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PHARMACOLOGICAL AND ANTIOXIDANT ACTIONS OF GARLIC AND/OR ONION IN NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) IN RATS

By
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Abstract

Non-alcoholic fatty liver disease (NAFLD) includes a broad spectrum of fat-induced liver injury, ranging from mild steatosis to cirrhosis and liver failure. This study investigates the hepatoprotective properties of garlic and onion in NAFLD rat model. Ninety male Sprague-Dawley rats were randomly divided into 9 groups; normal (I), NAFLD induced with high fat diet (HFD; II), NAFLD switched to regular diet (RD; III), NAFLD-HFD or NAFLD-RD treated with garlic (IV, V), onion (VI, VII) or the combined garlic+onion (VIII, IX) respectively. A NAFLD rat model was established by feeding the animals with a high-fat diet for 12 wk. These animals were then treated with garlic or/and onion or vehicle for 8 wk (weeks 13-20) and then killed to obtain serum samples and liver tissues. Liver histology, lipids, parameters of oxidative stress, TNF-α and TGF-β were measured. The liver in NAFLD-HFD showed typical steatosis, accompanied with mild to moderate lobular inflammatory cell infiltration. Serum levels of ALT, AST, ALP, leptin, cholesterol, triglycerides, TNF-α, TGF-β and hepatic MDA were significantly increased ($P<0.05$) compared with normal group. This was accompanied with reduction of hepatic GSH, GR, GPx, GST, SOD and serum adiponectin. These changes were to a less degree in NAFLD-RD group. Combined administration of garlic+onion produced a better and significant decrease in liver steatosis, serum liver enzymes, oxidative markers and lipid peroxidation versus each one alone. In the same time, NAFLD-induced inflammation was also mitigated via reduction of TNF-α and TGF-β. In addition, these results were better in the group IX versus group VIII.

Keywords: Fatty liver; garlic; onion, leptin; adiponectin; TGF-β; TNF-α; liver enzymes and antioxidants.

Introduction

The non-alcoholic fatty liver disease (NAFLD), deposition of fat in the liver due to causes other than alcohol, includes non-alcoholic steatohepatitis (NASH). NASH is a more severe form of NAFLD in which fatty infiltration of the liver is accompanied by the necroinflammatory activity, and is now recognized as one of the most common causes of chronic liver disease (Shifflet and Wu, 2009). NAFLD has also the potential to progress to hepatocellular carcinoma (HCC) or liver failure. NAFLD is strongly linked to the caloric over-consumption, physical inactivity, insulin resistance and genetic factors. Although significant progress in understanding pathogenesis of NAFLD has been achieved in years, the primary metabolic abnormalities leading to lipid accumulation within hepatocytes has remained poorly understood (Wei *et al*, 2008). Given the increasing prevalence of obesity worldwide, the deleterious effects of NAFLD, and more particularly NASH are becoming of increasing concern for
physicians (Shifflet and Wu, 2009).

The pathophysiology of NAFLD is described as a "two hit model". The first hit is supposed to be the increase of free fatty acids in hepatocytes, which results in a decrease of the β-oxidation. Down regulation of the β-oxidation further aggravates accumulation of

fatty acids and insulin resistance. The second step includes all mechanisms contributing to the development of inflammation and fibrosis (Day and James, 1998). In detail, increase of fatty acids enhances the expression of cytochrome peroxidase 2E1 (CYP2E1). CYP2E1 stimulates generation of proinflammatory cytokines, oxidative species and thereby enhances lipid peroxidation of the hepatocyte membrane (Esterbauer *et al*, 1991; Osmundsen *et al*, 1991). NAFLD represents a spectrum of disease from benign fatty liver to NASH and even cirrhosis. The disease seems to progress through a two- or even multiple-hit process, with successive liver insults leading from fatty infiltration to the inflammation and fibrosis. The interplay of various adipokines, the most important of which are leptin, adiponectin, tumor necrosis factor-alpha (TNF-α), has a key role in this process (Tsochatzis *et al*, 2009). Emerging data suggest that apoptosis plays a critical role in NAFLD-induced liver injury and in the progression from steatosis to NASH and cirrhosis (Alkhouri *et al*, 2011). Moreover, the degree of apoptosis is closely associated with the severity of NASH and the stage of fibrosis (Feldstein *et al*, 2003).

The role of natural foods in disease prevention has been studied extensively in recent years. Among these natural foods, garlic has attracted a great deal of attention. Garlic (*Allium sativum* L.) has long been used both for flavoring and for the potential benefits of preventing and curing ailments in many cultures (Rivlin, 2001). Preclinical and clinical studies reveal a close relationship between dietary habits and the occurrence of diseases. Previous studies have found that ingestion of garlic is inversely related to the incidence of hyperlipidemia, atherosclero-sis and thrombosis (Agarwal, 1996). Like other *Allium* vegetables, Welsh onion (*A. fistulosum* L.) proved a good source of flavonoids and of many kinds of sulfur compounds. These components have recently been suggested as having beneficial medicinal properties, including those on arteriosclerosis (Yamamoto and Yasuoka, 2010). However, few reports on the hypolipidemic activity of Welsh onion have been reported.

The aim of this study was to establish a NAFLD animal model through feeding a high-fat diet, and investigated the individual and combined action of garlic and onion on serum liver markers, pro-inflammatory cytokines, hepatic steatosis, apoptosis, and oxidative stress.

Materials and methods

Ninety male Sprague-Dawley rats, average weight 120g±20, were bred and maintained at the Schistosome Biology Supply Center (SBSC) of Theodor Bilharz Research Institute, Giza, Egypt. Animals were housed in a controlled temperature and light environment, and were given water and commercial chow *ad libitum*. The experiments were conducted at the animal unit according to the international ethical guidelines for the care and use of animals for research purposes.

The rats were randomly divided into 9 groups (10 rats each); normal control (I), NAFLD-induced with high-fat diet (HFD; II), NAFLD switched to regular diet (RD; III), NAFLD-HFD or NAFLD-RD treated with garlic (IV, V), onion (VI, VII) or combined garlic & onion (VIII, IX) respectively. Rats in the control group were maintained on the standard chow diet for 20 weeks. A rat model of NAFLD was induced by a high-fat diet (25 % fats + 1 % cholesterol + 0.25 % bile salts) for 12 weeks (Zulet *et al*, 1999). After 12 weeks, rats in the garlic/onion-treated NAFLD groups were treated with garlic (Tomex plus®; Atos Pharma; Egypt) at a dose of 500 mg/kg b.w. or onion (onion oil, CAP Pharm; Egypt) at a dose of 100 mg/kg body b.w. via oral gavage, for 8 weeks (starting from the 13th week to 20th week). Rats in NAFLD-RD

treated groups were switched and maintained on the standard chow diet (weeks 13-20). At end of the study, all rats were weighted and then sacrificed by decapitation after 12h of fasting. Blood samples were collected for biochemical assays. Liver was immediately removed and weighted after rinsed with ice-cold saline, and sampled for assessment of liver glutathione antioxidant-related enzymes, MDA, and for histological study. Liver weight index (%) was calculated as liver weight/body weight×100.

Biochemical analyses: Blood parameters including triglyceride (TG), total choleste- rol (TC), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were assayed spectrophotometrically by using the commercially available kits. Serum TNF-α and adiponectin (Assaypro, St. Louis, MO, USA), leptin (RayBiotech Inc., Norcross, GA, USA) and TGF-β levels (IBL International GMBH, Hamburg, Germany) were measured using mouse ELISA kits.

Assay of lipid peroxidation and oxidative stress: The liver tissue was homogenized in 4 volumes (w/v) of ice-cold 100 mM KH_2PO_4 buffer containing 1 mM EDTA, pH 7.4 and centrifuged at 10,000 g for one hour at 4°C. Supernatant was collected and kept at -80°C for subsequent analysis for determination of the liver levels of glutathione-related antioxidant enzymes and lipid peroxide-tion. GSH content was measured by the method of Ellman (1959). GST activity was measured using chlorodinitrobenzene (CDNB) as a substrate (Habig *et al*, 1974). The glutathione reductase (GR) activity was assayed by using oxidized glutathione as a substrate after Zanetti (1979). Glutathione peroxidase (GPx) catalyzes the oxidation of glutathione and its activity was measured after Paglia and Valentine (1967). Superoxide dismutase (SOD) activity was assayed spectropho-tometrically (Winterbourn *et al,* 1975). Degree of lipid peroxidation in liver tissue homogenates was determined in terms of thiobarbituric acid reactive substances (TBARS) and expressed by malondialdehyde (MDA) formation (Ohkawa *et al*, 1979).

Histological studies: Liver tissues sections were fixed in 10% buffered formaldehyde, and then embedded in paraffin wax. A 5 µm-thick section cut from a paraffin-embedded block was stained with H&E and Masson's tri-chrome. Steatosis was assessed by a the morphological semi-quantitative approach and graded as follows: mild= 5-30%, moderate= 30-60%, and severe > 60% of hepatocytes affected. The specimens were also examined for histological features including; ballooning degeneration, hepatocytes with cytoplasmic vacuolation, acidophilic necrosis, sinusoidal fibrosis and polymorph nuclear infiltration.

Immunohistochemical staining: Survivin polyclonal antibody was obtained from Santa Cruz (Santa Cruz, CA, USA). Immunohistochemical staining of 5 µm-thick paraffin-embedded liver sections was performed according to manufac-turer's protocol (Vectastain Elite ABC kit; Vector, Burlingame, CA, USA). Briefly, liver sections were deparaffinized in xylene and rehydrated in graded ethanol. After endogenous peroxidase and biotin were blocked, tissues were preincubated with 3% horse serum for 30min to prevent non-specific reactions. The sections were then incubated with primary antibodies diluted at 1:150 for 60 min. On negative control sections, the step with the primary antibodies was omitted. Polyclonal antibodies were detected using a bio-tinylated anti-goat or rabbit IgG diluted at 1:200 in 5% bovine serum albumin for 30 min. The sections were incubated with R.T.U. Vectastain Elite ABC reagent for 30 min, stained with diaminobenzidine for 5 min and counter stained with hemato-xylin before they were mounted. The liver sections were examined using a Zeiss light microscope

(Oberkochen, Germany). The number of positively stained cells with the highest expression recorded within 10 successive fields (x400) was counted/section⁄animal in a semi-quantita-tive way; the final value represented the mean of 80 readings per group. Zero percentage was given to unstained sections. Survivin expression sites (Baba *et al*, 2009) were examined intralobularly, in the periportal areas, in hepatocytes, bile duct epithelium and monocytes.

Statistical analysis: All the data were presented as means±SD. Differences among groups were assessed using one-way analysis of variance (ANOVA) followed by *post-hoc* LSD test. P-values of less than 0.05 were considered to be statistically significant. Calculations were performed with the SPSS, Version 16.0 statistical software package.

Results

Effects on hepatic gross manifestations: The liver weight and liver index in NAFLD-HFD or NAFLD-RD groups were significantly increased ($P<0.05$) over those of normal control group. However, the body weight of rats in both groups decreased significantly ($P<0.05$). Com-pared with NAFLD-HFD or NAFLD-RD groups, the liver weight and liver index were significantly decreased ($P<0.05$) in treated groups with either garlic or onion alone or in combination (Tab. 1).

The effects on hepatic biochemistry: Compared with normal control group, the serum levels of ALT, AST, ALP, cholesterol, triglyceri-des, TNF-α, TGF-β1 & leptin in NAFLD-HFD group increased significantly ($P<0.05$). These elevations were to a less extent in NAFLD-RD group. At the same time, the level of adiponectine was signi-ficantly decreased ($P<0.05$). Compared with NAFLD-HFD group, ALT, AST, ALP triglycerides, TNF-α and TGF-β1 serum levels decreased significantly ($P<0.05$) in the groups treated with either garlic or onion alone. The serum levels of cholesterol, leptin and TGF-β1 (in onion treated-group) also had decreasing tendency, but there was no significant difference ($P>0.05$). Compared with NAFLD-RD group, the serum levels of ALT, AST, ALP, cholesterol, triglyce-rides and TGF-β1 (in garlic group) were significantly increased in groups treated with either garlic or onion alone (Tab. 2 & Fig. 1). Serum level of adiponectine was insignificantly changed in either treated groups compared with NAFLD-HFD or NAFLD-RD groups.

Effects on oxidative stress and lipid peroxidation: Compared with normal control group, the hepatic GSH content and GST, GR, GPx and SOD activities in NAFLD-HFD group were significantly decreased ($P<0.05$), meanwhile, MDA level was significantly increased. These changes were to a less extent in NAFLD-RD group. Compared with NAFLD-HFD or NAFLD-RD groups, administration of either garlic or onion alone or both significantly decreased the effects of the oxidative stress and lipid peroxidation in the hepatic tissue by restoring GSH content, increasing GST, GR, GPx, SOD activities and decreasing of MDA level (Tab. 3).

In the garlic+onion combined group, all the measured serum and hepatic markers gave better results over those of each treated group, weather maintained on HFD or switched to regular diet. Moreover, the best results were obtained in all groups switched to regular diet.

Histological evaluation: After 20 weeks, in normal control group (Fig. 2A), liver lobules were distinct (preserved lobular architecture), the liver cell cords arranged regularly, whereas, the liver in NAFLD-HFD group (Fig. 2B) showed typical steatosis (65.0±2.69, micro and macro-steatotic changes), lobular infiltration (22.5±2.01) by mononuclear cell and lymphocyte, hepatocytes with cytoplasmic vacuolation (23.5±2.36), fat

droplets accumulation, spotty necrosis and focal necrosis. These findings suggest that the animal model of NAFLD was successfully established. However, the degree of hepatic injury including steatosis, hepato-cytes with cytoplasmic vacuolation and lobular inflammation were to a less degree in NAFLD-RD (30.1±6.54, 18.0±3.43 & 15.3±3.39 respectively; Tab. 4 & Fig. 2C). Treatment of NAFLD-induced rats with either garlic or onion alone or both, maintained on HFD or RD respectively, markedly attenuated steatosis (mean score: 20.0±3.57, 9.0±2.77, 42.0±6.96, 7.5±2.39, 12.5±3.59 & 2.5±0.83), hepato-cytes with cytoplasmic vacuolation (mean score: 15.0±2.11, 4.0±1.45, 13.5±2.59, 7.0±2.49, 7.0±3.0 & 2.5±0.83) and infla-mmation (mean score: 9.5±1.89, 3.0±0.82, 8.5±0.76, 2.5±0.83, 3.5±1.07 & 1.5±0.76) 8 weeks post treatment (Fig. 2D-I). In the garlic+onion combined group, steatosis and extension of lobular inflammation were relieved significantly over those of each treated group, and in all NAFLD-RD groups over those groups maintained on HFD (Tab. 4; Fig. 2H & I).

Immunohistochemical aspects: Control normal rats were negative for survivin polyclonal antibody (Tab. 4 & Fig. 3A), meanwhile, NAFLD induced rats showed positively survivin-stained hepatocyte cells indicating the presence of survivin cells (Fig. 3B). Switching to RD from HFD reduced percentage of hepatocytes expressing survivin (34.0±3.06 vs 43.0±1.86 respectively, Fig. 3C). The treatment of NAFLD-induced rats with either garlic or onion alone, maintained on HFD or RD, markedly reduced the percentage of hepatocytes expressing survivin (19.0±2.77, 7.5±2.39 for garlic, 26.0±3.93 & 8.5±3.17 for onion, res-pectively, Fig. 3D-G). Highest reduction in the expression of survivin was recorded in the group treated with both garlic and onion, maintained on HFD or RD (11.0±3.48 & 5.5±1.57 respectively (Tab. 4 & Fig. 3H & I).

Discussion

NAFLD represents a wide spectrum of disorders, the hallmark of which is hepatic steatosis. NAFLD was considered a benign condition, but is now increasingly recognized as a major cause of liver-related morbidity and mortality (Xiao *et al*, 2013). Insulin resistance is the basis for accumulation of free fatty acids and triglyceride storage in hepatocytes or steatosis. Oxidative stress from steatotic hepatocytes leads to lipid peroxidation, impaired mitochondrial and peroxisomal oxidation of fatty acids, and cytokine release (Parekh and Anania, 2007). The endotoxins and endotoxin-inducible cytokines, particularly TNF-α, are require-ed for the pathogenesis of NAFLD in experimental animals. Thus, the TNF-α plays an important role in NAFLD (Day, 2006). Garlic (*Allium sativum* L.) has a wide range of pharmacological effects including the antimicrobial, cardio-vascular, anti-inflammatory, anticancer, and immunomodulatory activity among many other effects. Onions (*A. cepa* L.) might be useful for preventing obesity-related breast, colorectal, laryngeal, and ovarian cancers (Galeone *et al*, 2006; Wang *et al*, 2012). To the present authors knowledge this may be the first report on the pharmacological effects of garlic and onion combination either maintained on HFD or switched to RD in a NAFLD-rat model.

In this study, a rat model of NAFLD induced by high fat diet was established. Decreased body weight, increased liver weight and liver weight index, elevated serum liver enzyme levels and altered liver histological conditions including steatosis, inflammation and fibrosis were observed in the NAFLD-HFD rats, and to a less extent in NAFLD-RD rats. These abnormalities were significantly

improved 8 weeks after treatment with either garlic or onion or both. Body mass significantly decreased with the high-fat diet and dietary restriction. This may be due to a metabolic imbalance of carbohydrate, protein, and fat. However, hepatic index was higher in the group with steatosis as compared to the garlic or onion-treated and control groups, which means that garlic or onion acts by decreasing fat accumulation in the liver and fat weight, and therefore decreases hepatic index. In our study, the elevated transaminase serum levels correlated strongly with NAFLD, are the result of leakage from damaged cells, therefore, reflect hepatocyte damage (Gholam *et al*, 2007). In agreement with previous reports (Assy *et al*, 2006), the present study also showed that the levels of serum triglycerides, cholesterol, leptin as well as TNF-α and TGF-β1 were significantly increased, whereas the levels of serum adiponectin remarkably decreased in the NAFLD group. The abnormalities were signi-ficantly improved, and hepatic steatosis was significantly decreased in all treated groups. Kuda *et al.* (2004) mentioned that garlic has anti-hyperlipidemic, hypo-cholesterolaemic and hypo triacylgly-ceride activities. Garlic may exert a lipid-lowering effect partly through reducing intestinal microsomal triglyceride transfer protein (MTP) gene expression, thus suppressing the assembly and secretion of chylomicrons from intestine to the blood circulation (Lin *et al*, 2002) or due to an increase in cholesterol degradation to bile acids and neutral sterols and mobilization of triacyl glycerols in treated rats (Kempaiah and Srinivasan, 2006). More-over, Rai *et al.* (2009) and Lii *et al.* (2012) reported that garlic's organosulfur compounds (such as diallyl trisulfide) display hypolipidemic effects by inhibi-ting fatty acids and cholesterol synthesis.

Oxidative stress is believed to play an important role in pathogenesis of NAFLD. It is likely to be involved in disease progression from steatosis to steato-hepatitis and potentially cirrhosis. It has been shown that chronic oxidative stress, generated through oxidation of cytotoxic free fatty acids, may lead to cytokine upregulation and depletion of hepatic antioxidant levels (Garcia-Ruiz *et al*, 1995). In addition, enhanced lipid peroxidation leads to the generation of by-products, such as MDA, which have been shown to further stimulate cytokine production. They are involved in hepatic stellate cells activation, fibrogenesis, and enhanced extracellular matrix protein deposition (Thong-Ngam *et al*, 2007). In this study, treatment of NAFLD-HFD or RD groups with garlic and/or onion restored the depleted hepatic antioxidant activities as GSH, SOD, GR, GST and GPx and decreased MDA levels in the liver. The lowered activities of hepatic antioxidant enzymes in hypercholes-terolemic rats were effectively countered by garlic (Kempaiah and Srinivasan, 2004). Gorinstein *et al* (2006) reported that dietary garlic was effective in reducing the oxidant stress, which was indicated by an increase of antioxidant activity and a decrease of lipids in the rats' blood. Onion bulbs contain more than 20 flavonoids other than quercetin (Slimestad *et al*, 2007). According to Jung *et al* (2011), Onion peel extract (OPE) was found to be composed of polyphenols at the level 60%, of these, 16% was quercetin. Thus, it was postulated that the higher potency of OPE might be attributed to the additive or synergistic effect of an array of phytochemicals. The antioxidant activity of *Allium* spp. has been attributed mainly to a variety of sulphur-containing compounds and the precursors (Nishimura *et al*, 2004). Scientific evidence shows that allicin, diallyl disulphide and diallyl trisulphide appeared to be the main antioxidative compounds (Kim *et al*, 1997). In addition, the antioxidant activity is also related to other bioactive compounds:

dietary fibers, microelements (especially Selenium) and polyphenols (Lanzotti, 2006). Nencini *et al* (2010) reported that that fresh *Allium* homogenates (leaves or bulbs) possess antioxidant properties and provide protection against ethanol-induced liver injury. Helen *et al* (2000) reported that onion oil is an effective antioxidant against the oxidative damage caused by nicotine as compared to vitamin E. In previous studies, the authors reported that outer dry layers of onion bulb have strong antiinflammatory and antioxidant activi-ties and proposed quercetin as the major component responsible for this activity (Coskun *et al*, 2005; Park *et al*, 2007).

Apoptosis is important for regulation of many physiological and pathological processes. Apoptosis signaling pathway can be abnormally activated in various pathological processes in the liver, including acute and chronic hepatitis, alcohol-induced liver disease, non-alcoholic fatty liver disease, cholestatic liver disease, fibrogenesis, liver cirrhosis and liver carcinogenesis. In NAFLD, Inflammatory response, oxidative stress and apoptosis serve as "following-hits" that contribute to the ongoing inflamma-tion (NASH). Emerging data suggest that apoptosis plays a critical role in NAFLD-induced liver injury and in the progression from steatosis to NASH and cirrhosis (Alkhouri *et al*, 2011). Liver regeneration is a complex process involving both proliferation and apoptosis. Survivin is a fascinating little protein that acts as a component of the chromosomal passenger complex, which is essential for cell division, and as an inhibitor of apoptosis. With dual roles in promoting cell proliferation and preventing apoptosis, it is considered a protein that interfaces life and death (Wheatley and McNeish, 2005). In this study, different treatment regimens tested decreased the expression of the survivin stained cells indicating the increase of apoptosis in hepatocyte cells. The maximum reduction was recorded in animals receiving both garlic and onion in combination, either maintained on HFD or RD (11.0±3.48 & 5.5±1.57 respectively). The results agreed with Colín-González *et al.* (2012) and Wang *et al.* (2012) who found that garlic and onion stimulate inhibition of cell proliferation and the increase of apoptosis.

In the present study, the combined administration of garlic and onion gave an enhancement effect in modulating the biochemical and histological markers related to NAFLD disease compared to either one alone, due to their synergistic antioxidative, anti-inflammatory and apo-ptotic effects. Organosulfur compounds present in garlic and onion are the most important contents responsible for most of their pharmacological effects. *A. sativum* may exert toxicity only at high doses and that there have been few reports of intoxications following the ingestion of garlic (Mikaili *et al*, 2013).

Conclusion

The current study is the first time that combined administration of garlic and onion has a modulating effect on the extent of fatty infiltration and on lipid peroxidation compared with the effect of either drug alone. Inducing apoptosis together with decreasing leptin, TGF-β1, TNF-α and reduction of oxidative stress remains the best targeted treatment to date for NAFLD. Since garlic and onion are common food supplements worldwide, so garlic and onion or their derivatives could be considered as one of the preventive measures in treating NAFLD. Further studies are ongoing to confirm their safety and quality to be used by clinicians as therapeutic agents.

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Table 1: Effect of garlic and/or onion on liver gross manifestations in NAFLD-induced rats.

		Body weight	Liver weight	Liver weight index (%)
	Normal	219.0±35.18	5.71±1.13	2.60±0.26
HFD	NAFLD Control	117.8±29.01[a]	9.01±2.16[a]	7.66±0.48[a]
HFD	Garlic	150.1±34.51[ab]	7.02±1.54[ab]	4.74±0.72[ab]
HFD	Onion	139.4±18.36[a]	7.51±1.38[a]	5.37±0.54[ab]
HFD	Garlic & Onion	179.0±25.69[abcd]	6.51±0.98[b]	3.64±0.18[abcd]
RD	NAFLD Control	135.0±29.25[a]	7.14±1.62[a]	5.31±0.74[a]
RD	Garlic	161.5±24.73[ab]	7.70±1.30[a]	4.76±0.31[ab]
RD	Onion	149.8±37.21[a]	6.91±1.26[a]	4.72±0.65[a]
RD	Garlic & Onion	195.5±21.79[bcd]	6.03±1.12[c]	3.08±0.39[abcd]

Rats/ group= 10 [a]Significant differences from normal at P<0.05. [b] from NAFLD control at P<0.05. [c]from garlic at P<0.05. [d] from onion at P<0.05.

Table 2: Effect of garlic and/or onion on liver enzymes and lipid profile in NAFLD-induced rats.

		ALT (U/L)	AST (U/L)	ALP (IU/L)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
	Normal	17.40±2.27	64.90±14.11	113.58±18.45	17.70±4.66	24.56±5.98
HFD	NAFLD Control	76.90±14.49[a]	157.00±21.48[a]	248.67±29.02[a]	95.95±29.52[a]	111.03±26.16[a]
HFD	Garlic	44.20±9.84[ab]	102.60±10.08[ab]	166.00±20.79[ab]	82.27±12.46[a]	75.53±12.62[ab]
HFD	Onion	47.70±8.11[ab]	108.00±10.68[ab]	165.94±41.94[ab]	87.40±18.01[a]	82.25±15.11[ab]
HFD	Garlic & Onion	36.10±6.19[abd]	89.40±9.00[abcd]	156.55±21.25[ab]	63.41±5.74[abcd]	62.39±16.81[abd]
RD	NAFLD Control	61.40±16.89[a]	119.30±13.32[a]	204.40±19.15[a]	83.82±22.12[a]	90.83±19.59[a]
RD	Garlic	38.70±10.87[ab]	96.80±10.77[ab]	129.40±16.31[b]	61.92±7.43[ab]	65.40±12.10[ab]
RD	Onion	43.80±7.18[ab]	102.10±9.73[ab]	133.19±16.80[b]	68.42±21.07[ab]	78.61±13.42[a]
RD	Garlic & Onion	27.50±5.19[abcd]	78.60±8.51[abcd]	117.70±10.26[b]	53.13±7.77[abd]	49.29±9.54[abcd]

Table 3: Effect of garlic and/or onion on glutathione-related antioxidant enzymes and lipid peroxidation in NAFLD-induced rats.

		GSH (umole/g liver)	GR (umole/ min/g liver)	GST (umole/min /g liver)	GPx (umole/ min/g liver)	SOD (umole/ min/g liver)	MDA (nmol/g liver)
	Normal	5.35±0.52	2.51±0.70	55.26±13.59	3.42±0.90	676.00±51.31	5.67±0.28
HFD	NAFLD Control	3.40±0.27[a]	1.32±0.58[a]	23.60±10.95[a]	1.77±0.58[a]	351.70±25.27[a]	28.06±4.09[a]
HFD	Garlic	3.89±0.39[ab]	1.84±0.49[ab]	38.55±10.46[ab]	2.67±0.50[ab]	452.40±89.49[ab]	18.70±2.06[ab]
HFD	Onion	3.73±0.30[a]	1.79±0.47[ab]	34.66±7.46[ab]	2.71±0.49[ab]	430.00±75.66[ab]	18.89±2.06[ab]
HFD	Garlic & Onion	4.19±0.24[abd]	2.12±0.47[b]	44.23±11.34[abd]	2.91±0.62[b]	544.10±63.52[abcd]	11.98±2.68[abcd]
RD	NAFLD Control	3.68±0.44[a]	1.63±0.56[a]	32.79±7.22[a]	2.56±0.54[a]	434.70±64.52[a]	16.17±1.67[a]
RD	Garlic	4.19±0.25[ab]	1.94±0.47[a]	41.75±11.62[a]	2.77±0.60[a]	473.30±85.56[a]	13.44±2.52[ab]
RD	Onion	4.28±0.35[ab]	1.77±0.58[a]	37.21±8.26[a]	2.82±0.60[a]	457.30±84.79[a]	14.11±2.66[a]
RD	Garlic & Onion	4.71±0.47[abcd]	2.29±0.46[bd]	48.67±10.03[bd]	3.11±0.48[b]	581.30±69.53[abcd]	9.68±1.63[abcd]

[a]Significant difference from normal at P<0.05. [b]Significant difference from NAFLD control at P<0.05. [c]Significant difference from garlic at P<0.05. [d]Significant difference from onion at P<0.05.

Table 4: Effect of garlic and/or onion on histological parameters in NAFLD-induced rats.

Group	Hepatic architecture		Steatosis	Type of steatosis		Inflammation	Hepatocytes with cytoplasmic vacuolation	Fibrosis		Survivin expression Percentage of positive stained with survivin	
	Preserved	Lost	% of cells	Micro-vesicular	Macro-vesicular	% of inflamed cells in 10 microscopic fields	% of cells in 10 microscopic fields	None	Present	% of positive cells	Intensity of stain in 10 HMF
Control-HFD	0/10	10/10	65.0±2.69	4/10	6/10	22.5±2.01	23.5±2.36	0/10	10/10	43.0±1.86	50% moderate 50% marked
Control-HFD/RD	6/10	4/10	30.1±6.54	5/10	5/10	15.3±3.39	18.0±3.43	4/10	6/10	34.0±3.06	60% moderate 40% marked
Garlic-HFD	6/10	4/10	20.0±3.57[a]	5/10	5/10	9.5±1.89[a]	15.0±2.11[a]	4/10	6/10	19.0±2.77[a]	30% moderate 70% mild
Garlic-HFD/RD	10/10	0/10	9.0±2.77b	7/10	3/10	3.0±0.82[b]	4.0±1.45[b]	8/10	2/10	7.5±2.39[b]	100% mild
Onion-HFD	4/10	6/10	42.0±6.96[a]	5/10	5/10	8.5±0.76[a]	13.5±2.59[a]	2/10	8/10	26.0±3.93[a]	80% moderate 20% marked
Onion-HFD/RD	8/10	2/10	7.5±2.39[b]	6/10	4/10	2.5±0.83[b]	7.0±2.49[b]	6/10	4/10	8.5±3.17b	90% mild 10% moderate
Garlic+Onion-HFD	10/10	0/10	12.5±3.59[ce]	5/10	5/10	3.5±1.07[ce]	7.0±3.0[c]	10/10	0/10	11.0±3.48[e]	70% mild 30% moderate
Garlic+Onion-HFD/RD	10/10	0/10	2.5±0.83[d]	5/10	5/10	1.5±0.76	2.5±0.83	10/10	0/10	5.5±1.57	50% mild 50% moderate

Data presented as mean ± SEM. Number of rats in each group= 10. [a] significant difference from NAFLD-HFD at P<0.05. [b] significant difference from NAFLD-RD at P<0.05. [c] significant difference from Garlic-HFD at P<0.05. [d] significant difference from Garlic-HFD/RD at P<0.05. [e] significant difference from Onion-HFD at P<0.05.

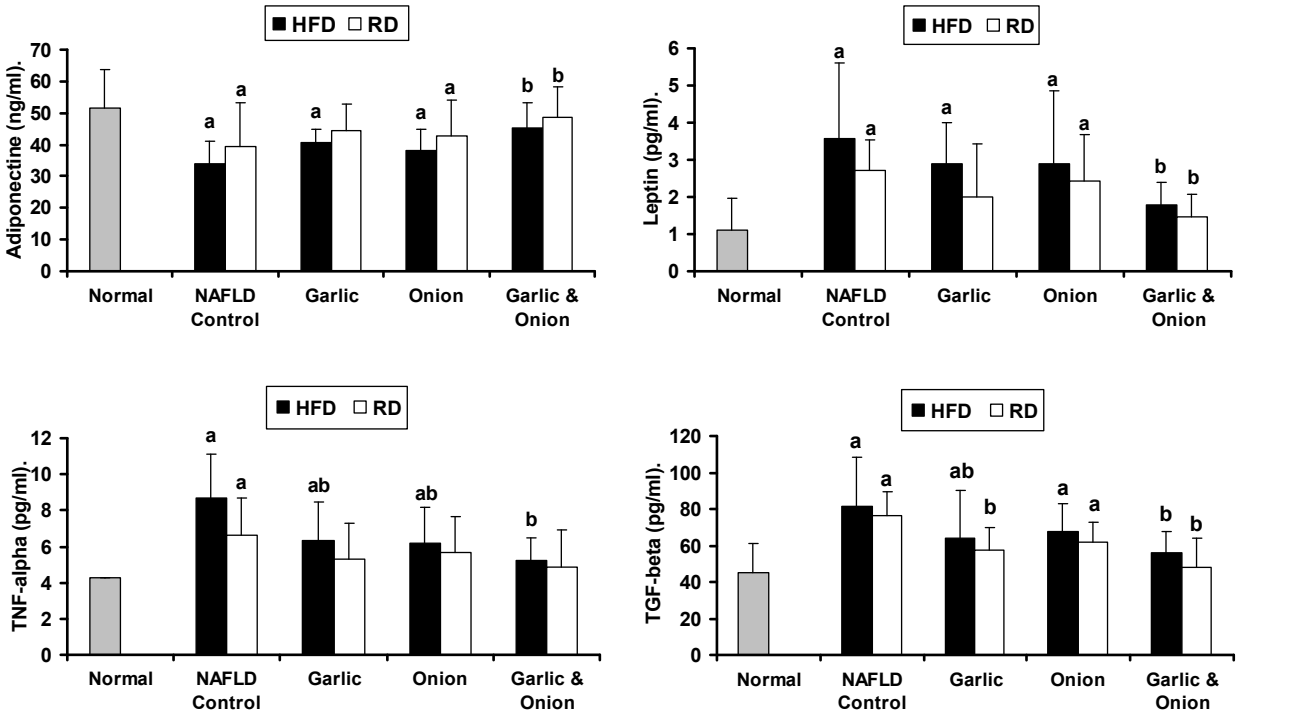

Fig. 1: Effect of garlic and/or onion on cytokines; adiponectine, leptin, TNF-α and TGF-β in NAFLD-induced rats. [a] Significant difference from normal at P<0.05. [b] Significant difference from the relative NAFLD control (HFD or RD) at P<0.05.

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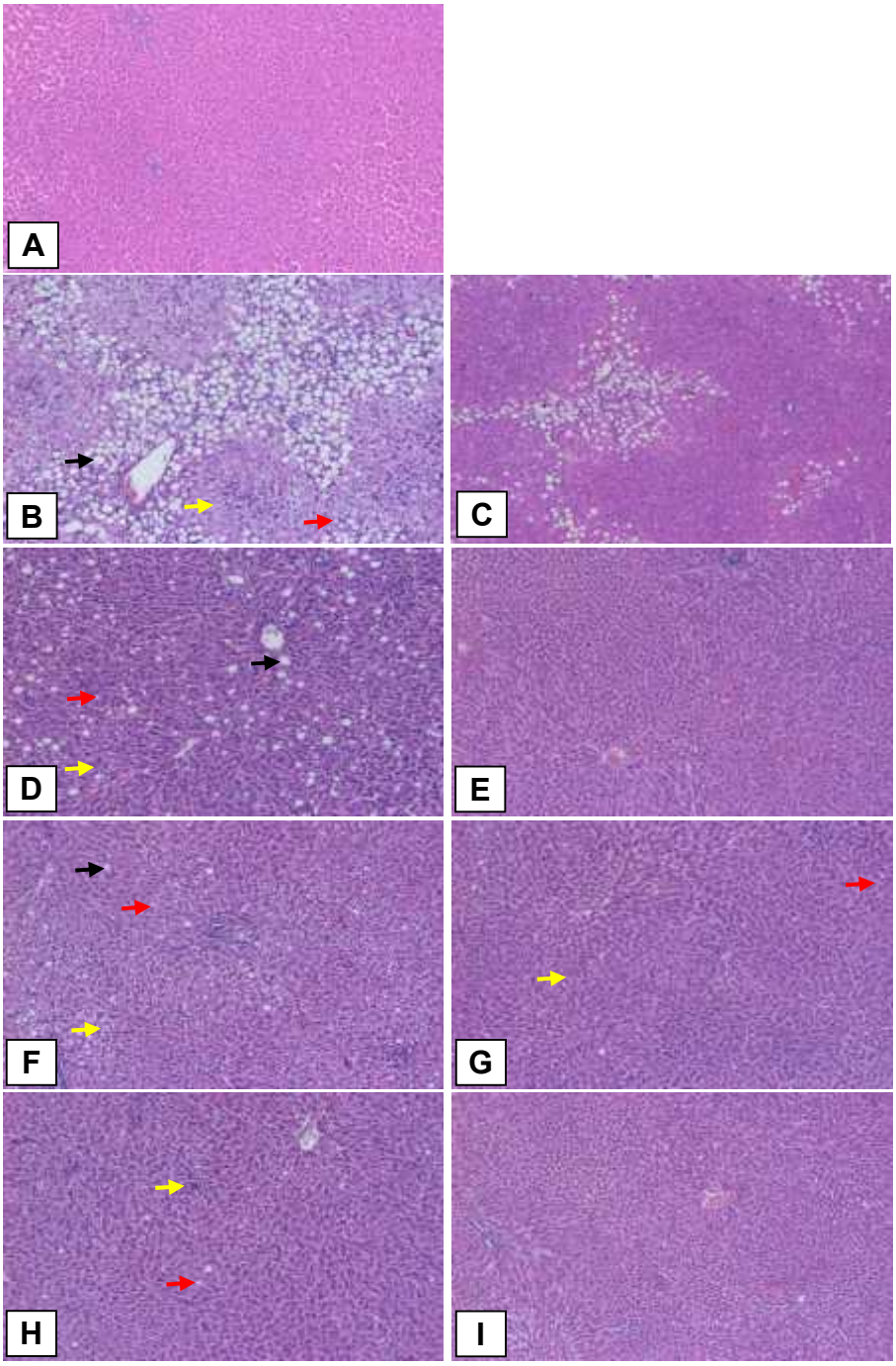

Fig. 2 (A-I): Liver sections from; normal untreated rats (A) showing liver lobules were distinct (preserved lobular architecture), liver cell cords arranged regularly, NAFLD-HFD rats (B) showing lost hepatic architecture, macro (black arrow) and micro (red arrow) 35% steatotic changes, many hepatocytes with cytoplasmic vacuoles (yellow arrow), and to a less extent in NAFLD-RD rats (C), garlic-HFD-treated rats (D) showing preserved hepatic architecture, macro (black arrow) and micro (red arrow) 8% steatotic changes, scattered lymphocytes between hepatocytes (yellow arrow), garlic-RD-treated rats (E) showing normal hepatic architecture, liver appear within normal limit, onion-HFD-treated rats (F) showing preserved hepatic architecture, macro (black arrow) and micro (red arrow) 15% steatotic changes, scattered lymphocytes between hepatocytes (yellow arrow), onion-RD-treated rats (G) showing preserved hepatic architecture, micro (red arrow) 3% steatotic changes, scattered lymphocytes between hepatocytes (yellow arrow), garlic+onion-HFD-treated rats (H) showing preserved hepatic architecture, micro (red arrow) 5% steatotic changes, scattered lymphocytes between hepatocytes (yellow arrow) and garlic+onion-RD-treated rats (I) showing normal hepatic architecture, liver appear within normal limit (H & E, x100).

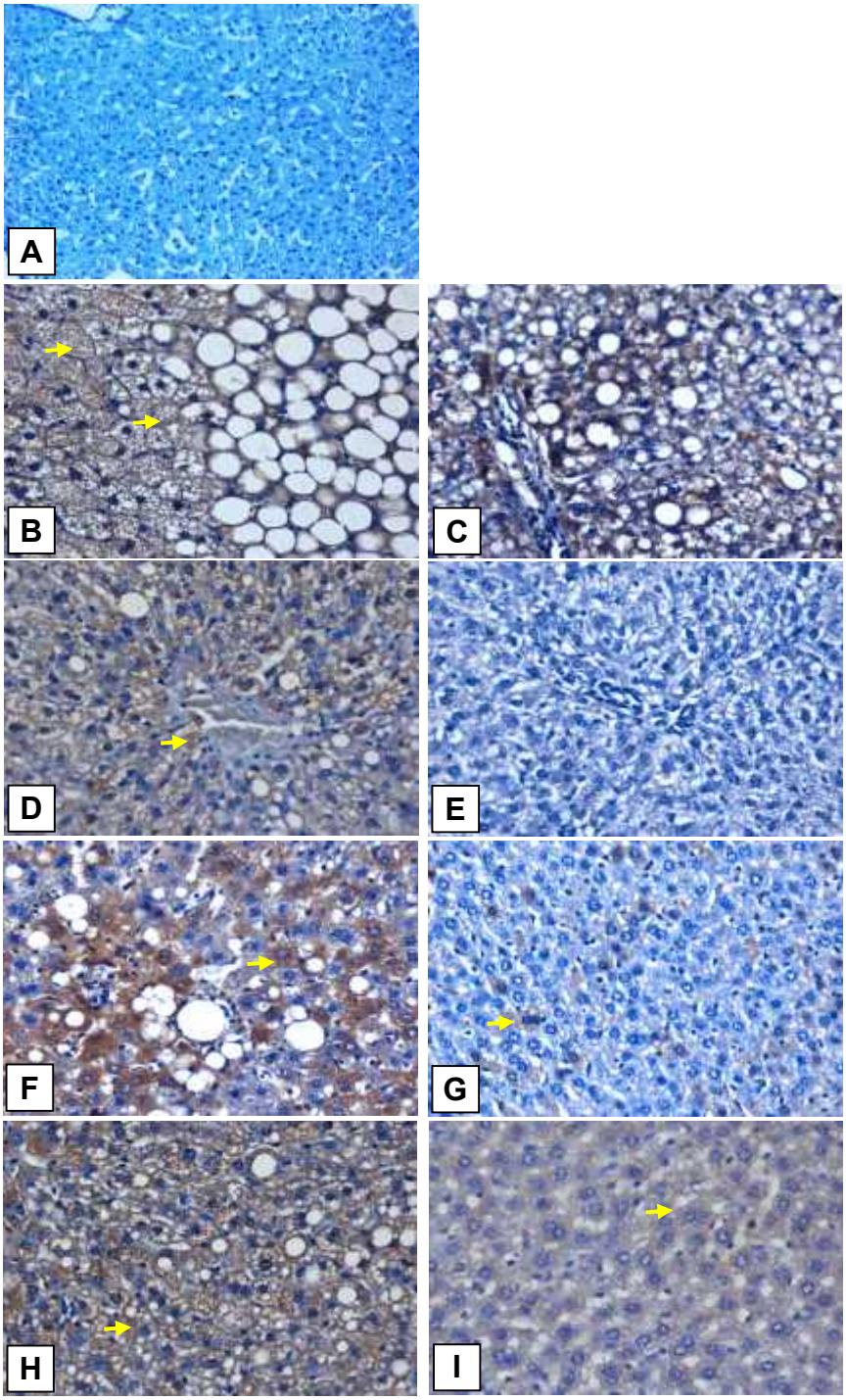

Fig. 3 (A-I): Liver sections from; normal untreated rats (A) negative for survivin polyclonal antibody, NAFLD-HFD rats (B) showing moderate cytoplasmic expression of polyclonal antibody survivin (50%) as brownish stains (arrows), and to a less extent in NAFLD-RD rats (C), garlic-HFD-treated rats (D) showing mild cytoplasmic expression of polyclonal antibody survivin (20%) as brownish stains (arrow), garlic-RD-treated rats (E) showing hepatocytes negative for polyclonal antibody surviving, onion-HFD-treated rats (F) showing moderate cytoplasmic expression of polyclonal antibody survivin (50%) as brownish stains (arrow), onion-RD-treated rats (G) showing scattered hepatocytes with cytoplasmic expression of polyclonal antibody survivin as brownish stains (arrow), garlic+onion-HFD-treated rats (H) showing mild cytoplasmic expression of polyclonal antibody survivin (20%) as brownish stains (arrow) and garlic+onion-RD-treated rats (I) showing faint scattered cytoplasmic expression of polyclonal antibody survivin (arrow) (IHC, DAB, x400).