



Original article

Enhanced hepatic glycogen synthesis and suppressed adenosine deaminase activity by lithium attenuates hepatic triglyceride accumulation in nicotine-exposed rats



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ABSTRACT

Reduced liver glycogen synthesis might signify increased glucose flux towards fat synthesis and triggers hepatic triglyceride accumulation and dysmetabolism. Adenosine deaminase (ADA) reduces adenosine content which increases glycogenolysis. In the present study, we evaluate the effect of modulating glycogen synthesis and ADA by lithium chloride (LiCl) on nicotine-induced dysmetabolism. Twenty four male Wistar rats ($n = 6/\text{group}$) were allotted into four groups namely; vehicle-treated (*po*), nicotine-treated (1.0 mg/kg; *po*), LiCl-treated (5.0 mg/kg; *po*) and nicotine + LiCl-treated groups. The treatments lasted for 8 weeks. Nicotine exposure resulted in reduced body weight gain, liver weight, visceral adiposity, glycogen content and synthase. Along with increased insulin resistance (IR), fasting plasma glucose, lactate, plasma and hepatic ADA, XO, UA, and triglyceride (TG), total cholesterol (TC), free fatty acid, lipid peroxidation and liver injury markers. However, plasma and hepatic glucose-6-phosphate dehydrogenase-dependent antioxidant defenses were not affected by nicotine exposure. Concurrent treatment with LiCl normalizes all alterations with exception of hepatic TC. This result shows that enhancement of hepatic glycogen synthesis and suppression of ADA/XO/uric acid pathway by lithium can salvage the liver from nicotine-induced TG accumulation.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is increasing rapidly worldwide due to wide spread obesity and metabolic complications. The worldwide prevalence of NAFLD is currently estimated to be 24% [1]. NAFLD is the leading cause of chronic liver disease which is an independent determinant of the development of type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD). Reports have suggested that NAFLD is the consequence of a complex interplay between dietary, environmental and metabolic factors. One of the pathophysiological mechanisms for the development of NAFLD is triglyceride (TG) accumulation which primes the liver for more forms of NAFLD especially in the presence of disrupted antioxidant capacity. Scientific evidences have shown that increased hepatic TG results in deteriorated antioxidant barrier accompanied with mitochondrial dysfunction [2–4]. The major substrates for hepatic TG synthesis are free fatty acid (FFA)

from hepatic de novo lipolysis, peripheral tissues, and dietary sources. Growing interest exists in the attempt to decipher the basis and import of fatty liver especially because of its association with insulin resistance (IR), a global endemic metabolic disease [2]. Certain factors are important to account for the global spread of metabolic derangement with NAFLD; western diet along with sedentary lifestyle [5] and nicotine exposure [6].

The liver glucoregulatory function is crucial in keeping up blood glucose and lipid homeostasis. Glycogen and TG synthesis or glycogenolysis and gluconeogenesis are hepatic pivotal homeostatic processes in either the postprandial state or postabsorptive state respectively

[7,8]. Disruption in hepatic glycogen metabolism as a result of metabolic dysfunction by insulin insensitivity and glucose deregulation has been associated with obesity and type 2 diabetes mellitus (T2DM) [9]. Therefore, attempts have been made to manipulate glycogen

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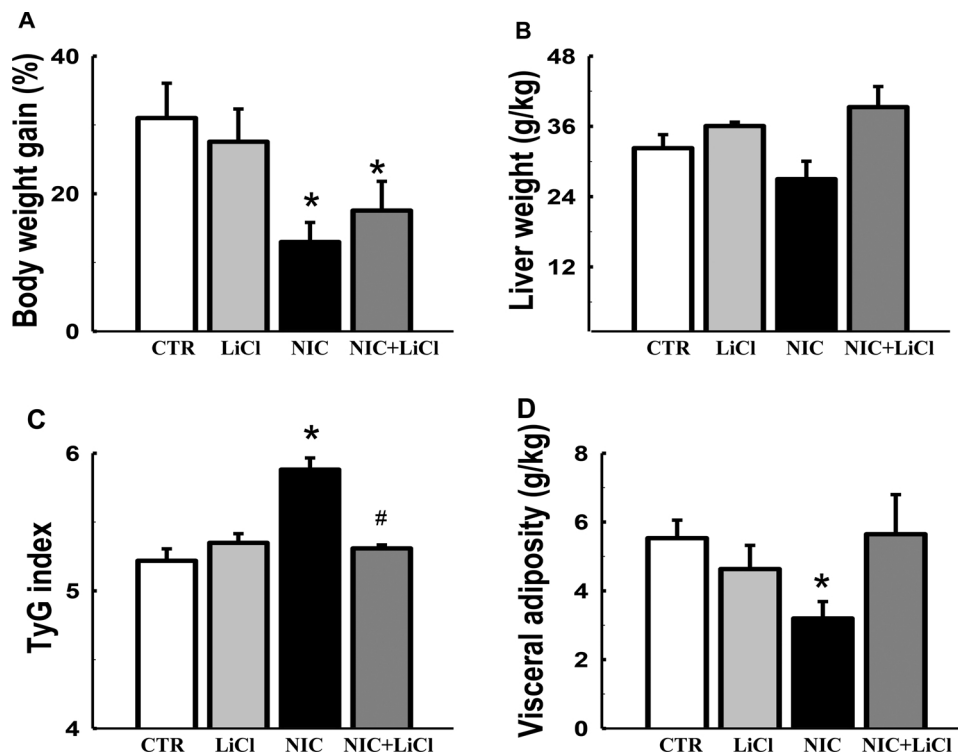


Fig. 1. Effect of lithium on a) body weight b) liver weight and c) TyG index d) visceral adiposity in nicotine-treated rats. There was decreased body weight gain, visceral adiposity and increased TyG index whereas liver weight was not affected in nicotine-treated male rats. However, lithium plus nicotine treatment led to decrease TyG index, whereas visceral adiposity and liver weight were normalized compared with control. Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* test. Values are expressed as mean $\pm$ SEM of 6 rats per group (* p < 0.05 vs. Con; # p < 0.05 vs. NIC).

synthesis to resolve the deleterious effects of glucose dysmetabolism. The rate limiting enzyme in the glycogenesis pathway is glycogen synthase, which catalyzes the addition of 1,4-linked glucose units from uridinediphosphate (UDP) glucose to an emerging glycogen chain [10]. Insulin enhances the activity of this enzyme, the failure of which results in defective glycogenesis that characterizes insulin resistant states. In IR, hepatic glucose flux can be diverted for lipogenesis in addition to hepatic TG delivery from the circulation. This results in hepatic TG accumulation and consequently fatty liver. However, glycogen synthesis, aside being a marker of IR and precursor of fatty liver, is also regarded as a major defect involved in the pathophysiology of type 2 diabetes *i.e.* reduced insulin-mediated non-oxidative glucose metabolism or reduced glycogen synthesis [11].

Purine metabolism is associated with clearance of adenosine, a regulatory molecule known to inhibit the inflammatory responses elicited by tissue macrophages. Conversely, adenosine level is known to be raised in inflammation and tissue damage. For instance, studies have shown that adenosine resists oxidative damage and oxidative stress elevates renal adenosine [12], it has also been reported that adenosine improves antioxidant capacity *via* activation of adenosine receptors (A_1 and A_{2A}) in the kidney [13] and in endothelium [14]. Interestingly, glycogen synthesis has been reported to be suppressed by adenosine *via* stimulation of glycogenolysis in hepatocytes [15].

Adenosine deaminase (ADA) is a ubiquitous mammalian enzyme involved in purine metabolism. It clears adenosine by converting it to inosine *via* an irreversible deamination reaction. ADA also stimulates immune responses in tissues [16] and its activity has been reported to be relatively high in the skeletal muscle, liver, fatty and lymphoid tissues [17]. Likewise elevated ADA activity had been reported in patients with T2DM [18]. Xanthine oxidase (XO) catalyzed the penultimate and final steps of purine metabolism, which produce uric acid from hypoxanthine [19]. XO is most expressed in liver and gut [20]. XO engages molecular oxygen as electron acceptor and with this generates superoxide anion and other reactive oxygen species as by-product. Pro-oxidant property of uric acid can be linked to elevated XO activity [21]. Accumulated reactive oxygen species is a key player in IR progression and establishment of NAFLD [22]. Oxidative stress is the result of the

complex interplay between the oxidative species and antioxidant species. Cellular antioxidant capacity is determined mainly by the G6PD-dependent glutathione system. G6PD is the rate limiting enzyme in the non-oxidative pentose phosphate pathway (PPP). It is also the main source of NADPH in the cytosol, which is required to fuel the glutathione system. Reduced glutathione (GSH) is produced through NADPH by the action of glutathione reductase. While glutathione peroxidase (GPx) catalyses the reaction that produces glutathione disulphide (GSSG). GSH/GSSG ratio is taken as an indicator of cellular antioxidant capacity, alteration in this ratio has been linked to metabolic perturbations [23].

Nicotine is the main alkaloid in tobacco and also one among the approximately 4000 compounds in cigarette smoke. Aside cigarette, nicotine exposure is acquired by nicotine replacement therapy (NRT) preparations for smoking cessation [24]. Nicotine is highly soluble in lipids and it rapidly crosses the blood-brain barrier [25]. It has been shown to be associated with reduced food intake and body weight but its suitability as a drug for body weight loss is inconclusive. Nicotine has been shown to decrease insulin sensitivity following prolonged exposure [26]. Long-term use of nicotine gum is associated with hyperinsulinaemia and insulin resistance. Furthermore, nicotine has been implicated in both type 1 and type 2 diabetes, sepsis [27], ulcerative colitis [28], renal ischemia-reperfusion injury [29] and liver disease [6]. The mechanism by which nicotine causes lipid dysmetabolism and hepatic fat accumulation is poorly understood. We have previously reported that nicotine-induced glucose deregulation is associated with increased glycogen synthase kinase-3 (GSK-3) [30].

Lithium a known inhibitor of GSK-3, an alkali metal and a trace element has been extensively used as a mood stabilizer in treatment of bipolar disorders for over five decades. Reports exist of its use in the treatment of a variety of disorders in humans, including bipolar affective disorders, migraine headache, neurodegenerative disorders, stroke, ischemia, arthritis, and leukopenia [31]. Long-term usage of lithium has been associated with acute lithium toxicity, chronic kidney disease and nephrogenic diabetes insipidus [32]. However, short-term use and at low doses, lithium can be renoprotective as corroborated by recent studies that demonstrated protective effects of lithium in experimental

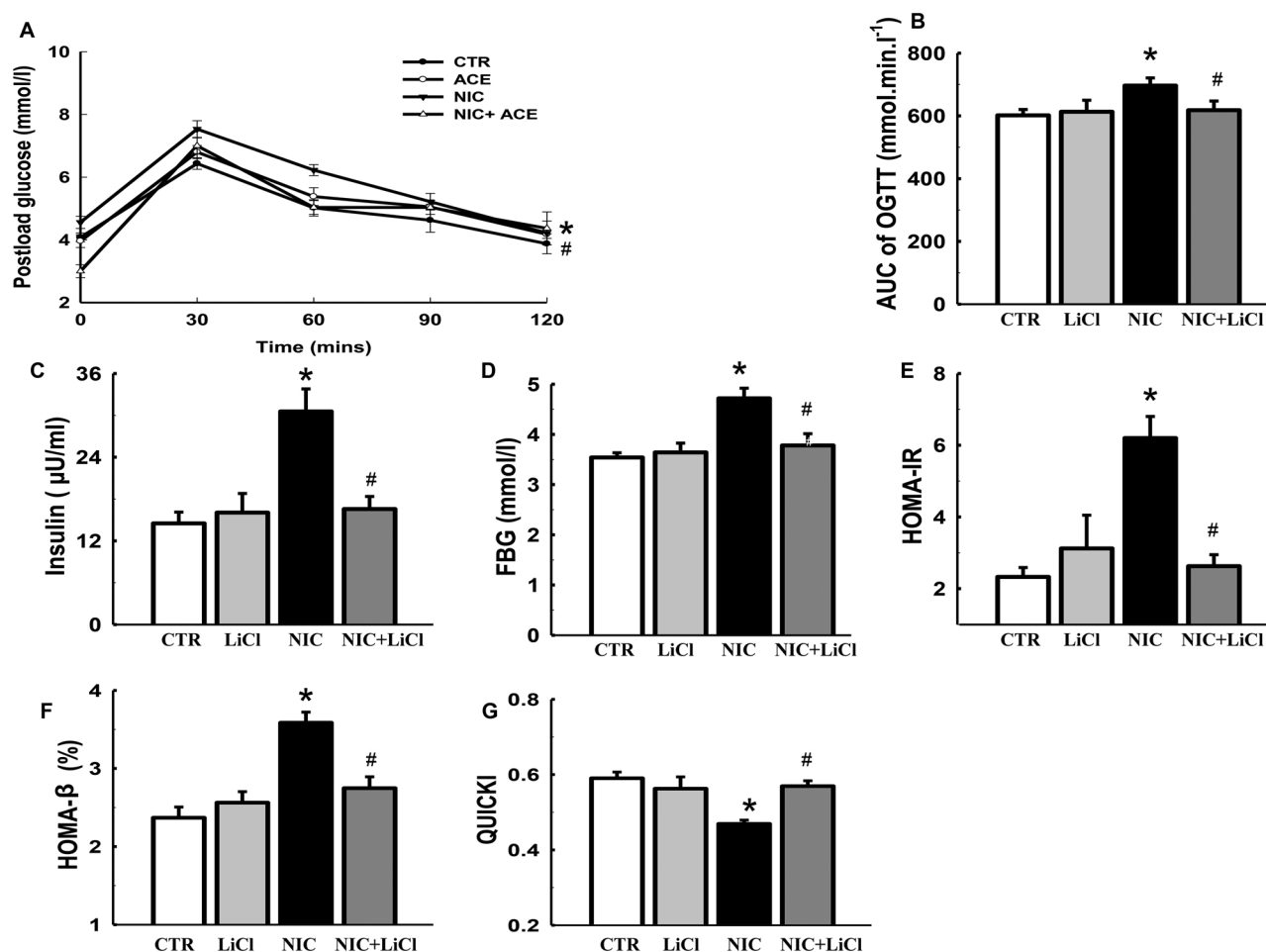


Fig. 2. Lithium improves glucoregulation; a) oral glucose tolerance test (OGTT), b) area under glucose curve (AUC) of OGTT, c) fasting insulin, d) fasting blood glucose, e) HOMA-IR, f) HOMA-β and g) QUICKI in nicotine-treated male rats. Nicotine treatment led to impaired glucoregulation as depicted by the increased fasting glycaemia, 1-h postload glycaemia, AUC of OGTT, HOMA-IR, insulinemia, HOMA-β and decreased QUICKI (insulin sensitivity) whereas lithium with or without nicotine treatment did not affect glucoregulation compared with control. In addition, lithium plus nicotine treatment led to reduced fasting glycaemia, 1-h postload glycaemia, AUC of OGTT, HOMA-IR, insulinemia, HOMA-β and increased QUICKI (insulin sensitivity) compared with nicotine-treated rats. Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* test. Values are expressed as mean ± SEM of 6 rats per group (**p* < 0.05 vs. Con; #*p* < 0.05 vs. NIC).

models of acute kidney injury induced by renal ischemia-reperfusion [33]. More so, lithium has been utilized to regulate the glucose transport system in metabolic tissues through its ability to minimize depletion of glycogen [34].

Till date, there is lack of information on the effect of lithium on hepatic lipid accumulation and ADA/XO/UA pathway. This study was therefore designed to elucidate the potential role of lithium on hepatic lipid accumulation and involvement of the ADA/XO/UA pathway in the development of nicotine-induced hepatic lipid accumulation and injury.

2. Materials and methods

2.1. Animals

The investigation was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Ethical Review Committee, and every effort was made to minimize both the number of animals used and their suffering. Ten weeks old male Wistar rats, weighing 120–125 g were used for the study. Rats had unrestricted access to standard rat chow and tap water. After 1 week of acclimatization, the animals were randomly assigned to four groups (n = 6/group). Rats were maintained under standard environmental conditions of temperature, relative humidity and dark/light cycle. Body weight, food and

water intakes were monitored weekly.

2.2. Treatment

Control group received distilled water (vehicle; *po*) daily, nicotine-treated group received (*po*)

1.0 mg/kg NIC (Alchem Inc., Minnesota, USA) daily, LiCl-treated group received (*po*) 5 mg Lithium Chloride (Science Company, Lakewood, CO, USA) daily. NIC + LiCl-treated group received (*po*) a combination of nicotine plus LiCl daily. The treatment lasted for 8 weeks.

2.3. Oral glucose tolerance test (OGTT) and insulin resistance (IR)

Glucose challenge test was performed, one week, prior to the sacrifice of the rats. The rats had 12-h overnight fast. Glucose (2 g/kg *bw*) was given (*po*). Blood was obtained from the tail before glucose load and then sequentially after 30, 60, 90 and 120 min. Terminal fasting blood glucose (FBG) was determined after the rats had 12-h overnight fast on the day of sacrifice. Blood glucose levels were determined with a glucometer (ONETOUCH®-LifeScan, Inc., Milpitas, CA, USA). Glucose tolerance was expressed as a function of the area under the OGTT curve (AUC) as previously described [35]. IR and β-cell function were determined using the homeostasis model assessment for IR (HOMA-IR =

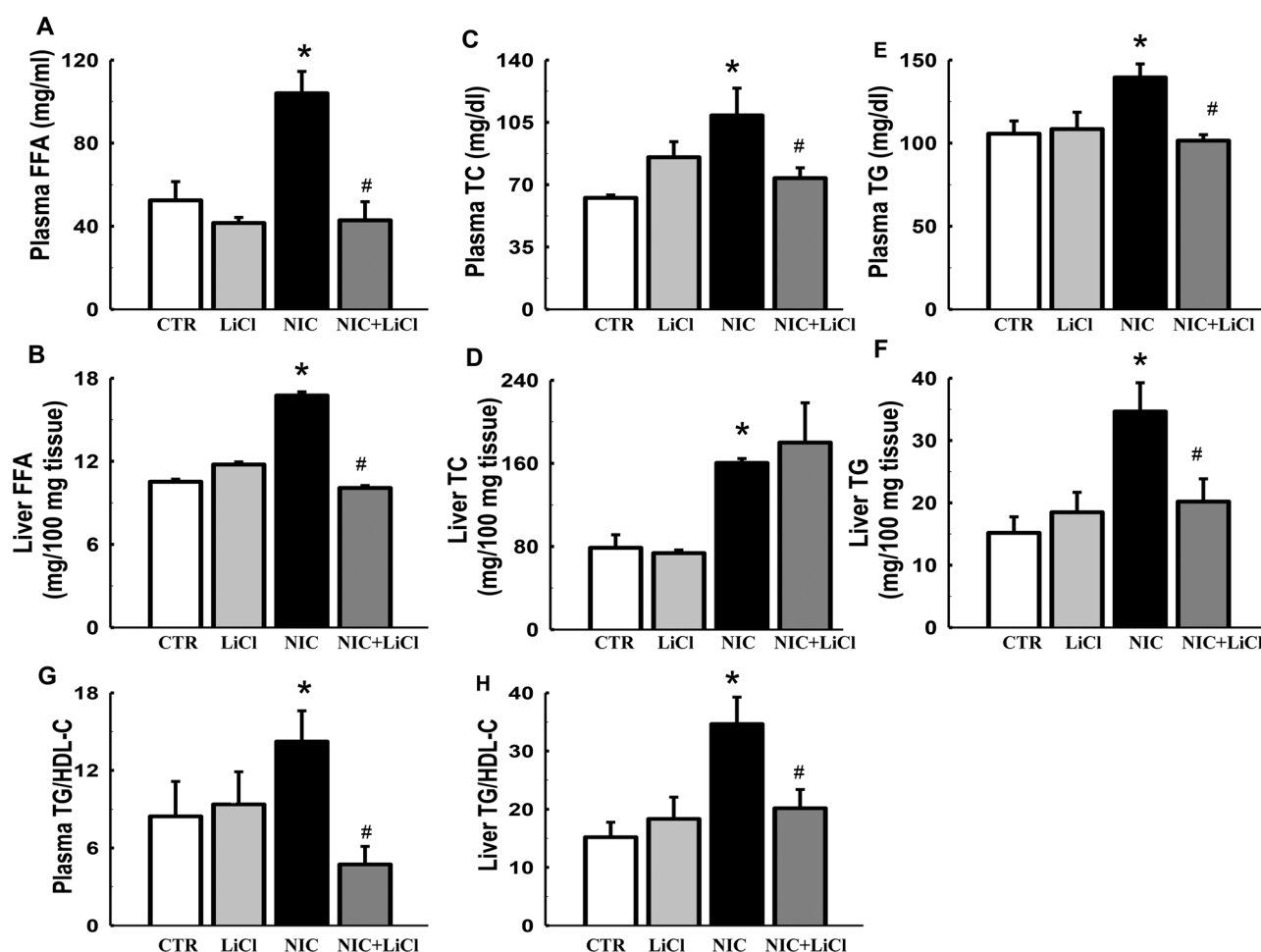


Fig. 3. Lithium improves lipid metabolism depicted by a) plasma FFA, b) hepatic FFA, c) plasma TC, d) hepatic TC, e) plasma TG, f) hepatic TG, g) plasma TG/HDL-C and h) hepatic TG/HDL-C in nicotine-treated male rats. Nicotine treatment led to impaired lipid metabolism that is revealed by increased plasma and hepatic TC, TG, FFA & TG/HDL-C compared with control. However, lithium plus nicotine treatment led to improved lipid metabolism by reducing plasma and hepatic TG, TG/HDL-C, plasma FFA, and plasma TC. Hepatic TC and FFA remained elevated in lithium plus nicotine-treated rats compared with control. Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* test. Values are expressed as mean $\pm$ SEM of 6 rats per group (* p < 0.05 vs. Con; # p < 0.05 vs. NIC).

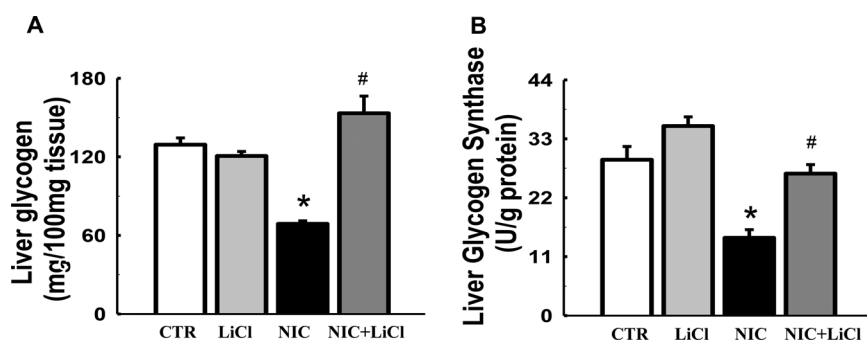


Fig. 4. Lithium improves glycogen synthesis in nicotine-treated male rats. Nicotine treatment led to decreased hepatic glycogen content and synthase activity compared with control. However, lithium plus nicotine led to increased hepatic glycogen content and synthase activity compared with nicotine-treated rats. Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* test. Values are expressed as mean $\pm$ SEM of 6 rats per group (* p < 0.05 vs. Con; # p < 0.05 vs. NIC).

fasting glucose (mmol/l)*fasting insulin (μ U/l)/22.5) and β -cell function ($\text{HOMA-}\beta = (20 \times \text{fasting insulin}) / (\text{fasting glucose} - 3.5)$) respectively while quantitative insulin sensitivity check index; QUICKI = $1 / [\text{Log}(\text{fasting insulin}) + \text{Log}(\text{fasting glucose})]$. Triglyceride-glucose index on the other hand is expressed as $\text{TyG} = \text{Ln}(\text{TG}(\text{mg/dl}) \times \text{FBG}(\text{mg/dl}) / 2)$ [36].

2.4. Sample preparation

At the end of treatment, the rats were anesthetized with pentobarbital sodium (50 mg/kg, *ip*). Blood sample was collected by cardiac

puncture into heparinized bottle and was centrifuged at 3000 rpm for 5 min. Plasma was stored frozen until needed for biochemical assay. The liver was excised, cleared of adhering connective tissues, blotted and weighed immediately. After weighing, 100 mg of liver tissue was carefully removed and homogenized with a glass homogenizer in cold phosphate-buffered.

2.5. Visceral adiposity

Following blood sample collection, all intra-abdominal fat depots including mesenteric, urogenital and retroperitoneal were dissected out

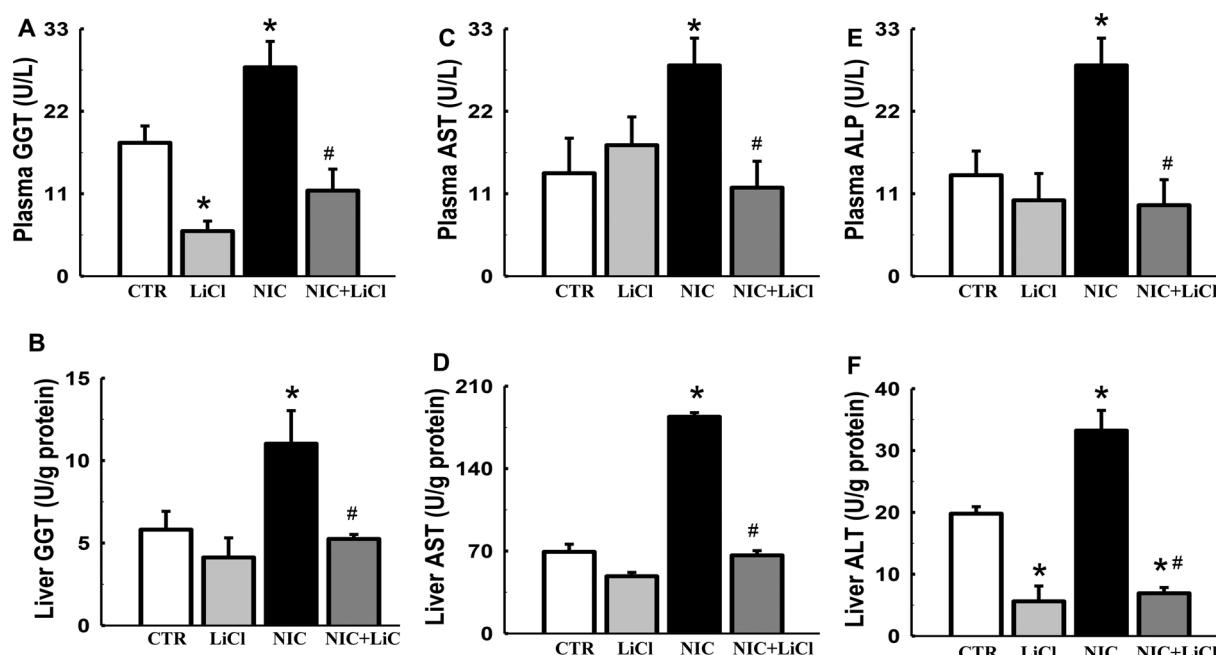


Fig. 5. Lithium attenuates hepatic injury as depicted by a) plasma gamma glutamyl transferase (GGT), b) hepatic GGT, c) plasma aspartate transaminase (AST), d) hepatic AST, e) plasma alkaline phosphatase (ALP), f) hepatic ALP in nicotine-treated male rats. There was increased plasma and hepatic GGT, ALP and AST activities in nicotine-treated rats compared with control. Lithium plus nicotine treatment led to decreased plasma and hepatic GGT, ALP and AST activities compared with nicotine-treated rats. Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* test. Values are expressed as mean $\pm$ SEM of 6 rats per group (* $p < 0.05$ vs. Con; # $p < 0.05$ vs. NIC).

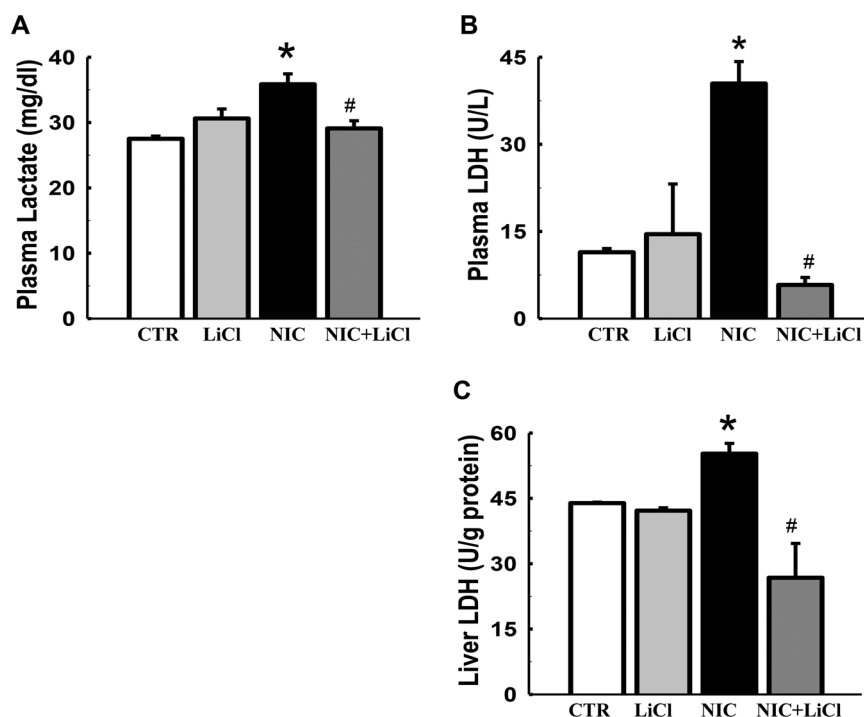


Fig. 6. Lithium suppresses lactate production in nicotine-treated male rats. There was increased plasma lactate, plasma and hepatic LDH in nicotine-treated male rats compared with control. However, lithium plus nicotine treatment resulted in decreased plasma lactate, plasma and hepatic LDH compared with nicotine-treated rats. Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* test. Values are expressed as mean $\pm$ SEM of 6 rats per group (* $p < 0.05$ vs. Con; # $p < 0.05$ vs. NIC).

and weighed immediately with an electronic weighing scale after dissection to avoid evaporative weight loss.

2.6. Biochemical assays

Plasma insulin was determined using ELISA kit from Ray Biotech, Inc. (Georgia, USA). Uric acid level, ADA and XO activities was measured by standardized enzymatic colorimetric method using assay kit obtained from Fortress diagnostics (Antrim, UK). Plasma and liver nitric

oxide was measured by quantification of nitrite using Griess Reagent and malondialdehyde (MDA), as marker of oxidative stress was measured by standardized enzymatic colorimetric methods using assay kits from Oxford Biomedical Research, Inc. (Oxford, MI, USA). Plasma and liver free fatty acids (FFA), lactate, glucose-6-phosphate dehydrogenase (G6PD), glutathione peroxidase, reduced glutathione (GSH), glutathione disulphide (GSSG), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), fasting triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) were measured

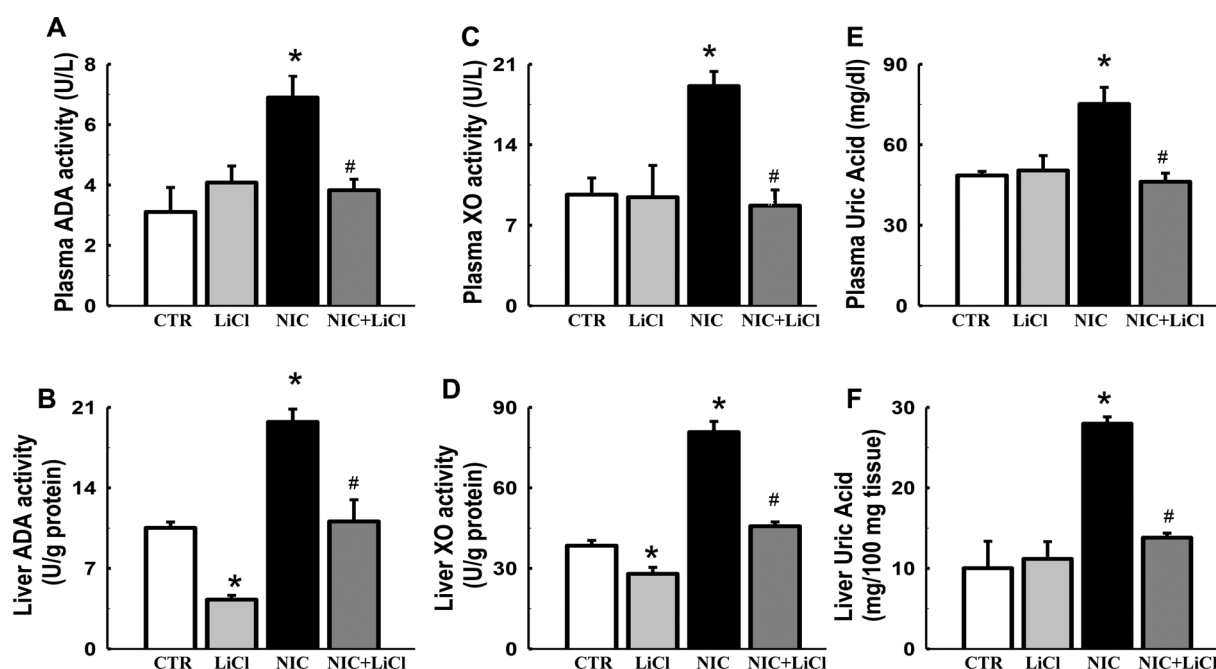


Fig. 7. Lithium decreases plasma and hepatic adenosine deaminase (ADA, a&b) xanthine oxidase (XO, c&d), uric acid (e&f) in nicotine-treated male rats. Nicotine treatment led to increased plasma and hepatic ADA, XO activities and uric acid levels compared with control. Lithium plus nicotine treatment led to decreased plasma and hepatic ADA, XO activities and uric acid compared with nicotine-treated rats. Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* test. Values are expressed as mean $\pm$ SEM of 6 rats per group (* p < 0.05 vs. Con; # p < 0.05 vs. NIC).

by standardized enzymatic colorimetric methods using reagents obtained from Randox Laboratory Ltd. (Antrim, UK). TG/HDL-C ratio was estimated as index of atherogenic dyslipidemia.

2.7. Statistical analysis

All data were expressed as means $\pm$ SEM. Statistical group analysis was performed with SPSS statistical software (Chicago, IL). One way analysis of variance (ANOVA) was used to compare the mean values of variables among the groups. Bonferroni's test was used to identify the significance of pair wise comparison of mean values among the groups. Statistically significant differences were accepted at p < 0.05.

3. Results

3.1. Lithium improves body weight, food intake, visceral adiposity, liver weight and TyG index in nicotine-exposed rats

Nicotine exposure led to a decrease in the body weight whereas lithium with or without nicotine treatment prevented body weight loss (Fig. 1a). Nicotine exposure led to decreased food intake whereas lithium plus nicotine treatment led to enhanced food intake (Fig. 1b). Nicotine exposure led to decreased visceral adiposity however lithium plus nicotine treatment did not affect visceral adiposity compared with control (Fig. 1c). Nicotine exposure decreased liver weight whereas lithium with or without nicotine treatment attenuate liver weight loss (Fig. 1d). Furthermore, nicotine exposure led to increased TyG index (a marker of metabolic obesity) which was however, ameliorated by lithium plus nicotine treatment (Fig. 1e).

3.2. Lithium improves glucose regulation in nicotine-exposed rats

Nicotine treatment led to impaired glucose regulation as depicted by the increased fasting glycaemia, 1-h & 2-h postload glycaemia, AUC of OGTT, HOMA-IR, insulinemia, HOMA- β and decreased QUICKI (insulin sensitivity) whereas lithium plus nicotine treatment did not affect glucose regulation compared with control. In addition, lithium plus

nicotine treatment led to reduced fasting glycaemia, 1-h & 2-h postload glycaemia, AUC of OGTT, HOMA-IR, insulinemia, HOMA- β and increased QUICKI (insulin sensitivity) compared with nicotine-treated rats (Fig. 2).

3.3. Lithium improves glycogen synthesis and attenuates lipotoxicity in nicotine-treated male rats

Nicotine exposure led to increases in plasma and hepatic FFA, TC, TG and TG/HDL-C ratio compared with control, whereas lithium plus nicotine treatment attenuates elevated plasma and hepatic FFA, TC, TG, and TG/HDL-C ratio compared with nicotine-exposed rats (Fig. 3). In addition nicotine exposure led to reduction in hepatic glycogen content and synthase activity compared with control whereas lithium plus nicotine treatment led to increases in hepatic glycogen content and synthase activity compared with nicotine-treated rats (Fig. 4).

3.4. Lithium suppresses hepatic dysfunction and lactate production in nicotine-treated male rats

Nicotine exposure led to significant increases in plasma and hepatic GGT, ALP and AST activities compared with control whereas lithium plus nicotine treatment decreases in GGT, ALP and AST activities compared with nicotine-treated rats (Fig. 5). Nicotine exposure led to increases in LDH activity and lactate level compared with control whereas lithium plus nicotine treatment suppressed LDH activity and lactate level compared with nicotine-treated rats (Fig. 6).

3.5. Lithium decreases plasma and hepatic adenosine deaminase, xanthine oxidase activities and uric acid in nicotine-treated male rats

Nicotine treatment led to increases in plasma and hepatic ADA, XO activities and uric acid levels compared with control whereas lithium plus nicotine treatment led to decreases in plasma and hepatic ADA, XO activities and uric acid levels compared with nicotine-treated rats (Fig. 7).

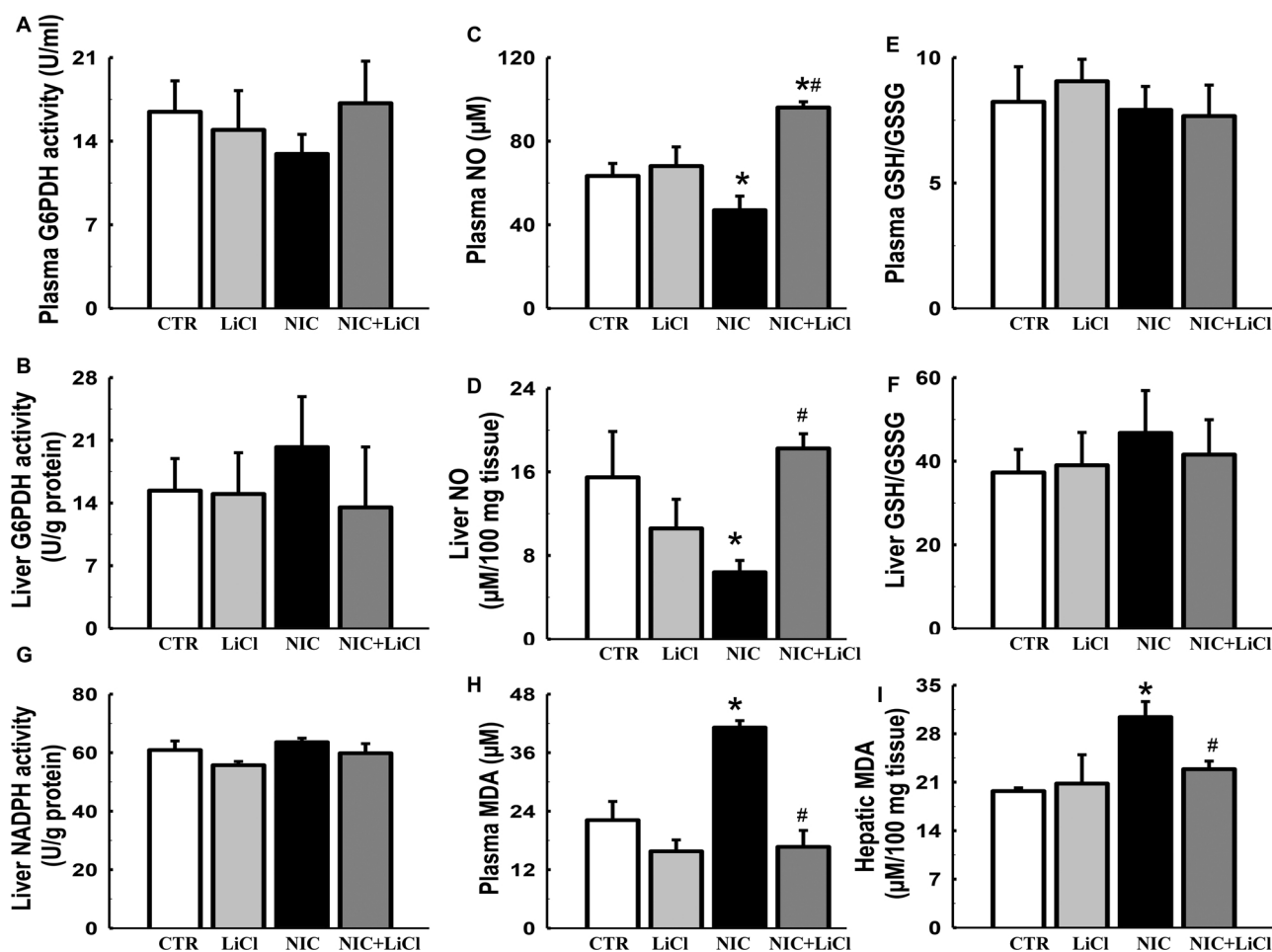


Fig. 8. Effect of lithium on a) Plasma glucose-6-phosphate dehydrogenase (G6PD) activity, b) hepatic G6PD, c) plasma nitric oxide (NO), d) hepatic NO, e) plasma reduced glutathione/glutathione disulfide (GSH/GSSG) ratio, f) hepatic GSH/GSSG ratio, g) hepatic nicotinamide adenine dinucleotide phosphate (NADPH), h) plasma malondialdehyde (MDA), i) hepatic MDA. Plasma and hepatic G6PD activity, GSH/GSSG ratio, and NADPH were not affected by the treatment. There was increased plasma and hepatic MDA level and decreased plasma and hepatic NO level in nicotine-treated rats compared with control. Lithium plus nicotine treatment led to decreased plasma and hepatic MDA level. More so, plasma and hepatic NO was increased in lithium plus nicotine-treated rats compared with nicotine-treated rats. Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* test. Values are expressed as mean $\pm$ SEM of 6 rats per group (**p* < 0.05 vs. CTR; #*p* < 0.05 vs. NIC).

3.6. Lithium attenuates oxidative stress biomarkers in nicotine-treated rats

Plasma and hepatic G6PD activity, GSH, GSSG, GSH/GSSG ratio and NADPH were not affected by the treatment (Fig. 8a & b, e–g) whereas there was increased plasma and hepatic MDA and decreased plasma and hepatic NO levels in nicotine-treated rats compared with control (Fig. 8c & d, h & i). Lithium plus nicotine treatment led to increased plasma and hepatic NO compared with nicotine-treated rats (Fig. 8c &

d). More so, plasma and hepatic MDA was decreased by lithium plus nicotine treatment compared with nicotine-treated rats (Fig. 8h & i, Table 2).

4. Discussion

The present study demonstrates that nicotine exposure causes disrupted glucose homeostasis, lipid dysmetabolism and fatty liver in male

Table 1

Effect of lithium on body weight, food and water intake in nicotine-treated rats.

	CTR	LiCl	NIC	NIC + LiCl
Body weight (g)	131.9 $\pm$ 9.0	129.7 $\pm$ 8.6	132.8 $\pm$ 5.5	130.9 $\pm$ 8.0
Before treatment	172.2 $\pm$ 12.5	165.4 $\pm$ 12.0	149.0 $\pm$ 7.1*	153.7 $\pm$ 10.6*
After treatment				
Food intake (g/kg/day)	73.4 $\pm$ 5.2	72.5 $\pm$ 4.3	73.9 $\pm$ 4.5	71.5 $\pm$ 4.1
Before treatment	73.1 $\pm$ 1.1	78.0 $\pm$ 1.4*	67.7 $\pm$ 3.3*	72.6 $\pm$ 4.2#
After treatment				
Water intake (ml/kg/day)	148.7 $\pm$ 12.3	149.5 $\pm$ 14.5	146.7 $\pm$ 14.9	148.3 $\pm$ 13.6
Before treatment	158.1 $\pm$ 10.6	114.0 $\pm$ 12.7*	110.2 $\pm$ 10.6*	115.7 $\pm$ 14.4*
After treatment				

Data are expressed as mean $\pm$ SEM of 6 rats per group. Data were analyzed by two-way ANOVA followed by Bonferroni *post hoc* test. (**p* < 0.05 vs. control; #*p* < 0.05 vs. NIC).

Table 2
Effect of lithium on pro-atherogenic lipid and anti-oxidant defense in nicotine-treated rats.

	CTR	LiCl	NIC	NIC + LiCl
HDL-C (mg/dl)	24.20 ± 2.37	19.67 ± 4.13*	14.69 ± 8.05*	18.48 ± 3.42*
TC/HDL-C	5.49 ± 1.92	5.01 ± 1.65	8.41 ± 1.05*	4.10 ± 1.71 [#]
GSH (mg/dl)	0.26 ± 0.07	0.27 ± 0.12	0.30 ± 0.11	0.28 ± 0.05
GSSG (mg/dl)	0.03 ± 0.01	0.03 ± 0.00	0.04 ± 0.05	0.04 ± 0.01

Data are expressed as mean ± SEM of 6 rats per group. Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* test. (*p < 0.05 vs. control; [#]p < 0.05 vs. NIC).

rats. The nicotine-induced systemic and hepatic metabolic impairments are accompanied by elevated plasma and hepatic ADA/XO/UA activities. However, despite histological and biochemical evidence of oxidative stress and hepatic injury, plasma and hepatic evaluation of the glutathione system in this study reveals that there was no alteration in the G6PD-dependent antioxidant capacity in nicotine-exposed rats. Furthermore, this study also indicates the protective role of lithium on nicotine-induced glucose dysregulation and hepatic lipid accumulation through decreased ADA/XO/UA-dependent pathway which was also accompanied by a normalized hepatic glycogen synthesis (Fig. 9).

Glucose deregulation is a hallmark of metabolic diseases, and the simple fasting blood glucose level (FBG) can be of great diagnostic or prognostic value. Also, IR markers like hyperinsulinemia, HOMA-IR, TG/HDL-C, TyG-index, 1-h postload glucose and AUC of OGTT are predictive of the development of metabolic syndrome and are linked to obesity and T2DM. In the present study, nicotine exposure led to glucose dysregulation indicated by elevated FBG, HOMA-IR, 1-h postload glucose, hyperinsulinemia, TG/HDL-C (Fig. 2) and β -cell dysfunction. Nicotine treatment also led to reduced body weight gain, food intake, visceral adiposity (Fig. 1; Table 1) with elevated plasma and hepatic FFA and marker of lipid peroxidation (MDA), which indicates negative energy balance and IR-associated FFA dysmetabolism. This result is in line with previous studies which elucidated the deleterious effects of nicotine exposure especially in precipitating glucose dysregulation and IR [29,36]. Studies have also shown that nicotine treatment produces weight loss and reduced food intake [29,37]. A study showed that nicotine *via* inhibition of AMP-activated protein kinase in the hypothalamus (AMPK) caused reduced appetite and adipocyte activation [38]. Studies in rodents revealed that nicotine caused lipolysis in adipocytes and lipogenesis in skeletal muscle by modulating lipoprotein lipase [39]. The mechanism(s) by which nicotine elicits dysmetabolism is not

fully established. However, the present study shows revealed that increased plasma and hepatic ADA/XO/UA activity and suppressed plasma and hepatic nitric oxide (NO) are associated with the nicotine-induced glucose dysregulation and IR. Increased ADA activity has been linked to metabolic derangement and immune system or inflammatory cytokine deregulation [40]. Likewise, XO is a known ROS and superoxide ion producer and increase in its activity is implicated in uric acid associated oxidative stress [41]. Elevated plasma uric acid is regarded as an indicator of inflammation and endothelial dysfunction as it is usually associated with reduced NO [42].

Maintenance of cellular integrity through the prevention of stress is expedient for cellular survival and optimum functionality. However, eukaryotic cells have evolved with mechanisms for preventing extreme intracellular levels of stressor species [43]. Cellular stress occurs when there is imbalance between the production of oxidative and reductive species indicating either oxidative or reductive stress. Oxidative species are important for immune response signaling and they are spontaneously released. The antioxidant systems help to establish homeostasis and they include enzymatic antioxidant species like glutathione peroxidase, G6PD, glutathione reductase, superoxide dismutase, the non-enzymatic antioxidant species like reduced glutathione (GSH), adenosine, ascorbic acid *etc.* The glutathione antioxidant system is G6PD-dependent and its capacity is critical for determinant of intracellular redox state [44] which has been measured by GSH/GSSG ratio [45]. In the present study, nicotine exposure did not alter the plasma and hepatic G6PD-dependent antioxidant capacity when compared with control. This shows that the nicotine-induced dysmetabolism is not associated with a depressed G6PD-dependent antioxidant capacity. The fact that both plasma and hepatic G6PD activity and GSH/GSSG ratio were not altered by nicotine exposure corroborates the unaltered intermediate NADPH in this study, since the main source of NADPH is G6PD (Fig. 8). Therefore, the nicotine-induced oxidative stress and peripheral and hepatic TG synthesis is not through NADPH-dependent pathway. This finding is not in line with the elevated NADPH expression detected in NAFLD [46]. More so, NADPH is said to be involved in *de novo* lipogenesis and cholesterol synthesis. Furthermore, report exist that NADPH is essential for antioxidant systems and reductive biosynthesis and is required by enzymes involved in the pathologies of metabolic disorders that generate ROS [47,48]. The unaltered NADPH concentration is in agreement with the fact that nicotine exposure did not affect G6PD-gluthathione antioxidant defenses in this study (Fig. 8G).

Glucose uptake, utilization and storage describes glucose metabolism and are ubiquitous functions of body cells. Glucose metabolism is characterized by both oxidative and non-oxidative metabolism. The oxidative metabolism requires oxygen and is utilized in the mitochondrion to generate ATP while the non-oxidative glucose metabolism is anaerobic in the cytoplasm and involves mainly glycogen synthesis and production of lactate [49]. Aside other glycolytic pathway branches, like the pentose phosphate pathway and hexose biosynthesis pathway, glucose can also be diverted to fat synthesis in tissues probably as a result of physiological response to unrestricted glucose delivery or pathological glucose dysregulation and IR. Glycogen synthesis is the metabolic process that forestalls glucose shortage between meals to ensure availability of energy for cellular activities [8,9]. The liver is the chief organ in glycogen metabolism that converts glucose to glycogen at

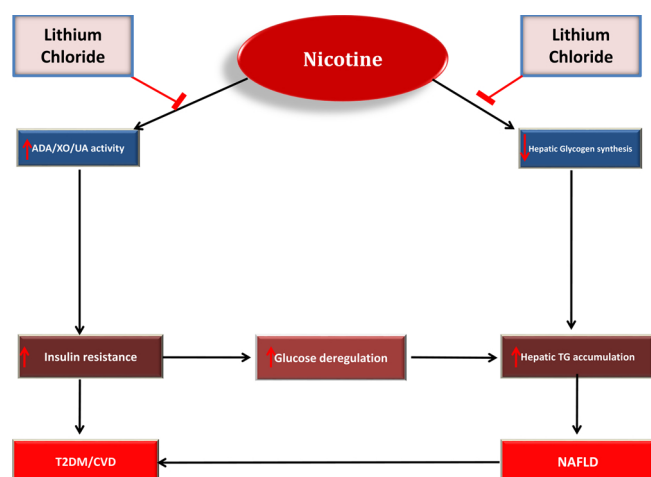


Fig. 9. Schematic diagram of the possible mechanism by which lithium ameliorates nicotine-induced dysmetabolism and fatty liver in male rats. ADA; adenosine deaminase, CVD; cardiovascular disease, NAFLD; non-alcoholic fatty liver disease, T2DM; type 2 diabetes mellitus, TG; triglyceride, XO; xanthine oxidase, UA; uric acid.

postprandial state by the action of mainly glucokinase (Hexokinase IV) and glycogen synthase. Hepatic IR is usually associated with fasting hyperglycemia and reduced hepatic glycogen synthesis [50]. The results of this study show that nicotine treatment induced a significant reduction in glycogen synthesis which was accompanied by elevated hepatic FFA, TG accumulation together with evidence of lipid peroxidation. This is likely as a result of diversion of glucose from glycogen synthesis to lipogenesis due to IR-induced reduction in glycogen synthase activity which was corroborated in this study by nicotine-induced depressed glycogen synthase activity (Fig. 4).

Lactate production is another non-oxidative product of glucose metabolism. It is produced by the action of lactate dehydrogenase (LDH) in a reversible reaction from pyruvate to divert glucose from the mitochondrial oxidative ATP production. Elevated blood lactate is regarded as predictive of IR, T2DM [50,51], mitochondrial insufficiency [52] and increased LDH activity is also regarded as indicator of oxidative stress. The finding that nicotine-induced hepatic TG accumulation and IR is associated with elevated blood lactate and LDH activity implicates the involvement of oxidative stress in nicotine-induced hepatic TG accumulation and IR. Taken together, the nicotine-induced hepatic TG accumulation in this study occurred along with increased plasma and hepatic ADA/XO/UA activity, LDH activity and lactate level. This result reveals that nicotine-induced hepatic injury might be through ADA/XO/UA-dependent pathway.

The finding that nicotine-induced hepatic TG accumulation is through ADA/XO/UA-dependent pathway implicates UA production in the pathogenesis of NAFLD. This is in agreement with several experimental studies that reported increase prevalence of metabolic syndrome is closely related to graded increases in hyperuricemia which strongly suggest elevated serum UA levels to be a predictor of metabolic syndrome, including impaired glucose tolerance, insulin resistance, lipid metabolism [53,54] and NAFLD [55]. The fact that UA has been reported to be capable of acting as a pro-oxidant causing oxidative damage is noteworthy and may be involved in the pathogenesis of NAFLD [56]. Besides the mechanism involving increased oxidative stress, an elevated UA concentration may itself also contribute to hepatic lipid accumulation because a previous report showed UA to promote TG accumulation in hepatocytes through inhibition of AMP-activated protein kinase (AMPK) [57]. AMPK and AMP deaminase (AMPD) within hepatocytes have been implicated in the development of hepatic steatosis. When AMPK activity is reduced excess fat infiltration occurs, while its stimulation can prevent steatosis through increased fat oxidation and inhibition of lipogenesis. AMPD has opposing effect on fat deposition within the hepatocytes. AMPD activation increases intracellular UA synthesis [58]. Intracellular UA inhibits AMPK activity [59] thereby causing excess fat infiltration and endoplasmic reticulum stress in the hepatocytes leading fatty liver due to fat oxidation and inhibition of lipogenesis.

Uric acid acts as a strong antioxidant outside the cell and as a pro-oxidant inside the cell where it stimulates NADPH oxidase enzyme causing increased intracellular oxidative stress, mitochondrial injury, and ATP depletion [60,61]. XO is abundantly expressed in the liver and generates hydroxyperoxide when catalyzing substrates. Therefore, serum UA elevation is considered to accompany increased accumulation of oxidative stress generated by hepatic XO. Although the endogenous production of UA is mainly from the liver, intestines, and other tissues like muscles, kidneys, and the vascular endothelium. Hyperuricemia is associated with impaired nitric oxide bioavailability. This interesting association is corroborated by our finding of reduced plasma and hepatic nitric oxide bioavailability and hyperuricemia in nicotine-treated rats. Nitric oxide is synthesised from L-arginine, which is regulated by arginase through catalytic degradation. This enzyme arginase that reduces nitric oxide precursor is activated by UA leading to reduction in nitric oxide, resulting in increased production of superoxide molecules, reactive oxygen species and endothelial dysfunction [62]. This is line with the findings of the present study where

nicotine-induced hepatic TG accumulation is associated with decreased plasma and hepatic NO bioavailability. Similarly, nicotine has been reported in our previous study to cause hyperuricemia, hyperinsulinemia and increased endothelial dysfunction mediators and oxidative stress in female Wistar rats [63].

Furthermore, several reports have demonstrated favorable effects of xanthine oxidase (XO) inhibitors on the disorders characteristic of metabolic syndrome further implicating the involvement of ADA/XO/UA-dependent pathway and/or UA production as observed in our rodent model of nicotine-induced TG accumulation. For example, administration of allopurinol or febuxostat which inhibits XO activities preventing UA production has been reported to prevent fructose-induced hyperinsulinemia, hypertension, and weight gain in rats [64], diabetic renal injury in streptozotocin-treated rats [65], and atherosclerosis development in apolipoprotein E knockout mice [66].

Furthermore, nicotine-induced hepatic TG accumulation and injury in the present study is accompanied by altered liver function as depicted by elevated alkaline phosphatase (ALP), aspartate transaminase (AST) and gamma-glutamyl transferase (GGT) activities in nicotine-exposed rats. In contrast to our finding, a previous study by McCullough has reported that a liver can remain fatty without disturbing liver function [67]. Similarly, another study also documented that in NAFLD, liver function test can be normal and the degree of abnormalities does not correlate with the degree of steatosis [68]. In consonance with our finding of elevated liver enzymes (ALT, AST & GGT) in nicotine-treated rats compared with control, a recent study indicated that the levels of ALT, AST, and GGT were significantly higher in NAFLD patients than in those without NAFLD [69].

The present investigation elucidates the role of lithium, an alkali metal and a known non-specific GSK-3 inhibitor [70] in nicotine-induced dysmetabolism. Lithium has been utilized to study the regulation of the glucose transport system in mammalian skeletal muscle aside been used for treatment of bipolar diseases [29,33]. The present study reveals that treatment of nicotine-exposed rats with lithium led to amelioration of the nicotine-induced dysmetabolism and hepatic injury. Various studies have elucidated the effects of lithium on ischemic/reperfusion injury even in the liver and revealed conflicting results of exacerbation [71]. However, there is dearth of studies on the role of lithium in hepatic fat accumulation.

Therefore, lithium ameliorates nicotine-induced dysmetabolism and hepatic TG accumulation by suppressing ADA and XO activities and UA production. This is in agreement with a report that XO inhibitor suppresses the development of nonalcoholic steatohepatitis in a rodent model [72]. The report of this study further confirms the salient/causative role of UA in the development of metabolic syndrome especially hepatic TG accumulation. The amelioration of nicotine-induced deleterious effects by lithium in this study is accompanied by restoration of hepatic glycogen synthesis and suppression of plasma and hepatic ADA/XO/UA activity (Fig. 9). Therefore, this study reveals that lithium suppressed ADA/XO/UA-dependent pathway and led to activation of glycogen synthase probably via its inhibitory effect on GSK-3, corroborating the earlier finding from our laboratory that GSK-3 activation might contribute to the nicotine-induced metabolic dysfunctions [30].

In conclusion, the present study demonstrates that nicotine-induced glucose dysregulation and hepatic TG accumulation and injury is associated with increased plasma and hepatic uric acid, ADA and XO activities, oxidative stress and depressed hepatic glycogen synthesis independent of G6PD-dependent antioxidant capacity. However, lithium protects the liver through suppression of plasma and hepatic ADA/XO/UA pathway, oxidative damage and augmentation of glycogen synthesis implicating the therapeutic importance of lithium against nicotine-induced metabolic dysfunction. Lithium would be a potential therapeutic intervention for patient with NAFLD particularly those associated with hyperuricemia.

Disclosure of interest

The authors have no conflicts of interest to declare.

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