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CASE REPORT

Hypolipidemic activity of *Semecarpus anacardium* in Streptozotocin induced diabetic rats

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Abstract Alterations in lipid metabolism and lipoprotein disturbances have played an important role in increasing the risk of cardiovascular mortality and morbidity in diabetes. A drug that has hypoglycemic activity can be used for the treatment of hyperlipidemia also. The present study was carried out to evaluate the hypolipidemic activity of *Semecarpus anacardium*. Male Wister rats weighing 250–270 g were injected with Streptozotocin at a dose of 50 mg/kg body weight and administered with *S. anacardium* (300 mg/kg body weight) and Metformin (500 mg/kg body weight) for 21 days. Control and drug control groups were also included in the study. After the experimental duration, serum was collected, liver and kidney were excised and used for the analysis of lipid and lipid metabolizing enzymes. The results of the study revealed that *S. anacardium* administration was able to decrease the levels of LDL, cholesterol, VLDL, TG, phospholipid and free fatty acid and increase the HDL levels and favorably modulate the lipid metabolizing enzymes in the liver and kidney. These results show that *S. anacardium* exerts hypolipidemic activity in diabetic rats.

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Introduction

Diabetes mellitus Type 1 is associated with increased morbidity and mortality, largely as a consequence of accelerated atherosclerotic disease. The key components of diabetic dyslipidemia are elevated serum VLDL–triglycerides (TG) and lowered high-density lipoprotein (HDL)-cholesterol [1]. In addition, excessive postprandial lipemia, preponderance of small dense low-density lipoprotein (LDL) and small dense HDL are also present. Small dense LDL is a strong risk factor for cardiovascular disease, which is highly atherogenic and serves as a marker for atherosclerosis. It is also associated with high TG/low HDL-cholesterol cluster. There is a stepwise decrease in LDL-size from normal to impaired glucose tolerance (IGT) and then to diabetes. This association is more striking in women than in men [2]. In diabetic dyslipidemia, not only the concentration of HDL-cholesterol is reduced but also its composition and distribution are changed. Changes in HDL are mediated via two pathways: plasma triglyceride elevation and a reduced ratio between lipoprotein lipase (LPL) and hepatic lipase. Both these lead to altered HDL composition and an enhanced catabolic rate.

Semecarpus anacardium (SA) Linn. that belongs to the family Anacardiaceae have been reported to have many therapeutic applications in the Indian system of medicine [3]. In traditional medicine, it is highly valued for the treatment of tumours. The fruits and oil have been claimed to be highly efficacious in the treatment of neuritis, leprosy, helmintic infection and other diseases [4]. Many compounds, mainly biflavonoids, phenolics, bhilawanols, and

sterols have been identified and on the basis of chemical and spectral data several biflavonoids have been characterized, viz. Semicarpuflavanone, Jeediflavonone, Galluflavanone, Nallaflavanone, Semecarpetin and Anacarduflavanone [5]. The milk extract of the nut when chemically analyzed was found to contain flavonoids, carbohydrates and traces of phenolic compounds [6]. TLC, HPLC and HPTLC analysis of the nut and milk extract confirmed the presence of the above compounds [7–10]. Studies carried out on marking nut preparation have also shown promising results in the treatment of cancers of the esophagus, urinary bladder and liver. Earlier studies from our laboratory have proved the anticancer and hepatoprotective activity of SA against aflatoxin B1-induced hepatocellular carcinoma in rats [11] and established its protective role in rheumatoid arthritis [12]. The nut also has hypoglycemic [13] and COX inhibitory property [14]. Effective treatment of diabetes should decrease the glycemic levels and in addition have favorable effect on lipid profile. So the present study was undertaken to prove the hypolipidemic activity of *S. anacardium* in diabetic rats.

Materials and methods

The drug *S. anacardium* (SA) nut extract contains purified nuts of *S. anacardium*, cow's milk in the ratio as indicated in the Formulary of Siddha Medicine [15]. 200 g of the nut were boiled with 500 ml of milk, which was repeated thrice, boiled till dehydration. The decoction was stored [6].

Animals

Male Albino rats of Wistar strain weighing 260 ± 10 g were used in this study. The animals were housed in polypropylene cages under a control environment with 12 h light/dark cycle and a temperature between 27 and 37°C, and were given a commercial diet with water ad libitum. All experiments involving animals were conducted according to NIH guidelines, after obtaining approval from the Institute's Ethical Committee (IAEC NO: 02/075/06).

Experimental design

Male albino Wistar rats weighing 250–270 g were divided into five groups of six animals each.

Group I Control animals—Normal healthy controls received olive oil (0.5 ml) orally by gastric intubation for 21 days daily

Group II Diabetes induced—(50 mg/kg b.wt.) Streptozotocin dissolved in 0.5 ml of 0.1 M citrate buffer pH 4.5

Group III SA treated—Three days after the induction of diabetes, SA (300 mg/kg body weight dissolved in 0.5 ml olive oil) was administered by gastric intubation for 21 days daily

Group IV Metformin treated—Three days after the induction of diabetes, Metformin (500 mg/kg body weight dissolved in 0.5 ml physiological saline) was administered by gastric intubation for 21 days daily

Group V Drug control—Animals received SA at a dose of 300 mg/kg b.wt. in olive oil (0.5 ml) orally by gastric intubation for 21 days daily

Biochemical analysis

After the experimental period, the animals were killed by cervical decapitation. Liver and kidney were excised immediately and immersed in ice-cold physiological saline. Ten per cent homogenate was prepared with fresh tissue in 0.01 M Tris–HCl buffer (pH 7.4) and were used for the assays. Blood was collected, serum was separated and used for the analysis.

Fractional precipitation of lipoproteins

Lipoproteins were fractionated by a dual precipitation technique of Wilson and Spiger [16]. Addition of heparin–manganese chloride to plasma precipitated very low-density lipoprotein (VLDL) and LDL and the content of cholesterol was measured. To a second aliquot of plasma, sodium dodecyl sulphate (SDS) was added which resulted in the aggregation of VLDL which flocculated on the top. The supernatant contained both HDL and LDL and the cholesterol content of this mixture was assayed.

(a) Total cholesterol (TC)—supernatant from SDS (containing $HDL_c + LDL_c$) = VLDL cholesterol.

(b) Supernatant from SDS–heparin–manganese chloride supernatant = LDL cholesterol.

HDL

Two millilitre of plasma was added to 0.18 ml heparin–manganese chloride reagent and mixed well. The solution was allowed to stand at 4°C for 30 min and then centrifuged at 2,000×*g* and maintained at 10°C for 30 min. The supernatant contained the HDL fraction. One millilitre of this was used for the estimation of cholesterol by the method of Parekh and Jung [17].

LDL and VLDL cholesterol assay

Two millilitre of plasma was added to 0.15 ml of SDS. The contents were mixed well and incubated at 37°C for 2 h. The contents were centrifuged in a refrigerated centrifuge at 10,000×*g* for 15 min. The supernatant contained the HDL and LDL fraction. Cholesterol was estimated from this fraction. Plasma lipoprotein cholesterol is expressed as mg/dl plasma. The lipids were purified by the Folch's wash procedure [18]. Cholesterol content was estimated by the method of Parekh and Jung [17]. Phospholipids (PL) were estimated by the method of Rouser et al. [19] after digesting the lipid extract with perchloric acid. Triglyceride in plasma was estimated by the method of Rice [20]. Plasma and tissue free fatty acid (FFA) were estimated by the method of Hron and Menahan [21].

Assay of lipid metabolizing enzymes

Total lipase was assayed by the method of Bier [22]. The LPL was assayed by the method of Schmidt [23]. LACT was assayed by the method of Legraud et al. [24] with modifications of Hitz et al. The activity of cholesterol ester synthetase and hydrolase was assayed by the method of Kothari et al. [25].

Statistical analysis

The values are expressed as mean ± SD for six rats in each group. Statistically significant differences between the groups were calculated using one-way analysis of variance (ANOVA), followed by Student-Newman–Keuls for multiple comparisons using statistical package for social sciences (SPSS) computer package. Values of $p < 0.05$ were considered to be significant.

Results

Effect of SA on lipid metabolism

The results of TC, HDL, LDL, VLDL, TG, PL, FFA and atherogenic index in serum are given in Table 1. When compared with Group I animals these were found to be increased ($p < 0.05$) in Group II animals. Cholesterol increased by 1.4-fold, LDL by 1.4-fold, VLDL by 1.75-fold, TG by 1.7-fold, PL by 1.4-fold, and FFA increased by 1.5-fold. HDL decreased by 0.67-fold. The atherogenic index increased by 3.12-fold in Group II animals indicating the alteration due to diabetes. All these were brought back to near normal levels upon administration of SA. The difference between the Metformin and SA treated groups were non significant for HDL, LDL, PL and FFA while VLDL, TG, TC, and atherogenic index showed a significance of $p < 0.05$. No significant difference was observed when Group V and Group I were compared.

The levels of TC, TG, PL, and FFA in the liver and kidney of the control and experimental animals are shown in Figs. 1 and 2. In liver of Group II animals, the values of cholesterol increased by 2.5-fold, PL by 1.4-fold, and FFA by 1.5 fold when compared to normal animals. In the kidney of Group II animals the values increased by 1.3-fold for TC, 1.4-fold for PL and 2.1-fold for FFA. TG decreased by 40 and 39% in Group II animals when compared to Group I animals. Upon SA and Metformin treatment, these alterations were brought back to near normal levels. The decrease in TC, PL, FFA and the increase in TG were more pronounced in Group III animals treated with SA than with Group IV animals treated with Metformin.

The effect of SA on the lipid metabolizing enzymes in the liver and kidney are shown in Tables 2 and 3. Significant increase in total lipase, LPL, CES, cholesteryl ester

Table 1 Lipid profile in serum of control and experimental animals

Parameters (mg/dl)	Group I (Control)	Group II (STZ)	Group III (STZ + SA)	Group IV (STZ + Metformin)	Group V (SA)
Cholesterol	94.24 ± 0.89	132.11 ± 13.8[a]*	103.51 ± 10.8[b]*	112.46 ± 10.2[b]* [c]*	93.18 ± 9.58[d] NS
HDL	38.14 ± 0.36	23.55 ± 2.68[a]*	31.83 ± 2.85[b]*	27.13 ± 2.98[b]* [c] NS	37.86 ± 3.58[d] NS
LDL	71.25 ± 0.69	100.43 ± 9.35[a]*	80.87 ± 7.85[b]*	87.76 ± 8.31[b]* [c] NS	70.58 ± 7.03[d] NS
VLDL	15.35 ± 1.43	26.98 ± 2.94[a]*	16.27 ± 1.58[b]*	18.60 ± 1.92[b]* [c]*	15.02 ± 1.77[d] NS
TG	76.79 ± 8.04	134.84 ± 14.4[a]*	81.35 ± 8.6[b]*	93.04 ± 9.44[b]* [c]*	75.11 ± 8.01[d] NS
PL	82.51 ± 8.41	116.93 ± 9.63[a]*	93.72 ± 9.11[b]*	98.33 ± 10.2[b]* [c] NS	83.48 ± 7.9[d] NS
FFA	61.04 ± 0.63	93.57 ± 9.86[a]*	66.35 ± 6.82[b]*	71.84 ± 6.83[b]* [c] NS	60.73 ± 6.14[d] NS
Athrogenic index	1.47 ± 0.09	4.60 ± 0.42[a]*	2.25 ± 0.19[b]*	3.12 ± 0.33[b]* [c]*	1.46 ± 0.18[d] NS

Values are expressed as mean ± SD for six animals. Comparisons are made between, [a] Group II vs. Group I, [b] Group III and IV vs. Group II, [c] Group IV vs. Group III, [d] Group V vs. Group I

NS non-significant

* Statistical significance at $p < 0.05$

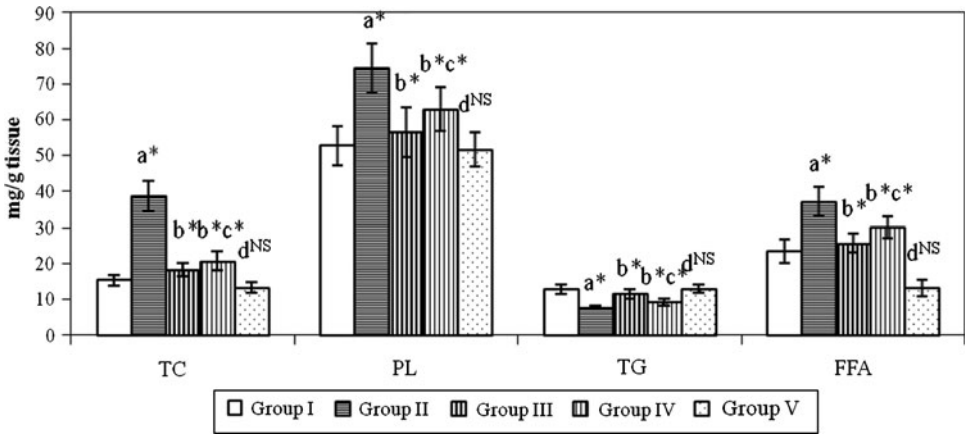

Fig. 1 Lipid profile in the liver of control and experimental animals. *TC* total cholesterol, *PL* phospholipids, *TG* triglycerides, *FFA* free fatty acids. Values are expressed as mean ± SD for six animals. Comparisons are made between, '*a*' Group II vs. Group I, '*b*' Group III and IV vs. Group II, '*c*' Group IV vs. Group III, '*d*' Group V vs. Group I. The symbol *asterisk* represents the statistical significance at $p < 0.05$, *NS* non-significant

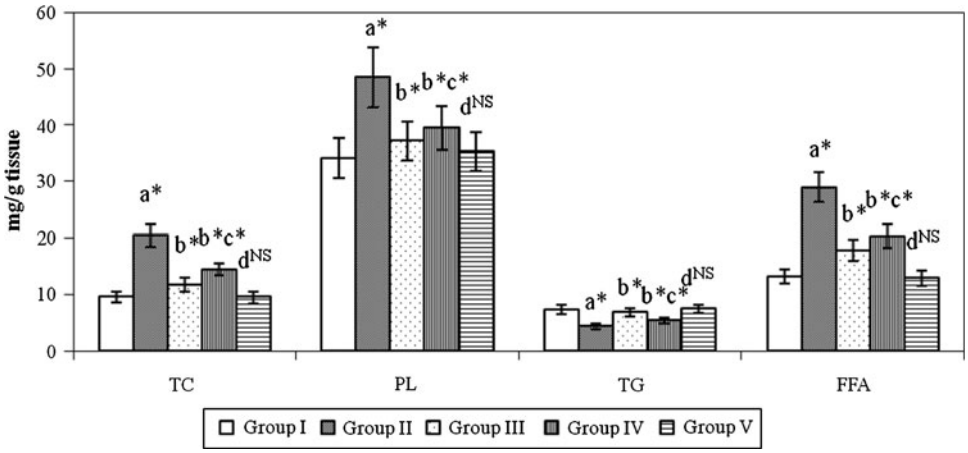

Fig. 2 Lipid profile in the kidney of control and experimental animals. *TC* total cholesterol, *PL* phospholipids, *TG* triglycerides, *FFA* free fatty acids. Values are expressed as mean ± SD for six animals. Comparisons are made between, '*a*' Group II vs. Group I, '*b*' Group III and IV vs. Group II, '*c*' Group IV vs. Group III, '*d*' Group V vs. Group I. The symbol *asterisk* represents the statistical significance at $p < 0.05$, *NS* non-significant

Table 2 Activities of lipid metabolizing enzymes in the liver of control and experimental animals

Parameters	Group I (Control)	Group II (STZ)	Group III (STZ + SA)	Group IV (STZ + Metformin)	Group V (SA)
Total lipase	9.55 ± 0.97	5.38 ± 0.59[a]*	8.39 ± 0.89[b]*	6.81 ± 0.73[b]* [c]*	9.14 ± 0.89[d] NS
LPL	11.28 ± 1.18	17.45 ± 1.9[a]*	12.68 ± 1.4[b]*	14.53 ± 1.51[b]* [c]*	10.94 ± 1.1[d] NS
CES	18.14 ± 2.01	26.52 ± 2.8[a]*	19.61 ± 2.3[b]*	22.43 ± 2.5[b]* [c]*	18.52 ± 1.96[d] NS
CEH	13.25 ± 1.4	19.19 ± 1.7[a]*	14.16 ± 1.35[b]*	17.24 ± 1.8[b]* [c]*	12.89 ± 1.18[d] NS
LCAT	15.38 ± 1.74	9.65 ± 0.98[a]*	13.41 ± 1.45[b]*	11.54 ± 1.24[b]* [c]*	15.34 ± 1.68[d] NS

Units: Total lipase—μmoles of *p*-nitrophenol liberate/h/mg protein, LPL—μmoles of free fatty acids liberated/h/mg protein, CES—μmoles of cholesterol esterified/h/mg protein, CEH—μmoles of cholesterol liberated/h/mg protein, LCAT—μmoles of cholesterol esterified/h/mg protein. Values are expressed as mean ± SD for six animals. Comparisons are made between, [a] Group II vs. Group I, *b* Group III and IV vs. Group II, [c] Group IV vs. Group III, [d] Group V vs. Group I

NS non-significant

* Statistical significance at $p < 0.05$

hydrolase (CEH) were found in liver and kidney of Group II animals, when compared to Group I animals, were as LCAT decreased significantly ($p < 0.05$) in both the tissues. Upon SA (Group II) and Metformin (Group IV) administration these altered values were significantly ($p < 0.05$) brought back to near normal levels. No difference was observed when Group V and Group I animals were compared.

Discussion

In Type 1 Diabetes, cardiovascular morbidity and mortality is two to four times higher than in non-diabetic population [26]. The insulin resistance-related factors including non-HDL and HDL-cholesterol and to a lesser extent HbA1c are related to the development of coronary artery disease [27]. The metabolism of different lipoproteins in Type 1

Table 3 Activities of lipid metabolizing enzymes in the kidney of control and experimental animals

Parameters	Group I (Control)	Group II (STZ)	Group III (STZ + SA)	Group IV (STZ + Metformin)	Group V (SA)
Total lipase	7.31 ± 0.74	13.62 ± 1.74[a]*	8.11 ± 0.83[b]*	10.47 ± 1.0[b]* [c]*	7.16 ± 0.69[d] NS
LPL	8.75 ± 0.88	16.15 ± 1.74[a]*	9.65 ± 0.98[b]*	13.31 ± 1.44[b]* [c]*	8.73 ± 0.86[d] NS
CES	15.86 ± 1.65	21.14 ± 2.23[a]*	17.10 ± 1.92[b]*	19.66 ± 2.14[b]* [c]*	15.18 ± 1.38[d] NS
CEH	10.63 ± 1.08	18.42 ± 1.94[a]*	11.96 ± 1.3[b]*	15.14 ± 1.63[b]* [c]*	10.95 ± 1.1[d] NS
LCAT	13.25 ± 1.4	6.31 ± 0.65[a]*	12.15 ± 1.3[b]*	9.68 ± 0.92[b]* [c]*	13.61 ± 1.44[d] NS

Units: Total lipase—μmoles of *p*-nitrophenol liberate/h/mg protein, LPL—μmoles of free fatty acids liberated/h/mg protein, CES—μmoles of cholesterol esterified/h/mg protein, CEH—μmoles of cholesterol liberated/h/mg protein, LCAT—μmoles of cholesterol esterified/h/mg protein. Values are expressed as mean ± SD for six animals. Comparisons are made between, [a] Group II vs. Group I, [b] Group III and IV vs. Group II, [c] Group IV vs. Group III, [d] Group V vs. Group I

NS non-significant

* Statistical significance at $p < 0.05$

Diabetes has been studied extensively which indicate abnormalities in every lipoprotein fraction [28].

The homeostatic balance between the intracellular free cholesterol and the esterified cholesterol maintains the amount of free cholesterol within the cell. The increased serum cholesterol seen in the present study may be due to the down regulation of the ABCG5 and ABCG8 sterol transporters in enterocytes and liver due to the deficiency of insulin, as reported earlier [29, 30].

Deficiency of insulin may be responsible for the increased LDL seen in the untreated diabetic rats. Oxidation of LDL results in lipid peroxidation and the subsequent formation of conjugated dienes and thiobarbituric acid reactive substances. Due to its impaired uptake and prolonged role in circulation, it exerts toxic effect [31]. Glycated LDL induces activation of signaling kinases such as protein kinase C or mitogen-activated protein kinases (MAPK) and enhances the DNA binding activities of several transcription factors such as NFκB, AP1 and STAT1/3 by increasing the formation of intracellular ROS [32]. The decreased LDL levels in the drug treated group may be due to the presence of flavonoids in SA, as flavonoids prevents lipid peroxidation and are potent inhibitors of the oxidative modification of LDL by macrophages [33]. SA may decrease the serum LDL by decreasing VLDL cholesterol synthesis and secretion as confirmed by Sharma et al. [34]. Since Metformin does not have the ability to scavenge free radicals due to the absence of flavonoids, administration of SA is considered to be more favorable than Metformin.

Insulin-dependent diabetes is often associated with an increased low-density lipoprotein (VLDL) fraction that is mostly of hepatic origin. The observed increase in serum VLDL in the untreated group may be due to the increased reuptake of VLDL from the liver or due to decreased affinity to LPL [35]. Reduced acyl Co-A cholesterol acyltransferase (ACAT) activity may lead to less cholesteryl ester available for VLDL packing, thereby causing decreased VLDL secretion from the liver. Low large and high small HDL plasma levels have been observed in adults with IGT or with Type 2 Diabetes mellitus [36]. The decreased serum HDL level in diabetic rats may be due to the decreased production of HDL by intestine and liver and or due to the glycation of HDL or its apoprotiens by high glucose rendering it more pro-atherogenic. Glycation of HDL increases its susceptibility to oxidation through reduced paraoxonase activity by glucose exposure [37] and lipoprotein C (LPC). SA may remove the excess cholesterol from peripheral tissues and prevent oxidative modification of LDL [34]. The concentration of triacyl glycerol in the serum is proportional to the susceptibility of lipoprotein to oxidation. The decreased triacyl glycerol in the liver and kidney tissues indicates increased mobilization of lipids from the tissues, decrease in fatty acid uptake and storage capacity [38].

Long term exposure of insulin-secreting pancreatic β cells to elevated concentrations of FFA alters glucose induced insulin secretion [39]. In addition, FFA decreases the activity of glucokinase, impairs glycolysis, increases the activity of acetyl CoA thus, increasing the glyconeogenic pathway [40]. Elevated fatty acid metabolites impair the insulin-substrate associated PI3-kinase activity and induce hepatic insulin resistance in transgenic mice with liver-specific overexpression of LPL. The increased FFA seen in untreated diabetic group might be due to defective lipogenesis or reduced LPL activity [41]. The decreased FFA in the drug treated group shows the lipid modulating activity of the SA. Insulin deficiency decreases the activity of LPL that results in hypertriglyceridemia and hypo LDL cholesterolemia. The decreased LPL catalytic activity seen in the untreated rats may be due to post-transcriptional/translational mechanisms leading to an accumulation of inactive LPL [42]. CEH is required to release free cholesterol from the intracellular stores of cholesterol esters and hydrolyze cholesterol esters. LCAT activity was found to be decreased in diabetic patients leading to

nonenzymatic glycation [43, 44]. The observed decrease in the lipid metabolizing enzymes and their increased activities after SA supplementation shows its favorable lipid modulating activity.

The effect of SA on controlled mobilization of serum TG, cholesterol and phospholipids is presumably mediated through controlling the tissue metabolism and improving the level of insulin secretion and action. The increased insulin out put in diabetic animals could activate LPL which hydrolyses LPL there by increasing the HDL fraction in the animals, lowering the intestinal absorption of cholesterol and enhancing the excretion of ingested cholesterol and inhibition of HMG CoA reductase [45]. In addition, the circulating flavonoids in plasma by the virtue of their high reactivity towards reactive oxygen species, ability to chelate transition metal cations [46] and regenerate α-tocopherol could contribute to the hypolipidemic activity of SA.

References

1. M.R. Taskinen, Diabetic dyslipidemia. Atheroscler. Suppl. **3**, 47–51 (2002)
2. R.S. Gray, D.C. Robbins, W. Wang, J.L. Yeh, R.R. Fabsitz, L.D. Cowan, T.K. Welty, E.T. Lee, R.M. Krauss, B.V. Howard, Relation of LDL size to the insulin resistance syndrome and coronary heart disease in American Indians. The Strong Heart Study. Arterioscler. Thromb. Vasc. Biol. **17**, 2713–2720 (1997)
3. M.N. Saraf, R.B. Ghooi, B.K. Patwardhan, Studies on the mechanism of action of *Semecarpus anacardium* in rheumatoid arthritis. J. Ethnopharmacol. **25**, 159–164 (1989)
4. K.M. Nadkarni, *Indian Materia Medica, Vol. I* (Popular Prakashan, Bombay, 1976), p. 1119
5. S.S.N. Murthy, New bioflavonoid from *Semecarpus anacardium* Linn. Clin. Acta Turc. **20**, 33–37 (1992)
6. T. Vijayalakshmi, V. Muthulakshmi, P. Sachdanandam, Effect of milk extract of *Semecarpus anacardium* nuts on glycohydrolases and lysosomal stability in adjuvant arthritis in rats. J. Ethnopharmacol. **58**, 1–8 (1997)
7. A.K. Sahoo, N. Narayanan, S. Sahana, S.S. Rajanb, P.K. Mukherjee, In vitro antioxidant potential of *Semecarpus anacardium* L. Pharmacologyonline **3**, 327–335 (2008)
8. S.G. Aravind, R. Arimboor, M. Rangan, N. Soumya, C. Madhavan, C. Arumughan, Semi-preparative HPLC preparation and HPTLC quantification of tetrahydroamentoflavone as marker in *Semecarpus anacardium* and its polyherbal formulations. J. Pharm. Biomed. Anal. **48**, 808–813 (2008)
9. Y.G. Shin, G.A. Cordell, Y. Dong, J.M. Pezzuto, A.V.N. Appa Rao, M. Ramesh, B. Ravi Kumar, M. Radhakishan, Rapid identification of cytotoxic alkenyl catechols in *Semecarpus anacardium* using bioassay-linked high performance liquid chromatography-electrospray/mass spectrometric analysis. Phytochem. Anal. **10**, 208–212 (1999)
10. P.K. Raveedran Nair, S.J. Melnick, S.F. Wnuk, M. Rapp, E. Escalon, C. Ramachandran, Isolation and characterization of an anticancer catechol compound from *Semecarpus anacardium*. J. Ethnopharmacol. **122**, 450–456 (2009)
11. B. Premalatha, P. Sachdanandam, *Semecarpus anacardium* L. Nut extract administration induces the in vivo antioxidant defence system in aflatoxin B1 mediated hepatocellular carcinoma. J. Ethnopharmacol. **6**, 131–139 (1999)
12. V.R. Ramprasath, P. Shanthi, P. Sachdanandam, Evaluation of antioxidant effect of *Semecarpus anacardium* Linn nut extract on the components of immune system in adjuvant arthritis. Vasc. Pharmacol. **42**, 179–186 (2005)
13. B. Arul, R. Kothai, A.J. Christina, Hypoglycemic and antihyperglycemic effect of *Semecarpus anacardium* Linn in normal and streptozotocin-induced diabetic rats. Methods Find. Exp. Clin. Pharmacol. **26**, 759–762 (2004)
14. C. Selvam, S.M. Jachak, A cyclooxygenase (COX) inhibitory biflavonoid from the seeds of *Semecarpus anacardium*. J. Ethnopharmacol. **95**, 209–212 (2004)
15. Formulary of Siddha Medicine, 2nd edn. (Indian Medicine Practitioners Co-operative Pharmacy and Stores Ltd., Madras, India, 1972), p. 197
16. D.E. Wilson, M.J. Spiger, A dual precipitation method for quantitative plasma lipoprotein measurement without ultracentrifugation. J. Lab. Clin. Med. **82**, 473–482 (1973)
17. A.C. Parekh, D.H. Jung, Cholesterol determination with ferric chloride-uranyl acetate and sulphuric acid ferrous sulphate reagents. Anal. Biochem. **42**, 1423–1427 (1970)
18. J. Folch, M. Lees, G.H. Sloane Stanley, A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. **226**, 497–509 (1957)
19. G. Rouser, S. Fkeischer, A. Yamamoto, Two dimensional then layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots. Lipids **5**, 494–496 (1970)
20. E.W. Rice, Triglycerides in Serum, in *Standard Methods of Clinical Chemistry*, 6th edn., ed. by P. Roderick, C.H. Mcdonald (Academic Press, New York, 1970), pp. 215–222
21. W.T. Hron, L.A. Menahan, A sensitive method for the determination of free fatty acids in plasma. J. Lipid Res. **22**, 377–381 (1981)
22. M. Bier, Enzymes of lipid metabolism. Lipases and esterases. Meth. Enzymol. **1**, 631–638 (1955)
23. A. Schmidt, Measurement of lipoprotein lipase and hepatic triglyceride lipase I human postheparin plasma. Meth. Enzymol. **72**, 325–337 (1974)
24. A. Legraud, R.J. Guillansseav, J. Land, Method colorimetric simole determination del'activit., de la lecithin cholesterol acyl transferase (LCAT) Plasmatique Interest on diabeteologic, in *Biologic Prospective*, ed. by G. Siest, M.M. Glateau, et al. (Masson, Paris, 1979), pp. 368–371
25. H.V. Kothari, B.F. Miller, D. Kritchevsky, Aortic cholesterol esterase characteristics of normal rat and rabbit enzymes. Biochem. Biophys. Acta **296**, 446–454 (1973)
26. K. Dahl-Jorgensen, J.R. Larsen, K.F. Hanssen, Atherosclerosis in childhood and adolescent type 1 diabetes: early disease, early treatment. Diabetologia **8**, 1445–1453 (2005)
27. S.S. Soedamah-Muthu, J.H. Fuller, H.E. Mulnier, V.S. Raleigh, R.A. Lawrenson, H.M. Colhoun, High risk of cardiovascular disease in patients with type 1 diabetes in the U.K.: a cohort study using the general practice research database. Diabetes Care **29**, 798–804 (2006)
28. K. Avramoglu, W. Qiu, K. Adeli, Mechanisms of metabolic dyslipidemia in insulin resistant states: deregulation of hepatic and intestinal lipoprotein secretion. Front. Biosci. **8**, d464–d476 (2003)
29. D.M. Maahs, A.K. Maniatis, K. Nadeau, R.P. Wadwa, K. McFann, G.J. Klingensmith, Total cholesterol and high-density lipoprotein levels in pediatric subjects with type 1 diabetes mellitus. J. Pediatr. **147**, 544–546 (2005)
30. L. Yu, J. Li-Hawkins, R.E. Hammer, K.E. Berge, J.D. Horton, J.C. Cohen, H.H. Hobbs, Overexpression of ABCG5 and ABCG8 promotes biliary cholesterol secretion and reduces fractional absorption of dietary cholesterol. J. Clin. Invest. **110**, 671–680 (2002)

31. R. Stocker, J. F. Keaney Jr, Role of oxidative modifications in Atherosclerosis. Physiol. Rev. **84**, 1381–1478 (2004)
32. J. Chen, J.L. Mehta, N. Haider, X. Zhang, J. Narula, D. Li, Role of caspases in Ox-LDL-induced apoptotic cascade in human coronary artery endothelial cells. Circ. Res. **94**, 370–376 (2004)
33. C.V. de Whalley, S.M. Rankin, J.R. Hoult, W. Jessup, G.M. Wilkins, J. Collard, D.S. Leake, Modification of low-density lipoproteins by flavonoids. Biochem. Soc. Trans. **18**, 1172–1173 (1990)
34. A. Sharma, R. Mathur, V.P. Dixit, Hypocholesterolemic activity of nut shell extract of *Semecarpus anacardium* (Bhilawa) in cholesterol fed rabbits. Indian J. Exp. Biol. **3**, 444–448 (1995)
35. N. Sambandam, M.A. Abrahani, S. Craig, O. Al-Atar, E. Jeon, B. Rodrigues, Metabolism of VLDL is increased in streptozotocin-induced diabetic rat hearts. Am. J. Physiol. Heart Circ. Physiol. **278**, H1874–H1882 (2000)
36. W.T. Garvey, S. Kwon, D. Zheng, S. Shaughnessy, P. Wallace, A. Hutto, K. Pugh, A.J. Jenkins, R.L. Klein, Y. Liao, Effects of insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear magnetic resonance. Diabetes **52**, 453–462 (2003)
37. C.C. Hedrick, S.R. Thorpe, M.X. Fu, C.M. Harper, J. Yoo, S.M. Kim, H. Wong, A.L. Peters, Glycation impairs high-density lipoprotein function. Diabetologia **43**, 312–320 (2000)
38. I. Ahmed, M.S. Lakhani, M. Gillett, A. John, H. Raza, Hypotriglyceridemic and hypocholesterolemic effects of anti-diabetic Momordica charantia (karela) fruit extract in streptozotocin-induced diabetic rats. Diabetes Res. Clin. Pract. **51**, 155–161 (2001)
39. J.D. McGarry, Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. Diabetes **51**(1), 7–18 (2002)
40. P. Iozzo, A.K. Turpeinen, T. Takala, V. Oikonen, J. Bergman, T. Grönroos, E. Ferrannini, P. Nuutila, J. Knuuti, Defective liver disposal of free fatty acids in patients with impaired glucose tolerance. J. Clin. Endocrinol. Metab. **89**, 3496–3502 (2004)
41. D. Pighin, L. Karabatas, C. Pastorale, E. Dascal, C. Carbone, A. Chicco, Y.B. Lombardo, J.C. Basabe, Role of lipids in the early developmental stages of experimental immune diabetes induced by multiple low-dose streptozotocin. J. Appl. Physiol. **8**, 1064–1069 (2005)
42. G. Shepherd, M.C. Cam, N. Sambandam, M.A. Abrahani, B. Rodrigues, Streptozotocin-induced diabetes enhances cardiac heparin-releasable lipoprotein lipase activity in spontaneously hypertensive rats. Hypertension **31**, 878–884 (1998)
43. A. Kiziltunc, F. Akçay, F. Polat, S. Kuşkay, Y.N. Sahin, Reduced lecithin: cholesterol acyltransferase (LCAT) and Na+, K+, ATPase activity in diabetic patients. Clin. Biochem. **30**(2), 177–182 (1997)
44. E. Nobecourt, M.J. Davies, B.E. Brown, L.K. Curtiss, D.J. Bonnet, F. Charlton, A.S. Januszewski, A.J. Jenkins, P.J. Barter, K.A. Rye, The impact of glycation on apolipoprotein A-I structure and its ability to activate lecithin: cholesterol acyltransferase. Diabetologia **50**, 643–653 (2007)
45. Y.B. Tripathi, R.S. Pandey, *Semecarpus anacardium* L, nuts inhibit lipopolysaccharide induced NO production in rat macrophages along with its hypolipidemic property. Indian J. Exp. Biol. **42**, 432–436 (2004)
46. H. El Hajji, E. Nkhili, V. Tomao, O. Dangles, Interactions of quercetin with iron and copper ions: complexation and autoxidation. Free Radic. Res. **40**, 303–320 (2006)