperature 24 - 28°C, relative humidity 60 - 70%) Animals described as fasted, were deprived of food for 16 h but had free access to water. Clearance for the handling of experimental animals was obtained from the Ethical committee (CPCSEA No: 659/02/a).

2.4 Alloxan-Induced diabetes

Diabetes was induced by a single ip injection of 120 mg/kg of alloxan monohydrate (S.D Fine – Chem. Ltd., Mumbai, India), in sterile saline [6]. After 72 h of alloxan injection, the diabetic rats (blood glucose level > 250 mg/dl) were separated and used for the study [7].

2.5 Methodology

The animals were divided in to 6 groups of 6 each. Group I served as normal healthy control. Group II (untreated diabetic control). Group III diabetic rats given *T. arjuna* bark extract (250 mg/kg body weight). Group IV diabetic rats given *T. arjuna* bark extract (500 mg/kg body weight). Group V control rats given *T. arjuna* bark extract (250 mg/kg body weight). Group VI control rats given *T. arjuna* bark extract (500 mg/kg body weight). The extract was administered for a period of 30 days.

2.6 Collection of blood, liver, kidney and adipose tissue from the rat

After the experimental regimen, the animals were sacrificed by cervical dislocation under mild chloroform anesthesia. Blood was collected on decapitation and serum was separated by centrifugation (for 20 min at 2000 rpm). The liver, kidney and adipose tissue were excised immediately and thoroughly washed in ice-cold saline. The serum and tissues were collected and used for biochemical experiments.

Estimation of biochemical parameters

Serum glucose was estimated by GOD/ POD method [8]. Tissue lipids [9], total cholesterol [10], free cholesterol [11], triglyceride [12], free

fatty acid [13] and phospholipids [14] were analysed in serum and tissues. High-density lipoprotein (HDL) cholesterol in serum was estimated [15] and low-density lipoprotein (LDL) cholesterol were calculated by Friedewald's formula: VLDL cholesterol = Triglyceride/5 and LDL cholesterol = Total cholesterol-(VLDL + HDL cholesterol) [16]. Protein in serum [17] and liver glycogen [18] were also estimated.

2.7 Acute toxicity study

The 50% ethanolic extract of *T.arjuna* stem bark was tested for its acute toxicity in rats. The study was designed on the basis of OECD (Organisation for Economic Co-operation and Development) guideline - 423, adopted on June 2001. To determine acute toxicity of a single oral administration of the extract, different doses of the extract (5, 50, 300, 2000 mg/kg body weight) were tried. The dose levels tested. 5, 50, 300, 2000 mg/ml suspensions were prepared in 1% W/V CMC (Carboxy Methyl Cellulose) and given at the dose of 1 ml /kg b.wt.

After administration of the extract, the animals were observed individually after dosing for 4 h and there after 14 days. The parameters noted were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsion. All animals were observed twice daily for mortality during the period of study.

2.8 Statistical evaluation

Statistical evaluation was done using one – way analysis of variance (ANOVA) followed by Duncans Multiple Range Test (DMRT). Statistical significance was set at $p<0.05$.

3. Results

In the acute toxicity study, the ethanol extract of *T.arjuna* did not show significant toxic signs when observed for the parameters during the first four hours and followed by daily observations for 14 days and no mortality was also observed, the substance is found to be safe at the tested dose level of 2000 mg/kg b.wt.

The levels of serum glucose, protein and liver glycogen of control and experimental animals are shown in Table 1. A significant ($p<0.05$) elevation in serum glucose with reduction in serum protein and liver glycogen were observed in diabetic rats, when compared with control rats. Oral administration of *T. arjuna* (250 and 500 mg/kg body weight) for 30 days showed significant reduction in glucose and a significant elevation in protein and liver glycogen in extract treated diabetic rats.

The levels of total cholesterol, free cholesterol, triglycerides, free fatty acids phospholipids, HDL, LDL and VLDL cholesterol of control and experimental animals in serum are given in the Table 2. A significant elevation in serum lipids and reduction in HDL cholesterol were observed in diabetic rats, when compared with control rats. Oral administration of *T.arjuna* (250 and 500 mg/kg body weight) for 30 days significantly reversed these values to near normal.

Table 3 shows the levels of lipids in the liver, kidney and adipose tissue in control and experimental rats. Cholesterol, phospholipids, triglycerides levels are significantly higher in tissues and significantly decreased in free fatty acids and free cholesterol in diabetic rats as compared to control rats. Oral administration of *T. arjuna* (250 and 500mg / kg body weight) was found to the near normal level.

The control rats treated with *T.arjuna* (250 and 500mg / kg body weight) did not produce any significant alteration in serum and tissue parameters when compared with normal values.

Table 1.
Effect of *Terminalia arjuna* stem bark on serum glucose, protein and liver glycogen in alloxan- induced diabetic rats

Parameters	Group 1 Control	Group II Diabetic	Group III Diabetic + TA 250mg/kg	Group IV Diabetic + TA 500mg/kg	Group V Control + TA 250mg/kg	Group VI Control + TA 500mg/kg
Serum						
Glucose (mg/dl)	98.33 ± 02.66^{b}	302.67 ± 22.35^{f}	125.60 ± 24.73^{e}	82.50 ± 04.72^{a}	106.67 ± 0.625^{b}	113.17 ± 14.25^{b}
Protein (g/dl)	5.47 ± 0.22^{d}	2.60 ± 0.11^{a}	6.06 ± 0.18^{e}	4.83 ± 0.67^{b}	5.35 ± 0.32^{d}	6.09 ± 0.05^{d}
Liver						
Glycogen (mg/g wet tissue)	24.06 ± 5.2^{e}	3.99 ± 0.51^{a}	18.690 ± 1.59^{b}	19.89 ± 3.50^{c}	21.93 ± 0.49^{e}	27.07 ± 2.61^{e}

Values are expressed as Mean ± SD (n=6)
Means followed by a common letter are not significantly differ at p<0.05 (DMRT).

Table 2.
Effect of *Terminalia arjuna* stem bark on serum lipid profile in alloxan induced diabetic rats

Parameters	Group 1 Control	Group II Diabetic	Group III Diabetic + TA 250mg/kg	Group IV Diabetic + TA 500mg/kg	Group V Control + TA 250mg/kg	Group VI Control + TA 500mg/kg
Serum						
Total cholesterol (mg/dl)	75.59 ± 1.24^{b}	174.10 ± 5.54^{f}	83.28 ± 1.79^{e}	76.62 ± 0.81^{c}	76.76 ± 0.64^{b}	75.01 ± 1.15^{b}
Free cholesterol (mg/dl)	5.79 ± 0.81^{b}	14.98 ± 0.73^{f}	10.84 ± 3.89^{e}	5.69 ± 0.82^{a}	7.06 ± 3.30^{b}	8.15 ± 0.84^{b}
Triglycerides (mg/dl)	101.31 ± 0.38^{c}	168.15 ± 5.41^{f}	131.77 ± 1.83^{e}	121.12 ± 0.61^{d}	99.43 ± 11.10^{c}	99.41 ± 1.02^{c}
Free Fatty acids (mg/dl)	57.48 ± 6.19^{d}	119.75 ± 5.13^{f}	26.37 ± 5.73^{b}	20.06 ± 2.39^{a}	60.89 ± 7.27^{d}	52.46 ± 4.13^{d}
Phospholipids (mg/dl)	78.20 ± 27.57^{b}	139.78 ± 13.43^{f}	86.95 ± 8.28^{c}	74.77 ± 3.68^{a}	94.07 ± 11.68^{b}	95.45 ± 12.66^{b}
HDL-C (mg/dl)	44.21 ± 2.88^{d}	28.84 ± 2.00^{a}	38.97 ± 3.73^{c}	31.44 ± 1.50^{b}	45.88 ± 1.55^{d}	46.87 ± 3.02^{d}
LDL-C (mg/dl)	27.01 ± 1.72^{c}	138.35 ± 2.81^{f}	38.45 ± 1.63^{e}	34.08 ± 2.91^{d}	24.86 ± 2.10^{c}	24.84 ± 1.59^{c}
VLDL-C (mg/dl)	20.26 ± 0.08^{b}	33.63 ± 1.08^{f}	26.35 ± 0.37^{e}	24.26 ± 0.14^{d}	21.82 ± 0.31^{b}	19.89 ± 0.20^{b}

Values are expressed as Mean ± SD (n=6)
Means followed by a common letter are not significantly differ at p<0.05 (DMRT).

Table 3.
Effect of *Terminalia arjuna* stem bark on lipid profile in liver, kidney and adipose tissue in alloxan-induced diabetic rats

Parameters	Group 1 Control	Group II Diabetic	Group III Diabetic + TA 250mg/kg	Group IV Diabetic + TA 500mg/kg	Group V Control + TA 250mg/kg	Group VI Control + TA 500mg/kg
Liver						
Cholesterol (mg/g tissue)	4.85 ± 0.40^{c}	7.82 ± 0.48^{f}	4.25 ± 1.62^{b}	4.24 ± 1.13^{a}	5.51 ± 0.60^{c}	5.78 ± 2.46^{c}
Phospholipids (mg/g tissue)	15.11 ± 1.16^{c}	21.25 ± 0.85^{f}	15.26 ± 2.58^{d}	9.91 ± 0.87^{a}	14.19 ± 1.78^{e}	16.41 ± 0.60^{c}
Triglyceride (mg/g tissue)	3.14 ± 0.60^{c}	6.82 ± 0.34^{f}	3.72 ± 0.29^{e}	2.12 ± 1.04^{a}	2.57 ± 0.78^{c}	3.59 ± 0.56^{c}
Free fatty acid (mg/g tissue)	8.02 ± 2.14^{c}	5.27 ± 1.22^{b}	11.83 ± 3.66^{f}	2.65 ± 0.39^{a}	9.12 ± 0.85^{c}	8.89 ± 0.61^{c}
Free cholesterol (mg/g tissue)	2.05 ± 0.41^{d}	0.69 ± 0.18^{a}	5.63 ± 1.91^{f}	3.26 ± 0.32^{e}	1.32 ± 0.33^{d}	1.45 ± 0.50^{d}
Kidney						
Cholesterol (mg/g tissue)	3.85 ± 0.24^{b}	8.68 ± 0.32^{e}	9.36 ± 1.94^{f}	6.11 ± 0.62^{c}	3.54 ± 0.70^{b}	5.67 ± 1.27^{b}
Phospholipids (mg/g tissue)	10.51 ± 0.47^{b}	22.90 ± 1.97^{f}	13.05 ± 4.19^{e}	6.37 ± 2.32^{a}	11.82 ± 1.49^{b}	12.44 ± 1.17^{b}
Triglyceride (mg/g tissue)	5.55 ± 0.61^{f}	5.93 ± 1.66^{e}	5.32 ± 0.33^{c}	4.88 ± 0.43^{a}	5.20 ± 0.47^{f}	5.48 ± 0.45^{f}
Free fatty acid (mg/g tissue)	6.61 ± 1.95^{d}	3.28 ± 0.64^{a}	13.15 ± 1.90^{f}	3.55 ± 0.75^{b}	6.93 ± 0.21^{d}	5.93 ± 0.67^{d}
Free cholesterol (mg/g tissue)	2.68 ± 0.75^{e}	0.74 ± 0.12^{a}	6.50 ± 1.95^{f}	1.17 ± 0.16^{b}	1.80 ± 0.18^{e}	1.90 ± 0.31^{e}
Adipose tissue						
Cholesterol (mg/g tissue)	7.58 ± 1.38^{b}	10.73 ± 0.74^{f}	8.48 ± 0.42^{c}	6.25 ± 1.22^{a}	8.56 ± 0.73^{b}	8.84 ± 0.46^{b}
Phospholipids (mg/g tissue)	9.13 ± 0.71^{c}	9.19 ± 0.96^{d}	8.66 ± 1.73^{b}	2.72 ± 0.93^{a}	9.80 ± 0.37^{c}	10.05 ± 0.98^{c}
Triglyceride (mg/g tissue)	3.82 ± 0.51^{e}	4.91 ± 1.11^{f}	1.99 ± 0.45^{b}	1.51 ± 0.54^{a}	3.32 ± 0.24^{e}	3.60 ± 0.63^{e}
Free fatty acid (mg/g tissue)	8.83 ± 0.57^{c}	1.68 ± 1.19^{a}	9.22 ± 0.57^{e}	3.18 ± 0.59^{b}	9.05 ± 0.43^{c}	9.58 ± 0.58^{c}
Free cholesterol (mg/g tissue)	1.78 ± 0.32^{f}	0.51 ± 0.09^{a}	1.74 ± 0.38^{e}	1.30 ± 0.54^{b}	1.32 ± 0.12^{f}	1.50 ± 0.78^{f}

Values are expressed as Mean ± SD (n=6)
Means followed by a common letter are not significantly differ at $p<0.05$ (DMRT).

4. Discussion

Alloxan produces oxygen radicals in the body, which causes pancreatic injury [19] and could be responsible for increased blood sugar seen in the animals. *Terminalia arjuna* bark extract (250 and 500 mg/kg body weight) decreases, the serum glucose in 30 days.

This result indicates hypoglycemic effect of the *T. arjuna* bark extract and this may be due to its protective role against pancreatic injury in diabetic rats. In this connection it may be added that *T. arjuna* showed hypoglycemic activity in rabbits and antihyperglycemic activity in man [20, 21]. This property of lowering of blood glucose level was seen in *Psidium guajava* stem bark in diabetic rats also [22]. The bark extract the treatment containing 500 mg bark extract/kg body weight have showed better efficacy than that with 250 mg/kg body weight.

Decreased serum protein level in alloxan induced diabetic rats is presumed to be due to increased protein catabolism and gluconeogenesis in diabetes [23]. On the administration of *T. arjuna*, the protein level was restored and was similar to that of normal rats. This is similar to the effect of "tarakeswara rasa" in diabetic rats [24].

Glycogen level was found to decrease in alloxan induced rats in the present study. This is line with the findings of others (24). In this work administration of *T.arjuna* bark extracts significantly improved the hepatic glycogen level in diabetic rats. The prevention of depletion of glycogen in the liver tissue is possibly due to an increase in glycogenesis and / or a decrease in glycogenolysis. So the *T.arjuna* extract would have stimulated glycogenesis and /or inhibited glycogenolysis [4].

In our experiment the higher level of serum and tissue lipids were observed in alloxan induced diabetic rats. Similar results have been reported by the other workers in alloxan diabetic rats [25, 26]. The abnormal high concentration of serum lipids in diabetes is believed to be mainly due to the increase in the mobilization of free fatty acids from the peripheral depots.

While insulin inhibits the hormone sensitive lipase, glucagon, cataecholamines and other hormones enhance lipolysis. The marked hyperlipidemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots [27].

The oral administration of *T. arjuna* bark extract down total cholesterol and LDL cholesterol to normal range and decreased triglyceride level and increased HDL cholesterol. This indicates hypolipidemic effect of the extract. Such hypolipidemic activity of *T.arjuna* has been observed by others also [28, 29, 30]. The mechanism involved in the reduction of serum LDL cholesterol is presumably due to an increase in the catabolism of LDL or a reduction in the synthesis of LDL [29].

The results obtained in the present study indicate that the ethanolic extract of *T.arjuna* stem bark exhibits significant antihyperglycemic and anti hyperlipidemic effect. It may be due to the presence of secondary metabolites such as tannins, flavonoids and saponins which on undergoing biotransformation through liver microsomal enzymes, produce active molecules cause this effects.

Among the two doses, 500 mg/kg body weight of the *T. arjuna* extract exhibited more hypoglycemic and hypolipidemic effect in the alloxan induced diabetic rats. The acute toxicity study of the extract shows no death of these rats even under high dose levels indicating the high margin of safety of this extract.

References

1. Hayat M, Mukhtar, Ansari SH, Ali M, Bhat ZA, Naved T. (2004) *Indian J. Exp. Biol.* 42: 1212 -15.

2. Kochupillai N. (1998) *Indian J. Diab. Dev. Countries.* 18: 69-70.

3. Murali YK, Ramesh Chandra, Murthy PS. (2004) *Indian J. Clin. Biochem.* 141-148.

4. Babu V, Gangadevi T, Subramonium A. (2003) *Indian J. Pharmacol.* 35: 290 - 296.

5. Tripathi VK, Singh B, Tripathi RK, Upadhyay, Pandey VB. (1996) *Oriental. J. Chem.* 1: 01 - 16.

6. Ravivijayavargia, Monika kumar, Sarita Gupta. (2000) *Indian J. Exp. Biol.* 38: 781 - 84.

7. Perfumi M, Tacconi R. (1996) *Indian J Pharmacol.* 34: 41.

8. Trinder P. (1969) *Clin. Biochem.* 6:24.

9. Folch J, Lees M, Slone, Stanely GH. (1957) *J Biol Chem.* 226: 497 - 509.

10. Parekh AC. (1970) *Anal. Chem.* 112: 1423 - 1427.

11. Sperry WM, Webb M. (1950) *J. Biol. Chem.* 187: 97 - 106.

12. Rice EW. (1970) In: Roderick MP(Ed) *Standard methods in Clinical Chemistr,* Academic press: NewYork; 6: 215 - 22.

13. Horn WT, Mehanan LA. (1981) *J. Lipid. Res.* 22: 377 - 381.

14. Rouser G, Fleischer S, Yamamoto A. (1970) *Phosphorus analysis of spots Lipids.* 5: 494 - 496.

15. Warnick GR, Nguyen T, Albers AA. (1985) *Clin. Chem.* 31: 217.

16. Friedewald WT, Ley RI, Stadrickson DS. (1972) *Clin. Chem.* 18: 499-502.

17. Lowry HD, Rosenberg NJ, Farr AL, Randall RS. (1951) *J. Biol. Chem.* 193: 265 -275.

18. Vander Vries J. (1954) *Biochem J.* 57: 410 - 416.

19. Halliwell B, Gutteridge JMC. (1985) *Free Radical in Biology and Medicine.* 215.

20. Shaila HP, Udupa SL, Udupa AL. (2000) *Pharm Pharmacol Commun.* 6 : 327 - 330.

21. Dwivedi S, Chansouria JPN, Somani PN, Udupa KN. (1989) *Alternative Medicine.* 32: 115 - 122.

22. Mukhtar HM, Ansari SH, Ali M, Naved T, Bhat ZA. (2004) *J. Nat. Rem.* 4: 150 - 154.

23. Palanivel R, Thangavel M, Selvendiran K, Sakthisekaran D. (2001) *Biomedicine.* 21: 23 - 30.

24. Vasanthakumari V, Shyamala Devi CS. (1997) *Indian J. Exp. Biol.* 35, 8: 909 - 911.

25. Stanely P. Mainzen Prince, Venugopal P. Menon, Gunasekaran G. (1999) *J. Ethnopharmacol.* 64: 53 - 57.

26. Eshrat Halim M. (2003) *Indian J Exp Biol.* 41: 636-640.

27. AL-Shamaony L, AL- Khazraj SM, Twaij HA. (1994) *J. Ethanopharmacol.* 43: 167-171.

28. Shaila HP, Udupa SL, Udupa AL. (1998) *Int. J. Cardiol.* 67(119-24).

29. Khanna AK, Ramesh Chander, Kapoor NL. (1996) *Phytotherapy.* 10: 663 - 665.

30. Chatterjee S. (2000) *Indian Drugs.* 37:3.