

Sodium acetate protects against nicotine-induced excess hepatic lipid in male rats by suppressing xanthine oxidase activity

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ABSTRACT

Fatty liver is the hepatic consequence of chronic insulin resistance (IR) and related syndromes. It is mostly accompanied by inflammatory and oxidative effects. Increased activity of xanthine oxidase (XO) exerts both inflammatory and oxidative effects and has been implicated in metabolic derangements including non-alcoholic fatty liver disease. Short chain fatty acids (SCFAs) elicit beneficial metabolic alterations in IR and related syndromes. In the present study, we evaluated the preventive effects of a SCFA, acetate, on nicotine-induced dysmetabolism and fatty liver. Twenty-four male Wistar rats ($n = 6/\text{group}$): vehicle-treatment (*p.o.*), nicotine-treated (1.0 mg/kg; *p.o.*), sodium acetate-treated (200 mg/kg; *p.o.*) and nicotine + sodium acetate-treated groups. The treatments lasted for 8 weeks. IR was estimated by oral glucose tolerance test and homeostatic model assessment of IR. Plasma and hepatic free fatty acid, triglyceride (TG), glutathione peroxidase, adenosine deaminase (ADA), XO and uric acid (UA) were measured. Nicotine exposure resulted in reduced body weight, liver weight, visceral adiposity, glycogen content and glycogen synthase activity. Conversely, exposure to nicotine increased fasting plasma glucose, lactate, IR, plasma and hepatic TG, free fatty acid, TG/HDL-cholesterol ratio, lipid peroxidation, liver function enzymes, plasma and hepatic UA, XO and ADA activities. However, plasma and hepatic glucose-6-phosphate dehydrogenase-dependent antioxidant defense was not affected by nicotine. Concomitant treatment with acetate ameliorated nicotine-induced effects. Taken together, these results indicate that nicotine exposure leads to excess deposition of lipid in the liver by enhancing XO activity. The results also imply that acetate confers hepatoprotection and is accompanied by decreased XO activity.

1. Introduction

Metabolic syndrome is characterized by obesity, adipose tissue accumulation, glucose dysregulation with hyperglycemia, dyslipidemia and hypertriglyceridemia among others [1]. This metabolic disorder affects millions of people and has contributed to high cardiovascular mortality and morbidity worldwide [2]. A key process in the development of metabolic syndrome is peripheral insulin resistance (IR) [3]. Particularly, hepatic IR plays a major role and results in the failure of insulin to suppress hepatic gluconeogenesis [4]. Moreover, the common consequence of IR is hyperglycemia and impaired glucose tolerance [3].

Increased adiposity, type 2 diabetes, and non-alcoholic fatty liver disease (NAFLD) are rapidly increasing worldwide [5]. NAFLD, a condition viewed as the hepatic expression of metabolic syndrome, is one of the most common causes of chronic liver diseases [6]. The dramatic increase in prevalence of metabolic syndrome, as well as NAFLD has

been linked to the global spread of the western diet coupled with a more sedentary lifestyle [7] and indiscriminate exposure to nicotine [8]. The aggressive form of this disease is commonly found in adults, but the number of young individuals affected is growing rapidly [9]. Increased production of triglycerides (TGs) by the liver is a hallmark of metabolic syndrome and type 2 diabetes, leading to increased plasma TG levels, accumulation of pro-atherogenic lipids and subsequently increased the risk of cardiovascular events [10,11]. Free fatty acids (FFAs) derived from various sources, including adipose tissue, de novo hepatic fatty acid synthesis, even FFA from dietary sources, are required as a major substrate for hepatic TG synthesis. Interest in the causes and consequences of lipid infiltration in the liver has risen in recent years because of the association between triglyceride accumulation in different tissues and the development of IR [12]. The pathophysiological mechanisms of NAFLD have not been well understood, but earlier studies have demonstrated that influx of triglycerides into hepatocytes

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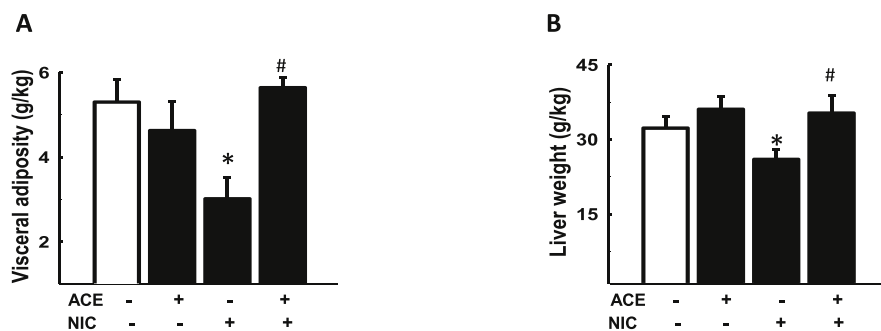


Fig. 1. Effect of sodium acetate on a) visceral adiposity and b) liver weight in nicotine-treated rats. There was decreased visceral adiposity and liver weight in nicotine-treated male rats. However, in sodium acetate plus nicotine treatment, visceral adiposity and liver weight were normalized compared 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).

Table 1
Effect of sodium acetate on body weight, food and water intake in nicotine-treated rats.

	CTR	ACE	NIC	NIC + ACE
Body weight (g)				
Before treatment	131.9 $\pm$ 13.7	130.2 $\pm$ 16.0	132.8 $\pm$ 8.8	129.4 $\pm$ 8.6
After treatment	176.5 $\pm$ 19.0	162.8 $\pm$ 19.4	149.0 $\pm$ 11.4*	152.6 $\pm$ 16.6
Food intake (g/kg/day)				
Before treatment	73.4 $\pm$ 10.4	72.5 $\pm$ 3.8	74.9 $\pm$ 6.9	70.6 $\pm$ 6.7
After treatment	73.1 $\pm$ 2.3	79.2 $\pm$ 2.5*	67.7 $\pm$ 2.1*	73.0 $\pm$ 3.7#
Water intake (ml/kg/day)				
Before treatment	148.7 $\pm$ 16.3	147.5 $\pm$ 14.5	145.8 $\pm$ 14.9	140.7 $\pm$ 3.6
After treatment	158.1 $\pm$ 4.4	133.4 $\pm$ 10.7	110.2 $\pm$ 10.6*	114.5 $\pm$ 13.9*

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).

results in over production of reactive oxygen species by β -oxidation, which causes an antioxidant/oxidant imbalance [13], with inactivation of some regulatory proteins and consequent tissue inflammation, IR and impaired mitochondrial function [14].

Xanthine oxidase (XO) is a cytosolic enzyme that is involved in purine metabolism, and catalyzes the modulation of hypoxanthine to xanthine, and then uric acid [15]. XO is highly expressed in the liver and intestine [15]. Increased activity of XO might be the underlying factor in the pro-oxidant property of uric acid which is demonstrated to contribute immensely to the development of cardiometabolic disorders [16] owing to its utilization of molecular oxygen to produce reactive oxygen species (ROS). In concordance, notable beneficial changes in inflammatory conditions with hyperuricemia have been reported when xanthine oxidase is inhibited by allopurinol [17]. Taken together, blockade of XO might significantly reduce UA production. This will have a biphasic effect: XO-induced ROS generation is reduced and UA proinflammatory effects are averted. Also, uric acid has been shown to enhance ectopic lipid accumulation in tissues especially following fructose exposure [18].

Nicotine, a major alkaloid of tobacco smoke and predominant chemical among over 4000 compounds in cigarette smoke, is widely used as nicotine replacement therapy (NRT) either as gum or patch preparations for smoking cessation [19]. Nicotine is highly lipid soluble and rapidly crosses the blood-brain barrier and it has been shown to decrease insulin sensitivity following prolonged use of tobacco products or nicotine gum [20,21]. Nicotine has further been implicated in both type 1 and type 2 diabetic patients, sepsis [22], ulcerative colitis, renal ischemia-reperfusion injury [23] and liver disease [24] including hepatic lipid dysmetabolism and accumulation. However, the mechanism by which nicotine causes lipid dysmetabolism and hepatic accumulation is poorly understood. We therefore investigated the effects of chronic nicotine exposure on XO activity, G6PD-dependent antioxidant defense and glycogen synthesis.

Short-chain fatty acids (SCFAs) are end-products from the fermentation of complex carbohydrates and/or digestible fibers largely by the colonic microbiota, especially by anaerobic bacteria [25]. Over 90% of the SCFAs present in the colon are constituted by acetate, propionate,

and butyrate [26]. Acetate constitutes about 60% of the SCFAs composition produced in the colon [27]. Administration of SCFA particularly acetate has been reported to positively impact colitis, airway disease, kidney disease and metabolic syndrome in diet-induced obese mice [28]. We therefore tested the hypothesis that SCFA, acetate would prevent deleterious hepatic metabolic phenotypes of chronic exposure to nicotine by suppressing xanthine oxidase activity.

2. Materials and methods

2.1. Animals

Eight to ten weeks old male Wistar rats, weighing 120–125 g were obtained from the animal house of the College of Health Sciences, University of Ilorin (Ilorin, Nigeria). Rats had unrestricted access to standard rat chow and tap water. After 1 week of acclimatization, the animals were randomly assigned to four groups (six animals per group). Rats were maintained under standard environmental conditions of temperature, relative humidity and dark/light cycle. 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 Board of University of Ilorin, (Ilorin, Nigeria) and every effort was made to minimize suffering and the number of animals used.

2.2. Treatment

Control group received distilled water (vehicle) daily, nicotine-treated group received 1.0 mg/kg NIC (Alchem Inc., Minnesota, USA) daily, acetate-treated group received 200 mg/kg sodium acetate (ACE; Science Company, Lakewood, CO, USA) daily. NIC + ACE-treated group received a combination of nicotine plus sodium acetate daily. All treatments were given by oral gavage and lasted for 8 weeks. Body weight, food and water intakes were monitored weekly.

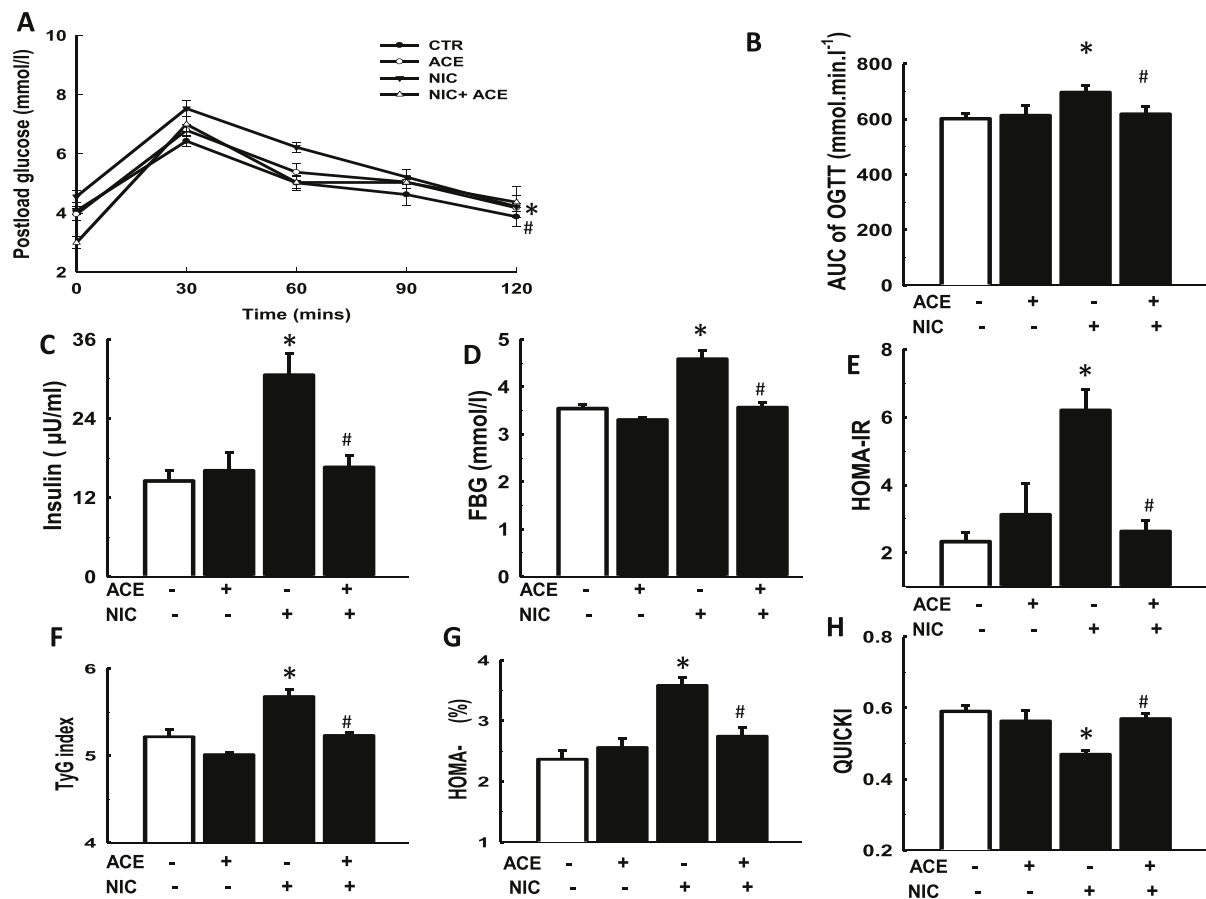


Fig. 2. Sodium acetate improves glucoregulation; a) oral glucose tolerance test (OGTT), b) area under glucose curve (AUC) of OGTT, c) fasting insulin, d) fasting blood glucose, e) HOMA-IR, f) TyG index g) HOMA-β and h) QUICKI in nicotine-treated male rats. Nicotine treatment led to impaired glucoregulation as depicted by the increased fasting glycaemia, 1-h postload glycaemia, AUC of OGTT, HOMA-IR, TyG index, plasma insulin level, HOMA-β and decreased QUICKI (insulin sensitivity) whereas acetate with or without nicotine treatment did not affect glucoregulation compared with control. In addition, acetate 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 $\pm$ SEM of 6 rats per group (* p < 0.05 vs. Con; # p < 0.05 vs. NIC).

2.3. Sample preparation

At the end of treatment, the rats were anesthetized with sodium pentobarbital (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. In addition, the liver was isolated, blotted and weighed immediately. After weighing, 100 mg of liver tissue was carefully removed and homogenized on ice with a glass homogenizer in phosphate buffer solution.

2.4. Visceral adiposity

Following blood sample collection, all visceral fat depots including abdominal, perirenal and retroperitoneal were isolated and weighed immediately with an electronic weighing balance.

2.5. Histopathological studies

For hematoxylin and eosin (H & E) stains, a section of the right lobe of liver was fixed in 10% formalsaline overnight and thereafter dehydrated, embedded in paraffin and sectioned at 5-μm thickness. The slides were observed under light microscope at magnification of X100.

2.6. Biochemical measurements

2.6.1. Glucoregulatory parameters

Oral glucose tolerance test (OGTT) was performed 48 h prior to the termination of the experiment. The rats had 12-h overnight fast and the fasting blood glucose was obtained. Thereafter, glucose (2 g/kg b.w.) was orally given and postload glucose level was determined 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). IR and β-cell function were determined using the homeostasis model assessment for IR (HOMA-IR = fasting glucose (mmol/l)*fasting insulin (μU/l)/22.5) and β-cell function (HOMA-β = (20*fasting insulin)/(fasting glucose-3.5) respectively while quantitative insulin sensitivity check index; QUICKI = 1/[Log (fasting insulin) + Log (fasting glucose)]. Triglyceride-glucose index on the other hand is expressed as TyG = Ln (TG (mg/dl) × FBG (mg/dl)/2) [18,29]. Plasma insulin level was determined using ELISA kits obtained from Ray Biotech, Inc. (Georgia, USA). Whereas, hepatic glycogen concentration, glycogen synthase (GS) and nicotinamide adenine dinucleotide phosphate (NADPH) activities were determined using standard spectrophotometric method and reagents obtained from Oxford Biomedical Research Inc. (Oxford, USA).

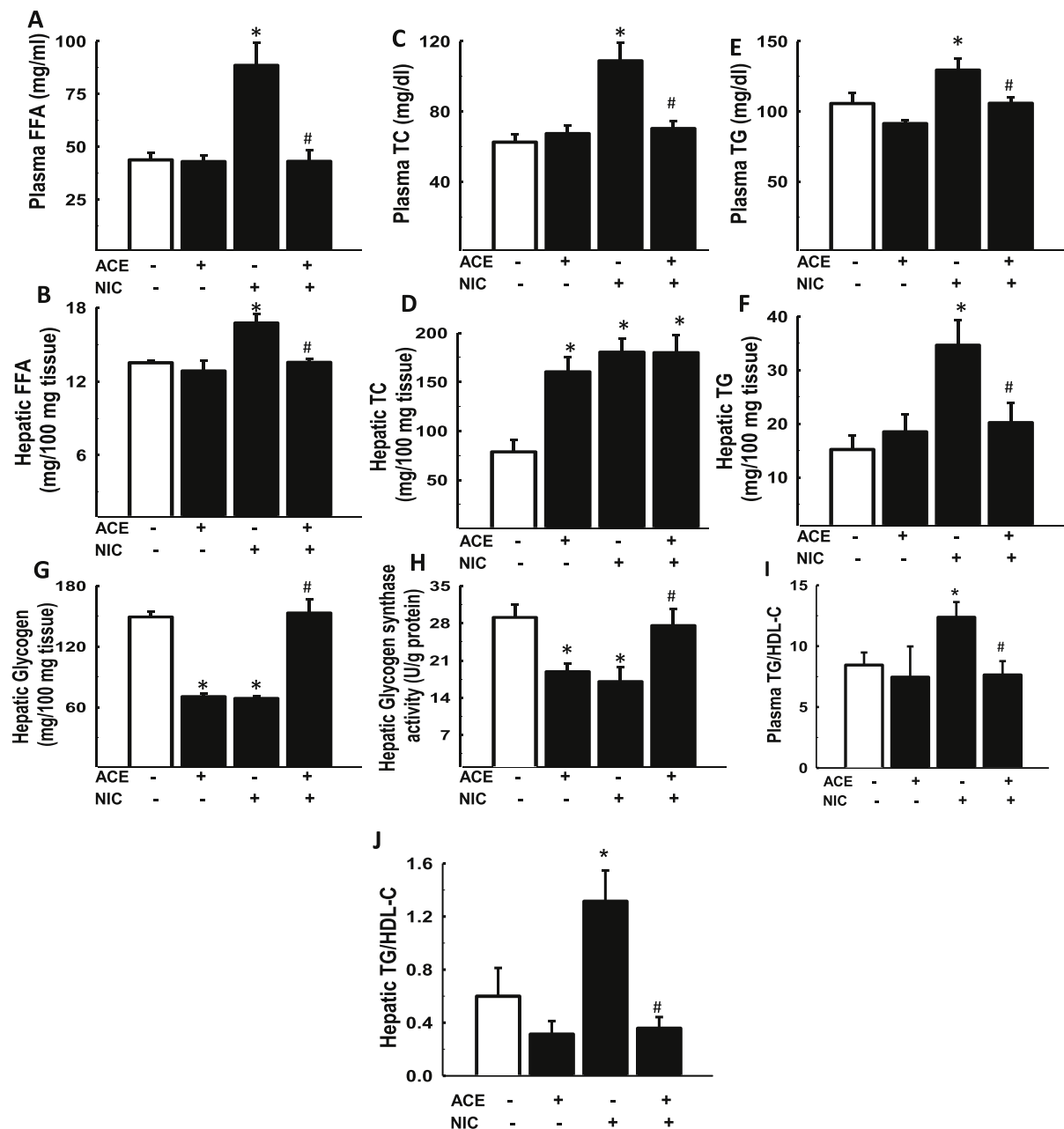


Fig. 3. Sodium acetate improves lipid metabolism depicted by a) plasma FFA, b) hepatic FFA, c) plasma TC, d) hepatic TC, e) plasma TG, f) hepatic TG, g) hepatic glycogen and h) hepatic glycogen synthase activity and plasma/hepatic TG/HDL-C ratio (i–j) in nicotine-treated male rats. Nicotine treatment led to impaired lipid metabolism that is revealed by increased plasma and hepatic TC, TG & FFA compared with control. However, acetate plus nicotine treatment led to improved lipid metabolism by reducing plasma and hepatic TG, FFA, and plasma TC. Hepatic TC remained elevated in acetate plus nicotine-treated rats compared with control. There was also decreased hepatic glycogen and glycogen synthase activity by acetate or nicotine treatment compared with control whereas acetate plus nicotine led to elevated hepatic glycogen and glycogen synthase activity compared with nicotine-treated rats. In addition, nicotine treatment led to increased plasma and hepatic TG/HDL-C ratio. However, sodium acetate led to reduced plasma and hepatic TG/HDL-C ratio in 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. Con; #*p* < 0.05 vs. NIC).

Table 2

Effect of sodium acetate on pro-atherogenic lipid and antioxidant defense in nicotine-treated rats.

	CTR	ACE	NIC	NIC + ACE
HDL-C (mg/dl)	22.16 $\pm$ 2.37	16.21 $\pm$ 3.47*	15.69 $\pm$ 8.05*	14.19 $\pm$ 2.17*
TC/HDL-C	5.49 $\pm$ 1.92	3.86 $\pm$ 0.47*	7.49 $\pm$ 1.05*	5.18 $\pm$ 0.55#
GSH	0.26 $\pm$ 0.07	0.17 $\pm$ 0.04	0.41 $\pm$ 0.09*	0.22 $\pm$ 0.02#
GSSG	0.03 $\pm$ 0.01	0.02 $\pm$ 0.00	0.08 $\pm$ 0.05*	0.04 $\pm$ 0.01#

Data are expressed as mean $\pm$ 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).

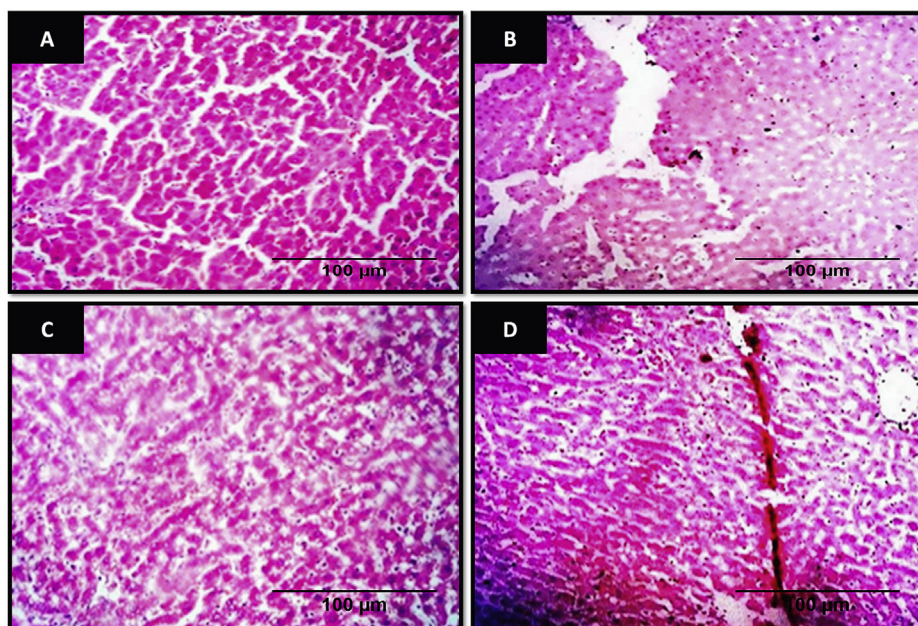


Fig. 4. Photomicrographs of the liver from male Wistar rats. Control group shows hepatic tissue with preserved architecture, Acetate-treated group shows normal hepatic tissue (b), Nicotine-treated rats shows hepatic fat accumulation (c) and Acetate plus nicotine-treated group shows hepatic tissue with improved hepatic cytoarchitecture (d). (H & E paraffin stain; transverse section).

2.6.2. Lipid profile

Fasting triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL), and free fatty acid (FFA) were estimated from the plasma and hepatic tissue homogenate by standardized colorimetric methods using reagents obtained from Randox Laboratory Ltd. (Co. Antrim, UK). Ratios of TG/HDL-C and TC/HDL-C were determined as atherogenic lipid.

2.6.3. Adenosine deaminase and xanthine oxidase activities and uric concentration

Plasma adenosine deaminase (ADA) and xanthine oxidase (XO) activities were determined by standard enzymatic colorimetric method using reagents from Randox Laboratory Ltd. (Co. Antrim, UK) while uric acid (UA) was determined using non-enzymatic kits from Randox Laboratory Ltd. (Co. Antrim, UK) and Oxford Biomedical Research Inc. (Oxford, USA) respectively, and in adherence to the manufacturer's procedures.

2.6.4. Redox biomarkers

Plasma and hepatic malondialdehyde (MDA) and glucose-6-phosphate dehydrogenase (G6PD) activity were determined by standard spectrophotometric method using assay kits obtained from Randox Laboratory Ltd. (Co. Antrim, UK) and in adherence to manufacturer's instruction. While oxidized glutathione (GSSG), reduced glutathione (GSH) and glutathione peroxidase (GPx) were determined by standard spectrophotometric method using reagent kits obtained from Oxford Biomedical Research Inc. (Oxford, USA). Reduced to oxidized glutathione ratio (GSH/GSSG) was calculated and used as an indicator of oxidative stress. Likewise, nitric oxide (NO) concentration was determined using reagent kits obtained from Oxford Biomedical Research Inc. (Oxford, USA) in adherence to manufacturer's procedures.

2.6.5. Liver enzymes and injury biomarkers

Plasma and hepatic gamma-glutamyl transferase (GGT), alanine transferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) aspartate transaminase (AST) activities were determined using standard spectrophotometric method and assay reagents obtained from Randox Laboratory Ltd. (Co. Antrim, UK). In addition, lactate concentration was determined using spectrophotometric method based on the oxidation of D-lactate to pyruvate by NAD⁺ and lactate dehydrogenase (LDH) activity was measured by standardized

enzymatic colorimetric method using assay reagents obtained from Randox Laboratory Ltd. (Co. Antrim, UK).

2.6.6. 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. Effect of sodium acetate on body weight, food intake, water consumption, liver weight, visceral adiposity and triglyceride-glucose index in nicotine-treated male rats

There was decrease in body weight gain, food intake, water intake and visceral adiposity in nicotine-treated rats compared with control whereas acetate with or without nicotine treatment did not affect body weight gain and visceral adiposity compared with control (Fig. 1A; Table 1). On the other hand, acetate treatment did not affect water intake but led to increased food intake compared with control (Table 1). However, acetate with nicotine treatment led to increased food intake compared with nicotine-treated rats whereas acetate plus nicotine resulted in decreased water intake. Liver weight was reduced in nicotine-treated rats compared with control whereas nicotine plus acetate treatment led to increased liver weight compared with nicotine-treated rats (Fig. 1B).

3.2. Sodium acetate improves glucoregulation in nicotine-treated male rats

Nicotine treatment led to impaired glucoregulation as shown by increased fasting glycaemia, 1-h postload glycaemia, AUC of OGTT, HOMA-IR, triglyceride-glucose (TyG) index, plasma insulin level, HOMA- β and decreased QUICKI (insulin sensitivity) whereas acetate with or without nicotine treatment did not affect glucoregulation compared with control. In addition, acetate plus nicotine treatment led to reduced fasting glycaemia, 1-h postload glycaemia, AUC of OGTT, HOMA-IR, TyG index, plasma insulin level, HOMA- β and increased QUICKI (insulin sensitivity) compared with nicotine-treated rats

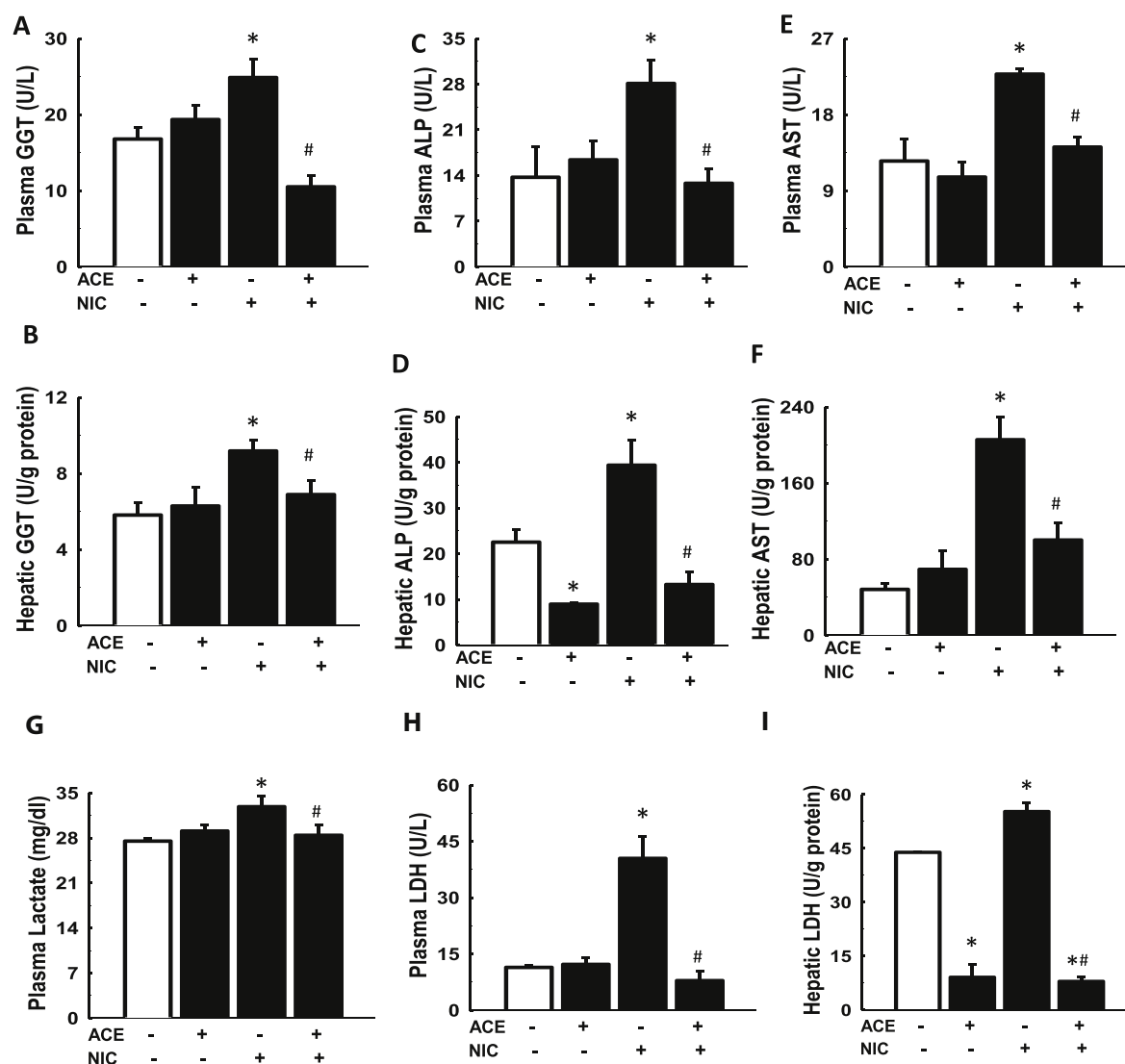


Fig. 5. Sodium acetate attenuates hepatic injury as depicted by a) plasma gamma glutamyl transferase (GGT), b) hepatic GGT, c) plasma alkaline phosphatase (ALP), d) hepatic ALP, e) plasma aspartate transaminase (AST), f) hepatic AST and plasma lactate and plasma/hepatic lactate dehydrogenase (LDH; g-i) in nicotine-treated male rats. There was increased plasma and hepatic GGT, ALP and AST activities in nicotine-treated rats compared with control. Acetate plus nicotine treatment led to decreased plasma and hepatic GGT, ALP and AST activities compared with nicotine-treated rats. Likewise, there was increased plasma lactate, plasma and hepatic LDH in nicotine-treated male rats compared with control. However, Acetate 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).

(Fig. 2A–H).

3.3. Sodium acetate improves plasma and hepatic free fatty acid, pro-atherogenic lipids, hepatic glycogen and glycogen synthase activity in nicotine-treated male rats

Nicotine treatment led to impaired lipid metabolism that is revealed by increased plasma and hepatic TC, TG, FFA, TC/HDL-C and TG/HDL-C compared with control (Fig. 3 A–J, Table 2). HDL-C was decreased across the treatment groups (Table 2) whereas acetate treatment did not affect plasma and hepatic FFA, plasma TC, TG and TC/HDL-C, plasma and hepatic TG/HDL-C but led to increased hepatic TC and TG respectively compared with control. However, acetate plus nicotine treatment led to improved lipid metabolism by reducing plasma and hepatic TG, FFA, TG/HDL-C, plasma TC and TC/HDL-C compared with nicotine-treated (Fig. 3A–J; Table 2). Hepatic TC remained elevated in acetate plus nicotine-treated rats compared with control (Fig. 3D).

There was also decreased hepatic glycogen and glycogen synthase activity by acetate or nicotine treatment compared with control whereas acetate plus nicotine led to elevated hepatic glycogen and glycogen synthase activity compared with nicotine-treated rats (Fig. 3G and H). H & E staining technique was used for the histology of liver to confirm hepatic fat accumulation microscopically. Nicotine-treated rats had hepatic fat accumulation whereas acetate plus nicotine showed improved cytoarchitecture (Fig. 4).

3.4. Sodium acetate decreases hepatic dysfunction and lactate production in nicotine-treated male rats

There was increased plasma GGT, ALP, AST, lactate and hepatic GGT, ALP and LDH activities in nicotine-treated rats compared with control. Acetate treatment did not affect plasma GGT, AST, ALP, LDH activities, increased hepatic GGT, AST, plasma lactate and decreased hepatic ALP and LDH. Acetate plus nicotine treatment led to decreased

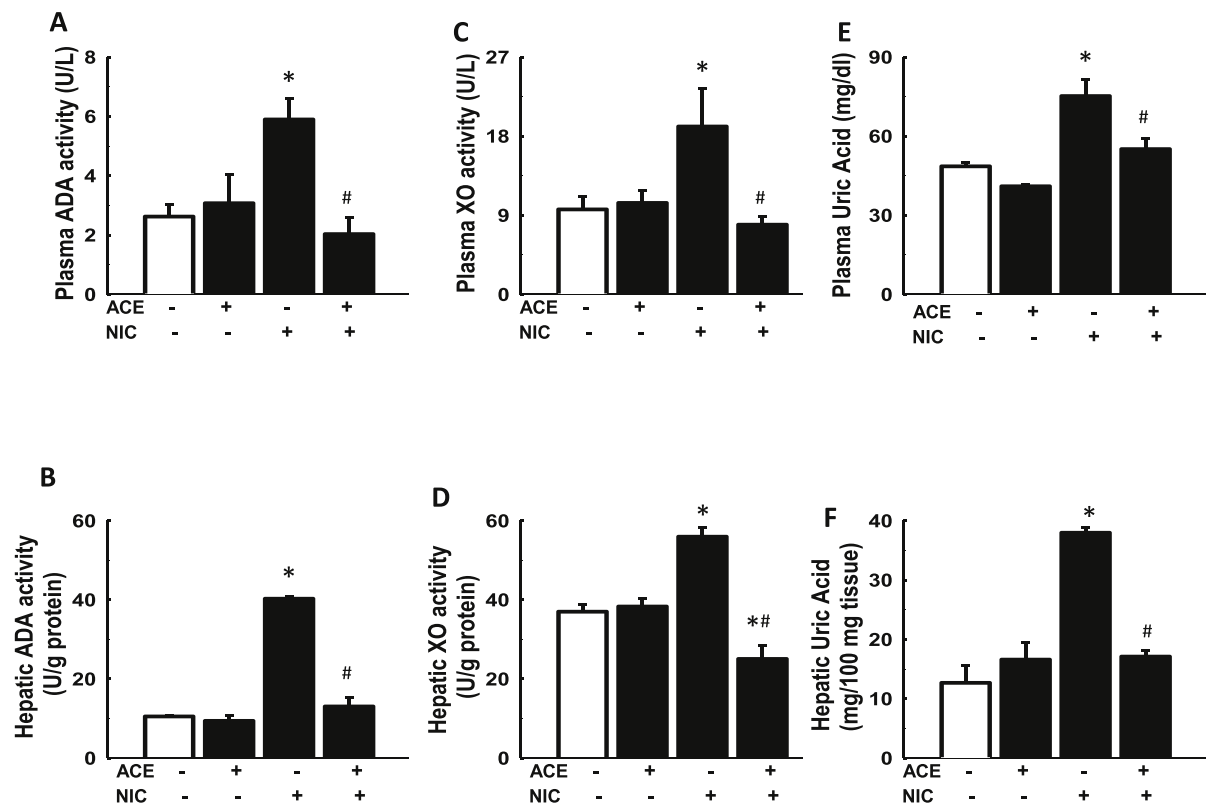


Fig. 6. Sodium acetate decreases plasma and hepatic adenosine deaminase (ADA, a&b) xanthine oxidase (XO, c&d), uric acid (e&f) in nicotine-treated male rats. Nicotine treatment led to increased plasma and hepatic ADA, XO activities and uric acid levels compared with control. Acetate treatment led to 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).

plasma and hepatic GGT, ALP, LDH activities and plasma lactate compared with nicotine-treated rats (Fig. 5).

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

Nicotine treatment led to increased plasma and hepatic ADA, XO activities and uric acid levels compared to control (Fig. 6A–F). Acetate treatment did not affect plasma and hepatic ADA and XO activities whereas uric acid level was decreased in plasma and hepatic tissue of acetate-treated rats compared with control (Fig. 6 E & F). However, acetate plus nicotine treatment led to decreased plasma and hepatic ADA, XO activities and uric acid level compared with nicotine-treated rats (Fig. 6B, D & F).

3.6. Sodium acetate did not alter plasma and hepatic G6PD, NADPH but decreases oxidative stress biomarkers in nicotine-treated male rats

Plasma and hepatic G6PD activity, GSH/GSSG ratio, hepatic NO and NADPH were not affected by the treatment (Fig. 7 A,B,D, E & F) whereas there was increased plasma and hepatic MDA level, plasma GSH, GSSG and decreased plasma NO level in nicotine-treated rats compared with control (Fig. 7C, H&I; Table 2). However, acetate treatment led to decreased plasma GSH, GSSG and MDA levels (Fig. 7H; Table 2) and increased plasma NO level compared with control (Fig. 7 C). Acetate plus nicotine treatment led to decreased plasma GSH, GSSG levels and MDA level (Fig. 7H; Table 2). In addition, plasma NO was increased in acetate plus nicotine-treated rats compared with nicotine-treated rats (Fig. 7C).

4. Discussion

This study elucidates the effects of SCFA, acetate on nicotine-induced metabolic dysfunction. Our data showed that nicotine exposure altered hepatic metabolism, which is indicated by fat accumulation and injury together with decreased glycogen synthesis. The study implicated increased activity of XO in nicotine-induced metabolic derangement which is found to be independent of glutathione antioxidant defense system. Impressively, acetate averted the glucose dysmetabolism and hepatic lipotoxicity and injury induced by nicotine exposure by attenuation of plasma and hepatic XO and enhancement of hepatic glycogen synthesis. Therefore, the evidences herein reveal that SCFAs, acetate possibly maintains systemic and hepatic metabolic homeostasis during nicotine exposure, and this effect is accompanied by decreased XO activity (Fig. 8).

Findings from previous studies agree with the fact that disruption of metabolic processes resulting in glucose deregulation and increased adiposity are precise precursors of CMD, T2DM, CVD and hepatic diseases like the immensely studied non-alcoholic fatty liver disease (NAFLD) [30,31]. The present study showed that nicotine induced impaired glucose homeostasis indicated by elevated fasting plasma glucose level, impaired insulin sensitivity, elevated HOMA-IR, TyG index, AUC of OGTT and 1-h postload glycaemia but this outcome is accompanied by reduced body weight gain, liver weight and adiposity. Although, body weight increase is considered a risk factor for metabolic diseases of which glucose deregulation is the hallmark, reduction in body weight and adiposity despite regular food and water intake with minimal stress and physical exertion needs critical attention. Body weight decrease can occur as a result of increased fat consumption and protein or muscle wasting. These conditions occur normally during chronic moderate and severe exercise or starvation but when they occur

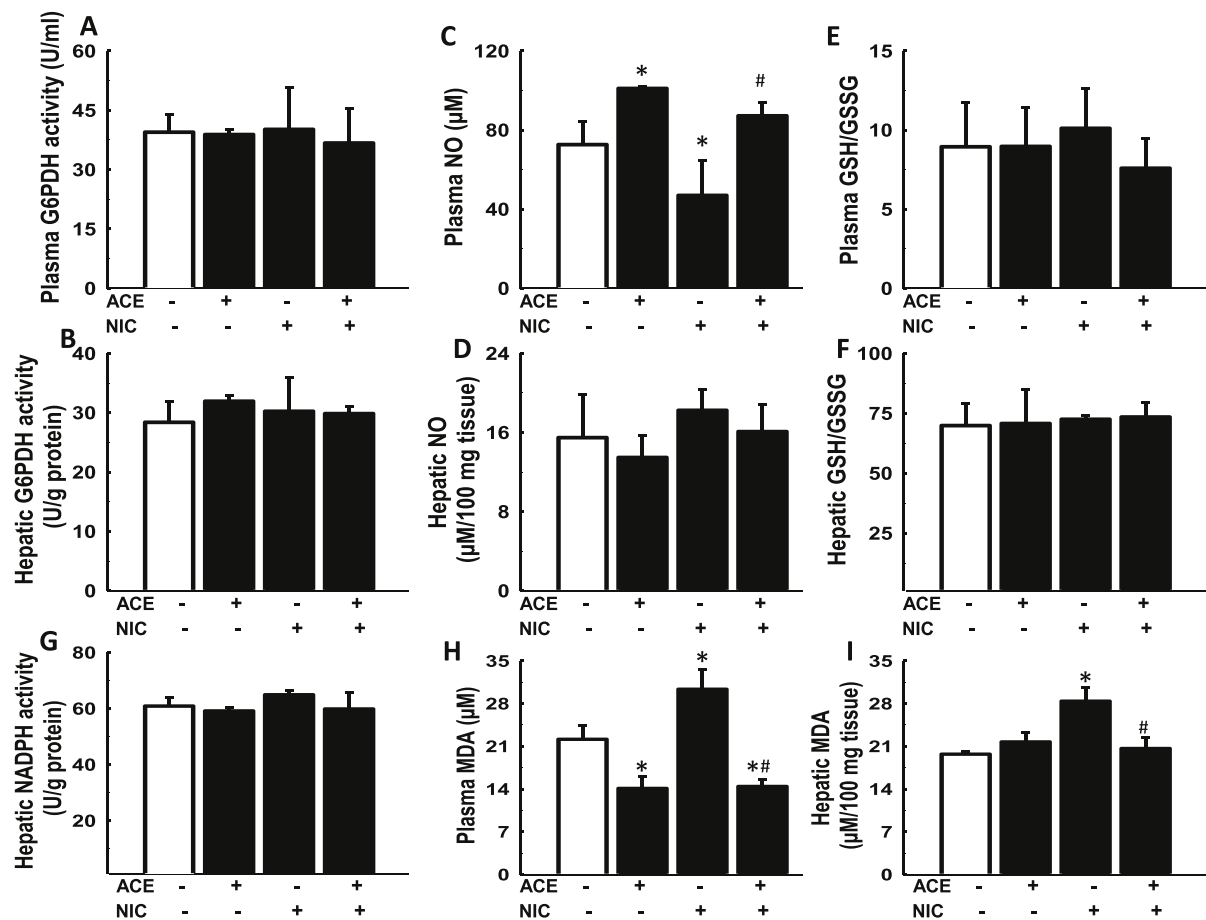


Fig. 7. Effect of sodium acetate on a) Plasma glucose-6-phosphate dehydrogenase (G6PD) activity, b) hepatic G6PD, c) plasma nitric oxide (NO), d) hepatic NO, e) plasma reduced glutathione/glutathione disulfide (GSH/GSSG) ratio, f) hepatic GSH/GSSG ratio, g) hepatic nicotinamide adenine dinucleotide phosphate (NADPH), h) plasma malondialdehyde (MDA), i) hepatic MDA. Plasma and hepatic G6PD activity, GSH/GSSG ratio, hepatic NO and NADPH were not affected by the treatment. There was increased plasma and hepatic MDA level and decreased plasma NO level in nicotine-treated rats compared with control. However, acetate treatment led to decreased plasma MDA level and increased plasma NO level compared with control. Acetate plus nicotine treatment led to decreased plasma MDA level. More so, plasma NO was increased in acetate 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. Con; # p < 0.05 vs. NIC).

without extreme physical exertion or food deprivation, they indicate disease states associated with excessive lipolysis in the adipose tissue and/or protein degradation in muscles. These two dysfunction events deliver FFA and alanine to the liver, excess of which results in lipotoxicity, perturbation in insulin signaling [32], fatty liver, hyperuricemia, and renal insult. In agreement, the insulin insensitivity and fatty liver caused by nicotine exposure in this study is therefore explicable because there was reduction in adiposity and body weight change and increased plasma and hepatic FFA, TG and TG/HDL-C, meaning that FFA from peripheral IR-induced lipolysis are delivered to the liver and stored as TG. Studies have shown that nicotine treatment can manifest anorexia. A study showed that nicotine via inhibition of AMP-activated protein kinase in the hypothalamus (AMPK) caused reduced appetite and adipocyte activation [33]. Therefore, a central alteration of energy intake and peripheral tissue metabolism could have been the underlying factor in the nicotine-induced dysmetabolism that is evident in this study.

The relationship between elevated peripheral IR and hepatic steatosis or injury does not only depend on FFA-induced TG accumulation but on oxidative stress and stimulation of inflammatory responses [34]. Increased XO activity has been implicated in metabolic derangements and is associated with oxidative stress, inflammation and insulin resistance [35–37]. Increased XO activity has biphasic impact on cellular integrity: It leads to overproduction of UA which promotes inflammatory responses via stimulation of inflammasomes, tissue injury

via reduction in nitric oxide (NO) [38], superoxide generation and endothelial dysfunction. It also produces ROS as a byproduct while utilizing molecular oxygen. In this study, there was marked elevation in plasma and hepatic levels of XO and UA, which corroborate the nicotine-induced oxidative damage regardless of the unaltered G6PD-dependent antioxidant capacity (GSH/GSSG) in the same treatment. The elevated levels of liver function enzymes show probable damage to the liver and antioxidant inadequate response of cellular antioxidant systems. G6PD is the rate limiting enzyme in the oxidative pentose phosphate pathway (PPP). It is the main source of NADPH in the cytosol [39] and NADPH is used to generate GSH (reduced glutathione) by the action of glutathione reductase. GSH is converted in the same cascade to GSSG (oxidized glutathione) by glutathione peroxidase as it catalyzes conversion of H_2O_2 to H_2O . GSH/GSSG ratio is taken as a marker of cellular antioxidant capacity [40] and nicotine exposure herein maintained this ratio compared with the control. NADPH can also be used up in lipid synthesis where superoxide is generated but our study reveals again that hepatic NADPH is not affected by nicotine exposure. Therefore, nicotine-induced deleterious hepatic effects in this study showed elevated FFA, TG accumulation and elevated XO activity without alteration of G6PD-dependent antioxidant system.

Glycogen synthesis is one of the two main non-oxidative glucose metabolisms, the second being glycolytic production of lactate [41]. The liver is a glucoregulatory organ that converts glucose to glycogen at saturation by the action of glucokinase (Hexokinase IV) [42] and GS.

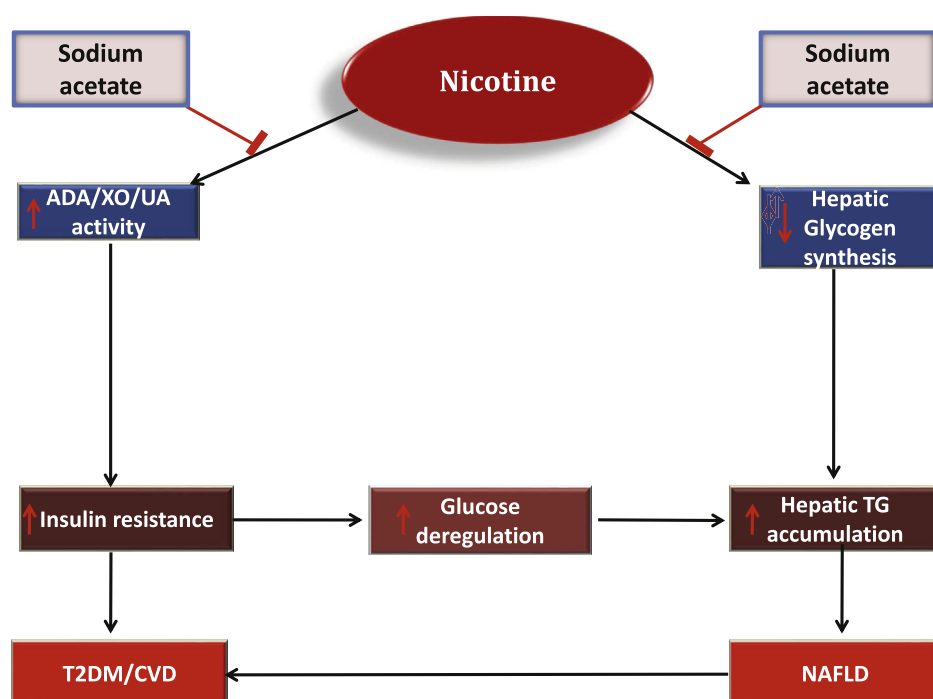


Fig. 8. Schematic diagram of the possible mechanism by which sodium acetate protect against nicotine-induced dysmetabolism and fatty liver in male rats. ADA; adenosine deaminase, CVD; cardiovascular disease, NAFLD; non-alcoholic fatty liver disease, T2DM; type 2 diabetes mellitus, TG; triglyceride, XO; xanthine oxidase, UA; uric acid.

When this hepatic system is disturbed as a result of IR or liver steatosis, glycogenesis is impaired. Hepatic IR is usually associated with fasting hyperglycemia and reduced hepatic glycogen synthesis [43]. Therefore, alterations in hepatic glycogen synthesis may be representative of peripheral glucose deregulation, fatty liver and hepatic injury in two steps: first, elevated hepatic glucose and FFA delivery due to hyperglycemia and peripheral IR-induced lipolysis can be diverted to lipid synthesis leading to hepatic fat accumulation (lipotoxicity) and injury. Second, lipotoxicity-induced hepatic IR will result in glycogen depletion as a result of GS inactivation. The result of the present study showed that nicotine exposure caused depletion of hepatic glycogen synthesis indicated by reduced glycogen content and GS activity corroborating the glucose dysregulation, peripheral IR and fatty liver associated with nicotine exposure. These effects also provide evidence for nicotine-induced hepatic IR. The other form of non-oxidative glucose metabolism, glycolytic lactate production, is recently gaining the focus of many studies. Blood lactate is being proposed as a marker of mitochondrial oxidative insufficiency and an independent predictor of IR, T2DM and CVD [44–46]. The finding of the present study that nicotine-induced dysmetabolism is accompanied by elevated blood lactate, LDH and hepatic LDH activity is in line with previous studies [45,46]. When the mitochondrial oxidative capacity especially that of the skeletal muscles reduces due to mitochondrial dysfunction or increased LDH activity as a result of IR, lactate is elevated and released into the circulation to engage in pyruvate cycle (Cori cycle) [44,46].

SCFAs, predominantly butyrate, propionate and acetate, are by-products of intestinal microbiota fermentation that involves in energy homeostasis and their physiological relevance is well documented. Among the SCFAs, butyrate enjoys early attention and the most investigated by scientists in the recent times [47,48]. However, acetate, the most abundant of these SCFAs is also rapidly gaining focus in several scientific studies. Interestingly, our current study demonstrated that acetate ameliorated the glucose dysregulation induced by nicotine exposure and prevented hepatic TG accumulation which is accompanied by suppressed plasma and hepatic XO activity with consequent attenuation of UA, which according to the findings herein prevented overt damage to the hepatic tissue at least as indicated by liver function enzymes and the histology of the liver. These therapeutic effects of acetate reveal the fact that nicotine might induce gut dysbiosis and that

acetate through mechanism(s) yet to be elucidated can suppress XO. An important event herein that demonstrates the effectiveness of acetate in the reversal of glucose dysregulation is the replenishing of hepatic glycogen. Normalization of glycogen synthesis is an indication of restored or maintained glucose homeostasis.

5. Conclusion

The present study demonstrates that nicotine-induced dysmetabolism characterized by glucose dysregulation and hepatic fat accumulation is associated with increased plasma and hepatic XO activity and depressed hepatic glycogen synthesis independent of glutathione antioxidant capacity. However, acetate protects the liver from nicotine-induced dysmetabolism and oxidative damage by suppressing XO activity. Therefore, nicotine might be injurious to human health through gut dysbiosis and acetate may begin to gain attention as safer preventive or therapeutic agent. We hereby recommend that the future study should focus on the course-relationship effect of acetate and XO activity.

Author contribution

EOD, LAO and AOS conceived and designed the research. EOD and TEO conducted the experiments. LAO and AOS contributed to the new reagents and analytical kits. EOD, TEO, EDA, KSO and LAO analyzed and interpreted the data. EOD, EDA, KSO and LAO drafted the manuscript. EOD, EDA, TEO, KSO, LAO and AOS read, revised and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- [1] S.M. Grundy, J.I. Cleeman, S.R. Daniels, K.A. Donato, R.H. Eckel, B.A. Franklin, et al., Diagnostic and management of metabolic syndrome: an American heart association/national heart, lung, and blood institute scientific statement, *Curr. Opin. Cardiol.* 21 (2006) 1–6.
- [2] C.M. Povel, J.W. Beulens, Y.T. van der Schouw, M.E. Dollé, A.M. Spijkerman, W.M. Verschuren, et al., Metabolic syndrome model definitions predicting type 2 diabetes and cardiovascular disease, *Diabetes Care* 36 (2) (2013) 362–368.
- [3] C.K. Roberts, A.L. Hevener, R.J. Barnard, Metabolic syndrome and insulin resistance: underlying causes and modification by exercise training, *Comp. Physiol.* 3 (1) (2013) 1–58.
- [4] R. Meshkani, K. Adeli, Hepatic insulin resistance, metabolic syndrome and cardiovascular disease, *Clin. Biochem.* 42 (13–14) (2009) 1331–1346.
- [5] I.R. Wanless, J.S. Lentz, Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors, *Hepatology* 12 (1990) 1106–1110.
- [6] N. Chalasani, Z. Younossi, J.E. Lavine, A.M. Diehl, E.M. Brunt, K. Cusi, M. Charlton, A.J. Sanyal, American gastroenterological association; American association for the study of liver diseases; American College of gastroenterology. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American gastroenterological association, American association for the study of liver diseases, and American College of gastroenterology, *Gastroenterol.* 142 (2012) 1592–1609.
- [7] J.S. Lim, M. Mietus-Snyder, A. Valente, J.M. Schwarz, R.H. Lustig, The role of fructose in the pathogenesis of NAFLD and the metabolic syndrome, *Nat. Rev. Gastroenterol. Hepatol.* 7 (2010) 251–264.
- [8] E.P. Conceicao, N. Peixoto-Silva, C.R. Pinheiro, E. Oliveira, E.G. Moura, P.C. Lisboa, Maternal nicotine exposure leads to higher liver oxidative stress and steatosis in adult rat offspring, *Food Chem. Toxicol.* 78 (2015) 52–59.
- [9] J.D. Browning, J.D. Horton, Molecular mediators of hepatic steatosis and liver injury, *J. Clin. Invest.* 2 (114) (2004) 147–152.
- [10] G.M. Reaven, Are triglycerides important as a risk factor for coronary disease? *Heart Dis. Stroke* 2 (1) (1993) 44–48 1993.
- [11] M.P. Reilly, D.J. Rader, The metabolic syndrome: more than the sum of its parts? *Circulation* 108 (2003) 1546e51 2003.
- [12] A.R. Saltiel, R. Khan, Insulin signalling and the regulation of glucose and lipid metabolism, *Nature* 414 (2001) 799–806.
- [13] C. Elliot, A.J. Vidal-Puig, Lipotoxicity, an imbalance between lipogenesis de novo and fatty acid oxidation, *Int. J. Obes.* 28 (2004) S22–S28.
- [14] S.K. Mantena, A.L. King, K.K. Andringa, H.B. Eccleston, S.M. Bailey, Mitochondrial dysfunction and oxidative stress in the pathogenesis of alcohol- and obesity induced fatty liver diseases, *Free Radic. Biol. Med.* 44 (2008) 1259–1272.
- [15] A.K. Mandal, D.B. Mount, The molecular physiology of uric acid homeostasis, *Annu. Rev. Physiol.* 77 (2015) 323e345.
- [16] W. Doeberner, N. Schoene, M. Rauchhaus, F. Leyva-Leon, D.V. Pavitt, D.A. Reaveley, G. Schuler, A.J. Coats, S.D. Anker, R. Hambrecht, Effects of xanthine oxidase inhibition with allopurinol on endothelial function and peripheral blood flow in hyperuricemic patients with chronic heart failure: results from 2 placebo-controlled studies, *Circulation* 4 (2002) 2619–2624.
- [17] E.E. Kelley, N.K. Khoo, N.J. Hundley, U.Z. Malik, B.A. Freeman, M.M. Tarpey, Hydrogen peroxide is the major oxidant product of xanthine oxidase, *Free Radic. Biol. Med.* 48 (2010) 493–498.
- [18] K.S. Olaniyi, L.A. Olatunji, Oral ethinylestradiol-levonorgestrel normalizes fructose-induced hepatic lipid accumulation and glycogen depletion in female rats, *Can. J. Physiol. Pharmacol.* (2019), <https://doi.org/10.1139/cjpp-2019-0037>.
- [19] C. Silagy, D. Mant, G. Fowler, M. Lodge, Meta-analysis on efficacy of nicotine replacement therapies in smoking cessation, *Lancet* 343 (1994) 139–142.
- [20] B. Eliasson, M.R. Taskiran, U. Smith, Long-term use of nicotine gum is associated with hyperinsulinemia and insulin resistance, *Circulation* 94 (1996) 878–881.
- [21] Y. Wu, P. Song, W. Zhang, J. Liu, X. Dai, Z. Liu, et al., Activation of AMPK (2 in adipocytes is essential for nicotine-induced insulin resistance in vivo, *Nat. Med.* 21 (4) (2015) 373–382.
- [22] H. Wang, H. Liao, M. Ochani, M. Justiniani, X. Lin, L. Yang, et al., Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis, *Nat. Med.* 10 (2004) 1216–1221.
- [23] C. Sadiq, G. Teske, G. Stokman, C. Kubjak, N. Claessen, F. Moore, et al., Nicotine protects kidney from renal ischemia/reperfusion injury through the cholinergic anti-inflammatory pathway, *PLoS One* 2 (5) (2007) e469.
- [24] P. Seoane-Collazo, P.B. de Morentin, J. Fernø, C. Diéguez, R. Nogueira, et al., Nicotine improves obesity and hepatic steatosis and ER stress in diet-induced obese male rats, *Endocrinology* 155 (5) (2014) 1679–1689 2014.
- [25] J.M. Wong, R. de Souza, C.W. Kendall, A. Emam, D.J. Jenkins, Colonic health: fermentation and short chain fatty acids, *J. Clin. Gastroenterol.* 40 (2006) 235–243.
- [26] P.B. Mortensen, M.R. Clausen, Short-chain fatty acids in the human colon: relation to gastrointestinal health and disease, *Scand. J. Gastroenterol.* 216 (1996) 132–148.
- [27] G. Frost, M.L. Sleeth, M. Sahuri-Arisoylu, B. Lizarbe, S. Cerdan, L. Brody, et al., The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism, *Nat. Commun.* 5 (2014) 3611.
- [28] K.M. Maslowski, A.T. Viera, A. Ng, J. Kranich, F. Sierro, D. Yu, et al., Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43, *Nature* 461 (2009) 1282–1286.
- [29] F. Guerrero-Romero, et al., The product of triglycerides and glucose, a simple measure of insulin sensitivity. Comparison with the euglycemic-hyperinsulinemic clamp, *J. Clin. Endocrinol. Metab.* 95 (7) (2010) 3347–3351.
- [30] D.J. Samuvel, K.P. Sundararaj, A. Nareika, M.F. Lopes-Virella, Y. Huang, Lactate boosts TLR4 signaling and NF-kappaB pathway-mediated gene transcription in macrophages via monocarboxylate transporters and MD-2 up-regulation, *J. Immunol.* 182 (4) (2009) 2476–2484 2009.
- [31] R.A. DeFronzo, Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard Lecture 2009, *Diabetologia* 53 (7) (2010) 1270–1287.
- [32] C. Saponaro, M. Gaggini, F. Carli, A. Gastaldelli, The soluble balance between lipolysis and lipogenesis: a critical point in metabolic homeostasis, *Nutrients* 7 (2015) 9453–9474.
- [33] P.B. Martínez de Morentin, A.J. Whittle, J. Fernø, et al., Nicotine induces negative energy balance through hypothalamic AMP-activated protein kinase, *Diabetes* 61 (2012) 807–817.
- [34] S. Satapati, B. Kucejova, J.A. Duarte, J.A. Fletcher, L. Reynolds, N.E. Sunny, et al., Mitochondrial metabolism mediates oxidative stress and inflammation in fatty liver, *J. Clin. Invest.* 125 (12) (2015) 4447–4462.
- [35] A.A. Jadhav, A. Jain, Elevated adenosine deaminase activity in overweight and obese Indian subjects, *Arch. Physiol. Biochem.* 118 (1) (2012) 1–5.
- [36] A.M. Feoli, F.E. Macagnan, C. Piovesan, L. Bodanese, I.R. Siqueira, Xanthine oxidase activity is associated with risk factors for cardiovascular disease and inflammatory and oxidative status markers in metabolic syndrome: effects of a single exercise session, *Oxi. Med. Cell. Longev.* (2014) 587083 2014.
- [37] A. Kushiya, Y. Nakatsu, Y. Matsunaga, T. Yamamotoya, K. Mori, K. Ueda, et al., Mediators inflamm, (2016), p. 8603164, <https://doi.org/10.1155/2016/8603164>.
- [38] Y.J. Choi, Y. Yoon, K.Y. Lee, T. Hien, K.W. Kang, K.C. Kim, et al., Uric acid induces endothelial dysfunction by vascular insulin resistance associated with the impairment of nitric oxide synthesis, *FASEB J.* 28 (7) (2014) 3197–3204 2014.
- [39] R.F. Kletzien, P.K. Harris, L.A. Foellmi, Glucose-6-phosphate dehydrogenase: a "housekeeping" enzyme subject to tissue-specific regulation by hormones, nutrients, and oxidant stress, *FASEB J.* 8 (2) (1994) 174–181 1994.
- [40] Zitka, S. Skalickova, J. Gumulec, M. Masarik, V. Adam, J. Hubalek, et al., Redox status expressed as GSH:GSSG ratio as a marker for oxidative stress in paediatric tumor patients, *Oncol. Lett.* 4 (6) (2012) 1247–1253.
- [41] M.M. Adeva-Andany, M. González-Lucán, C. Donapetry-García, C. Fernández-Fernández, E. Ameneiros-Rodríguez, Glycogen metabolism in human, *BBA Clin.* 5 (2016) 85–100.
- [42] M. Adeva-Andany, N. Pérez-Felpete, C. Fernández-Fernández, C. Donapetry-García, C. Pazos-García, Liver glucose metabolism in human, *Biosci. Rep.* 36 (6) (2016) e00416.
- [43] V.T. Samuel, Z.X. Liu, X. Qu, B.D. Elder, S. Bilz, D. Befroy, et al., Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease, *J. Biol. Chem.* 279 (31) (2004) 32345–32353.
- [44] S.O. Crawford, P.R. Hoogeveen, F.L. Brancati, B.C. Astor, C.M. Ballantyne, Association of blood lactate with type 2 diabetes: the atherosclerosis riskin communities carotid MRI study, *Int. J. Epidemiol.* 39 (2010) 1647–1655.
- [45] S.P. Juraschek, G.P. Shantha, A.Y. Chu, E.R. Miller, E. Guallar, et al., Lactate and risk of incident diabetes in a case-cohort of the atherosclerosis risk in communities (ARIC) study, *PLoS One* 8 (1) (2013) e51113.
- [46] S.P. Juraschek, J.K. Bower, E. Selvin, G.P. Subash Shantha, R.C. Hoogeveen, C.M. Ballantyne, et al., Plasma lactate and incident hypertension in the atherosclerosis risk in communities study, *Am. J. Hypertens.* 28 (2) (2015) 216–224 2015.
- [47] S. Khan, G.B. Jena, Protective role of sodium butyrate, a HDAC inhibitor on beta-cell proliferation, function and glucose homeostasis through modulation of p38/ERKMAPK and apoptotic pathways: study in juvenile diabetic rats, *Chem. Biol. Interact.* 213 (2014) 1–12.
- [48] O.C. Badejogbin, E.D. Areola, K.S. Olaniyi, O.A. Adeyanju, O.I. Adeosun, Sodium butyrate recovers high fat diet-fed female Wistar rats from glucose dysmetabolism and uric acid-associated cardiac tissue damage, *Naunyn Schmiedeberg's Arch. Pharmacol.* (2019), <https://doi.org/10.1007/s00210-019-01679-2>.