

Therapeutic effect of L-carnitine on sialic acid, soluble fas (sFas) and other biochemical variables in hyperinsulinemic rats

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Abstract

Background. Rats fed “high-fructose diet” (60 g/100 g diet) (FRU) were used as a model of insulin resistance in these context. **Objective.** The aim of this study was to evaluate the modulator effect of L-carnitine (CAR) on oxidative stress, lipid profile, inflammatory (sialic acid) and apoptotic (soluble Fas) markers in blood of rats fed “high-fructose diet”. **Materials.** Male Wistar rats of body weight 120 – 160 g were divided into 5 groups of 6 rats each. Groups 1 and 2 animals received starch as control diet, while groups 3, 4 and 5 rats were fed a “high-fructose diet”. Groups 2, 4 and 5 animals additionally received intraperitoneal CAR (300 mg/kg body weight) for 30 days. Group 4 rats received CAR on first day of the experiment (to study the prophylactic effect), while group 5 rats received CAR on after 15th day of the experiment (to study the therapeutic effect of CAR). **Methods.** At the end of the experimental period, levels of glucose, insulin, lipid profile, sialic acid and sFas in serum and glycosylated Hb (HbA1c) in whole blood were determined, in addition total antioxidant capacity (TAC) and malondialdehyde (MDA) were assayed. **Results.** Fructose feeding to rats caused significant elevations in the serum levels of glucose, insulin, triacylglycerol, HDL-c, sialic acid, sFas and MDA, while the level of serum TAC was significantly reduced as compared to controls. Intraperitoneal administration of CAR to fructose-fed rats, alleviated the effect of fructose and these rats showed near normal levels of the parameters studied. **Conclusion.** Exogenous CAR to fructose-fed rats improves insulin resistance, reduces lipo- and glucotoxicity and attenuates oxidative stress, inflammatory and apoptotic markers. These effects suggest that CAR supplementation may have some benefits in patients suffering from insulin resistance. [Life Science Journal. 2009; 6(2): 87 – 93] (ISSN: 1097 – 8135).

Keywords: L-carnitine (CAR); fructose-fed rats; insulin resistance; sialic acid; soluble fas

1 Introduction

Rats consuming a “high-fructose diet” induces insulin resistance accompanied by deleterious metabolic consequences including hyperinsulinemia, hyperglycemia, glucose intolerance, hypertriglyceridemia and hypertension in rats and these metabolic effects are similar to those observed in the human multimetabolic syndrome X and in which a cluster of disorders are described^[1,2]. Studies

have reported that hyperglycemia and insulin resistance could also promote inflammation by increased oxidative stress^[3,4] and alteration in lipid metabolism in rat tissues^[5].

Furthermore, the serum sialic acid (N-acetyl neuraminic acid) concentration is one of the markers for acute-phase response^[6] and an inflammatory marker and predictor of microvascular complications in type 2 diabetic patients^[7].

Inflammatory and resident cells (endothelial and vascular smooth muscle cells) release different proteins (sFas and sFas L) that can generate a chronic inflammatory re-

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sponse and to play an important role in apoptotic signaling^[8]. Oxidative stress is a critical part of the apoptotic agent, antioxidants can inhibit or delay oxidative stress induced apoptosis^[9].

L-carnitine (CAR, β -hydroxy- γ -trimethyl amino butyrate), has been described as a conditionally essential nutrient for humans. It is formed by biosynthesis from lysine and methionine in the body. CAR may act as antioxidant either having a primary antioxidant activity (inhibiting free radical generation, scavenging the initiating free radicals, and terminating the radical propagation reactions) or more likely, functioning as a secondary antioxidant (repairing oxidized polyunsaturated fatty acids esterified in membrane phospholipids^[10]). CAR functions to transport long-chain fatty acids across the inner mitochondrial membrane into the matrix for β -oxidation and has effects on oxidative metabolism of glucose in tissues^[11]. Considering all these, the present study was to evaluate the therapeutic effect of CAR on oxidant-antioxidant balance, lipid profile, inflammatory (sialic acid) and apoptotic (sFas) markers in blood of rats fed "high-fructose diet".

2 Materials and Methods

2.1 Chemicals and drugs

CAR, other chemicals and solvents were of analytical grade and were purchased from Sigma Chemical Company.

Animals and treatment: Adult male Wistar rats of body weight ranging from 120 – 160 g were used in this study. They were purchased from the breeding unit of the Egyptian Organization for Biological Products and Vaccines (Helwan, Egypt). They were housed 2/cage under controlled condition 12 h light/12 h dark cycle. All animals received standard pellet diet for one week and water ad libitum. The procedure used in this study was approved by National Institute of Diabetes and Endocrinology (NIDE), Cairo, Egypt.

Insulin resistance was induced by feeding high-fructose diet (60 g/100 g). After acclimatization, the animals were divided into 5 groups consisting of 6 rats each and were maintained as follows:

Group 1: CON/control animals received the control diet containing corn starch (60%) as a sole source of carbohydrate and tap water ad libitum.

Group 2: (CON + CAR)/control animals received the control diet and intraperitoneal CAR (300 mg/kg body wt/day).

Group 3: FRU/fructose-fed animals received the fructose enriched diet (60%) and tap water ad libitum.

Group 4: (FRU + CAR on 1st day)/fructose-fed animals received the fructose-diet and intraperitoneal CAR (300 mg/kg body wt/day) on 1st day of experimental period (prophylactic or prevention effect).

Group 5: (FRU + CAR on 16th day)/fructose-fed animals received the fructose-diet and intraperitoneal CAR (300 mg/kg body wt/day) on 16th day of experimental period (therapeutic effect).

The diet composition was given in Table 1. The animals were maintained in their respective groups for 30 days and body weight changes were measured weekly.

Table 1. Composition of diet (g/100 g)^[5]

Ingredients	Control diet	High-fructose diet
Corn starch	60	–
Fructose	–	60
Casein (fat free)	20	20
Methionine	0.7	0.7
Groundnut oil	5	5
Wheat bran	10.6	10.6
Salt mixture*	3.5	3.5
Vitamin mixture**	0.2	0.2

*: The composition of mineral mix (g/kg) contained 30.5 g MgSO₄·7H₂O, 65.2 g NaCl, 105.7 g KCl, 200.2 g KH₂PO₄, 3.65 g MgCO₃, 38.8 g Mg(OH)₂·3H₂O, 40.0 g FeC₆H₅O₇·5H₂O, 512.4 g CaCO₃, 0.8 g KI, 0.9 g NaF, 1.4 g CuSO₄·5H₂O, 0.4 g MnSO₄ and 0.05 g CONH₃. **: One kilogram of vitamin mix contained 3.0 g thiamine mononitrate, 3.0 g riboflavin, 3.5 g pyridoxine HCl, 15 g nicotinamide, 8.0 g d-calcium pantothenate, 1.0 g folic acid, 0.1 g d-biotin, 5 mg cyanocobalamin, 0.6 g vitamin A acetate, 25 g α -tocopherol acetate and 10 g chlorine chloride.

2.2 Blood sampling and processing

On day 29th, rats were fasted overnight, about 4 ml of blood was drawn from the animal retina under ether anesthesia. A part of blood sample was taken on EDTA as whole blood sample for determination of glycosylated hemoglobin (HbA1c) using Chromatographic–Spectrophotometric methods^[12]. Another part of blood collected was taken on a plain tube without anticoagulant for separation of serum by centrifugation at 3000 r.p.m. for 10 min. Serum glucose was assayed by glucose oxidase method^[13], total cholesterol was determined by the enzymatic method^[14], triacylglycerol was assayed by peroxidase-coupled method^[15] and HDL-c by the enzymatic method after precipitation of other lipoproteins with MgCl₂ and dextran sulphate^[16]. LDL-c was calculated by the Friedwald formula^[17]. Atherogenic index was calculated from ratio of total cholesterol/HDL-c^[18]. The remaining part of serum was stored at – 80 °C until

analysis of insulin, malondialdehyde (MDA), sialic acid, total antioxidant capacity (TAC) and sFas. Levels of serum insulin was assayed by monoclonal immunoradiometric assay using kit supplied by Diagnostic Products Corporation (DPC)^[19]. Lipid peroxidation product, MDA concentration was determined fluorometrically with excitation at 515 nm and emission at 550 nm after isobutyl alcohol extraction^[20]. Serum sialic acid was measured by a spectrophotometric assay^[21]. Total antioxidant capacity was determined according to the method of Koracevic *et al*^[22]. Plasma sFas concentration was assessed using the enzyme-linked immunosorbent assay (ELISA) kit (Research & Diagnostic Systems, Minneapolis, U.S.A.) according to the manufacturer's instructions. Model Assessment (HOMA) correlates positively with insulin-resistance, and was calculated according to Matthews *et al*^[23].

2.3 Statistical analysis

All results were expressed as the mean \pm SD. Statistical analysis was performed with Statistical Package for the Social Science for Windows (SPSS, version 11.0, Chicago, IL, USA). The data were analyzed by one-way analysis of variance (ANOVA). To compare the difference among the groups, post hoc testing was performed by the Bonferroni test. Pearson's correlation analysis was used to determine the correlation among the parameters assessed. The *P*-value less than 0.05 was considered statistically significant^[24].

3 Results

The body weights of the animals increased progressively during the experimental period. The percentage change in the body weight gain in rats of studied groups did not vary significantly as compared to controls.

The levels of serum glucose, insulin, HOMA index and glycosylated hemoglobin are shown in Table 2. There were significant elevations in glucose, insulin levels and HOMA index after 30 days of fructose-fed rats

(FRU group) ($P < 0.0001$ for each) compared to control animals.

CAR supplementation to fructose-fed rats (group 5) caused a significant reduction in the levels of glucose, insulin and HOMA index ($P < 0.0001$ for each) as compared to fructose diet fed rats, while administration of CAR from the first day of the experiment (group 4) has no statistically effect on insulin level but it induced pronounced decrease in serum glucose level ($P < 0.003$) and HOMA index ($P < 0.0001$) compared to FRU-group. Rats fed control diet, CAR administration had no marked effects on the studied parameters.

Table 3 demonstrates that high-fructose diet (FRU) induced marked elevation in serum level of triacylglycerol which amounted (161.0 ± 1.5 , $P < 0.0001$) as compared to control diet fed rats. Whereas the levels of cholesterol, low density lipoprotein cholesterol (LDL-c) and atherogenic index did not show any significant alteration in FRU group compared to control rats. Significantly decreases in triacylglycerol and atherogenic index ($P < 0.003$, 0.005 respectively) and marked increase in HDL-c level ($P < 0.005$) were observed in group 5 as compared to group 3. The level of triacylglycerol in group 4 showed slight significant decrease ($P < 0.01$) as compared to group 3. For animals fed control diet, exogenous CAR caused a significant reduction in atherogenic index only ($P < 0.003$) as compared to control group.

Fed high-fructose diet to rats induced significant increases in serum levels of sialic acid (1.75 ± 0.51), sFas (27.6 ± 1.9) and MDA (15.33 ± 2.4) ($P < 0.0001$ for each), while TAC level represent a marked reduction (0.53 ± 0.22 , $P < 0.0001$) as compared to rats fed control diet (Table 4). In fructose-fed rats the serum sialic acid levels positively correlated with serum triacylglycerol ($r = 0.048$), and serum insulin ($r = 0.342$), while negative correlation was observed between sialic acid and not only glucose ($r = -0.386$), but also sFas ($r = -0.468$). Good correlation was observed between sialic acid and HDL-c ($r = 0.939$, $P = 0.005$) in high fructose feed rats (Figure 1). The significant negative correlation was ob-

Table 2. Serum levels of glucose, insulin, HOMA index and HbA1c in control and experimental animals (Means \pm SD)

Parameters	Experimental groups				
	1	2	3	4	5
F. Glucose (mg/dl)	83.8 \pm 19.0	66.3 \pm 17.6	134.2 \pm 17.4 ^a	92.8 \pm 12.7 ^b	66.0 \pm 15.2 ^c
Insulin (μ IU/ml)	16.0 \pm 2.3	14.2 \pm 1.9	35.8 \pm 4.4 ^a	32.5 \pm 4.3	21.3 \pm 3.3 ^c
HOMA index	3.23 \pm 0.76	2.26 \pm 0.40	11.82 \pm 2.75 ^a	7.64 \pm 0.92 ^c	3.38 \pm 0.68 ^c
HbA1c (%)	4.2 \pm 0.12	4.1 \pm 0.12	4.3 \pm 0.1	4.2 \pm 0.24	4.2 \pm 0.098

^a: $P < 0.0001$ vs. CON, ^b: $P < 0.003$ vs. FRU, ^c: $P < 0.0001$ vs. FRU.

Table 3. Serum lipid profile in control and experimental animals (Means ± SD)

Parameters	Experimental groups				
	1	2	3	4	5
Cholesterol (mg/dl)	70.7± 7.2	62.0 ± 7.4	81.5 ± 10.8	75.2 ± 9.7	70.7 ± 10.4
Triacylglycerol (mg/dl)	69.2± 7.1	90.2 ± 13.1	161.6 ± 60.5 a	104.7 ± 25.5 b	98.0 ± 14.2 c
HDL-c (mg/dl)	31.2± 1.2	34.2 ± 1.7	37.2 ± 1.5 d	38.3 ± 3.6	43.0 ± 3.9 e
LDL-c (mg/dl)	25.8 ± 1.3	24.0 ± 0.63	26.7 ± 1.9	25.2 ± 2.4	26.2 ± 2.0
T. cholesterol/HDL-c	2.3 ± 0.23	1.8 ± 0.18 d	2.18 ± 0.29	1.97 ± 0.14	1.65 ± 0.27 e

^a: $P < 0.0001$ vs. CON, ^b: $P < 0.01$ vs. FRU, ^c: $P < 0.003$ vs. FRU, ^d: $P < 0.003$ vs. CON, ^e: $P < 0.005$ vs. FRU

Table 4. Serum levels of sialic acid, sFas, TAC and MDA in control and experimental animals (Means ± SD)

Parameters	Experimental groups				
	1	2	3	4	5
Sialic acid (mmol/L)	0.32± 0.067	0.25 ± 0.055	1.75 ± 0.51a	0.76 ± 0.31	0.35 ± 0.06 b
sFas (ng/ml)	19.1 ± 1.1	16.2 ± 1.8	27.6 ± 1.9 a	23.8 ± 2.1	17.2 ± 3.5 b, e
TAC (mM/L)	0.82± 0.068	0.69 ± 0.085	0.53 ± 0.22 a	0.69 ± 0.071	0.75± 0.095 c
MDA (nmol/ml)	7.56± 1.57	8.32 ± 2.68	15.33 ± 2.4 a	10.8 ± 2.97 d	5.85± 1.62 b

^a: $P < 0.0001$ vs. CON, ^b: $P < 0.0001$ vs. FRU, ^c: $P < 0.024$ vs. FRU, ^d: $P < 0.008$ vs. FRU, ^e: $P < 0.008$ vs. FRU + CAR on 1st day.

served between sFas and triacylglycerol in FRU group ($r = -0.832$, $P = 0.04$) (Figure 2). Intraperitoneal injection of CAR to fructose-fed rats improved these parameters toward the control levels after 2 weeks of treatment. In fructose-fed rats treated with CAR on 16th day, there was negative correlation between sialic acid and triacylglycerol ($r = -0.583$), glucose ($r = -0.106$) and insulin ($r = -0.72$), while sFas level correlated with serum sialic acid ($r = 0.435$), fasting glucose ($r = 0.200$) and negative correlation with HDL-c ($r = -0.98$), also there was a positive correlations between serum insulin significantly positive correlated with HDL-c level ($r = 0.928$, $P = 0.008$) (Figure 3).

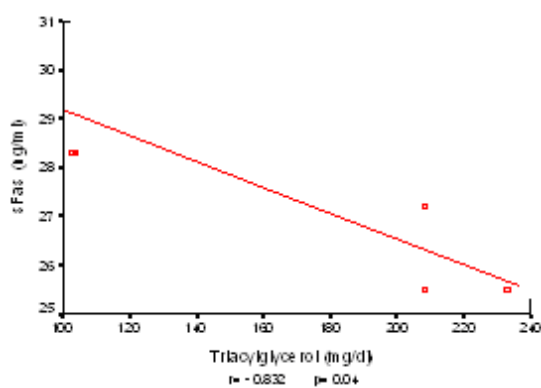


Figure 2. Correlation between serum sFas and serum triacylglycerol in fructose- fed rats.

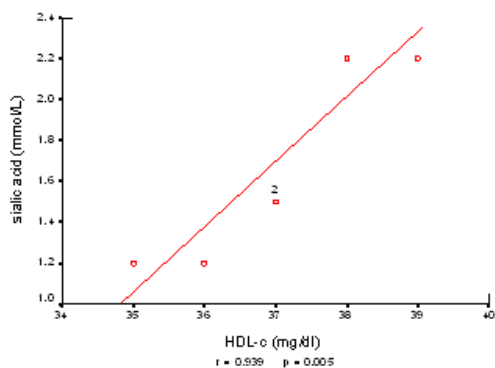


Figure 1. Correlation between serum sialic acid and HDL-c in fructose-fed rats.

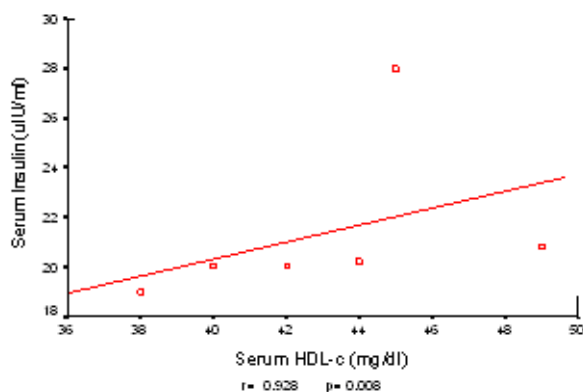


Figure 3. Correlation between serum insulin and HDL-c in fructose-fed rats treated with CAR.

4 Discussion

The development of insulin resistance in high fructose fed rats is well documented in the literature^[2], and has been established in the present study. The degree of insulin resistance was higher in fructose-fed rats (FUR group) as indicated by significant elevation of serum glucose, insulin levels and higher value of HOMA index as compared with control fed rats. Insulin resistance has been attributed to low level of insulin-stimulated glucose oxidation due to modifications in the post-receptor cascade of insulin action^[25]. Several metabolic hypotheses has been advanced to explain insulin resistance in fructose-fed rats. It has been shown that chronic fructose feeding alters the activity of several enzymes regulating hepatic carbohydrate metabolism, including decreasing the activity of glucokinase and increasing glucose-6-phosphatase activity leading to hepatic insulin resistance^[26].

The present study demonstrated non significant changes in HbA1c in the studied groups, this may be due to insufficient of time duration of the experiment. Rajasekar et al^[27] illustrate that rats fed high-fructose diet for six weeks showed significant alterations in the RBC membrane composition, which membrane-bound ATPases were significantly lower while MDA and lipid hydroperoxide were significantly higher in high fructose fed rats than those of control rats. Consequently, this explain the unaffected HbA1c level in FRU group. High fructose fed rats reduced the adverse effects of fructose load on glucose and insulin levels, where glucose and HOMA were improved. CAR reduces intramitochondrial acyl-CoA/CoA ratio, promotes oxidative glucose utilization, lowers intracellular glucose levels, and improves insulin sensitivity^[5].

Varying the type of dietary carbohydrate has a profound influence on insulin action^[28] and could cause changes in the metabolism of lipids^[29]. Fructose when fed as the sole source of dietary carbohydrate induces dyslipidaemia which observed in the present study. The results revealed significant elevation of serum levels of triacylglycerol and HDL-c. These results may be attributed to the induction of various lipogenic enzymes by feeding fructose and can be associated with impaired insulin action. In this study, there were no significant alterations in total cholesterol and LDL-c concentration in fructose-fed rats compared to control diet fed animals. These findings are consistent with those of Dai and McNeill^[30] who found no significant alteration in serum cholesterol in fructose-fed rats. These results agree with those of Nandhini et al^[29]. On the contrary, Hallfrisch et al^[31] demonstrated higher plasma cholesterol and LDL-c

levels in rats fed fructose for 4 weeks. Nandhini et al^[29] reported that hypertriglyceridemia may be due to reduction of lipoprotein lipase activity which is an important enzyme responsible for hydrolysis of triglyceride, this leads to hypertriglyceridemia.

In the present study, there was impairment of the antioxidant defense system in rats fed high-fructose diet. This indicated by pronounced decrease in serum total antioxidant capacity (TAC), while lipid peroxidation as MDA was statistically increased compared to control rats. These findings are in agreement with the suggestion of Ceriello et al^[32] who found that acute hyperglycemia provokes oxidative capacity. It is well known that fructose itself can create oxidative stress by its metabolism^[5]. They reported also that in fructose-fed rats, free radical production can be enhanced during hyperinsulinemia and hyperglycemia by mechanisms such as autoxidation of glucose, enhanced glycation, and altered polyol pathway^[33]. These free radicals can catalyzed lipid peroxidation. Other potential mechanisms of oxidative stress include the reduction of antioxidant defense, MDA is one of the well-known secondary products of lipid peroxidation after exposure to reactive oxygen species and free radicals, it may be used to evaluate oxidative damage^[34].

The dyslipidemia and production of free radicals resulting from fructose-fed rats was attenuated by CAR supplementation in the present study. The intraperitoneal injection of CAR to fructose-fed rats improved the antioxidant defense system by increasing the total antioxidant capacity and decreased the MDA levels toward the normal values. This improvement is more pronounced in the group 5 than the values of group 4 of the experimental period.

Reduction of MDA may be due to the enhancement of the fatty acid transport by CAR into mitochondria for energy production, thereby lowering the availability of lipids for peroxidation. The modulatory effect of CAR on dyslipidemia was indicated by decreased the triacylglycerol level and increased the HDL-c level significantly compared with FUR group. The rise in serum HDL-c concentration by CAR treated rats after two weeks of fructose feeding (group 5) (therapeutic effect) may be due to delayed clearance and increased synthesis of HDL constituents, stimulation of lipoprotein lipase levels to rise in HDL production and reduction of VLDL constituents^[29]. In fructose-fed rats treated with CAR (group 5), serum insulin significantly positive correlated with HDL-c level (Figure 3).

Rajasekar and Anuradha^[5] reported that exogenous CAR improves insulin sensitivity, reduces both lipo and gluco-toxicity and attenuated oxidative stress in skeletal

muscle. The benefits could be attributed to its effect on glucose disposal, antioxidative mechanisms and lipid profile and oxidative stress after two weeks of fructose-fed rats. As mention above, under certain conditions increased generation of ROS and/or reduction of the antioxidative capacity leads to enhanced ROS activity and oxidative stress which cause cellular injury and tissue damage by promoting several cellular reactions (e.g. lipid peroxidation, DNA damage)^[35,36].

The present results revealed that feeding high-fructose diet to rats induced significant elevations in the levels of both inflammatory (sialic acid) and apoptotic (sFas) markers compared to those fed control diet. With regard to the result of sFas, its increasing level may be associated with hypertension which induced by chronic fructose feeding: this results was in agreement of reports of Park *et al*^[3] who reported that rats fed high-fructose diet represent an animal model for insulin resistance and hypertension. Ceriello *et al*^[37] reported that high serum glucose may produce myocardial damage and cardiac cell apoptosis through a formation of nitrotyrosine, which leads to increase the fas/fas-ligand system in uncontrolled apoptosis.

It has been reported that sFas concentration are increased and sFas-L are decreased in subjects of high cardiovascular risk compared with healthy subjects and also demonstrated that sFas and sFas-L concentrations are related to different cardiovascular risk factors such as diabetes, metabolic syndrome or hypertension, suggesting that these proteins may be novel markers of vascular injury^[38,39]. The significant negative correlation was observed between sFas and triacylglycerol in fructose-fed rats (Figure 2).

With respect to the inflammatory marker, the present research indicates that there is a significant increase in serum sialic acid concentration in rats fed high fructose compare to control diet fed rats. Sialic acid is a component of cell membranes^[11] and elevated levels may indicate excessive cell membrane damage but more specifically to the cells of vascular tissue. Sialic acid can be used as a measurement of acute phase response because many of the proteins of the immune response are actually glycoproteins which have sialic acid as the terminal sugar of their oligosaccharide chain^[40]. In high fructose feeding rats, good correlation was observed between sialic acid and important cardiovascular risk factors such as HDL-c. Several research studies have shown that the concentration of sialic acid in serum is elevated in pathological states when there is damage to tissue, tissue proliferation and inflammations^[41].

5 Conclusion

It is concluded that intraperitoneal administration of CAR to fructose-fed rats alleviated the oxidative stress, lipid profile, inflammatory (sialic acid), apoptotic (sFas) markers and may be helpful in overcoming of these abnormalities as therapeutic effect.

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