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Concomitant administration of atorvastatin with N-acetylcysteine mitigates liver injury and steatosis in experimental non-alcoholic fatty liver disease

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ABSTRACT

Although numerous efforts have been directed toward searching for new treatments against non-alcoholic fatty liver disease (NAFLD), there are no approved pharmacologic agents up to date. This study evaluates the therapeutic effect of concomitant administration of atorvastatin (ATO) and N-Acetylcysteine (NAC) with/without diet control. Nine groups of rats were divided into: normal, rats fed on high-fat diet for 12 weeks (NAFLD-HFD model), HFD-rats switched to regular diet (NAFLD-RD model), NAFLD-HFD or -RD rats treated with either ATO or NAC orally with 30 or 500 mg/kg/day, respectively, or both for 8 weeks. NAFLD-HFD rats exhibited remarkable steatosis with lobular inflammation, hepatocytes vacuolation, and fibrosis, as well as significant changes in lipid profile, oxidative stress, and adipocytokines and these manifestations were less prominent in the HFD-RD group. ATO and NAC combination with diet control has the added benefits on ameliorating lipid levels, liver enzymes, oxidative stress, hepatic steatosis ($9.01\% \pm 1.66\%$ *vs.* $13.21\% \pm 2.20\%$ for ATO and $25.60\% \pm 2.11\%$ for NAC), inflammation, hepatocyte vacuolation, and fibrosis versus each drug separately. Conclusion: ATO and NAC concomitant therapy has a greater effect on NAFLD as compared to monotherapy and is recommended for further investigation in clinical trials.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is globally the most common liver disease and its prevalence had progressively increased over the last decade (Dajani and Abu Hammour, 2016). NAFLD is a clinical aspect of liver damage, categorized into simple with only hepatic steatosis, and nonalcoholic steatohepatitis (NASH), where intralobular inflammation, hepatocyte ballooning degeneration, and hepatic steatosis are observed. NASH is a progressive disease and may develop into liver fibrosis, cirrhosis, and hepatocellular carcinoma (Foster *et al.*, 2011). NAFLD/NASH is robustly linked with obesity, insulin resistance, hypertension, and dyslipidemia, and it is considered as a hepatic manifestation of the metabolic syndrome (Souza *et al.*, 2012). Adding on, oxidative stress, as a result of an increased level of fatty acids within the hepatocytes, is thought to be responsible for the progression of NAFLD pathogenesis (Khoshbaten *et al.*, 2010), justifying the study of several antioxidants in NAFLD treatment.

Life style modification by diet and exercise is currently the main treatment for NAFLD/NASH; however, finding of therapeutic options is requisite because NAFLD patients often have difficulty maintaining healthy life styles (Takahashi *et al.*, 2015). Until then, treatment has focused on therapeutic medicines used for associated co-morbidities (e.g., hypertension, diabetes mellitus, hyperlipidemia, etc.), and possibly liver-protecting agents (Dajani and Abu Hammour, 2016). Although several studies revealed that treatment with atorvastatin (ATO), an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, is efficient and secure for NAFLD/NASH patients (Nseir and Mahamid, 2013); however, there are limited and conflicting reports about the statins effect on liver histology (Chalasani *et al.*, 2012) in addition to the limited number of patients involved in these investigations.

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N-Acetylcysteine (NAC) is a glutathione precursor, limits the reactive oxygen species that causes hepatocellular injury and its antioxidative properties have been tested clinically and in animal models of NAFLD (Ali *et al.*, 2016; Khoshbaten *et al.*, 2010; Uzun *et al.*, 2009), with encouraging results. Since NAFLD/ NASH has a complicated pathogenesis, so combining agents that reduce cholesterol/load of fatty acids (e.g., ATO) with agents that reduce oxidative stress and cell injury (e.g., NAC) is reasonable, and is the best therapeutic strategy to investigate moving forward. Besides, to the best of our information, there is no report examining the ATO plus NAC combination strategy. Accordingly, the current research was conceived in rats for investigating the concomitant therapeutic impact of ATO plus NAC with and without diet control versus each drug separately on lipid profile, oxidative stress, adipocytokines, liver steatosis, inflammation, and fibrosis. Herein, we used the animal model to avoid the host confusing variables, environmental factors, including diet and lifestyle, as well as the availability and ease of histological examination.

MATERIALS AND METHODS

Animals

One hundred-eight male Sprague-Dawley rats, average weight 120 ± 20 g, were kept at the animal house of Theodor Bilharz Research Institute (TBRI), Giza, Egypt. Rats were accommodated in individual cages under controlled temperature (25°C) and 12/12-hour light/dark cycle, with *ad libitum* access to food and water, 1 week before initiation of the experiment. All the animal experiments were carried out according to the international guidelines for the care and use of laboratory animals and were approved by the Institutional Review Board of TBRI (TBRI-IRB/FWA00010609/Feb. 2018).

Experimental design

Nine groups, each of 12 rats, were randomly classified into: normal control, rats fed on high-fat diet (NAFLD-HFD model), HFD-rats switched to regular diet (NAFLD-RD model), NAFLD-HFD or -RD rats treated with ATO, NAC or the combined ATO + NAC, respectively. Rats in the control group were maintained on the standard chow diet for 20 weeks. NAFLD-rats were induced by feeding a HFD of [25% fats + 1% cholesterol (Win lab Laboratory chemicals) + 0.25% bile salts (Alpha chemika, India)] for 12 weeks according to Zulet *et al.* (1999), with slight modification. After 12 weeks, rats in the ATO/NAC-treated NAFLD groups were treated with ATO (Ator®; Egyptian International Pharmaceutical Industries Company, EIPICO, 10th of Ramadan City, Egypt) at a dose of 30 mg/kg/day b.w. (Roglans *et al.*, 2002) or NAC (Sigma-Aldrich Chemical Company, St. Louis, MO) at a dose of 500 mg/kg/day b.w. (Samuhasaneeto *et al.*, 2007) via oral gavage, for 8 weeks (starting from the 13th week to the 20th week). Rats in the NAFLD-RD treated groups were switched and maintained on the standard chow diet (weeks 13–20). At the end of experiment and after overnight fasting, rats of all the groups were weighted and then killed with an intraperitoneal injection of ketamine (80 mg/kg). Blood samples were collected and sera were separated for biochemical assessment, and the livers were immediately removed, weighted, and sampled for assay of oxidative stress markers, and for histopathological examination. Liver weight index (%) = Liver weight/body weight × 100.

Study of biochemical markers

Liver function tests, including serum alanine and aspartate transaminases (ALT and AST), gamma glutamyl transferase (γ-GT), and alkaline phosphatase (ALP), as well as the lipid profile; total cholesterol (TC), triglycerides (TG), and high-density lipoprotein (HDL) were assayed spectrophotometrically using the available commercial kits. Low-density lipoprotein (LDL) was calculated using Friedewald formula: [LDL = TC − HDL − TG/5 (mg/dl)]. Very low-density lipoprotein (VLDL) was calculated by formula [VLDL = TC − (HDL + LDL) (mg/dl)]. Serum transforming growth factor-β1 (TGF-β1; IBL International GMBH, Hamburg, Germany), tumor necrosis factor-α (TNF-α; Assaypro, St. Louis, MO), adiponectin (RayBiotech Inc., Norcross, GA), and leptin (Assaypro, St. Louis, MO) levels were measured using ELISA kits.

Study of lipid peroxidation and oxidative stress

Liver samples (1 gm each) were homogenized (Pro Scientific Inc., U.S.A. homogenizer) in ice-cold 100 mM KH_2PO_4 buffer (1:4 w/v; pH 7.4) containing 1 mM Ethylenediaminetetraacetic acid (EDTA) and centrifuged at 10,000 g for one hour at 4°C. Aliquots of the collected supernatant were used for subsequent analysis of reduced glutathione (GSH) content, superoxide dismutase (SOD) activity and degree of liver lipid peroxidation [expressed by malondialdehyde (MDA) formation] spectrophotometrically using the available commercial kits.

Histological examinations

Specimens of liver tissues were fixed in 10% buffered formaldehyde, embedded in paraffin wax, then sectioned (5 μm thick) and stained with hematoxylin-eosin (H&E). Steatosis was examined morphologically by semi-quantitative analysis, at high power microscopic fields of ×400/section, using the computerized image analysis system (Axiovision version 4.8, Zeiss Germany), and graded as the following: mild = 5%−30%, moderate = 30%−60%, and severe >60% of hepatocytes affected (Kleiner *et al.*, 2005). Also, histological changes, such as ballooning degeneration, vacuolation, acidophilic necrosis, sinusoidal fibrosis, and polymorph nuclear infiltration were examined.

Statistical analysis

Data are expressed as the mean ± SEM and analyzed using the one-way ANOVA test followed by Tukey's *post hoc* test (SPSS, software package version 16.0, Chicago, IL). Data are considered statistically significant if $p < 0.05$.

RESULTS

Hepatic gross manifestations

The body, liver weights, and liver index in the NAFLD-rats were increased significantly when compared to the corresponding values of normal controls. Compared with the NAFLD untreated group, a significant decrease ($p < 0.05$) in body, liver weights, and index was observed in rats treated with ATO alone or ATO + NAC with the shift of data toward normal values in these parameters in groups treated with both drugs weather maintained on HFD or RD (Table 1).

Biochemical and oxidative stress markers

Following treatment of HFD-group with ATO for 8 weeks with or without reverting to RD, serum lipids (Table 2), liver enzymes (Fig. 1), and adipocytokines (Fig. 2) were significantly improved ($p < 0.05$) as compared with the parallel-untreated groups. Moreover, GSH, SOD, and MDA were significantly ameliorated when ATO treated group reverted into RD (Fig. 3). Significant improvements ($p < 0.05$) in TC, TG, LDL (Table 2), ALT, AST, γ-GT (Fig. 1), adiponectin, leptin, TNF-α, and TGF-β (Fig. 2) were observed in the group treated with NAC alone. In addition, administration of NAC alone or in combination with ATO significantly decreased the effects of the oxidative stress and lipid peroxidation by restoring GSH content, increasing SOD activity and decreasing of MDA level (Fig. 3). In the ATO/NAC combined groups, most of the measured serum and hepatic markers gave better and significant results over those of each individual-treated group, weather maintained on HFD or switched to RD.

Histology

After 20 weeks, in the normal control group (Fig. 4A), the liver lobules were intact with preserved lobular architecture, the liver cell cords arranged regularly with no fat aggregation, or inflammatory infiltration, or hepatocyte ballooning. The liver in NAFLD/HFD model group showed notable steatosis with abundant fat deposition (Fig. 4B), steatosis was predominantly macrovesicular (up to 65% of the lobules), with isolated single droplets and nuclear eccentricity; nevertheless, microvesicular steatosis were also noted in some hepatocytes. Hepatocytes ballooning degeneration, as a result of accumulation of intracellular fluid, was characterized by swollen cells. Based on the observations of the number of lymphocytes, moderate inflammation was found in the lobular and portal areas with moderate fibrosis in the form of thin fibrous bands (Fig. 4B); however, in NAFLD/RD, steatosis involved up to 37% of the lobules (Fig. 4C) with moderate inflammation. The degree of hepatic damage as manifested by steatosis, inflammatory infiltration, hepatocyte ballooning, and fibrosis were moderately alleviated in ATO group (Fig. 4D and E) and partially improved in NAC group (Fig. 4F and G). In the ATO + NAC treated group, steatosis and lobular inflammation were mitigated significantly over those of each drug separately, and in all NAFLD-RD groups more than those groups maintained on HFD (Fig. 4H and I) where steatosis (<20%) and extension of lobular inflammation were below the lower limit for the diagnosis of steatohepatitis (Table 3). In addition, all the treated groups showed that lipid deposits were smaller than untreated groups, and no signs of cirrhosis were observed in all examined groups (Fig. 4A−I).

DISCUSSION

The data of this study showed that HFD aggravated blood liver enzymes, lipid profile, inflammatory, and oxidative stress markers in rats. Moreover, the histological profile showed liver steatosis, lobular inflammatory infiltrates, hepatocytes ballooning, and fibrosis. In agreement with a previous study (Hernández-Guerra *et al.*, 2006), ATO significantly increased HDL, lowered TC, TG, LDL, and VLDL. As a non-HDL cholesterol-decreasing agent, ATO catalyzes the conversion of HMG-CoA into mevalonate, an important step in the cholesterol biosynthesis, leading to lower liver cholesterol production, high plasmatic clearance of LDL cholesterol and up-regulation of hepatocyte LDL receptors (Taylor *et al.*, 2011). The current study also revealed that NAC significantly lowered TC, TG, and LDL. Similar results were obtained in mice where treatment with NAC

Table 1. Effect of atorvastatin and N-acetylcysteine alone or in combination on liver gross manifestations in non-alcoholic fatty liver disease-induced rats.

	Groups	Body weight (g)	Liver weight (g)	Liver weight index (%)
HFD	Normal	167.50 ± 7.68	5.16 ± 0.31	3.13 ± 0.18
	NAFLD control	272.83 ± 5.16[a]	15.25 ± 0.63[a]	5.58 ± 0.52[a]
	NAFLD + ATO	190.41 ± 9.14[b]	8.03 ± 0.36[ab]	4.22 ± 0.32[ab]
	NAFLD + NAC	257.33 ± 7.10[a]	13.35 ± 0.72[a]	5.17 ± 0.69[a]
	NAFLD + ATO + NAC	171.41 ± 5.54[bd]	6.14 ± 0.46[bcd]	3.58 ± 0.12[bd]
RD	NAFLD control	247.25 ± 6.39[a]	10.48 ± 0.78	4.13 ± 0.25[a]
	NAFLD + ATO	174.75 ± 6.47[b]	7.32 ± 0.51[ab]	4.24 ± 0.31
	NAFLD + NAC	231.25 ± 5.82[a]	9.37 ± 0.68[a]	4.18 ± 0.35
	NAFLD + ATO + NAC	162.20 ± 7.54[bd]	5.10 ± 0.34[bcd]	3.14 ± 0.23[bcd]

Values are presented as mean ± SEM ($n = 12$), analyzed by one-way ANOVA followed by Tukey's *post hoc* test. [a]$p < 0.05$ significant difference from normal, [b]$p < 0.05$ significant difference from corresponding NAFLD control group, [c]$p < 0.05$ significant difference from corresponding NAFLD + ATO, [d]$p < 0.05$ significant difference from corresponding NAFLD+NAC.

Table 2. Effect of atorvastatin and N-acetylcysteine alone or in combination on lipid profile in non-alcoholic fatty liver disease-induced rats.

	Groups	TG (mg/dl)	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL
HFD	Normal	85.65 ± 4.13	98.49 ± 4.20	46.22 ± 3.95	35.14 ± 2.89	17.13 ± 1.36
	NAFLD control	191.45 ± 9.57[a]	222.61 ± 10.21[a]	28.12 ± 1.06[a]	156.12 ± 3.68[a]	38.37 ± 2.04[a]
	NAFLD + ATO	146.30 ± 4.06[ab]	157.13 ± 5.38[ab]	39.25 ± 3.07[b]	88.62 ± 3.99[ab]	29.26 ± 1.52[ab]
	NAFLD + NAC	155.25 ± 3.85[ab]	162.64 ± 4.13[ab]	25.23 ± 3.07[a]	106.36 ± 4.51[ab]	31.05 ± 2.96[a]
	NAFLD + ATO + NAC	123.65 ± 4.36[abcd]	132.38 ± 5.46[abcd]	43.35 ± 4.11[bd]	64.30 ± 3.69[abcd]	24.73 ± 2.28[b]
RD	NAFLD control	150.65 ± 3.68[a]	170.04 ± 4.17[a]	33.61 ± 1.38[a]	106.30 ± 3.93[a]	30.13 ± 1.59[a]
	NAFLD + ATO	125.65 ± 3.41[ab]	136.81 ± 3.83[ab]	45.50 ± 2.75[b]	66.18 ± 3.39[ab]	25.13 ± 1.18[ab]
	NAFLD + NAC	131.70 ± 2.15[ab]	149.04 ± 4.11[ab]	37.58 ± 3.02	85.12 ± 3.20[ab]	26.34 ± 1.76[a]
	NAFLD + ATO + NAC	103.00 ± 3.70[abcd]	109.21 ± 5.12[abcd]	47.35 ± 4.02[b]	41.26 ± 1.93[bcd]	20.60 ± 1.05[bd]

HDL: high-density lipoprotein; TG: Triglyceride; TC: Total cholesterol; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein. Values are presented as mean ± SEM ($n = 12$), analyzed by one-way ANOVA followed by Tukey's *post hoc* test. [a]$p < 0.05$ significant difference from normal, [b]$p < 0.05$ significant difference from corresponding NAFLD control group, [c]$p < 0.05$ significant difference from corresponding NAFLD + ATO, [d]$p < 0.05$ significant difference from corresponding NAFLD + NAC.
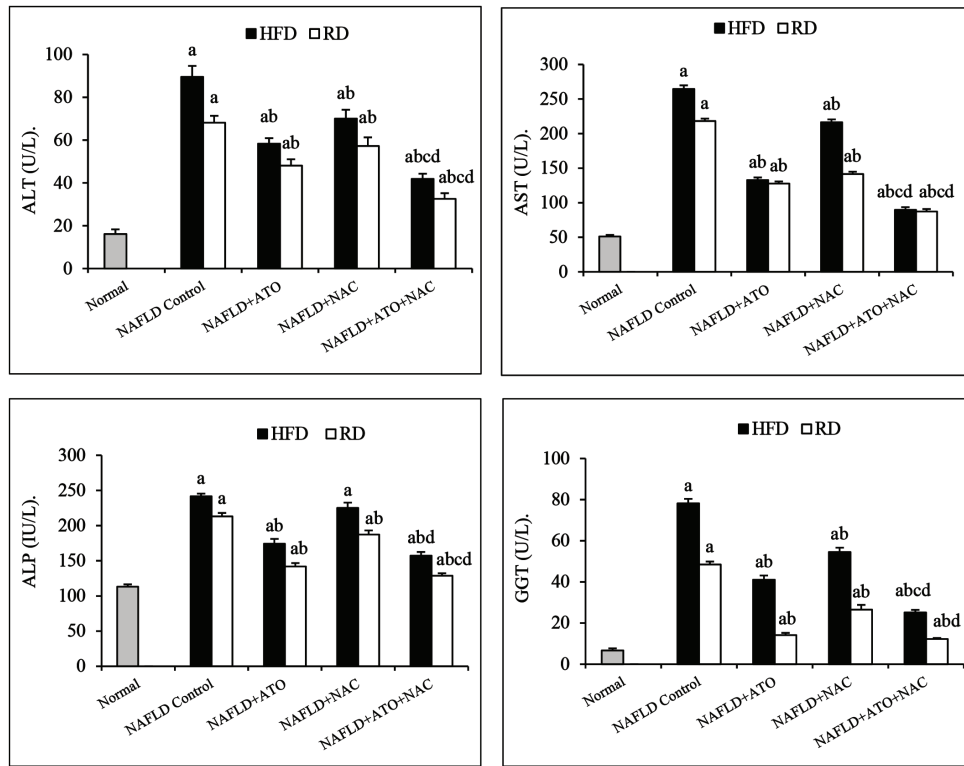
Figure 1. Effect of atorvastatin and N-acetylcysteine alone or in combination on serum liver enzymes in NAFLD-induced rats. Values are presented as mean ± SEM ($n = 12$). [a]$p < 0.05$ versus normal, [b]$p < 0.05$ versus corresponding NAFLD control group, [c]$p < 0.05$ versus NAFLD + ATO-treated group, [d]$p < 0.05$ versus NAFLD+NAC-treated group.
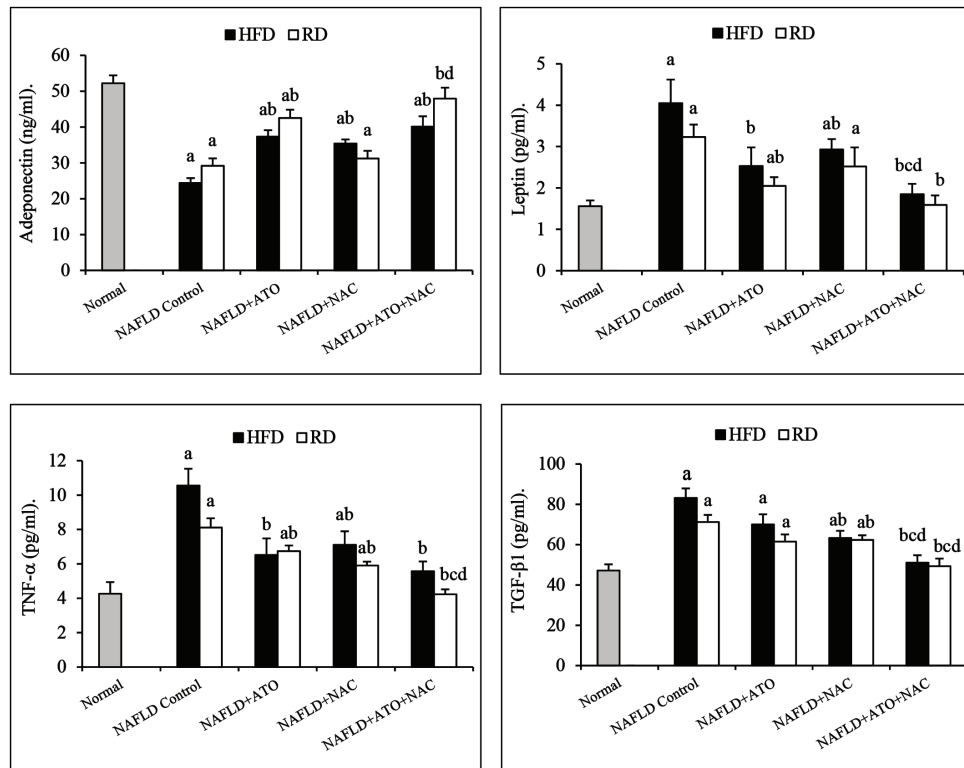

Figure 2. Effect of atorvastatin and N-acetylcysteine alone or in combination on adipocytokine markers in NAFLD-induced rats. Values are presented as mean ± SEM ($n = 12$). [a]$p < 0.05$ versus normal, [b]$p < 0.05$ versus corresponding NAFLD control group, [c]$p < 0.05$ versus NAFLD + ATO-treated group, [d]$p < 0.05$ versus NAFLD + NAC-treated group.
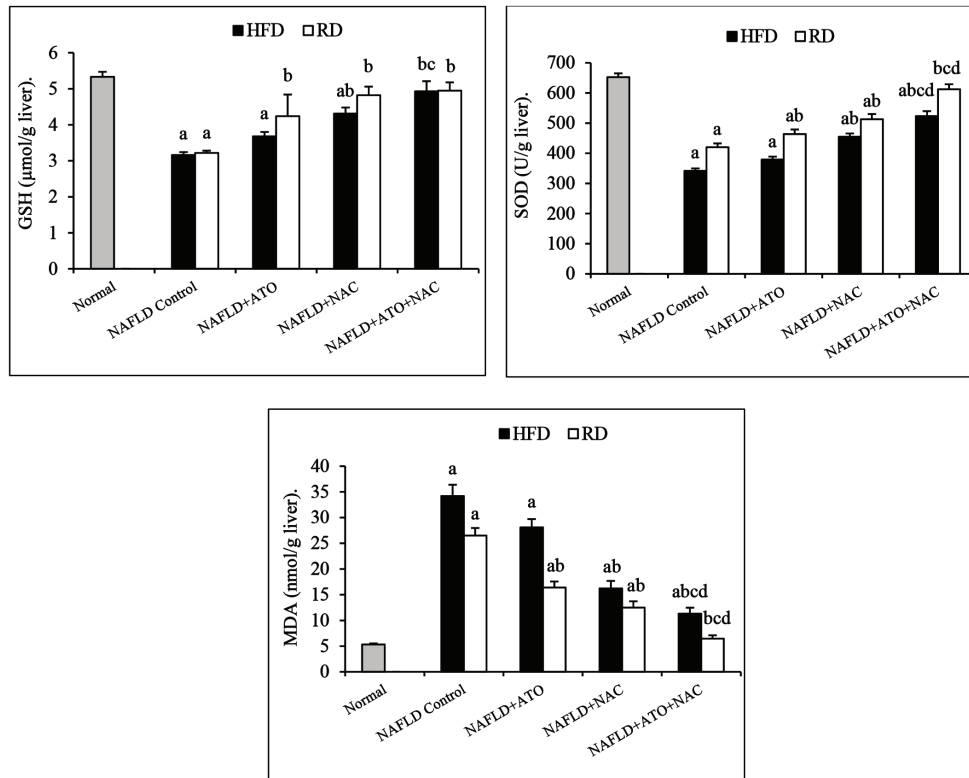
Figure 3. Effect of atorvastatin and N-acetylcysteine alone or in combination on oxidative stress markers and lipid peroxidation in NAFLD-induced rats. Values are presented as mean ± SEM, (n = 12). [a]$P < 0.05$ versus normal, [b]$P < 0.05$ versus corresponding NAFLD control group, [c]$P < 0.05$ versus NAFLD+ATO-treated group, [d]$P < 0.05$ versus NAFLD+NAC-treated group.

almost completely blocked lipid accumulation in the liver, which was evidenced by lower liver levels of triglyceride and cholesterol (Ma *et al.*, 2016).

Reactive oxygen species as a result of oxidative stress have contributed to impaired liver cellular function leading to cell death and necrosis (Stärkel *et al.*, 2003). This impaired cellular function could be monitored by determining serum transaminases (ALT and AST) which are considered among the most sensitive biochemical markers investigated in the diagnosis of liver diseases and the best indicators of liver necrosis (Amacher *et al.*, 2013). In this study, the elevated transaminase serum levels in HFD rats reflect the hepatocyte damage that was investigated histopathologically in this study. Eight weeks after the treatment with either ATO or NAC alone or both, these abnormalities were significantly improved, suggesting that ATO and/or NAC could alleviate the HFD-induced liver injury. This favorable impact on serum transaminases could reflect on the amelioration of liver histopathology and less fatty infiltration in hepatocytes as shown in the present investigation.

It is assumed that adipokines and cytokines, such as adiponectin, leptin, and TNF-α, have an important role in pathogenesis and severity of NAFLD (Giby and Ajith, 2014). The effect of HFD on leptin and adiponectin level in this study was in accordance with Ali *et al.* (2016), who reported that leptin levels were significantly higher in NAFLD/NASH rats; meanwhile, adiponectin levels were significantly lower when compared to controls. In disagreement with the results of Mäuser

et al. (2007) who mentioned that ATO decreased adiponectin synthesis in mature adipocytes, data of this study showed that ATO partially restored the depleted adiponectin levels in HFD group; meanwhile, it significantly lowered the leptin level. The same findings were recorded in groups treated with NAC. NAC was able to reduce high levels of leptin while increasing adiponectin levels in female mice prenatally exposed to a HFD (Berry *et al.*, 2018). Also, it could improve the obesity-related disturbed adipocytokine metabolism through alleviating the TNF-α-induced oxidant-antioxidant imbalance in adipocytes (Araki *et al.*, 2006). In spite of several small, short term, and uncontrolled trials on statins have documented a reduction in hepatic steatosis, the majority of these did not give a report on liver histology; however, there is agreement that statins do not have a positive impact on liver histology in NASH but can be safely prescribed to mend their increased cardiovascular risk (Chalasani *et al.*, 2012). In this study, ATO alone was not able to significantly reduce the hepatic oxidative stress indicators. Nevertheless, histopathology examination displayed that ATO might decrease significantly both steatosis (~23% *vs.* 65% for HFD-group and 13% *vs.* 37% for HFD-RD group) and liver portal inflammation as shown by diminution in the number of inflammatory foci and their density. Adding on, ATO could markedly attenuate the serum TNF-α with partial decline in TGF-β. In agreement with our study, Martın-Castillo *et al.* (2010) reported that ATO therapy decreased TC and TG, liver steatosis, inflammation, and hepatocellular damage in hyperlipidemic chickens. The function of statins in mitigating
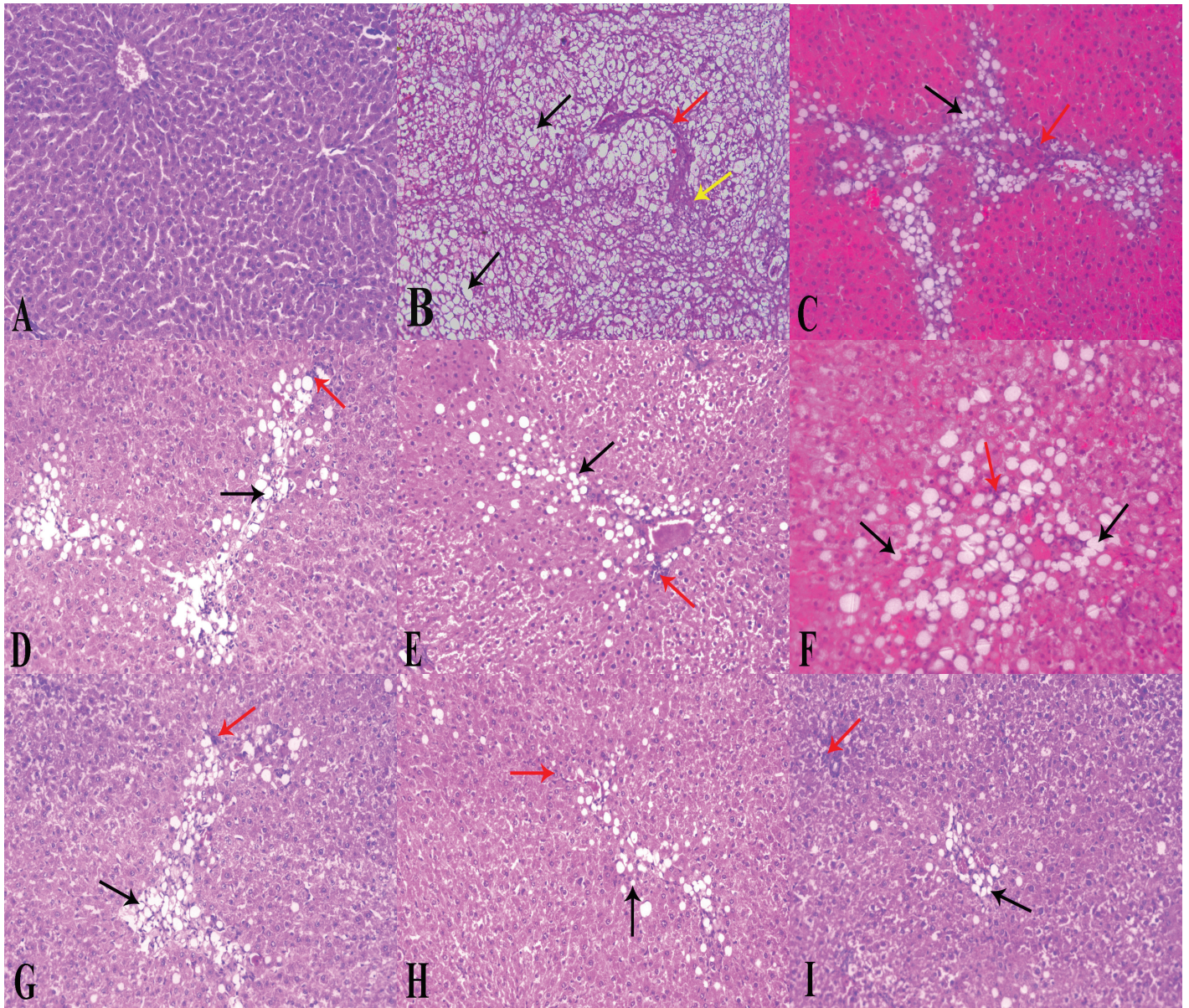
Figure 4. Liver sections showing: (A) normal control, intact hepatic lobular architecture and normal morphological appearance; (B) NAFLD/HFD, partial disturbed hepatic lobular architecture, severe micro and macro steatotic changes about 80% (black arrow), fibrous bands porto-portal and porto-central (red arrow) and moderate interlobular inflammatory cells (yellow arrow); (C) NAFLD/RD, moderate micro and macro steatotic changes (black arrow), mild interlobular inflammation (red arrow); (D) ATO/HFD, mild micro and macro steatotic changes (black arrow), mild interlobular inflammation (red arrow); (E) ATO/RD, improved steatotic changes (black arrow) and mild interlobular inflammation (red arrow); (F) NAC/HFD, mild−moderate micro and macro steatotic changes (black arrow), moderate interlobular inflammation (red arrow); (G) NAC/RD, mild micro steatotic changes (black arrow) and mild interlobular inflammation (red arrow); (H) ATO + NAC/HFD, scattered micro steatotic changes (black arrow) and mild interlobular inflammation (red arrow); and (I) ATO + NAC/RD, enhancement improvements in steatotic changes (black arrow) and interlobular inflammation (red arrow) and almost normal morphological appearance. Mild = 5%−30%, moderate = 30%−60%, and severe >60% of hepatocytes affected. The hematoxylin and eosin images were captured under magnification ×200.

inflammation is assumed to be as a result of suppression of neutrophils chemotaxis and reduction of pro-inflammatory cytokines (Dunzendorfer *et al.*, 1997) or through lowering both stress-activated c-Jun N-terminal kinase (JNK) activation and the hepatic expression of TGF-β (Wang *et al.*, 2013).

In the current study, NAC markedly recovered the reduction in hepatic GSH, abolished lipid peroxidation, and suppressed TNF-α, TGF-β as well as the inflammation, resulting in a significant decline in cellular damage, hepatocyte

injury, and fibrosis as displayed histopathologically. These amendments are in agreement with those previously reported by Baumgardner *et al.* (2008). Oxidative stress, regardless of its etiology, plays a major role in hepatic fibrogenesis (Tsukamoto, 1993). Previous studies reported that NAC blocked experimental fibrosis/cirrhosis through abolishing oxidative stress and decreasing TGF-β (Pereira-Filho *et al.*, 2008; Galicia-Moreno *et al.*, 2009). Herein, the first report on ATO and NAC combination therapy showed enhanced ameliorations in hepatic inflammation,

Table 3. Effect of atorvastatin and N-acetylcysteine alone or in combination on liver histological parameters in non-alcoholic fatty liver disease-induced rats.

| | Groups | Hepatic architecture | | % Steatosis | Steatosis type | | Inflammation | Hepatocyte vacuolation | Fibrosis | |
		preserved	lost		Micro-vesicular	Macro-vesicular			Non	Present
HFD	NAFLD control	2/8	6/8	64.80 ± 6.23	3/8	5/8	17.50 ± 1.32	36.21 ± 3.11	2/8	6/8
	NAFLD + ATO	3/8	5/8	23.21 ± 2.62[a]	4/8	4/8	10.85 ± 1.46[a]	14.22 ± 2.21[a]	5/8	3/8
	NAFLD + NAC	4/8	4/8	47.00 ± 6.14	4/8	4/8	9.04 ± 0.93[a]	18.60 ± 2.20[a]	6/8	2/8
	NAFLD + ATO+NAC	6/8	2/8	17.28 ± 2.55[abc]	5/8	3/8	6.77 ± 1.20[ab]	9.44 ± 1.36[abc]	7/8	1/8
RD	NAFLD control	5/8	3/8	37.31 ± 3.22	5/8	3/8	16.30 ± 1.32	24.56 ± 3.43	3/8	5/8
	NAFLD + ATO	6/8	2/8	13.21 ± 2.20[a]	3/8	5/8	10.62 ± 1.24[a]	10.75 ± 1.16[a]	6/8	2/8
	NAFLD + NAC	6/8	2/8	25.60 ± 2.11[a]	4/8	4/8	9.00 ± 1.34[a]	15.32 ± 1.31[a]	7/8	1/8
	NAFLD + ATO + NAC	7/8	1/8	9.01 ± 1.66[abc]	6/8	2/8	6.07 ± 1.02[ab]	8.44 ± 1.37[ac]	8/8	0/8

Values are presented as mean ± SEM ($n = 8$), analyzed by one-way ANOVA followed by Tukey's *post hoc* test. [a]$p < 0.05$ compared with corresponding NAFLD control group, [b]$p < 0.05$ compared with NAFLD + ATO-treated group, [c]$p < 0.05$ compared with NAFLD + NAC-treated group.

fibrosis, and oxidative stress markers over each drug separately. Furthermore, they alleviated hepatic steatosis, concomitant with modulation of liver function, and lipid profile, leading to a reduction in the severity of hyperlipidemia in NAFLD rats. These dual effects were more manifested under dietary control and this confirms that diet control and personal lifestyle adjustments are crucial for the control of hyperlipidemia. These results strengthen the utility of this combination therapy in modulating metabolic profile and protecting liver in hyperlipidemic rats.

CONCLUSION

The current study demonstrated that the diet control is still the first intervention for NAFLD treatment. Combination of ATO with NAC under diet control has the added benefits on reducing the lipid levels, liver enzymes, oxidative stress markers, hepatic steatosis, inflammation, and fibrosis versus each drug separately. Accordingly, this combination strategy is recommended for further investigation in clinical trials.

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CONFLICT OF INTEREST

The author declares no conflicts of interest.

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