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Protective Effect of L-carnitine on Metabolic Disorders, Oxidative Stress, Antioxidant Status and Inflammation in a Rat Model of Insulin Resistance

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ABSTRACT

The high-fructose diet induces insulin resistance, hyperinsulinaemia, hyperglycemia, alterations in lipid metabolism and oxidative stress in rat tissues which Oxidative stress plays a vital role in pathology associated with insulin resistance. The present study was aimed to explore the effect of L-carnitine (CAR) on insulin resistance, inflammation, oxidative stress, antioxidant status and lipid metabolism in male rats fed with high fructose diet. Insulin resistance was induced by feeding high fructose diet (60 g/100 g). Sixty male albino rats were divided into four groups containing 15 rats each. Group I: (Control group) rats received the control diets. Group II: (fructose-fed group) rats received fructose-enriched diet (60 g/100 g diet). Group III: (fructose+CAR group) animals received high fructose diet and were administered CAR (300 mg kg^{-1} b.wt. day^{-1}, orally). Group IV: (control+CAR group) rats received the control diet and were administered CAR. After 45 and 60 days of treatment blood samples and liver tissue were collected for determination of serum glucose, insulin, insulin resistance, pyruvate, lactate, leptin, total cholesterol, triacylglycerols, phospholipids, free fatty acids, sialic acid, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and nitric oxide in addition to L-malondialdehyde (L-MDA). Moreover, antioxidant enzymes (SOD,CAT and GPx) in liver tissues were also determined. The obtained results revealed that, high fructose diet induce a significant increased in serum glucose, insulin, insulin resistance, lipid profiles, pyruvate, lactate, lipten, TNF-α, IL-6, sialic acid, MDA and nitric oxide concentrations and decrease serum phospholipids with marked reduction in CAT, SOD and GpX activities in liver tissues compared to rats fed normal diet. L-carnitine treatment to high fructose fed rats reduced the effects of fructose and associated with significant normalization of all serum parameters level and was able to improve dyslipidemia, inflammation and insulin resistance, attenuated the increased MDA and enhanced antioxidant status in liver tissues. These results suggest that, L-carnitine is effective in improving the high fructose induced oxidative stress, inflammation and insulin resistance in male rats. Also, the administration of of L-carnitine to rats fed a high fructose diet prevents the development of oxidative stress and its associated complications include hyperglycemia, hyperinsulinemia and dyslipidemia.

Key words: L-carnitine, high fructose diet, insulin resistance, oxidative stress, inflammatory markers

INTRODUCTION

Rats consuming a high-fructose diet induces insulin resistance accompanied by deleterious metabolic consequences including hyperinsulinemia, hyperglycemia, glucose intolerance,

hypertriglyceridemia and hypertension and these metabolic effects are similar to those observed in the human multi metabolic syndrome X and in which a cluster of disorders are described. Hyperglycemia and insulin resistance could also promote inflammation by increased oxidative stress and alteration in lipid metabolism in rat tissues (Rajasekar and Anuradha, 2007).

Fructose metabolism occurs in the liver, which has a great capacity to uptake and phosphorylates it. This nutrient can be transformed into glucose and glycogen but this pathway is very inefficient. So, the liver choice is to produce pyruvate, which is transferred to mitochondria and is transformed in fatty acids. These fatty acids are used as the mainly liver energy source, stored as triglycerides depots or released in the blood stream as VLDL and NEFA. This characteristic makes fructose a highly lipogenic nutrient (Botezelli *et al*., 2012). The influx of triglycerides into hepatocytes leads to an overproduction of reactive oxygen species by beta-oxidation, which causes an anti-oxidant/oxidant imbalance. The elevation of pro-oxidant species causes membrane and DNA damage and the inactivation of some regulatory proteins, which causes tissue inflammation and induces insulin resistance, apoptosis, cellular mutations and other effects (Lelliott and Vidal-Puig, 2004).

Moreover, insulin resistance in rat feeding high fructose diet is associated with an over abundance and higher ectopic lipid deposition of lipids especially FFA and TG in liver, muscle and adipose tissue which may generate toxic lipid-derived metabolites, such as diacylglycerol, fatty acyl CoA and ceramides. The presence of these metabolites in the intracellular environment leads to a higher serine/thre onine phosphorylation of insulin receptor substrate-1 (IRS-1), which has been shown to reduce insulin signaling (Shulman, 2000).

Carnitine (β-hydroxy-γ-trimethyl amino butyrate), has been described as a conditionally essential nutrient for humans. CAR may acts 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 (Liu *et al*., 2004). Moreover, CAR 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 (Broderick *et al*., 1992). Accordingly, this study was performed to investigate the ameliorative effect of L-carnitine on glucose, insulin resistance, lipid metabolism, biomarkers of oxidative stress, antioxidant status and some inflammatory markers in rats fed high-fructose diet for 60 days.

MATERIALS AND METHODS

Experimental animals: Sixty white male albino rats of 8-10 weeks old and weighting 150-200 g were used in this study. Rats were housed in separated metal cages and kept at constant environmental and nutritional conditions throughout the period of experiment. The animals were fed on constant ration and water was supplied *ad libitum*.

Chemicals and drugs used: All chemicals were of analytical grade and obtained from standard commercial suppliers. The drug and chemicals used in the present study were:

- Fructose was obtained as bottle contains (D (+) fructose) 250 g in the Crystalline form. It was manufactured by El Nasr Pharmaceutical company and purchased from El-Gomhouria Co. For Trading Chemicals, Medicines And Medical Appliances, Egypt. Rats fed fructose-enriched diet daily (60 g/100 g diet) for 60 days (Rajasekar *et al*., 2005)

- L-carnitine was obtained as a capsule form (one capsule contains 350 mg L-carnitine) and manufactured by MEPACO (Arab Co. For Pharmaceuticals & Medicinal Plant). Carnitine was dissolved in Propylene glycol and administered to rats at a dose of (300 mg kg^{-1} b.wt. day^{-1}, orally) for 60 days

Ration and additives: There are two type of ration were prepared freshly daily throughout the course of experiment:

- A control ration (g/100 g)
- High fructose ration (60/100g of control diet)

The composition of two type of ration is presented in Table 1.

Experimental design: After acclimatization, the animals were divided into four groups containing 15 rats each, placed in individual cages and classified as follows:

- **Group I (control group):** Rats received control diet, served as control non-treated for all experimental groups
- **Group II (fructose-fed group):** Rats received fructose enriched diet (60 g fructose/100 g diet) for 60 days
- **Group III (fructose+L-carnitine group):** Rats received daily fructose enriched diet (60 g fructose/100 g of diet) and were administered L-carnitine (300 mg kg^{-1} b.wt. day^{-1}, orally) for 60 days
- **Group IV (control+L-carnitine group):** Rats received the control diet and were administered L-carnitine for 60 days

Table 1: Composition of control diet (g 100 g^{-1}) (NRC, 1995)

Ingredient	Control diet	High fructose diet
Fructose	-	60
Soya bean meal (44% C.P. or 49% C.P)	24	24
Ground yellow corn	36.3	36.3
Ground whole wheat	22	22
Wheat bran	10	10
Soya bean oil	3	3
Ca carbonate	0.5	0.5
Salt NaCl	1	1
Dry yeast	1	1
Mineral and vit mixture *	2	2
Methionine	0.2	0.2

*The composition of mineral mix (g kg^{-1}) contained 30.5 g $MgSO_4$·$7H_2O$, 65.2 g NaCl, 105.7 g Kcl, 200.2 g KH_2PO_4, 3.65 g $MgCO_3$, 38.8 g Mg $(OH)_2$·$3H_2O$, 40.0 g $FeC_6H_5O_7$·$5H_2O$, 512.4 g $CaCO_3$, 0.8 g KI, 0.9 g NaF, 1.4 g $CuSO_4$·$5H_2O$, 0.4 g $MnSO_4$ and 0.05 g $CONH_3$. 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 pantothenat, 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 choline chloride

Sampling: Random blood samples and liver tissue specimens were collected from all animals groups (control and experimental groups) two times along the duration of experiment at 45 and 60 days from the onset of L-carnitine administration.

Blood samples: Blood samples for serum separation were collected by ocular vein puncture after overnight fasting in dry, clean and screw capped tubes and serum were separated by centrifugation at 2500 rpm for 15 min to processed serum who used directly for glucose determination and then kept in a deep freeze at -20°C until used for subsequent biochemical analysis.

Liver tissue samples: At the end of the each experimental period, rats were sacrificed by cervical decapitation. The liver specimen was quickly removed and weighted, then perfused with cold saline to exclude the blood cells and then blotted on filter paper and stored at -20°C. Briefly, liver tissues were cut, weighed and minced into small pieces, homogenized with a glass homogenizer in 9 volume of ice-cold 0.05 mM potassium phosphate buffer (pH 7.4) to make 10% homogenates. The homogenates were centrifuged at 5,000 rpm for 15 min at 4°C then the supernatant was used for the determination of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx).

Biochemical analysis: Serum glucose, insulin, insulin resistance, total cholesterol, triacylglycerols (TG), phospholipids, Free Fatty Acids (FFA), pyruvate, lactate, sialic acid, L-Malondialdehyde (L-MDA), Leptin, Tumor necrosis factor-alpha (TNF-α), Interleukin-6 (IL-6) and Nitric Oxide (NO), liver superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were determined using methods described by Trinder (1969), Wilson and Miles (1977), Matthews *et al.* (1985), Meiattini *et al.* (1978), Bucolo and David (1973), Takayama *et al.* (1977), Matsubara *et al.* (1983), Sutherland *et al.* (1995), Noll (1988), Simpson *et al.* (1993), Ohkawa *et al.* (1979) and Beall *et al.* (1992), DRG® Interleukin-6 (rat) (EIA-NO.4845) (Montgomery and Dymock, 1961; Nishikimi *et al.*, 1972; Aebi, 1984; Fossati *et al.*, 1980; Paglia and Valentine, 1967).

Statistical analysis: The obtained data were statistically analyzed by one-way analysis of variance (ANOVA) followed by the Duncan multiple test. All analyses were performed using the statistical package for social science. The 20 values of $p<0.05$ were considered to be significant.

RESULTS

The obtained data in Table 2 revealed a significant increase in serum glucose, insulin, insulin resistance index, pyruvate, lactate and leptin concentrations in rats feeding High-fructose diet all over the periods of the experiments compared to rats fed control diet. Administration of L-carnitine in rats fed high fructose diet resulted in a significant decrease in the concentrations of serum glucose, insulin, insulin resistance index, pyruvate and lactate with a non significant decrease in serum leptin level all over the experimental periods as compared to untreated fructose-fed rats.

The obtained data presented in Table 3 revealed a significant increased in the concentrations of serum total cholesterol, triacylglycerols, NEFA, sialic acid, IL-6 and TNF-α with significant decrease in phospholipids concentration in rats feeding High-fructose diet all over the periods of the experiments compared to rats fed control diet. L-carnitine treatment to rats fed high fructose diet resulted in a significant decrease in serum total cholesterol, triacylglycerols, NEFA, sialic acid, IL-6 and TNF-α concentrations with a significant increase in serum phospholipids all over the periods of the experiment as compared to untreated fructose-fed rats.

Table 2: Effect of L-carnitine administration on some serum biochemical parameters in high fructose fed rats and their control

	Glucose (mg dL^{-1})		Insulin (μU mL^{-1})		IR	
	45	60	45	60	45	60
Animal groups	(Days)					
Control	90.00±1.81^{c}	89.20±2.42^{b}	25.39±0.80^{b}	25.46±0.49^{c}	5.65±0.25^{b}	5.62±0.23^{b}
Fructose	113.80±4.57^{a}	117.80±7.36^{a}	33.11±1.42^{a}	31.91±2.12^{a}	9.37±0.66^{a}	9.41±1.13^{a}
Fructose+L-CAR	95.30±3.46^{bc}	95.40±2.80^{b}	30.84±1.90^{a}	26.27±1.61^{bc}	7.29±0.58^{ab}	6.23±0.54^{b}
Control+L-CAR	110.20±8.87^{ab}	98.20±1.24^{b}	29.94±2.03^{ab}	30.82±1.75^{ab}	8.31±1.15^{a}	7.47±0.41^{ab}
	Pyruvate (mmol dL^{-1})		Lactate (mmol dL^{-1})		Leptin (pg mL^{-1})	
	45	60	45	60	45	60
Animal groups	(Days)					
Control	78.68±4.40^{b}	69.09±2.25^{c}	4.27±0.03^{a}	4.20±0.09^{b}	134.46±3.81^{c}	133.98±14.12^{b}
Fructose	94.86±1.34^{a}	94.99±6.20^{a}	4.57±0.05^{a}	4.64±0.06^{a}	181.24±6.40^{a}	178.34±5.87^{a}
Fructose+L-CAR	76.28±3.01^{b}	78.80±2.317^{bc}	3.62±0.37^{b}	4.31±0.04^{b}	161.94±6.02^{ab}	169.75±2.80^{a}
Control+L-CAR	83.92±5.88^{ab}	85.61±4.49^{ab}	4.45±0.05^{a}	4.60±0.03^{a}	143.41±12.19^{bc}	168.84±6.73^{a}

Data are presented as (Mean±SE), SE: Standard error, Mean values with different superscript letters in the same column are significantly different at $p<0.05$

Table 3: Effect of L-carnitine administration on serum lipid profile and inflammatory markers in high fructose fed rats and their control

	T. cholestrol (mg dL^{-1})		Triacylglycerols (mg dL^{-1})		Phospholipids (mg dL^{-1})		FFA (MmEq L^{-1})	
	45	60	45	60	45	60	45	60
Animal groups	(Days)							
Control	108.60±2.18^{b}	108.40±1.21^{bc}	110.00±4.02^{b}	108.±2.35^{b}	102.77±5.36^{ab}	123.02±6.42^{ab}	264.04±14.14^{c}	264.69±4.81^{c}
Fructose	124.20±3.44^{a}	123.40±1.08^{a}	137.80±6.37^{a}	160.0±7.36^{a}	91.57±6.01^{b}	91.91±3.51^{c}	336.20±10.37^{a}	337.30±11.60^{a}
Fructose+L-CAR	111.60±2.01^{b}	105.00±1.70^{c}	108.00±2.0^{bc}	111.0±2.83^{b}	111.24±1.73^{a}	127.95±2.10^{a}	284.28±12.85^{bc}	301.03±5.99^{b}
Control+L-CAR	111.00±2.83^{b}	111.60±1.83^{b}	95.90±3.43^{c}	109.0±2.21^{b}	105.16±8.27^{ab}	110.03±4.66^{b}	318.00±7.49^{ab}	319.60±10.08^{ab}
	Sialic acid (mg dL^{-1})		IL-6 (pg mL^{-1})		TNF-α (pg mL^{-1})	
	45	60	45	60	45	60
Animal groups	(Days)					
Control	26.56±1.27^{b}	26.89±1.57^{b}	62.53±4.40^{c}	78.58±3.07^{ab}	54.83±3.60^{b}	46.38±1.67^{c}
Fructose	39.75±1.17^{a}	35.68±1.78^{a}	105.22±3.58^{a}	97.53±7.84^{a}	73.72±3.62^{a}	65.56±1.30^{a}
Fructose+L-CAR	28.19±2.26^{b}	28.95±1.27^{b}	81.73±5.16^{b}	66.37±9.89^{b}	65.19±2.92^{ab}	57.34±0.71^{b}
Control+L-CAR	32.12±2.13^{b}	38.32±1.70^{a}	75.43±5.34^{bc}	72.19±2.26^{b}	57.95±7.13^{b}	69.04±2.18^{a}

Data are presented as (Mean±SE), SE: Standard error, Mean values with different superscript letters in the same column are significantly different at $p<0.05$

The obtained results in Table 4 revealed that a significant increased in serum L-malondialdehyde and nitric oxide concentratios and associated with significant decrease in liver tissues catalase, superoxide dismutase and glutatione peroxidase concentration in rats feeding high-fructose diet all over the periods of the experiments when compared to rats fed control diet. L-carnitine administration to rats fed a high fructose diet resulted in a significant decrease in serum L-malondialdehyde and nitric oxide levels with significant increase liver superoxide dismutase and glutatione peroxidase activities and also resulted in a non significant increase in liver catalase activity all over the periods of the experiment as compared to untreated fructose-fed rats.

Table 4: Effect of L-carnitine administration on liver CAT, SOD and GPx activities, serum MDA and NO levels in high fructose fed rats and their control

	CAT (mmol g^{-1} tissue)		SOD (U g^{-1} tissue)		GPX (nmol g^{-1} tissue)	
	45	60	45	60	45	60
Animal groups	(Days)					
Control	55.54±1.94^{a}	57.76±2.47^{a}	54.77±0.98^{b}	58.21±1.50^{a}	29.13±0.83^{a}	25.67±0.93^{a}
Fructose	39.30±2.31^{b}	37.48±3.00^{e}	49.60±0.92^{c}	50.40±0.39^{e}	25.81±0.51^{b}	16.77±1.10^{b}
Fructose+L-CAR	42.54±2.55^{b}	44.25±3.20^{bc}	59.97±1.38^{a}	56.49±0.81^{ab}	23.74±0.83^{b}	23.14±0.32^{a}
Control+L-CAR	47.43±3.51^{b}	47.91±4.20^{b}	49.79±1.18^{c}	53.88±0.52^{b}	17.31±0.76^{c}	23.72±1.18^{a}

	MDA (nmol mL^{-1})		NO (µmol L^{-1})	
	45	60	45	60
Animal groups	(Days)			
Control	64.76±4.57^{e}	87.20±3.48^{b}	8.25±0.49^{b}	9.33±0.31^{bc}
Fructose	95.30±3.05^{a}	113.79±1.36^{a}	12.52±0.98^{a}	13.79±0.98^{a}
Fructose+L-CAR	79.53±2.98^{b}	105.66±1.05^{a}	8.84±0.41^{b}	8.02±0.69^{c}
Control+L-CAR	72.67±6.59^{bc}	82.089±4.23^{b}	9.43±0.94^{b}	10.45±0.62^{b}

Data are presented as (Mean±SE), SE: Standard error, Mean values with different superscript letters in the same column are significantly different at $p<0.05$

DISCUSSION

Consumption of a high-fructose diet promotes development of three pathological characteristics associated with metabolic syndrome: Visceral adiposity, dyslipidemia and insulin resistance (Stanhope and Havel, 2008). Insulin resistance is not only an early and major feature in development of non Insulin Dependent Diabetes Mellitus (NIDDM) but also associated with hyperlipidemia, hypertension, obesity, enhanced oxidative stress, endothelial dysfunction and cardiovascular disease, so called insulin-resistance syndrome (syndrome X, metabolic syndrome) (Oudot *et al*., 2009).

Serum glucose, insulin and Insulin resistance levels increased significantly in fructose fed rats as compared to normal control group. These results came in accordance with Nandhini *et al*. (2005) who recorded that, feeding rats with high fructose diet for 30 days significantly elevated plasma glucose, insulin and insulin resistance index levels. These results could be due to that, fructose consumption does not directly promote insulin secretion from pancreatic cells due to the low concentrations of the fructose transporter GLUT5 in ß cells (Elliott *et al*., 2002) but the glucose produced as a result of fructose metabolism stimulates insulin release and the high fructose diet induced insulin resistance which prevents the insulin from effectively metabolizing glucose. As a consequence, increased amounts of glucose circulate throughout the body (hyperglycemia). Insulin resistance can also lead to compensatory hyperinsulinemia, where the body attempts to balance the reduced effects of insulin by producing and releasing more insulin (Suga *et al*., 2000). Several metabolic hypotheses has been advanced to explain insulin resistance in fructose fed rats. It has been shown that, Insulin resistance may occur due to a defect in insulin binding caused by decreased of insulin receptor number or affinity or defects at the level of effectors molecules such as glucose transports and enzymes involved in glucose metabolism (Kim *et al*., 2000). Insulin resistance in rat feeding high fructose diet may be associated with an overabundance and higher ectopic lipid deposition especially FFA and TG in liver, muscle and adipose tissue which may generate toxic lipid-derived metabolites, such as diacylglycerol, fatty acyl CoA and ceramides. The

presence of these metabolites in the intracellular environment leads to a higher serine/threonine phosphorylation of insulin receptor substrate-1 (IRS-1), which has been shown to reduce insulin signaling (Shulman, 2000). Another cofactor, elevated cytokines and oxidative stress are responsible for IR observed in fructose-fed rats (Mooney *et al*., 2001). Also, fructose feeding decreases the efficacy of insulin extraction by the liver which retards insulin clearance from the circulation (Suga *et al*., 2000). L-carnitine (L-CA) treatment to the high fructose-fed rats resulted in significant reduction in serum glucose, insulin and insulin resistance levels as compared to fructose-fed rats. Similarly, Ghanem (2010) reported that, intraperitoneal injection of 300 mg of CA kg^{-1} b.wt. day^{-1} to fructose-fed rats (60% of diet) improved the levels of serum glucose, insulin, IR index towards the control levels. These results might be attributed to that, exogenous carnitine mitigated lipid abnormalities in fructose-fed rats which one of causes of insulin resistance by removing toxic lipid-derived metabolites which reduce insulin signaling, also L-CA increases the utilization of glucose by activating pyruvate dehydrogenase and by decreasing the intra-mitochondrial acetyl CoA/CoA ratio, so promotes oxidative glucose utilization, lowers intracellular glucose levels and improves insulin sensitivity (Rajasekar and Anuradha, 2007).

Serum lactate and pyruvate concentrations were significantly increased in fructose fed rats. Similarly, Rajasekar and Anuradha (2007) recorded that, high-fructose feeding rats (60 g/100 g diet) for of 3-4 weeks showed significant increases in lactate and pyruvate concentrations. These results might be due to that, fructose metabolism bypasses the regulatory step catalyzed by Phospho-fructokinase (PFK). Thus fructose continuously enters the glycolytic pathway at the level of glyceraldehydes and dihydroxyacetone phosphate and produces glucose, lactate and pyruvate continuously in an unregulated manner. The elevated circulating levels of gluconeogenic substrates pyruvate, lactate and glycerol in the fructose-fed animals confirm impaired glycolysis. Increased gluconeogenic substrate availability could be suggested to be the major cause of the fasting hyperglycemia in fructose-fed rats (Mayes, 1993). The fructose-fed rats treated with L-carnitine showed a significant decrease in serum lactate and pyruvate levels. These results are in a harmony with the data of Rajasekar and Anuradha (2007) who observed that, the levels of lactate and pyruvate was significantly decreased after administration of L-carnitine (300 mg of CA kg^{-1} b.wt. day^{-1}, i.p.) for 3-4 week in rats fed high fructose diet. These results may be attributed to that, the effect of L-CA to return gluconeogenesis in fructose-fed rats to normal as seen in the control rats may be through the suppression of gluconeogenic enzyme activity. In addition, enhanced oxidative utilization of glucose by L-CA may have occurred as a result of increased flux of glucose through PFK. This enzyme is reported to be inhibited in the fructose-fed insulin resistant rats (Mayes, 1993). In addition to, L-Carnitine can remove of short and medium-chain fatty acids formed as a consequence of normal metabolism, preventing a toxic accumulation of these compounds in the mitochondria and leading to an increase of free CoA. An increase in free CoA results in activation of the pyruvate dehydrogenase complex and subsequently improves coupling between glycolysis and glucose oxidation (Mate *et al*., 2010).

A significant increase in serum leptin concentration was observed in fructose fed rats. Similarly, Hu *et al*. (2012) reported that, elevation in serum leptin levels was observed in rats given 10% fructose in drinking water for 8 week. These results may possibly due to that, leptin is one of a number of hormones that signals the brain that enough food has been consumed. High consumption of fructose causes lipid abnormalities and high triacylglycerols promote leptin resistance by preventing leptin from crossing the blood brain barrier, because the main target of leptin action is the leptin receptor that is located in the hypothalamus. In order for circulating leptin

to reach its receptor in the hypothalamus, it must first cross the blood-brain barrier (Banks *et al.*, 2004). Treatment with L-carnitine to high fructose-fed rats exhibited a non significant decrease in serum leptin level as compared to fructose-fed rats. Similarly, Alshammari (2011) revealed that, a decreased serum leptin was lower in L-carnitine administration before the physical activity. Leptin secretion was independent of the L carnitine administration and was not exclusively regulated by the adiposity degree. Because of exogenous L-carnitine mitigated lipid abnormalities in fructose-fed rats, therefore lowering triacylglycerols which allow circulating leptin to reach its receptor in the hypothalamus and cross the blood-brain barrier (Rajasekar and Anuradha, 2007).

Regarding serum lipid profile, high-fructose feeding to normal rats resulted in significant increase in the concentrations of serum total cholesterol, triacylglycerols (TGs) and free fatty acids (FFAs) and significant decrease in serum phospholipids level compared with normal control group. Similarly, Rajasekar *et al.* (2005) showed that, significant increase in the concentrations of plasma TG (63%), Phospholipids (24%) and FFA (38%) in fructose-fed rats (60 g/100 g diet). These results could be attributed to that, the unregulated fructose metabolism generates both glycerol and acyl portions of acyl-glycerol molecules, the substrates for triglycerides (TG) synthesis. Increase in acyl CoA carboxylase and sterol regulatory element binding protein, binds to sterol responsive elements found on multiple genes and activates a cascade of enzymes involved in lipid biosynthesis pathway such as HMG-CoA reductase and fatty acid synthase. The activity of this protein in liver is reported to be enhanced in insulin resistant fructose fed mice (Miyazaki *et al.*, 2004) and this explains the increased levels of cholesterol and fatty acids during fructose feeding. Increased delivery of FFA to muscle interferes with glucose utilization, through the principles of Randle cycle, this can attenuate insulin signaling and exacerbate IR. Conversely, diminished insulin-stimulated glucose disposal could lead to impaired FFA re-esterification and thereby to higher circulating FFA concentrations (Randle, 1998). FFA could directly increase reactive oxygen species via peroxidation reactions and via mitochondrial production (Bakker *et al.*, 2002). The major targets of damaging free radicals are the cellular and membrane phospholipids. The oxidative tissue damage can release the membrane lipids such as Free Fatty Acids (FFA) and phospholipids into blood. Also, fructose feeding can lead to a decrease in the ability of insulin to stimulate the activity of lipoprotein lipase (LPL). This reduction in the activity of LPL; can be ascribed to the insulin resistance induced by fructose. Besides, it could be possible that the activity of hepatic lipase, that hydrolyses triglycerides and phospholipids from lipoprotein, is blocked in these rats (Takagawa *et al.*, 2001). L-carnitine treatment to fructose-fed rats showed a significant reduction in serum total cholesterol, triacylglycerols and free fatty acid concentrations and significantly increased serum phospholipids level. Also, Rajasekar and Anuradha (2007) observed that, administration of (300 mg of CA kg^{-1} b.wt. day^{-1}, i.p.) for a period of 3-4 weeks caused significant reduction in the levels of FFA, cholesterol and TG in plasma, liver and muscle of fructose-fed rats (60% of diet) for a period of 3-4 weeks. These results may be due to that, exogenous L-carnitine mitigated lipid abnormalities in fructose-fed rats. One obvious mechanism of the TG lowering effect is the role of L-carnitine to increase the influx of fatty acids as acylcarnitine into mitochondria. This reduces the substrate availability for the synthesis of TG in the liver. The effects of L-carnitine on lipid metabolism may also be related to its effect on glucose utilization and improvement of insulin action. L-carnitine increases the utilization of glucose by activating pyruvate dehydrogenase and by decreasing the intra-mitochondrial acetyl CoA/CoA ratio (Ferrannini *et al.*, 1988). Also, improved insulin action by exogenous carnitine could regulate the key enzymes of lipid metabolism and normalize lipid levels in the circulation (Rajasekar *et al.*, 2005). L-carnitine increases the synthesis of phospholipids

required for membrane formation and integrity and plays a role in the membrane repair by reacylation of phospholipids (Kashiwagi *et al*., 2001).

Regarding the pro-inflammatory cytokines the obtained results revealed significant increase in the concentrations of serum TNF-α and IL-6 in high fructose feeding rats compared with normal control group. Similarly, Sivakumar and Anuradha (2011) reported that, a significant increase in TNF-α and IL-6 was observed in rats fed on high fructose diet (60% of diet)for 60 days. These results may be related to that, high fructose diet consumption may lead to Steatosis or fat overload in the hepatocytes which results in activation of stress/inflammatory pathways that trigger the resident macrophages in the liver (Kupffer cells) for inflammatory reactions (Popa *et al*., 2007). Novel non-oxidative lipid metabolites like ceramides, diacylglycerol and long chain fatty acyl CoA have been hypothesized to be potential candidates for this lipotoxic effect of fructose. Thus the rise in TNF-α and IL-6 in fructose-fed rats could be related to NF-KB activation which in turn is attributed to the rise in Reactive Oxygen Species (ROS) levels and oxidative stress. NF-KB activation increased the expression of a large number of pro-inflammatory cytokines and stimulation of inflammatory cascade have been observed in fructose-fed rats (Kuhad *et al*., 2009). Increase in leptin production is another reason of inflammation in high fructose diet, increases in leptin levels (in response to caloric intake) function as an acute pro-inflammatory response mechanism to prevent excessive cellular stress induced by overeating. When high caloric intake overtaxes fat cells ability to grow larger or increase in number in step with caloric intake, the ensuing stress response leads to inflammation at the cellular level and ectopic fat storage (i.e., the unhealthy storage of body fat within internal organs, arteries and/or muscle) (Wabitsch *et al*., 1996). Dysregulated production of IL-6 and TNF-α have been found to inhibit hepatic insulin receptor activation and downstream insulin signaling *in vivo*, which impair insulin action at the cellular level. Furthermore, TNF-α promotes cell injury through overproduction of oxidants that cause damage to critical cellular components (Lorenzo *et al*., 2008). Treatment with L-carnitine to fructose-fed rats resulted in significant reduction in serum TNF-α and IL-6 levels. These results are nearly similar with those of Famularo and de Simone (1995) who recorded that, L-carnitine cause reduction in the serum levels of pro-inflammatory cytokines, specifically interleukin IL-1b, IL-6 and TNF-α. Also, Malaguarnera *et al*. (2013) who observed that, L-carnitine supplementation induces decrease plasma TNF-α level. The real mechanism underlying this is not clear but we can assume that L-carnitine can interfere with processes involved in β-oxidation and accumulation of lipotoxic metabolites that might contribute to mitochondrial dysfunction and insulin resistance. L-carnitine could act through mechanisms that are independent of the putative detoxifying role and use it as antioxidants to protect cellular structures against damage from oxygen free radicals and from reactive products of lipid peroxidation (Malaguarnera *et al*., 2013).

A significant increase in serum sialic acid concentration was observed in fructose fed rats. These results are nearly similar with Mahfouz *et al*. (2010) who reported that, rats fed high fructose diet (60% of diet) for 30 days induced significant increases in serum sialic acid levels. Under condition of fructose consumption 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 (Maxwell, 1995). Sialic acid is a component of cell membranes and elevated levels may indicate excessive cell membrane damage but more specifically to the cells of vascular tissue. Also, 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

(Pickup, 2004). The release of sialic acids which are derivatives of neuraminic acid located on the terminal residues of glycolipids of cell membranes could ensue as a result of the breakdown of cell membranes and/or lipid peroxidation. The released sialic acid reaches the plasma and this could be reflected by the elevated plasma sialic acid concentracion (Schutter *et al.*, 1992).

Treatment with L-carnitine to high fructose-fed rats resulted in significant decrease in serum sialic acid level. These results are in a harmony with the data of Mahfouz *et al.* (2009) who found that, intraperitoneal injection of L-carnitine to fructose-fed rats improved serum levels of sialic acid toward the control levels after 2 weeks of treatment. These results might be attributed to the Protection of cellular membranes through L-carnitine that it may exert a direct effect on the membrane. It may prevent cell damage by stabilizing the membrane against free radical-induced injury and also may prevent mitochondrial injury, thus increasing energy production and decreasing the leakage of free radicals (Binienda *et al.*, 1999). In addition to increases the synthesis of phospholipids required for membrane formation and integrity and plays a role in the membrane repair by reacylation of phospholipids (Kashiwagi *et al.*, 2001).

The obtained results revealed that, high fructose fed rats resulted in significant increase in serum malondialdehyde (MDA) concentration compared with control group. Similarly, Ghibu *et al.* (2013) reported that, a significant increase in the lipid peroxidation, expressed as MDA was observed in rats fed on fructose-enriched diet (60% of diet) for 12 weeks. Polyunsaturated fatty acids in membrane phospholipids are the major targets for free radicals (formed on account of increased blood pressure and/or glycaemia) which are capable of inducing a chain reaction of lipid peroxidation. These reactions in lipid membranes give rise to the formation of end products which are used to detect free radical damage. One of the important end products measured as an indicator of lipid peroxidation is known to be MDA (De Zwart *et al.*, 1999). Administration of L-carnitine to fructose-fed rats resulted in significant decrease in serum malondialdehyde concentration. These results are nearly consistent with Sayed-Ahmed *et al.* (2004) who recorded that, administration of L-carnitine at 500 mg kg^{-1} for 10 days in Sprague-Dawley rats attenuated the increased MDA level. L-carnitine increases the synthesis of phospholipids required for membrane formation and integrity and plays a role in the membrane repair by reacylation of phospholipids (Kashiwagi *et al.*, 2001). It has been suggested that, L-carnitine may exert a direct effect on the membrane. It may prevent cell damage by stabilizing the membrane against free radical-induced injury and also may prevent mitochondrial injury, thus increasing energy production and decreasing the leakage of free radicals (Binienda *et al.*, 1999).

The obtained results revealed that, rats fed on a HFD showed significant increase in serum Nitric Oxide (NO) concentration. These results are nearly Consistent with Bagul *et al.* (2012) who reported that, a significant increase in nitric oxide level was observed in rats fed 65% fructose for a period of 8 weeks. Feeding high fructose diet to rats induced elevation in the expression of inducible nitric Oxide Synthase (iNOS) is assumed to be one of the candidates that mediate inflammation involved insulin resistance. This report elucidate the elevation of nitric oxide which result of increasing the iNOS expression associated with insulin resistance which induced by feeding high fructose diet to rats. Inducible nitric oxide synthase has been implicated in many human diseases associated with inflammation. iNOS deficiency was shown to prevent insulin resistance. The role of iNOS in hyperglycemia and hepatic insulin resistance remains to be investigated (Fujimoto *et al.*, 2005). Administration of L-carnitine to rats fed a high fructose diet resulted in significant decrease in serum nitric oxide level. Similarly, Ghanem (2010) relieved that, administration of L-Carnitine (300 mg kg^{-1} b.wt. day^{-1} i.p.) to rats fed high fructose diet for 30 day,

showed significant decrease in iNOS expression and pronounced increases in both IGF-1 mRNA expression and IRS-1 receptors. These results could be attributed to that, L-Carnitine reduced the activity of angiotensin-converting enzyme which might result in lower Ang II and NADPH oxidase dependent ROS production, thus improving NO bioavailability owing to a lower depletion by ROS. Also, L-Carnitine increased the activity of antioxidant which SOD scavenges superoxide ions and improves NO activity (Faraci and Didion, 2004). So, L-Carnitine is antioxidant that interferes with the chain reaction of lipid peroxidation and stabilizes the cell membrane. Its use has proven to be beneficial to reduce oxidative stress that has been shown to damage endothelial relaxation and reduce the level of NO (Cunningham *et al*., 1996).

Regarding, antioxidant enzymes the obtained results showed significant decrease in catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in liver tissues of rats fed on a High Fructose Diet (HFD). These results are nearly similar to those noticed by Rajasekar *et al*. (2005) who reported that, enzymatic antioxidants SOD, CAT and GPx were significantly lower in fructose-fed rats (60 g/100 g diet) for 30 days. Fructose feeding itself can induce oxidative stress by a number of mechanisms; the increased catabolism of fructose could be associated with the cellular energy depletion that can increase the susceptibility of cells to lipid peroxidation. Further, down-regulation of HMP shunt enzymes in the presence of fructose could lead to decreased generation of reducing equivalents NADPH (Fields *et al*., 1992). Another mechanism, feeding of fructose rich diet presented bigger triacylglycerols content. High concentrations of liver triglycerides lead to an increase of the activity of fatty-acyl-coA oxidase activity and also stimulate the liver to produce energy through beta-oxidation. NEFA can induce insulin secretion by pancreatic islets, leading to a hyperinsulinemic state, as presented by the fructose fed group. High insulin levels can downregulate the Malonil-CoA activity, reducing the mitochondrial NEFA transport, leading to an excessive NEFA oxidation at peroxisomes and endoplasmic reticulum (Jurgens *et al*., 2005). Fatty oxidation in these cytoplasmic organelles areas releases high amounts of reactive oxygen species, which can damage mitochondrial membranes (rich in polyunsaturated fat), producing lipid peroxidation metabolites. These metabolites are extremely toxic to the cells and are released into the blood stream, as observed in the fructose group (Esterbauer *et al*., 1991). The chronic imbalance of reactive oxygen species production can impair the ability of the antioxidant system to reduce the levels of these radicals, which attenuates its protective function (Girard *et al*., 2006). Moreover, the increase fructose catabolism could be associated with the cellular energy depletion that can increase the susceptibility of cells to lipid peroxidation (Rajasekar *et al*., 2005). Reactive Oxygen Species (ROS) can themselves reduce the activity of antioxidant enzymes such as CAT, SOD and GPx (Datta *et al*., 2000). Administration of L-carnitine to rats fed a high fructose diet resulted in significant significant increase in liver catalase, glutathione peroxidase and superoxide dismutase activities compared to fructose-fed rats. Similarly, Rajasekar *et al*. (2005) reported that, a significant increase in the activities of both enzymatic and non-enzymatic antioxidant were observed in fructose-fed rats treated with CA (300 mg kg^{-1} b.wt. day^{-1}, i.p. in 0.89% saline) for 30 days. Carnitine plays a role in chelating free Fe^{2+} ions and hence could reduce free radical generation. Furthermore, Carnitine by virtue of its ability to enhance ATP production could reduce susceptibility to oxidative damage. Scavenging of ROS is determined by antioxidant enzymes such as SOD and CAT. Catalase is considered to be the most important enzyme involved in detoxification of H_2O_2 and protection of hepatocytes from oxidative stress. De Bleser *et al*. (1999) who observed that, the inhibited activities and expressions of SOD and CAT induced by H_2O_2 were attenuated by L-carnitine. The enhancement of β-oxidation

induced by L-carnitine would generate ATP, thereby reversing H_2O_2 initiated depletion of ATP in cells and attenuating cell injury. ATP was considered to be a critical event in lethal cell injury produced by oxygen radicals (Hyslop *et al*., 1988).

CONCLUSION

This study demonstrates that, the administration of L-carnitine to rats fed a high fructose diet enhanced the levels of antioxidants status and prevents the development of oxidative stress and its associated complications include hyperglycemia and hyperinsulinemia. Also, the results indicate that, L-carnitine is effective in improving the dyslipidemia, inflammation and insulin resistance induced in high fructose-fed rats and may have implications in the treatment of insulin resistance and its metabolic complications.

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