Egyptian Journal of Basic and Clinical Pharmacology
December 2011, Vol. 1, No. 1: 1-8
http://www.ejbcp.eg.net/

Original Article

Molecular Mechanisms Underlying the Protective Effect of Telmisartan in Non-Alcoholic Fatty Liver Disease: Role of Proinflammatory Enzymes

Mohamed Ibrahim[1], Adel Saad[2], Soha Abdelwahab[3] and Hend Abdelghany[4]

[1]Department of Pharmacology, Faculty of Medicine, [2]Department of physiology, Faculty of Medicine, [3]Department of histology, Faculty of Medicine, [4]Department of biochemistry, Faculty of Medicine, Minia University, Egypt.

A B S T R A C T

Background: Non-alcoholic fatty liver disease (NAFLD) is an increasingly recognized condition that may progress to end stage liver cell failure. There is as yet no clearly established therapy for prevention or treatment of NAFLD. Telmisartan, an angiotensin II receptor blockers and partial agonist on peroxisome proliferative activated receptor-gamma (PPAR-γ), showed a promising protective effect in NAFLD.
Aim: The present study explored the mechanism(s) of the protective effect of telmisartan on NAFLD with a focus on the role of the proinflammatory enzymes: induced nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2).
Methods: Adult male Wistar rats were assigned into three groups and treated for 8 weeks as follow: group 1 fed normal diet and served as normal control group; group 2 fed high cholesterol diet; group 3 fed high cholesterol diet plus telmisartan.
Results: Administration of telmisartan in cholesterol-fed rats attenuated the development of NAFLD as evidenced by significant decrease in liver index and confirmed by histopathological examination. The effect of telmisartan was accompanied with significant decrease in hepatic tissue expression of the proinflammatory enzymes: iNOS and COX-2.
Conclusion: Telmisartan inhibited the proinflammatory mediators, iNOS and COX-2, reported to be involved in the progression of NAFLD indicating that telmisartan might serve as a potential therapy for NAFLD.

Key Words: Non alcoholic fatty liver, telmisartan, iNOS, COX-II.

Corresponding Author: Mohamed Abdellah Ibrahim **Email:** mabdellah69@yahoo.com

1. INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) describes a spectrum of liver clinicopathological changes extending from simple steatosis through non-alcoholic steatohepatitis (NASH) to fibrosis *(Farrell and Larter, 2006)*. It is the most common cause of chronic liver injury worldwide. It has been documented in 10 to 15 percent of normal individuals and 70 to 80 percent of obese individuals *(Bellentani et al. 2000)*.

It was initially believed that NAFLD is a completely benign disorder; but histological follow-up studies showed that progression to fibrosis occurs in about one third of patients *(Bellentani et al. 2000)*. However, the mechanisms that mediate the transition from steatosis to NASH and to fibrosis remain unknown. Several mediators and mechanisms have been suggested. The most recent prevailing concept is "multiple-hit" hypothesis *(McCllough, 2006)*. The starting point is insulin resistance that leads to a reversible accumulation of fat in hepatocytes *(Chitturi et al., 2006)*, followed by oxidative stress and upregulation of pro-inflammatory mediators that activate inflammatory pathway *(Anstee et al. 2006)*.

Low grade inflammation was suggested as a leading cause for conversion of NAFLD from simple steatosis to statohepatitis and cirrhosis *(Tipoe et al. 2009)*. Among the pro-inflammatory mediators that had been upregulated in NAFLD are induced nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) *(Hsieh et al. 2009; Tipoe et al. 2009)*. Both iNOS and COX-2 are inducible mediators in the pathogenesis of many inflammatory diseases *(Majano et al. 1998; Pata et al. 2003; Koeppel et al. 2007)*.

Several therapies, including diet and antioxidants have been tried to treat patients with NAFLD *(Fan et al. 2003; Miele et al. 2006)*. However, no satisfying drug therapy has been established for NAFLD as yet. Recently, angiotensin II, the principle effector of the renin-angiotensin system, has been reported to have a crucial role in pathogenesis of hepatic steatosis and hepatic fibrosis *(Yoshiji et al. 2001;*

Sugimoto et al. 2006; Georgescu, 2008; Nabeshima et al. 2009). The action of angiotensin II is mainly mediated by the angiotensin II type 1 receptor (AT1), which is expressed in hepatic stellate cells *(Bataller, et al. 2003)*. Hence, drugs that modulate the effect of angiotensin II represent possible targets for treatment of NAFLD. Telmisartan is distinguished among other members of angiotensin II type 1 receptor blockers (ARBs) by its partial agonistic activity on peroxisome proliferative activated receptor-gamma (PPAR-γ) which is known to have anti-inflammatory, anti-oxidant and adipocyte differentiating effects *(Yamagishi and Takeuchi, 2005)*. Recent studies reported that telmisartan controlled the development of NAFLD *(Sugimoto et al. 2006; Fujita et al. 2007)*.

The promising initial results of telmisartan in prevention of NAFLD justified further experiments to explore its mechanism of action. Our study investigated the effect of telmisartan on the series of events involved in the pathogenesis of NAFLD. The study is the first that addressed the effect of telmisartan on the proinflammatory enzymes: iNOS and COX-2.

2. MATERIALS AND METHODS

2.1. Materials:

Telmisartan was obtained from Boehringer Ingelheim, International (GmbH, Germany); Cholesterol was obtained from sigma chemical (St Louis, MO, USA); Triglyceride, Malondialdehyde and ALT kits were obtained from Biodiagnostic (Cairo, Egypt);. Epitope specific rabbit antibodies to iNOS and COX-2 were purchased from Thermo Fisher Scientific Anatomical Pathology (CA, USA) and other chemicals were obtained from EL-Gomhoria company (Cairo, Egypt).

2.2. Animals:

Adult male Wistar rats weighing 140-150 g were obtained from animal house (Faculty of Medicine, Minia University). They were housed in controlled environment and allowed free access to standard laboratory chow (El-Nasr Company, Abou-Zaabal, Cairo, Egypt) and water. All experimental protocols were approved by the ethics committee of Minia University.

2.3. Experimental protocol:

After acclimatization for 1 week, the rats were assigned into three experimental groups, 7 rats each and treated for 8 weeks as follow: Group-1: Fed normal diet, received physiological saline and served as normal control group; group-2: Fed 2%-cholesterol diet, received physiological saline and served as high cholesterol (positive control) group; group-3: Fed 2% cholesterol diet and received telmisartan (5 mg/kg/day) by gavage. Administartion of telmisartan was started from the first day of the experiment and continued for 8 weeks. The doses and schedules of the study were chosen according to our preliminary study and in consistence with previous reports *(Ping et al., 2006; Fujita et al. 2007; In Suk et al. 2007; Zheng et al. 2008)*. All experimental protocols wer approved by the ethical committee of The Faculty of Medicine, Minia University.

At the end of the experimental period, rats were anesthetized by inhalation of ether and blood samples were collected from abdominal aorta and processed for biochemical measurements. Then, rats were sacrificed and their livers were rapidly collected, blotted dry, weighed and divided into 2 parts. One part was put in 10% formalin for histopathological examination and immunohistochemical assay of iNOS and COX-2. The second part was kept at -70°C and used for biochemical measurements.

2.4. Measurement of liver index:

Liver index was calculated from the equation: (liver weight/body weight) ×100 *(Ping et al., 2006)*.

2.5. Biochemical measurements:

2.5.1. Serum levels of alanine amine transferasenase (ALT):

Serum level of ALT was measured using biochemistry automatic analyzer (Hitachi7600).

2.5.2. Liver tissue content of triglycerides:

Triglyceride was assayed in hepatic tissue using commercially available kits after lipid extraction as described by *(Folch et al. 1957)*.

2.5.3. Liver tissue content of lipid peroxidation products: malondialdehyde (MDA):

MDA was determined using a commercially available kit according to the manufacturer instructions.

2.5.4. Liver tissue content of NO:

Liver tissue content of NO was measured as total nitrite (NOx), the stable degradation products of NO, after reduction of nitrate to nitrite by copper-cadmium alloy and measuring total nitrite (nitrite + nitrate) using Griess Reagent *(Green et al. 1982)*.

2.6. Histopathological examination:

For the histological study, rat liver specimens were taken 5 mm away from the edge of the largest hepatic lobe, fixed with 10% formaldehyde; embedded in paraffin wax, stained with hematoxylin and eosin (HE) and then observed under the light microscope. Masson's trichrome staining was applied to detect collagen as an index for fibrosis in the lesions. Fibrosis was scored according to the following scoring: 1 = thickened perivenular collagen and a few thin collagen septa; 2 = thin septa with incomplete bridging between portal regions; 3 = thin septa and extensive bridging; 4 = thickened septa with complete bridging of portal regions and a nodular appearance *(Rivera et al., 2001)*. The severity of liver steatosis (the % of liver cells containing fat) was assessed as follow: 1+, ≤ 25% of cells; 2+, 26-50% of cells; 3+, 51-75%; 4+, > 75% of cells *(Nanji et al. 2001)*. Inflammation was scored as the number of inflammatory cells per square millimeter. At least three different sections were examined per sample of liver. The pathologist was unaware of the treatment protocol.

2.7. Immunohistochemical examination

For immunohistochemical examination, five μm thick sections were prepared from different animal groups. Immunohistochemistry was performed as previously

described. 26 Sections were deparaffinized, rehydrated and endogenous peroxidase activity was blocked with H_2O_2/ methanol. Sections were pre-treated in citrate buffer (pH 6.0) in microwave. Sections were incubated at room temperature with monoclonal anti iNOS and anti COX-2 antibodies for 40 min. Semiquantitative immunohistochemical assay of iNOS and COX-2 was performed. The number of positive expressive cells was calculated as percentage of total cells *(Ibrahim et al. 2009)*.

2.8. Statistical Analysis of the Data

Data are shown as mean ± S.E.M. The results were analyzed by one way analysis of variant (ANOVA) followed by *t*-test or turkey test with $P < 0.05$ selected as the criterion for statistical significance.

3. RESULTS

3.1. Effect of telmisartan on liver index:

Liver index was significantly increased in cholesterol-fed group compared with normal control group. In telmisartan-treated group, liver index was significantly decreased compared with cholesterol-fed group (Table 1).

3.2. Effect of telmisartan on serum ALTP:

There was significant elevation of serum ALT in cholesterol-fed group compared with normal control group. Both AST and ALT activities were decreased to normal values in telmisartan-treated group (Table 1).

3.3. Effect of telmisartan on liver triglycerides:

Liver contents of triglycerides were significantly increased in cholesterol-fed group compared with the control value. Administration of telmisartan resulted in significantl decease in liver triglycerides compared with either control or cholesterol-fed groups (33.6% and 59.2% below normal control and cholesterol-fed values, respectively) (Table 1).

3.4. Effect of telmisartan on liver content of MDA:

MDA in the livers of animals fed cholesterol diet was significantly higher than animals fed normal control diet. Treatment with telmisartan significantly decreased MDA to normal values (Table 1)

3.5. Effect of telmisartan on liver NO:

NO in the liver tissues of cholesterol-fed group was significantly higher than normal control group. Treatment with telmisartan significantly decreased NO (Table 1)

3.6. Effect of telmisartan on histopathological changes:

In the cholesterol-fed group, there was significant increase in microvesicular steatosis, inflammatory cells and fibrosis. The degree of hepatic steatosis, inflammation and fibrosis were significantly decreased by 72.7%, 87.1, 61.4, respectively, in telmisartan-treated group compared to cholesterol-fed group (Figure 1, 2 and Table 2).

3.7. Effect of telmisartan on liver tissue expression of iNOS and COX-2

Hepatic tissue expression of iNOS and COX-2 was increased in cholesterol-fed group compared with normal control group. Administration of telmisartan significantly decreased expression of iNOS. and COX-2 expression by 58.3 % and 72.4%, respectively, compared with cholesterol-fed group (Figure 3, 4 and Table 3).

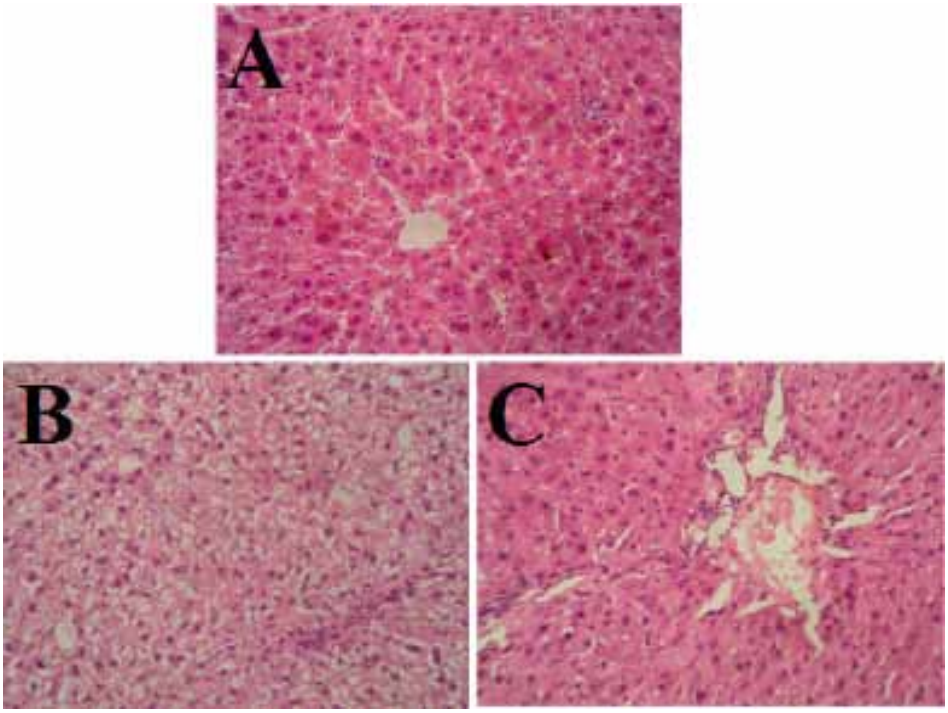

Figure 1: Effect of telmisartan on histopathological changes. Photomicrograph of the liver tissue (H&E 100x). (A) represents a section from control group with normal liver tissue, (B) a section from 2% cholesterol-fed group shows steatohepatitis after 8 weeks of 2% cholesterol diet, hepatocytes showing vacuolated cytoplasm and eccentric nuclei, notice inflammatory cell Infiltration, (C) a section from telmisartan-treated group shows liver tissue back to nearly normal appearance in telmisartan treatment rats.

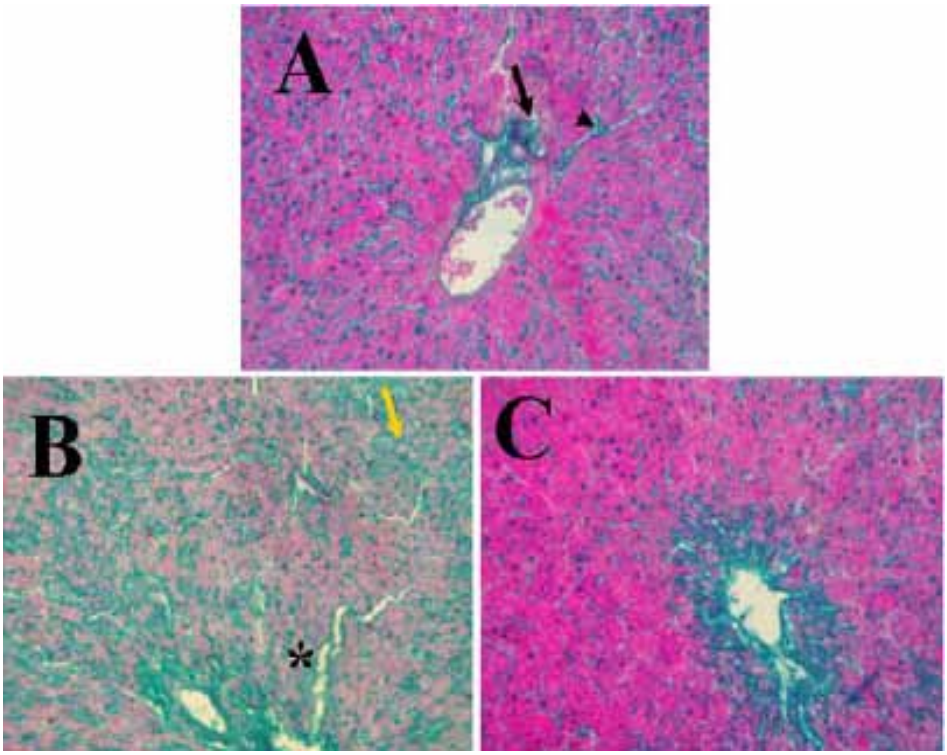

Figure 2: Effect of telmisartan on fibrotic changes.
Photomicrograph of Masson trichrome staining for collagen fibers in hepatic tissue .(A) represents a section from control group shows normal liver tissue with collagen fibers green in color around the portal tract (arrow) and around blood sinusoids (arrow head), (B) 2% cholesterol fed group shows increase expression of collagen after development of liver steatosis, especially around hepatocytes (green arrow) and around blood sinusoids (asterisk), (C) telmisartan-treated group shows collagen with almost normal pattern of distribution within the liver tissue.

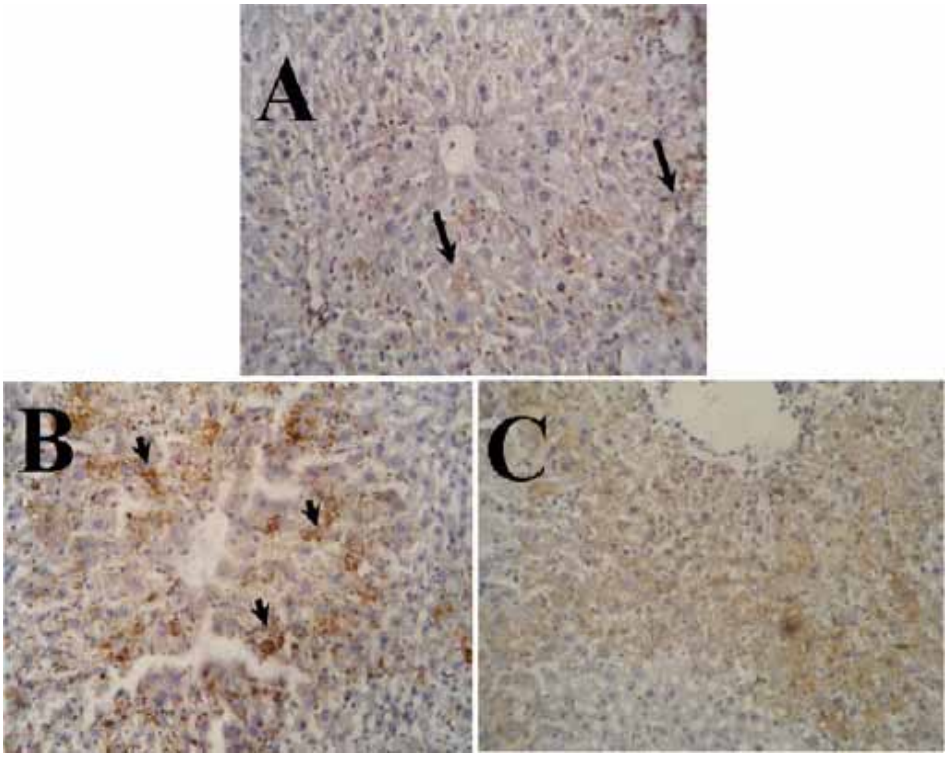

Figure 3: Effect of telmisartan on iNOS immunostaining expression in hepatic tissue. Photomicrograpgh of immunohistochemical staining for iNOS. It shows (A) control group: Normal liver tissue with low cytoplasmic expression of iNOS in the hepatocytes away from central venule (arrow), (B) 2% cholesterol-fed group: Enhanced expression expression of iNOS after development of liver steatosis, notice the positive signals in all hepatocytes (arrow head), (C) telmisartan-treated group: Down regulation of iNOS expression after telmesartan treatment.

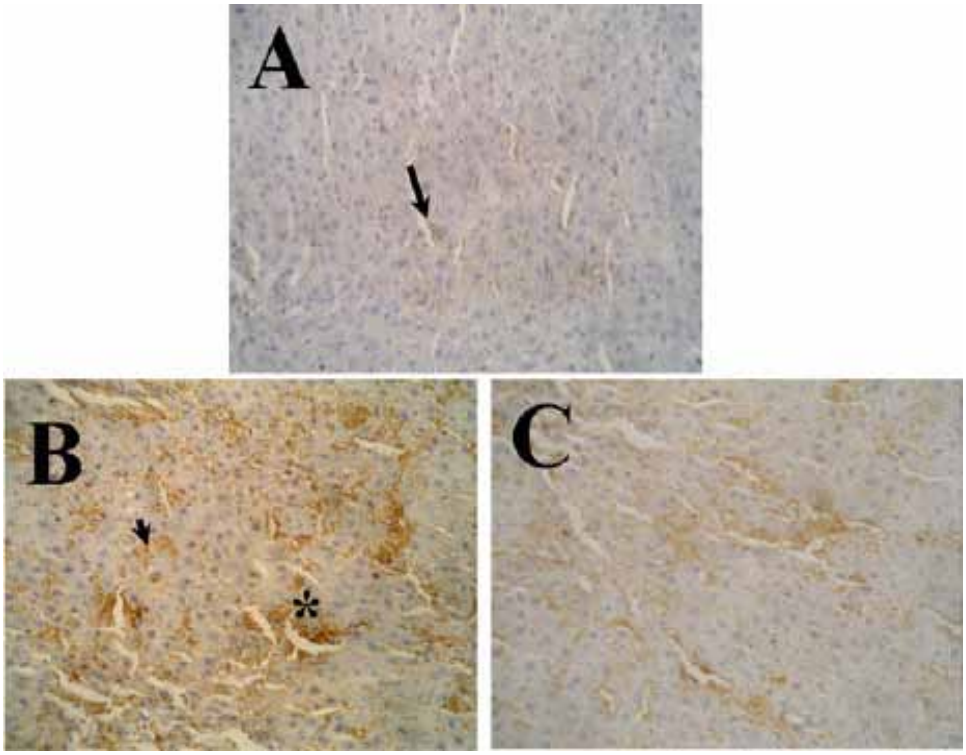

Figure 4: Effect of telmisartan on COX-2 immunostaining expression in hepatic tissue. Photomicrograph of immunohistochemical staining for COX-2. It shows (A) Control group: Normal liver tissue with low cytoplasmic expression of Cox-2 in the endothelial cells (arrow), (B) 2% cholesterol-fed group: Increased expression of cox-2 after development of liver steatosis, positive signals are extended to Kupffer cells (arrowhead) as well as endothelial cells (asterisk), (C) telmisartan-treated group: Down regulation of Cox-2 expression after Telmisartan treatment.

Table 1: Effect of telmisartan on liver index, serum ALT and liver tissue contents of triglycerides, MDA and NO.

	Liver index (g/g %)	Serum ALT (U/L)	Liver TG (mg/g tissue)	Liver MDA (µM/g tissue)	Liver NOx (µM/g)
Control	2.3 ± 0.1	43.2 ± 2.7	12.8 ± 2.1	7.6 ± 0.7	53. 7 ± 2.4
Choles-fed	2.7 ± 0.1[a]	254.8 ± 12[a]	20.8± 3.6[a]	16.5 ± [a]1.9	95.7 ± 6.1[a]
Telm-Tr.	2.1 ± 0.1[ab]	47.8[b] ± 4.7	8.50 ± [ab] (1.09)	10.34 ± [b] (1.04)	61.0 ± 2.4 [b]

Values are the mean ± SEM (number = 7 rats in each group). a $P < 0.05$) compared with control group, b $P < 0.05$ compared with cholesterol-fed group. Choles-fed, cholesterol-fed; Telm-Tr, telmisartan-treated; ALT, alanine transferase; TG, triglycerides; TG, triglycerides; MDA, malondialdehyde; NOx, total nitrite.

4. DISCUSSION

Excessive accumulation of triglycerides in hepatocytes, followed by lipid peroxidation and release of inflammatory mediators are potential players in the pathogenesis of liver injury in NAFLD *(Bellentani et al., 2000; Farrel et al. 2006)*.

The present study investigated the effect of telmisartan on the multiple steps involved in the pathogenesis of NAFLD. The study demonstrated that, 2%-cholesterol diet successfully induced steatohepatitis as evidenced physically by significant increase in liver weigh/body weight ratio (liver index) and confirmed histopathologically by increased deposition of fat droplets in hepatocytes, inflammatory cell infiltration and expression of collagen. Administration of telmisartan significantly attenuated both physical and histopathological evidences of steatohepatitis. A similar finding has been reported *(Fan et al. 2003; Benson et al. 2004; Schupp et al. 2005; Fujita et al. 2007)*.

ALT is a useful screening test for detecting liver injury *(Hennes et al. 1990)*. When the hepatocyte is injured, plasma membrane is disrupted and the leakage through extracellular fluid of the enzyme occurs where they can be detected at abnormal levels in the serum. Our results showed that high cholesterol diet elevated serum ALT level. Similar finding has been reported *(George et al. 2003)*. Rats receiving telmisartan showed almost normalization of serum ALT.

In a trial to explore the mechanism of the protective effect of telmisartan, we measured hepatic tissue contents of triglycerides, lipid peroxidation product (MDA), NO and the proinflammatory enzymes: iNOS and COX-2.

High cholesterol diet led to a significant increase in triglycerides in liver tissues compared with normal control group. It has been reported that the increase in hepatic trglycerides was due to failure of the liver to synthesize apolipoprotein required for packaging and exporting fat

from the liver thus accumulates triglycerides in the liver *(Thong-Ngam et al. 2007)*. In telmisartan-treated rats, there was a significant decrease in liver triglycerides. Similar findings had been reported *(Adaramoye et al. 2005; Yokozawa et al. 2006)*. Reduction of the hepatic triglyceride biosynthesis and redistribution of cholesterol among the lipoprotein molecules are the suggested mechanisms of the triglyceride lowering effect of telmisartan *(Fujita et al. 2007)*. Due to its structural resemblance to pioglitazone, telmisartan influences the expression of PPAR-γ target genes that are involved in the metabolism of carbohydrates and lipids as well as in inflammation *(Fujita et al. 2007)*. Previous studies showed that telmisartan decreased hepatic triglyceride accumulation and circulating lipid concentrations through its modulation of PPAR receptors. Hepatic PPAR-α activation may provide an explanation for telmisartan's antidyslipidemic actions observed in recent clinical trials. PPAR-γ activation by telmisartan exerts selective recruitment of nuclear factors resulting in insulin sensitization. Decreased availability of precursor substrates, including free fatty acids and glucose, diminishes hepatic synthesis and export of esterified lipids and caused a decrease in hepatic triglyceride accumulation and circulating lipid concentrations *(Clemenz et al., 2008; Rong et al., 2008)*. Moreover, Zanchi et al., reported that telmisartan prevented the glitazone-induced weight gain via decreasing the food consumption *(Zanchi et al. 2007)*. In our study, telmisartan decreased the body weight by 5.9% and 19.4% compared with normal control and cholesterol-fed groups, respectively (data not shown). The multiple effects of telmisartan that include insulin sensitization, antidyslipidemia and decreased body weight explains, at least partially, why telmisartan decreased liver tissue content of TG even below the values of normal control group.

Lipid peroxidation serves as a marker of cellular oxidative stress and had long been recognized as a major causative factor of oxidative damage in chronic diseases *(In Suke et al. 2007)*. Our results showed that telmisartan significantly reduced MDA in liver tissues. Previous studies suggested that telmisartan has a lipid peroxidation chain-breaking antioxidant effect *(Yamagishi and Takeuchi 2005)*.

NO is produced from L-arginine by one of three NO synthases (NOS) enzymes: Two constitutive; neuronal type (nNOS: Type 1 [NOS-1]) and or endothelial type (eNOS; type 3 [NOS-3]) and one inducible (iNOS: Type 2 [NOS-2]) *(Knowles and Moncada 1994)*. NO is a multifunctional molecule and its role in liver injury is an issue of debate *(Reiss and Komatsu 1998)*. In the present study, induction of NAFLD in rats was accompanied with significant elevation of NO content in liver tissues. Telmisartan significantly reduced NO content in liver tissue that might be due to its antioxidant effect *(Yamagishi et al. 2005)*. Emerging evidence shows that NO regulates fatty acids metabolism in mammals. At physiological levels, NO enhances lipolysis in and inhibits synthesis of fat in target tissues (e.g., liver and adipose tissue). However, at pathological levels, NO inhibits nearly all enzyme-catalyzed reactions through protein oxidation *(Jobgen et al. 2006)*.

Our results showed significant increase in the expression of iNOS and COX-2 in liver tissues of cholesterol-fed group. Previous studies reported that the presence of necroinflammation necroinflammation in fatty liver was accompanied by a significant increase in COX-2 and iNOS *(Tipoe et al. 2009; Wan et al. 2000)*. A recent study showed that overexpression of iNOS and COX-2 in fatty liver was due to increase in lipid peroxidation, interleukin-2 and tumor necrosis factor-α *(Yuan et al. 2006)*.

For the first time, the current study showed that telmisartan significantly attenuated overexpression of iNOS and COX-2 in hepatic tissue in NAFLD. In link with our finding, it has been reported that telmisartan reduced COX-2 expression in renal tissue in danorubicin-induced nephrotoxicity *(Arozal et al. 2010)* Similarly, in our previous study we have found that the protective effect of telmisartan in doxorubicin-induced nephrotoxicity and cardiotoxicity was accompanied with significant decrease in iNOS expression in both cardiac and renal tissues *(Ibrahim et al., 2009)*.

5. CONCLUSION

In addition to its effect on triglyceride and lipid peroxidation; telmisartan protected against the progression of NAFLD in rats via decreasing the expression of iNOS and COX-2 in hepatic tissue. These finding suggest that telmisartan might serve as a potential therapy for NAFLD. However, clinical studies are recommended to confirm its effect in human.

6 REFERENCES

Adaramoye, O. A., Nwaneri, V. O., Anyanwu, K. C., et al. 2005. Possible anti-atherogenic effect of kolaviron (a Garcinia kola seed extract) in hypercholesterolaemic rats. Clinical and Experimental Pharmacology & Physiology 32(1-2):40-46.

Anstee, Q. M., and Goldin, R. D. 2006. Mouse models in non-alcoholic fatty liver disease and steatohepatitis research. International Journal of Experimental Pathology 87(1):1-16.

Arozal, W., Watanabe, K., Veeraveedu, P. T., et al. 2011. Telmisartan prevents the progression of renal injury in daunorubicin rats with the alteration of angiotensin II and endothelin-1 receptor expression associated with its PPAR-ã agonist actions. Toxicology 279(1-3):91-99.

Bataller, R., Sancho Bru, P., Gines, P., et al. 2003. Activated human hepatic stellate cells express the renin-angiotensin system and synthesize angiotensin II. Gastroenterology 125(1):117-125.

Bellentani, S., Saccoccio, G., Masutti, F., et al. 2000. Prevalence of and risk factors for hepatic steatosis in Northern Italy. Annals of Internal Medicine 132(2):112-117.

Benson, S. C., Pershadsingh, H. A., Ho, C. I., et al. 2004. Identification of telmisartan as a unique angiotensin II receptor antagonist with selective PPARgamma-modulating activity. Hypertension 43(5):993-1002.

Chitturi, S., Abeygunasekera, S., Farrell, G. C., et al. 2002. NASH and insulin resistance: Insulin hypersecretion and specific association with the insulin resistance syndrome. Hepatology (Baltimore, Md.) 35(2):373-379.

Clemenz, M., Frost, N., Schupp, M., et al. 2008. Liver-specific peroxisome proliferator-activated receptor á target gene regulation by the angiotensin type 1 receptor blocker telmisartan. Diabetes 57(5):1405-1413.

Fan, J. G., Zhong, L., Xu, Z. J., et al. 2003. Effects of low-calorie diet on steatohepatitis in rats with obesity and hyperlipidemia. World Journal of Gastroenterology 9(9):2045-2049.

Farrell, G. C., and Larter, C. Z. 2006. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. Hepatology (Baltimore, Md.) 43(2 Suppl 1):S99-S112.

Folch, J., Lees, M., and Sloane Stanley, G. H. 1957. A simple method for the isolation and purification of total lipides from animal tissues. Journal of Biological Chemistry 226(1):497-509.

Fujita, K., Yoneda, M., Wada, K., et al. 2007. Telmisartan, an angiotensin II type 1 receptor blocker, controls progress of nonalcoholic steatohepatitis in rats. Digestive Diseases and Sciences 52(12):3455-3464.

George, J., Pera, N., Phung, N., et al. 2003. Lipid peroxidation, stellate cell activation and hepatic fibrogenesis in a rat model of chronic steatohepatitis. Journal of Hepatology 39(5):756-764.

Georgescu, E. F. 2008. Angiotensin receptor blockers in the treatment of NASH/NAFLD: Could they be a first-class option? Adv.Ther. 25(11):1141-1174.

Green, L. C., Wagner, D. A., Glogowski, J., et al. 1982. Analysis of nitrate, nitrite and [15N]nitrate in biological fluids. Analytical Biochemistry 126(1):131-138.

Hennes, H. M., Smith, D. S., Schneider, K., et al. 1990. Elevated liver transaminase levels in children with blunt abdominal trauma: A predictor of liver injury. Pediatrics 86(1):87-90.

Hsieh, P. S., Jin, J. S., Chiang, C. F., et al. 2009. COX-2-mediated inflammation in fat is crucial for obesity-linked insulin resistance and fatty liver. Obesity 17(6):1150-1157.

Ibrahim, M. A., Ashour, O. M., Ibrahim, Y. F., et al. 2009. Angiotensin-converting enzyme inhibition and angiotensin AT1-receptor antagonism equally improve doxorubicin-induced cardiotoxicity and nephrotoxicity. Pharmacological Research 60(5):373-381.

Jobgen, W. S., Fried, S. K., Fu, W. J., et al. 2006. Regulatory role for the arginine-nitric oxide pathway in metabolism of energy substrates. The Journal of Nutritional Biochemistry 17(9): 571-588.

Knowles, R. G., and Moncada, S. 1994. Nitric oxide synthases in mammals. The Biochemical Journal 298(Pt 2):249-258.

Koeppel, T. A., Mihaljevic, N., Kraenzlin, B., et al. 2007. Enhanced iNOS gene expression in the steatotic rat liver after normothermic ischemia. European Surgical Research 39(5):303-311.

Majano, P. L., Garcia Monzon, C., Lopez Cabrera, M., et al. 1998. Inducible nitric oxide synthase expression in chronic viral hepatitis. Evidence for a virus-induced gene upregulation. The Journal of Clinical Investigation 101(7):1343-1352.

McCullough, A. J. 2006. Pathophysiology of nonalcoholic steatohepatitis. Journal of Clinical Gastroenterology 40(3 Suppl 1):S17-29.

Miele, L., Gabrieli, M. L., Forgione, A., et al. 2006. Lo stress ossidativo nella sindrome metabolica e nella steatoepatite non alcolica. E possibile un ruolo delle vitamine nella pratica clinica? [Oxidative stress in metabolic syndrome and nonalcoholic steatohepatitis. Is it possible a role for vitamins in clinical practice?]. Recenti Progressi in Medicina 97(1):1-5.

Nabeshima, Y., Tazuma, S., Kanno, K., et al. 2009. Deletion of angiotensin II type I receptor reduces hepatic steatosis. Journal of Hepatology 50(6):1226-1235.

Nanji, A. A., Jokelainen, K., Tipoe, G. L., et al. 2001. Dietary saturated fatty acids reverse inflammatory and fibrotic changes in rat liver despite continued ethanol administration. The Journal of Pharmacology and Experimental Therapeutics 299(2):638-644.

Pata, C., Yazar, A., Altintas, E., et al. 2003. Serum levels of intercellular adhesion molecule-1 and nitric oxide in patients with chronic hepatitis related to hepatitis C virus: Connection fibrosis. Hepato-Gastroenterology 50(51):794-797.

Reiss, C. S., and Komatsu, T. 1998. Does nitric oxide play a critical role in viral infections? Journal of Virology 72(6):4547-4551.

Rivera, C. A., Bradford, B. U., Hunt, K. J., et al. 2001. Attenuation of CCl(4)-induced hepatic fibrosis by GdCl(3) treatment or dietary glycine. American Journal of Physiology. Gastrointestinal and Liver Physiology 281(1):G200-7.

Rong, X., Li, Y., Ebihara, K., et al. 2009. An adipose tissue-independent insulin-sensitizing action of telmisartan: A study in lipodystrophic mice. Journal of Pharmacology and Experimental Therapeutics 331(3):1096-1103.

Schupp, M., Clemenz, M., Gineste, R., et al. 2005. Molecular characterization of new selective peroxisome proliferator-activated receptor gamma modulators with angiotensin receptor blocking activity. Diabetes 54(12):3442-3452.

Son, I. S., Kim, J. H., Sohn, H. Y., et al. 2007. Antioxidative and hypolipidemic effects of diosgenin, a steroidal saponin of yam (Dioscorea spp.), on high-cholesterol fed rats. Bioscience, Biotechnology and Biochemistry 71(12):3063-3071.

Sugimoto, K., Qi, N. R., Kazdova, L., et al. 2006. Telmisartan but not valsartan increases caloric expenditure and protects against weight gain and hepatic steatosis. Hypertension 47(5):1003-1009.

Thong Ngam, D., Samuhasaneeto, S., Kulaputana, O., and Klaikeaw, N. 2007. N-acetylcysteine attenuates oxidative stress and liver pathology in rats with non-alcoholic steatohepatitis. World Journal of Gastroenterology 13(38):5127-5132.

Tipoe, G. L., Ho, C. T., Liong, E. C., et al. 2009. Voluntary oral feeding of rats not requiring a very high fat diet is a clinically relevant animal model of Non-Alcoholic Fatty Liver Disease (NAFLD). Histol.Histopathol. 24(9):1161-1169.

Wan, G., Ohnomi, S., and Kato, N. 2000. Increased hepatic activity of inducible nitric oxide synthase in rats fed on a high-fat diet. Bioscience, Biotechnology and Biochemistry 64(3):555-561.

Xu, P., Zhang, X. G., Li, Y. M., et al. 2006. Research on the protection effect of pioglitazone for Non-Alcoholic Fatty Liver Disease (NAFLD) in rats. Journal of Zhejiang University Sciences B 7(8):627-633.

Yamagishi, S., and Takeuchi, M. 2005. Telmisartan is a promising cardiometabolic sartan due to its unique PPAR-gamma-inducing property. Medical Hypotheses 64(3):476-478.

Yokozawa, T., Cho, E. J., Sasaki, S., et al. 2006. The protective role of Chinese prescription Kangen-karyu extract on diet-induced hypercholesterolemia in rats. Biological & Pharmaceutical Bulletin 29(4):760-765.

Yoshiji, H., Kuriyama, S., Yoshii, J., et al. 2001. Angiotensin-II type 1 receptor interaction is a major regulator for liver fibrosis development in rats. Hepatology (Baltimore, Md.) 34(4 Pt 1):745-750.

Yuan, G. J., Zhou, X. R., Gong, Z. J., et al. 2006. Expression and activity of inducible nitric oxide synthase and endothelial nitric oxide synthase correlate with ethanol-induced liver injury. World Journal of Gastroenterology 12(15):2375-2381.

Zanchi, A., Dulloo, A. G., Perregaux, C., et al. 2007. Telmisartan prevents the glitazone-induced weight gain without interfering with its insulin-sensitizing properties. American Journal of Physiology. Endocrinology and Metabolism 293(1):E91-E95.

Zheng, S., Hoos, L., Cook, J., et al. 2008. Ezetimibe improves high fat and cholesterol diet-induced non-alcoholic fatty liver disease in mice. European Journal of Pharmacology 584(1):118-124.