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Research Article

Assessment of Ficus Spp. in Improving the Metabolic Syndrome Secondary to Hypercholesterolemia in Rats Fed with High-Fat Diet

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ABSTRACT

The ethanolic extract of Ficus religiosa and F. microcarpa (Fam. Moraceae) as well as the hexane extract of F. microcarpa and F. mysorensis leaves have been examined for improving the metabolic syndrome secondary to hypercholesterolemia in rats. The evaluation was done through measuring hepatic glucose, glycogen, protein and vitamin C and E levels. The more pronounced extract will be phytochemically screened and identified for the most abundant compounds using PC, TLC, MS, IR and 1HNMR techniques. Rats fed with high-fat-diet and orally administered with cholesterol (30 mg/0.3ml 0.7% tween/animal) five times/week for nine consecutive weeks. It has been recorded a significant decrease ($p<0.001$) in hepatic glucose, glycogen, total protein, and vitamin E and C levels. Orally treatment with leaves extract (500mg/kg body weight) at the same time of cholesterol induction and with the same duration revealed an improvement of the selected parameters by variable degree. F. religiosa recorded the most potent effect. The screening of this plant revealed the presence of carbohydrate, amino acids, carotenoids, triterpenes, flavonoids, alkaloids, coumarins, tannins and saponins. In conclusion, the ethanol extract of F. religiosa leaves succeeded to improve the metabolic disturbance associated with hypercholesterolemia and recorded antioxidant effect.

Keywords: Ficus religiosa, Moraceae, hypercholesterolemia, metabolic disturbance.

INTRODUCTION

Hyperlipidaemia is one of the most important risk factors involved in the development of cardiovascular disease, obesity, cholestasis and overall mortality[1]. Lowering the lipids and cholesterol levels, by a drug, exercise or dietary interventions could reduce the risk of coronary heart diseases; however some patients cannot tolerate their adverse effects[2].

The metabolic syndrome develops in an individual with any three of the following risk factors: obesity, diabetes, inflammation, hypertension, dyslipidemia, and thrombosis[3]. The metabolic syndrome is more common in western societies than the underdeveloped countries. Individuals in western societies usually consume a high calorie diet that lacks essential nutrients. Moreover, the lifestyle of these societies is considered sedentary. These dietary and environmental factors coupled with the sedentary lifestyle predispose them to metabolic syndrome risk factors[3].

Recently, reducing blood LDL-cholesterol, inhibiting cholesterol synthesis[4,5] and blocking the absorption of dietary cholesterol[6] are of great challenge. Current interest in natural products has stimulated the search for new cholesterol-lowering agents from these sources[7,8].

Ficus is a genus of about 800 species of woody trees and shrubs collectively known as figs[9]. Abdel-Hameed[9] added that Ficus spp. (Moraceae) are considered as anticancer, anti-inflammatory, antioxidant and antihyperglycemic agents due to its being rich with phenolic compounds; especially flavonoids. Researches on Ficus has focused on its edible part (fruits) followed by aerial roots and barks, while the leaves are rarely studied[9]. Ficus religiosa has been reported as antibacterial[10], anticonvulsive[11], anti-diabetic[12] and antinephropathic[13].

The aim of the present work is to evaluate the role of some Ficus spp. for improving the metabolic syndrome secondary to hypercholesterolemia that are represented by disturbance in glucose and glycogen levels as well as the linked role of vitamins (E&C) associated with this disease.

MATERIALS AND METHODS

Plant material: Ficus microcarpa, F. religiosa and F. mysorensis fresh leaves were collected in May and June 2008, from Orman Garden, Giza, Egypt. Specimens of the plants were identified by Dr. Trease Labib Consultant of Plant Taxonomy at the Ministry of Agriculture and director of Orman Botanical Garden, Giza, Egypt. The collected leaves were air-dried, powdered and kept in tightly-closed containers until needed. Voucher specimens of Ficus leaves; FMiL, FRL and FMyL-2008, respectively were deposited at Pharmacognosy Dept., National Research Center, Cairo, Egypt as references.

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Table 1: Glucose level in normal and hypercholesterolemic rats treated with different *Ficus spp.* leaves extracts

Groups	Mean ± SD	% Change	% Improvement
Normal control	$17.11^{ab} \pm 1.39$	---	---
Hypercholesterolemia	$6.34^{e} \pm 1.04$	-62.94	---
Cont. + *F. microcarpa* ethanol ext.	$16.33^{abc} \pm 1.93$	-4.55	---
Chol. + *F. microcarpa* ethanol ext.	$13.65^{d} \pm 1.27$	-20.22	42.72
Cont. + *F. microcarpa* hexane ext.	$14.80^{bcd} \pm 1.20$	-13.50	---
Chol. + *F. microcarpa* hexane ext.	$14.50^{cd} \pm 1.43$	-15.25	47.69
Cont. + *F. religiosa* ethanol ext.	$16.49^{a} \pm 1.08$	-3.62	---
Chol. + *F. religiosa* ethanol ex	$15.31^{bcd} \pm 2.69$	-10.52	52.42
Cont + *F. mysorensis* hexane ext.	$16.45^{abc} \pm 1.36$	-3.85	---
Chol + *F. mysorensis* hexane ext.	$14.81^{abcd} \pm 1.52$	-13.44	49.50
	$15.23^{abcd} \pm 3.26$	-10.98	51.95

Data are means ±SD of eight rats in each group.
Glucose was expressed as mg/g tissue.
Statistical analysis is carried out using one way analysis of variance (ANOVA) using CoStat computer program.
Unshared superscript letters between groups are the significance values at p< 0.001.

Table 2: Glycogen level in normal and hypercholesterolemic rats treated with different *Ficus spp.* leaves extracts

Groups	Mean ± SD	% Change	% Improvement
Normal control	$12.93^{ef} \pm 1.33$	---	---
Hypercholesterolemia	$5.57^{h} \pm 0.80$	-56.92	---
Cont. + *F. microcarpa* ethanol ext.	$14.39^{bcd} \pm 0.79$	+10.48	---
Chol. + *F. microcarpa* ethanol ext.	$11.92^{fg} \pm 1.16$	-7.81	49.11
Cont. +*F microcarpa* hexane ext.	$17.08^{a} \pm 1.15$	+32.09	---
Chol. + *F.microcarpa* hexane ext.	$13.20^{cde} \pm 1.11$	+2.08	59.01
Cont. + *F.religiosa* ethanol ext.	$13.51^{b} \pm 0.78$	+4.48	--
Chol. + *F. religiosa* ethanol ext.	$15.61^{def} \pm 1.46$	+20.72	77.64
Cont + *F. mysorensis* hexane ext.	$14.74^{bc} \pm 0.84$	+13.99	---
Chol + *F. mysorensis* hexane ext.	$11.42^{g} \pm 1.14$	-11.67	45.24
Chol. +Drug	$12.15^{efg} \pm 1.26$	-6.03	50.88

Data are means ±SD of eight rats in each group.
Glycogen was expressed as mg/g tissue.
Statistical analysis is carried out using one way analysis of variance (ANOVA) using CoStat computer program.
Unshared superscript letters between groups are the significance values at p< 0.001.

Table 3: Protein level in normal and hypercholesterolemic rats treated with different *Ficus spp.* leaves extracts

Groups	Mean ± SD	% Change	% Improvement
Normal control	$152.40^{ab} \pm 5.00$	---	---
Hypercholesterolemia	$130.50^{e} \pm 4.70$	-14.37	---
Cont. + *F. microcarpa* ethanol ext.	$157.80^{a} \pm 6.30$	+3.54	---
Chol. + *F. microcarpa* ethanol ext.	$143.40^{bc} \pm 3.58$	-5.90	8.46
Cont. +*F microcarpa* hexane ext.	$154.40^{ab} \pm 6.00$	+1.29	---
Chol. + *F.microcarpa* hexane ext.	$148.50^{cd} \pm 4.47$	-2.55	11.81
Cont. + *F.religiosa* ethanol ext.	$154.80^{ab} \pm 6.20$	+1.57	---
Chol. + *F. religiosa* ethano	$149.00^{bc} \pm 6.20$	-2.23	12.13
Cont + *F. mysorensis* hexane ext.	$153.30^{ab} \pm 3.80$	+0.59	---
Chol + *F. mysorensis* hexane ext.	$147.00^{bc} \pm 4.44$	-3.54	10.82
Chol. +Drug	$139.00^{d} \pm 5.76$	-8.79	5.57

Data are means ±SD of eight rats in each group.
Protein was expressed as mg/g tissue.
Statistical analysis is carried out using one way analysis of variance (ANOVA) using CoStat computer program.
Unshared superscript letters between groups are the significance values at p< 0.001.

Leaves extraction: Air-dried powdered leaves of Ficus religiosa, Ficus microcarpa and Ficus mysorensis (500g) were extracted with 70% ethanol, yield 20.00, 22.00 and 10.00%, respectively. Other specimens (500g) were extracted with hexane yield 5.00, 10.00 and 5.00%, respectively. These extracts were evaporated to dryness under vacuum at 40°C for further determinations.

Plant selection: These six leaves extract were tested in our previous study[8] for their hypolipidemic effect by in vitro measuring the rate limiting enzyme of cholesterol biosynthesis; β-hydroxy- β- methyl glutaryl Co A

Table 4.Vitamin C level in normal and hypercholesterolemic rats treated with different *Ficus spp.* leaves extracts

Groups	Mean ± SD	% Change	% Improvement
Normal control	2.61^{e}± 0.35	---	---
Hypercholesterolemia	1.73^{f}± 0.24	-33.72	---
Cont. + *F. microcarpa* ethanol ext.	3.87^{b}± 0.68	+48.27	---
Chol. + *F. microcarpa* ethanol ext.	2.55^{e}± 0.24	-2.29	31.41
Cont. +*F microcarpa* hexane ext.	2.72^{de}± 0.25	+4.21	---
Chol. + *F.microcarpa* hexane ext.	2.97^{cd}± 0.29	+13.79	47.50
Cont. + *F. religiosa* ethanol ext.	2. 69^{de}± 0.25	+3.06	---
Chol. + *F. religiosa* ethanol ext.	3.21^{c}± 0.31	+22.98	56.70
Cont + *F. mysorensis* hexane ex	4.57^{a}± 0.76	+75.09	---
Chol + *F. mysorensis* hexane ext.	2.48^{de}± 0.18	-4.98	28.73
Chol. +Drug	2. 29^{e}± 0.22	-12.26	21.45

Data are means ±SD of eight rats in each group.
Vitamin C was expressed as µg/mg protein.
Statistical analysis is carried out using one way analysis of variance (ANOVA) using CoStat computer program.
Unshared superscript letters between groups are the significance values at p< 0.001.

Table 5.Vitamin E level in normal and hypercholesterolemic rats treated with different *Ficus spp.* leaves extracts

Groups	Mean ± SD	% Change	% Improvement
Normal control	2.45d^{e}± 0.26	---	---
Hypercholesterolemia	1.77^{f}± 0.29	-27.75	---
Cont. + *F. microcarpa* ethanol ext.	2. 89^{b}± 0.39	+17.95	---
Chol. + *F. microcarpa* ethanol ext.	2.08d^{e}± 0.28	-15.10	12.65
Cont. +*F. microcarpa* hexane ext.	2. 29^{de}± 0.32	-6.53	---
Chol. + *F. microcarpa* hexane ext.	2.25^{cd}± 0.27	-8.16	19.59
Cont. + *F.religiosa* ethanol ext.	2.36^{de}± 0.24	-3.67	---
Chol. + *F. religiosa* ethanol ext.	2. 89^{c}± 0.38	+17.95	45.71
Cont + *F. mysorensis* hexane ext.	1.83^{a}± 0.29	-25.30	---
Chol + *F. mysorensis* hexane ext.	2.34^{de}± 0.33	-4.48	23.26
Chol. +Drug	2.35^{e}± 0.43	-4.08	23.67

Data are means ±SD of eight rats in each group.
Vitamin E was expressed as µg/mg protein.
Statistical analysis is carried out using one way analysis of variance (ANOVA) using CoStat computer program.
Unshared superscript letters between groups are the significance values at p< 0.001.

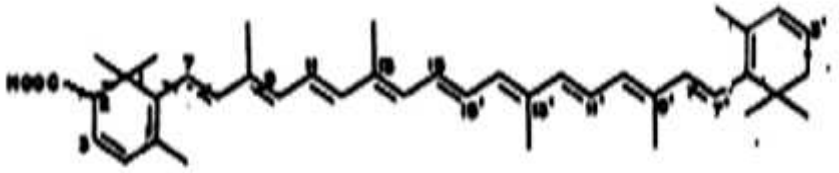

Fig.1 Dehydro-β-carotene-2-carboxylic acid

reductase. In addition, the same extracts were evaluated in vivo for their hypolipidemic effect through measuring the lipid profile of hypercholesterolemis rats fed with high fat diet[5,7]. The most potent extracts for their in vitro and in vivo hypolipedemic effect will be evaluated for improving the metabolic syndrome secondary to hypercholesterolemia that are represented by disturbance in glucose and glycogen levels as well as the linked role of vitamins (E&C) associated with this disease. These extracts were the ethanolic extract of F. religiosa and F. microcarpa as well as the hexane extract of F. microcarpa and F. mysorensis. The most potent extract will be phytochemically screened and identification of the most abundant compounds have been carried out using PC, TLC, MS, IR and ^{1}HNMR techniques.

Animals: Male Wistar strain albino rats (100: 120g) were selected for this study. They were obtained from the animal house, National Research Center, Egypt. All animals were kept in a control environment of air and temp (25–30oC) with access of water and diet ad libitum. Animals were left 14 days in this environment for acclimatization. Anesthetic procedures complied with the ethical guidelines of the Ethical Committee of the Federal Legislation and National Institutes of Health Guidelines in USA were approved by the Medical Ethical Committee of the National Research Centre in Egypt.

Table 6. Phytochemical screening of *Ficus religiosa* (L) leaves extract

Constituents	Prevalence
Carbohydrates	++
Carotenoids	++
Protein	++
Sterols& or triterpenes	-
Flavonoids	+
Alkaloids & or nitrogenous compounds	+
Coumarins	+
Tannins	+
Saponins	++

(+): Present.
(-): Absent.
(++): Appreciably present.

Table 7: Analysis of carbohydrate contents of *Ficus religiosa* leaves

Authentic sugars	Rt (min)	**%** sugar
Glucuronic acid	-	5.87
Galacturonic acid	5.91	-
Glucose	7.17	3.59
Xylose	8.40	6.85
Galactose	8.50	-
Fructose	9.67	-
Arabinose	9.70	-
Unidentified	11.16	-
Total		10.44

Nutrition: Control groups were fed with standard diet (El-Kahira Co. for Oil and Soap) which consisted of (g/kg) caseine: 240.0; dl-methionine: 2.0; groundnut vegetable oil: 1.0; mineral mixture 40.0; vitamin mix: 80.0; and cornstarch: 658.0.

Cholesterol and cholesterol treated groups were fed with standard diet containing 150 g sheep grease per kg diet[14].

Administration regimens: Administration protocol was five times in a week for nine consecutive weeks. Leaves extract were administrated orally at a dose 500 mg/kg body weight[15]. Cholesterol (Sigma, US) was orally given at a dose 30 mg/0.3 ml of 0.7% tween/ animal[16]. Lipanthyl drug (Mina Pharm., Egypt) was orally given at a dose 50 mg/kg body weight[17]. The dose of lipanthyl drug was calibrated to exactly contain 50mg of fenofibrate/kg body weight.

Animal grouping: Eighty eight male rats were divided into eleven groups (eight rats each) as follows: Group 1: normal healthy control rats. Groups 2–5: normal healthy rats administrated with leaves extract. Group 6: cholesterol administrated group. Groups 7–10: cholesterol treated groups; forced with cholesterol and leaves extract. Group 11: rats treated with cholesterol and lipanthyl drug at the same time and for the same duration.

Sample preparations: Liver tissue was homogenized in normal physiological saline solution (0.9% NaCl) (1:9w/v). The homogenate was centrifuged at 4oC for 5 min at 3000 rpm. The supernatant was used for estimation of hepatic protein, glucose, glycogen and vitamins E and C.

Biochemical assays: Liver total protein was assayed according to Bradford[18]. The Coomassie Brilliant blue dye reacts with Bradford reagent to give a blue complex which is measured colorimetrically at 595 nm. Hepatic glucose was estimated colorimetrically at 505 nm by the method of Trinder[19]. Hepatic glycogen content was estimated by the method of Nichoals et al. [20], the green color formed was read at 610 nm against blank. The method adapted by Jogata and Dani[21] was used for estimation of hepatic vitamin C using Folin reagent and the developed colour was read at 760 nm. Vitamin E was measured by the colourimetric assay of Angustin et al.[22]. The method is based on the oxidation of xylene-extracted tocopherols of the liver homogenate by ferric chloride and the pink complex of ferrous ions, bathophenanthoroline, was measured at 536 nm.

Phytochemical screening: The ethanol extract of Ficus religiosa (L) extract was chemically screened for sterols and triterpenes[23], flavonoids[24], carbohydrates and tannins[25], coumarins[26] and saponins[27], protein and carotenoids[28]. The rich abundant contents (carotenoids, carbohydrates and protein) will be further examined using PC, TLC, MS, IR and 1HNMR techniques.

Investigation of the carotenoids content: The ethanolic extract of Ficus religiosa (L) was subjected to PC examination for the detection of carotenoid using Whatmann No. 1 sheets with the two developing solvent systems n- butanol - acetic acid-water (4: 2: 1 v/v) and acetic acid- water (15: 85 v/v). The chromatograms were examined under UV light before and after exposure to ammonia vapor and spraying with AlCl3 solution; no change in the color of the spots detected in the extract which eliminates the possibility of flavonoid presence of the selected spot. On the other hand, the chloroformic

extract of the pigment gave blue color with Antimony trichloride in CHCl3. Also, the chloroformic extract of the isolated pigment gave dark blue color with conc. H2SO4 at the junction between two solutions which distinguish it from anthocyanin pigment[28]. The Rf values in the fore-mentioned systems are 0.83 and 0.91, respectively. Further purification of the carotenoid carried out by TLC (silica gel, Fluka) using different solvent systems pet. ether (40-60°C)-benzene (9:1), benzene: ETOAc 8:2 & ether -n-hexane (3:7) and the Rf values were 0.88,0.75 and 0.64, respectively. Estimation of the pigment has been carried out by applying 1g of the crude extract on PC (3MM) using the above mentioned solvent systems. The carotenoid band had been eluted and weighed.

Identification of carotenoid pigment: The pigment gave orange color in day light & dark color under UV. The purified pigment was subjected to UV spectral analysis, MS⁄ MS (Data dependent mixture triple play technique), IR and 1HNMR determinations. The spectroscopic data of these compounds were compared with the published data[29].

Investigation of the carbohydrate content: The isolated carbohydrate was neutral to litmus paper indicating the absence of uronic acid units. Also it gave negative gel formation test upon testing with 2% potassium hydroxide indicating that the isolated substances is mucilage and eliminating the presence of pectin.

For HPLC analysis, 5 mg of hydrolysate of Ficus religiosa as well as of individual authentic reference sugars were separately dissolved in 1 ml of deionized water. Samples (75µl) had been injected into HPLC. Qualitative identification of the hydrolysate was carried out by comparing the retention time of peaks with those of authentic compounds, while the quantitative determination was carried out based on the peak area measurement of the HPLC chromatograms[30].

HPLC analysis of amino acids: HPLC analysis was used to determine amino acids of crude extract of Ficus religiosa L. The analysis was performed on a model Eppendorf-Germany LC 3000 Amino Acid Analyzer. The flow rate was 0.2 ml/ min, the pressure of buffer was from 0 to 50 bars, the pressure of reagent was from 0 to 150 bars and the reaction temperature was 50°C.

Statistical analysis: All data were expressed as mean ± SD of eight rats in each group. Statistical analysis was carried out using one-way analysis of variance (ANOVA) by Costat Computer program.

RESULTS

Hepatic glucose, glycogen and protein level in normal and hypercholesterolemic rats: Normal control rats recorded insignificant changes in glucose and protein levels after treatment hypercholesterolemic rats with F. microcarpa ethanol extract, F. microcarpa hexane extract, F. religiosa ethanol extract and F. mysorensis hexane (Tables 1 and 3), while glycogen recorded significant increase after treatment with different extracts under investigation (Table 2). In hyperlipidemic rats, the hepatic glucose, glycogen and protein levels were significantly decreased by 62.94, 56.92 and 14.37%, respectively (Tables1, 2 and 3).

Table 8: Total amino acids of the crude extract of *Ficus religiosa* L. leaves

Amino Acids	Relative %
Essential amino acids	
Threonine	0.159
Valine	0.513
Isoleucine	0.423
Leucine	0.219
Phenylalanine	0.248
Lysine	0.456
Non-essential amino acids	
Aspartic acid	0.890
Glutamic acid	0.299
Glycine	0.621
Histidine	0.15 1
Arginine	0.329
Alanine	0.114
Proline	5
Tyrosine	1

- *Total amino ac are expressed in g /0.5g of the crude extract.*

Treatment of hypercholesterolemic rats with the ethanolic extract of F. microcarpa improved glucose, glycogen and protein levels by 42.72, 49.11 and 8.46%, respectively. Treatment with F. microcarpa hexane extract showed enhancement by 47.69, 59.01 and 11.81%, respectively. The ethanol extract F. religiosa recorded amelioration in glucose, glycogen and protein levels by 52.42, 77.64 and 12.13%, respectively, while F. mysorensis hexane extract showed improvement by 49.50, 45.42 and 10.82%, respectively (Tables 1, 2 and 3). Treatment with lipanthyl drug recorded improvement in glucose, glycogen and protein levels by 51.95, 50.88 and 5.57%, respectively.

Vitamins C and E in normal and hypercholesterolemic rats: Normal control rats recorded insignificant changes in vitamin C and E levels after treatment with F. microcarpa hexane extract and F. religiosa ethanol extract, while significant changes were observed after treatment with F. microcarpa ethanol extract and F. mysorensis hexane extract (Table 3 and 4). Hypercholesterolemic rats recorded significant decrease in vitamin C and E levels by 33.72 and 27.75%, respectively (Tables 4 and 5). Vitamin C recorded improvement after treatment of hypelipidemic rats with F. microcarpa ethanol extract, F. microcarpa hexane extract, F. religiosa ethanol extract and F. mysorensis hexane extract by 31.41, 47.50, 56.70 and 28.73%, respectively (Table 4). Treatment with lipanthyl drug recorded improvement by 21.45%. Hyperlipidemic rats recoded enhancement in vitamin E level after treatment with F. microcarpa ethanol extract, F. microcarpa hexane extract, F. religiosa ethanol extract and F. mysorensis hexane extract by 12.65, 19.59, 45.71 and 23.26%, respectively (Table 5). Treatment with lipanthyl drug recorded improvement by 23.67%.

Phytochemical screening of Ficus religiosa leaves ethanol extract: Phytochemical screening of the ethanol extract of Ficus religiosa leaves revealed the presence of carbohydrates, flavonoids, coumarins, saponins and tannins (Table 6).

Carotenoid contents of Ficus religiosa leaves ethanol extract: One gram of the crude extract applied to PC (3MM) using the above mentioned solvent systems yield 6% carotenoid pigment.

UV-visible absorption spectrum of the carotenoid pigment: The UV-visible absorption spectrum for the isolated pigment in methanol gives three absorption maxima at 466, 493, 523.

MS⁄MS spectrum of the carotenoid pigment: The mass spectrum of the pigment displayed an [M+] at m/z 576, corresponding to molecular formula C41H5202 with a high degree of unsaturation. A peak at m/z 470 (M-106) is characteristic of a carotenoid[29]. Identification of a carboxylic group present in the pigment is concluded from the fragments (532) M-44 and (492) M-84. The peak at m/z (520) M-56 represents one cyclic end of pigment which does not have any substitution, whereas the peaks at m/z M-68, (492) M-84 and (411) M-165 correspond to the other cyclic end carrying a carboxylic group at position 2. Similarly, the peaks at (442) M-134 represent fragment originating from the noncarboxylic end of the pigment.

IR spectrum of the carotenoid pigment: The IR spectrum of the isolated pigment indicated the presence of a carboxylic group characterized by the peaks at 3424.96 for OH group, 1741.41 for carbonyl group, 29232.56 for C-H aliphatic stretching and 1636.30 for C=C olefinic bonds stretching.

^{1}HNMR data of the carotenoid pigment: The ^{1}HNMR spectrum of the pigment (300 MHz in CDCl3) recorded olefinic protons at δ 5-7 regions and methyl groups and aliphatic protons at δ 0.8 to 2.6 regions, respectively. The four sets of 18 olefinic hydrogens and their chemical shifts are as follows: δ 6.5 to 6.7 8 integrating for six hydrogens, 6.3 to 6.45 for four hydrogens, 6.05 to 6.28 for six hydrogens, and 5.35 to 5.5 8 for two hydrogens.

From the four mentioned spectral data we can conclude that the identified pigment is a polar carotenoid, it is a C40 carotenoid with a molecular weight of 576 and with one carboxylated end and it has olefinic double bonds. By comparing the above spectral analysis with the reported data of Medicharla et al.[29], it could be concluded that this pigment is: Dehydro-β-carotene-2-carboxylic acid (Fig.1).

Carbohydrate contents of Ficus religiosa leaves extract: Acid hydrolysis of cold hydrolysate of Ficus religiosa L. leaves analyzed by HPLC revealed the presence of xylose (3.59%) and glucose (6.85%) (Table 7).

Total amino acids contents of Ficus religiosa L. leaves extract: The percentage of total protein of the crude extract was found to be 1.50% w/w of the crude extract. Table 8 showed that proline (5%) is the most abundant amino acid followed by tyrosine (1%), glysine (0.89%) and aspartic acids (0.621%). The amounts of essential amino acids are small compared with those of the non-essential amino acids.

DISCUSSION

As hyperlipidemia is mainly associated with obesity, diabetes and at certain time with thrombosis and hypertension, therefore a condition of metabolic syndrome is established[3]. The present results revealed that hypercholesterolemia is associated with a diabetic condition through the observed significant decrease in hepatic glucose. The depletion of hepatic glucose is mainly due to the higher blood glucose level. Treatment of different extracts ameliorates this disturbance in glucose level by variable degrees with more potent effect of F. religiosa. These results run parallel with the reported hypoglycemic effect of Morus spp[31]. Liver glycogen level may be considered as the best marker for assessing anti-hyperglycemic activity of any drug[32]. In the present study, the decrease in hepatic glycogen of hypercholesterolemic rats are in accordance with the finding of[31] and support the suggestion of increased glucose output during diabetes associated with dislipidemia. Several investigators have attributed hepatic glycogen loss to the loss of glycogen synthetase activating system in diabetic animals[33] and/or increased activity of glycogen phosphorylase and glucose -6- phosphatase[31-33]. In our study, the observed decrease in glycogen content may be attributed to the enhanced glycogen breakdown, decreased glucokinase and increased glucose-6-phosphatase activity. During oxidative stress, auto-oxidation and the presence of an excess of hydroxyl radical damaged carbohydrate contents[34]. These effects are regarded as an important risk factor in the acceleration of chronic diseases[35] and give an additional support of the disturbance in glucose and glycogen level.

Protein, as one of the major structural and functional component of the cell membrane, is the target of oxidative modification by free radicals. There is extensive evidence that lipid peroxidation and protein oxidation lead to loss of membrane integrity, an important factor in acceleration of chronic diseases[36]. In the present study, hypercholesterolemic rats recorded significant decrease in hepatic protein level. Romero et al.[37] showed that increase in serum total protein content can be deemed as a useful index of the severity of cellular dysfunction in liver diseases. This finding supports the role of the observed reduction in hepatic protein content. Treatment with different extracts enhanced the protein level. This was in line with the finding of Sharma and Shukla[38] who stated that stimulation of protein synthesis is a contributory self healing mechanism, which accelerates the regeneration process in the liver.

With regard to vitamin C and coinciding with the present results, Frei et al.[39] reported that peroxyl radicals are trapped by ascorbate and thus the level of the enzyme and vitamin decreased during the free radical scavenging process. Also, the reduction of vitamin E after oxidative stress state occurs since the vitamin acts as a soluble antioxidant to protect biological membranes against oxidative stress which leads to maintenance of cell function[40]. In addition, Palsamy et al.[41] observed significant reduction in Vit. C and E in diabetic patients and rats.

The phytochemical screening of Ficus extract supports its role as antioxidant agent. In accordance with this observation, Abdel-Hameed[9] postulated the presence of phenolic compounds, flavonoids, stilbenes and 2-arylbenzofurans in the aerial root and stem bark of some Ficus spp. In addition, Sudhahar et al.[42] mentioned that

triterpenes have antihyperlipidaemic, antiatherosclerotic, anti-inflammatory and antioxidant activity. Moreover, saponins exhibit a hypocholesterolaemic effect by both improving lipid profile and modulating oxidative stress[43].

Carotenoids present in F. religiosa support our observations in improving the metabolic disorder associated with hypercholesterolemia. Sluijs et al.[44] found that consumption of carotenoids (beta-carotene, alpha-carotene, and lycopene) may help improve certain risk factors involved in metabolic syndrome. The same author added that, higher carotenoid intake was linked to smaller waistlines, less belly fat, lower measures of adiposity, and lower levels of triglycerides.

The present results revealed the presence of different amino acids in F. religiosa extract. Recent research of Hamed et al.[45] discussed the role of essential and non-essential amino acids in the living body where, threonine balances the protein level in the body and promotes the immune system. It aids in the synthesis of glycine and serine; two amino acids that help in the production of collagen, elastin and muscle tissue. It also speeds up wound healing after injury by boosting the immune system. Threonine, in combination with the amino acids aspartic acid and methionine, helps liver digest fat and fatty acids, a process that reduces the accumulation of fat in the liver. Histidine is important for the synthesis of red and white blood cells. Alanine removes toxic substances released from the breakdown of muscle proteins during intensive exercise. Glutamine and aspartic acid aid the functioning of all cells, RNA and DNA (the carriers of genetic code). Ad-ditional benefit of aspartic acid is the protection of the liver from damages that can be caused by excess am-monia in the bloodstream. Proline plays a role in intracellular signaling.

F. religiosa ethanol extract also revealed the presence of glucuronic acid (5.87%), glucose (3.59%) and xylose (6.85%). Glucuronic acid is often linked to the xenobiotic metabolism of substances resulting from glucuronidation are known as glucuronides (or glucuronosides) and are typically much more water-soluble than the non-glucuronic acid-containing substance from which they were originally synthesized[46]. The human body uses glucuronidation to make a large variety of substances more water-soluble, and, in this way, allow for their subsequent elimination from the body. Sometimes toxic substances are also less toxic after glucuronidation[47]. Jiang et al.[46] found that glucose, arabinose, xylose, galactose and galacturonic acid extracted and fractioned from the roots of Coptis Chinensis recorded antidiabetic, antioxidant and antihypercholesterolemic effects in diabetic mice induced by high-fat diet and injected with streptozotocin. Cao et al.[48] added that a mixture of mannose, arabinose, galactose, xylose, and rhamnose isolated from Lentinus edodes Mycelia had a potential antitumor material for laryngeal carcinoma.

In conclusion, Ficus microcarpa, Ficus religiosa and Ficus mysorensis improved the metabolic disturbance secondary to hypercholesterolemia and recorded antioxidant effect. Ficus religiosa ethanol extract showed the most potent effect due to the presence of carbohydrate, amino acids, carotenoids, triterpenes, flavonoids, alkaloids, coumarins, tannins and saponins.

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