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The Protective Role of Enalapril on Kidney Functions in Fructose Induced Metabolic Syndrome in Rats *

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Metabolic syndrome is an entity characterized by insulin resistance, hyperinsulinemia, hypertension, dyslipidemia, and obesity. In experimental models of fructose-induced metabolic syndrome, hypertension, hypertriglyceridemia, and insulin resistance were seen in rats. Enalapril, an angiotensin-converting enzyme inhibitor, inhibits the enzyme dipeptidyl carboxypeptidase, which hydrolyes angiotensin-I. This study aims to determine the potential protective roles of enalapril on kidney functions, plasma lipid levels, and some intracellular pathway markers in an experimental model of metabolic syndrome induced by fructose in rats. 28 Wistar albino male rats (8 weeks old) were included in this study, and they were divided into four equal groups. Rats were sacrificed after eight weeks. Blood samples were taken for kidney and liver function tests and lipid levels; kidney tissue samples were collected for Western blot analysis. Transforming growth factor beta (TGF-β), tumor necrosis factor-alpha (TNF-α), nuclear factor kappa B (NF-κB), interleukin-6 (IL-6), and mothers against decapentaplegic-3 (SMAD-3) protein levels were quantified by Western blotting. Administration of enalapril on high fructose-fed rats showed significant improvement in serum glucose, total cholesterol (TC), low density lipoprotien (LDL), triglyceride, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatinine levels but did not affect high density lipoprotein (HDL) and blood urea nitrogen (BUN) parameters. Administration of enalapril on high fructose-fed rats caused a decrease in TGF-β, IL-6, and SMAD-3 protein levels but did not affect NF-κB, TNF-α. In conclusion, feeding with high fructose causes aggravative and destructive effects on kidney and liver function tests, but enalapril administration showed significant improvement in these parameters.

Key Words: *Fructose, enalapril, rat*

Sıçanlarda Fruktoz ile İndüklenmiş Metabolik Sendrom Modelinde Enalapril'in Böbrek Fonksiyonları Üzerindeki Koruyucu Etkisi

Metabolik sendrom, insülin direnci, hiperinsülinemi, dislipidemi, hipertansiyon ve obezite ile karakterize bir tablodur. Yüksek fruktoz tüketimiyle oluşturulmuş deneysel metabolik sendrom modellerinde ratlarda hipertansiyon, hipertrigliseridemi ve insülin direnci görülmüştür. Bir anjiotensin dönüştürücü enzim inhibitörü olan Enalapril, anjiyotensin-I'i hidrolize eden dönüştürücü enzim dipeptidil karboksipeptidazı inhibe eder. Bu çalışmanın amacı, ratlarda fruktoz ile oluşturulmuş deneysel metabolik sendrom modelinde, bir anjiotensin dönüştürücü enzim inhibitörü olan enalapril'in böbrek fonksiyonları, plasma lipid düzeyleri ve bazı hücre içi yolak belirteçleri üzerine potansiyel koruyucu etkilerini ortaya koymaktır. Çalışmaya 8 haftalık, 28 adet Wistar Albino ırkı erkek sıçanlar alındı. Bu sıçanlar rastgele 4 gruba ayrıldı ve 8 hafta sonunda sakrifiye edildi. Kan örnekleri; böbrek fonksiyon testleri, karaciğer fonksiyon testleri ve lipid düzeyleri için, böbrek doku örnekleri ise Western blot analizler için toplandı. Böbrek dokusunda transforme edici büyüme faktörü beta (TGF-β), tümör nekroze edici faktör alfa (TNF-α), nükleer faktör kappa B (NF-κB), interlökin-6 (IL-6), mothers against decapentaplegic-3 (SMAD-3) protein düzeyleri Western blot ile ölçüldü. Yüksek fruktozla beslenen sıçanlarda enalapril uygulaması serum glikoz, total kolesterol (TC), düşük yoğunluklu lipoprotein (LDL), trigliserid, aspartat aminotransferaz (AST), alanin aminotransferaz (ALT) ve kreatinin düzeyleri üzerinde olumlu etki gösterirken, HDL, BUN parametreleri üzerine anlamlı etki göstermemiştir. Enalapril uygulaması yüksek fruktoz ile beslenen sıçanlarda sadece fruktoz ile beslenen sıçanlara göre TGF-β, IL-6, SMAD-3 protein düzeylerini anlamlı şekilde düşürürken, NF-κB, TNF-α protein düzeylerinde anlamlı değişikliğe yol açmamıştır. Sonuç olarak yüksek fruktozla beslenme karaciğer ve böbrek fonksiyon testleri üzerinde kötüleştirici ve yıkıcı etkiler gösterirken enalapril uygulmasının bu etkileri iyileştirdiği görülmüştür.

Anahtar Kelimeler: *Fruktoz, enalapril, sıçan*

Introduction

Metabolic syndrome is an entity characterized by insulin resistance, hyperinsulinemia, hypertension, dyslipidemia, and central obesity (1). Metabolic syndrome (MS) has reached epidemic proportions world-wide including cardiovascular damage, nonalcoholic fatty liver disease, and increased incidence of chronic kidney disease (CKD) (2).

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Fructose is commonly found in added sugar in our food, in the form of high-fructose corn syrup and sucrose. Dietary fructose consumption has been predicted as an enviromental factor which causes obesity and anomalies caused by metabolic syndrome. In experimental models of fructose induced metabolic syndrome hypertension, hypertriglyceridemia, hyperinsulinemia and insulin resistance were seen in rats (3). In addition to insulin resistance and hypertriglyceridemia, hypertension is induced in rats fed with a high fructose diet, and this was described by Hwang et al. for the first time (4). It was found that plasma angiotensin-2 (AT-2) levels and systolic blood pressure increased significantly in rats fed the fructose diet. In these animal models, the use of angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor antagonists (ARB) has been shown to prevent the development of hypertension (5).

Nuclear factor kappa B (NF-κB), an accepted intracellular pathway, has been shown to be an obesity-dependent pathway in metabolic syndrome (6). Activation of the NF-κB pathway can lead to increased AT-2 and the release of TGF-β (transforming growth factor beta) and inflammatory cytokines (Interleukin-6 [IL-6], Tumor necrosis factor-alpha [TNF-α]) and increased intracellular pathways (Mothers against decapentaplegic-3 [SMAD-3]), thereby causing fibrosis (7-9). Activation of the TGF-β signaling pathway, which proceeds in a cascade, begins with the binding of the ligand to its receptors. There are three types of receptors on the cell membrane: TGF-β type I (TβRI), TGF-β type II (TβRII), and TGF-β type III (TβRIII). TβRI and TβRII receptors have serine/threonine kinase properties. Binding of TGFβ to the type II receptor causes the kinase activity of this receptor to emerge and phosphorylation of the glycine-serine region in the structure of the type I receptor. In this way, activated TβRI phosphorylates SMAD proteins in the cytosol. SMAD 2 and 3 TGF-β, called receptor regulatory SMAD; SMAD 1, 5 and 8 are involved in the signaling pathway of bone morphogenic protein and anti-mullerian hormone and are activated by phosphorylation by ligand/receptor complexes (10, 11). As a result of these pathways, TGF-β induces the formation of fibrosis in the lung, kidney and liver, and understanding TGF-β and its family pathways is important for clinical studies to be conducted for the regulation of the signaling activities of serine/threonine kinase receptors and SMADs (12).

Enalapril, an angiotensin-converting enzyme (ACE) inhibitor, reduces RAS activity in the body, and has both cardioprotective and renal protective effects (1). The protective effects of enalapril treatment on kidney are hemodynamic (ameliorating glomerular capillary and intraglomerular pressure) as well as a non-hemodynamic (reduction of mesangial proliferation, gene expression upregulating, inflammatory cell infiltration and ameliorating renal fibrotic changes) mechanism (13).

The aim of this study was to determine the potential protective roles on kidney functions, plasma lipid levels and some intracellular pathway markers of enalapril in an experimental model of metabolic syndrome induced by fructose in rats.

Materials and Methods

Research and Publication Ethics: All animal procedures were approved by the Animal Experimentation Ethics Committee of Fırat University (Decision No: 114-2015/59 Elazığ, Türkiye). All procedures involving rats were conducted in strict compliance with the relevant laws, the Animal Welfare Act, Public Health Services Policy, and guidelines established by the Institutional Animal Care and Use Committee of the Institute.

Animals and Study Design: Twenty-eight (8 week-old; 200-220 g body weight) male Wistar-Albino rats were used from the Fırat University Laboratory Animal Research Center (Elazığ, Türkiye). The rats were randomly divided according to body weight, which was similar, into four equal groups containing seven rats each. The rats were fed either i): a standard diet (rat chow) as control (Control), ii): a fructose diet (Fructose) (containing 60% fructose), iii): a standard diet with/ administrated Enapril into drinking water (10 mg/kg body weight per day) (Enapril), iv): a combination of fructose and enapril (Fructose+Enapril) for 8 weeks.

Laboratory Analyses: At the end of the experiment, all rats were killed by cervical dislocation. Blood samples and tissues from kidney were taken and processed for biochemical and Western blot examination.

Serum glucose, total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), LDL cholesterol (LDL-C), triglyceride (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN) and creatinine concentrations were measured by an automatic analyzer (Samsung LABGEO PT10, Samsung Electronics Co, Suwon, Korea). Repeatability and device/method precision of LABGEOPT10 was established according to the IVR-PT06 Guideline.

Western Blot Analyses: The protein levels of IL-6, NF-κB, TGF-β, TNF-α and SMAD-3 in kidney tissue were determined by Western blotting according to the previously described method (14).

To determine levels of the proteins in kidney Western blot analysis, the samples were homogenized in in 1:10 (w/v) in 10 mM Tris-HCl buffer at pH 7.4, comprising 0.1 mM NaCl, 0.1 mM phenylmethylsulfonyl fluoride, and 5 μM soybean (soluble powder; Sigma, St. Louis, MO) as trypsin inhibitor. The sample (20 μg of protein per lane) was mixed with sample buffer, boiled for 5 min, and separated by sodium dodecyl sulfate-polyacrylamide (12%) gel electrophoresis under denaturing conditions, and then electroblotted onto a nitrocellulose membrane (Schleicher and Schuell Inc., Keene, NH). Nitrocellulose blots were washed in PBS and blocked with 1% bovine serum albumin in PBS for 1 h prior to application of the primary antibodies (IL-6, NF-κB, TGF-β, TNF-α and SMAD-3 Abcam, Cambridge, UK). Primary antibody was previously diluted (1:1000 or

1:5000) in the same buffer containing 0.05% Tween-20. The nitrocellulose membrane was incubated overnight at 4°C with protein antibody. The blots were washed and incubated with horseradish peroxidase-conjugated goat antimouse IgG, the secondary antibody (Abcam). Specific binding was detected using diaminobenzidine and hydrogen peroxide as substrates. Protein load was controlled using a monoclonal mouse antibody against β-actin antibody (Sigma, St. Louis, MO). Protein levels were quantified densitometrically using an image analysis system (Image J; National Institute of Health, Bethesda, MD).

Statistical Analysis: Data were analyzed using the IBM SPSS version 22 software. The changes among groups were analyzed using one-way analysis of variance (ANOVA) followed by the Tukey post hoc test, and P<0.05 was considered statistically significant. The data were presented as mean ± standard error.

Results

The effects of enalapril administration on biochemical parameters in rats with metabolic syndrome induced by a diet with high levels of fructose are presented in Table 1. While enalapril administration in rats fed with high fructose affected serum glucose, total cholesterol, LDL, triglyceride, AST, ALT, and creatinine levels, it did not significantly affect HDL, BUN parameters.

The mean body weight of the animals at the end of the 8th week were as follows: control group 397.1±40.2 grams, fructose group 404.1±31.6 grams, enalapril group 382.7±28.3 grams, fructose+enalapril group was 370.7±37.9 grams (Figure 1). No statistical significans was found between body weight of the rats at the end of 8 weeks despite the mean weight of the fructose group was higher (P>0.05). Serum glucose levels in rats fed with high doses of fructose were found to increase by 30% compared to the control group (P<0.0001). Although enalapril administration caused a decrease in blood glucose levels in rats fed with high fructose, this decrease was not statistically significant (P>0.05). While the serum triglyceride level in rats fed with high doses of fructose increased by 38% compared to the control group (P<0.05), enalapril administration caused a decrease in blood triglyceride levels in rats fed with high fructose, and this decrease was statistically significant (P<0.05). While the serum creatinine level in rats fed with high doses of fructose increased by 130% compared to the control group (P<0.0001), enalapril administration caused a decrease in blood creatinine level in rats fed with high fructose and this decrease was statistically significant (P<0.0001). In addition, although creatinine in the group fed with high fructose and given enalapril was lower in than the control group, this decrease was not statistically significant (P>0.05). Although enalapril administration caused a decrease in blood total cholesterol, ALT, AST, BUN, and LDL levels in rats fed high fructose, this decrease was not statistically significant (P>0.05).

The studied IL-6, NF-κB, TGF-β, TNF-α, and SMAD-3 protein levels were higher in the fructose group than the control group. Enalapril administration caused a significant decrease in IL-6, TGF-β, SMAD-3 levels in the high fructose group compared to the group given only fructose (P<0.01). The group receiving high fructose displayed 97.3%, 43.0%, 115.9% in IL-6, TGF-β and SMAD-3 protein levels compared to control, respectively. Enalapril administration to fructose fed group resulted in a significant decrease in IL-6, TGF-β, and SMAD-3 protein levels (24.6%, 32.9%, 35.0%, respectively). There was no noteworthy difference observed when comparing TNF-α and NF-κB protein levels between groups (Figure 2; P>0.05).

Table 1. The effect of Enalapril on weight and biochemical parameters in rats fed with fructose

Items	Groups			
	Control	Fructose	Enalapril	Fructose + Enalapril
Glucose, mg/dL	94.71±6.80	123.40±12.82***	97.83±9.41$^{\#\#}$	107.80±7.92
Weight, gram	397.1±40.2	404.1±31.6	382.7±28.3	370.7±37.9
T-C, mg/mL	60.00±2.52	67.86±5.01**	59.71±4.89$^{\#\#}$	62.57±3.05
HDL, mg/dL	27.43±2.70	22.71±2.87	27.80±4.71	25.25±4.57
LDL, mg/dL	21.33±3.20	36.50±3.87***	22.33±4.03$^{\#\#\#}$	32.50±5.07**
TG, mg/dL	63.43±12.16	87.17±8.45**	56.86±11.77$^{\#\#\#}$	70.17±8.30$^{\#}$
AST, U/L	235.43±35.29	361.50±62.00**	212.00±37.84$^{\#\#\#}$	334.14±73.32*
ALT, U/L	56.40±5.81	68.67±5.43**	60.20±2.28$^{\#}$	65.67±3.27*
BUN, mg/dL	18.73±1.91	20.30±1.99	17.53±0.84	19.23±1.90
Creatinine, mg/dL	1.03±0.21	2.30±0.33***	1.14±0.13$^{\#\#\#}$	1.49±0.21$^{**\#\#\#}$

T-C: Total Cholesterol; HDL High-density lipoprotein; LDL: Low-density lipoprotein; TG: Triglycerides; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; BUN: Blood urea nitrogen.

The data presented as mean and standard error. Mean values within same row with are statistically different for * P<0.05, ** P<0.01, *** P<0.001 as compared to control group; # P<0.05, ## P<0.01, ### P<0.001 as compared to fructose group.

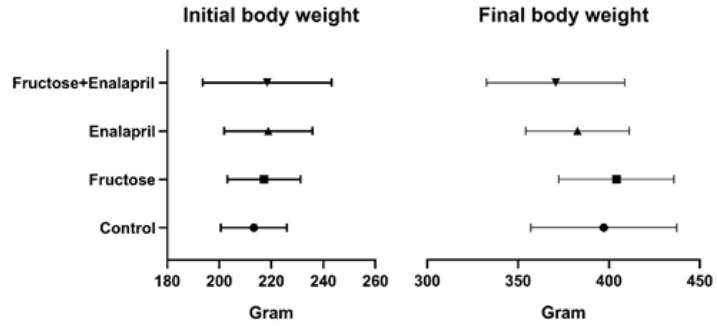

Figure 1. Initial and final weight of the rats at the end of eight weeks

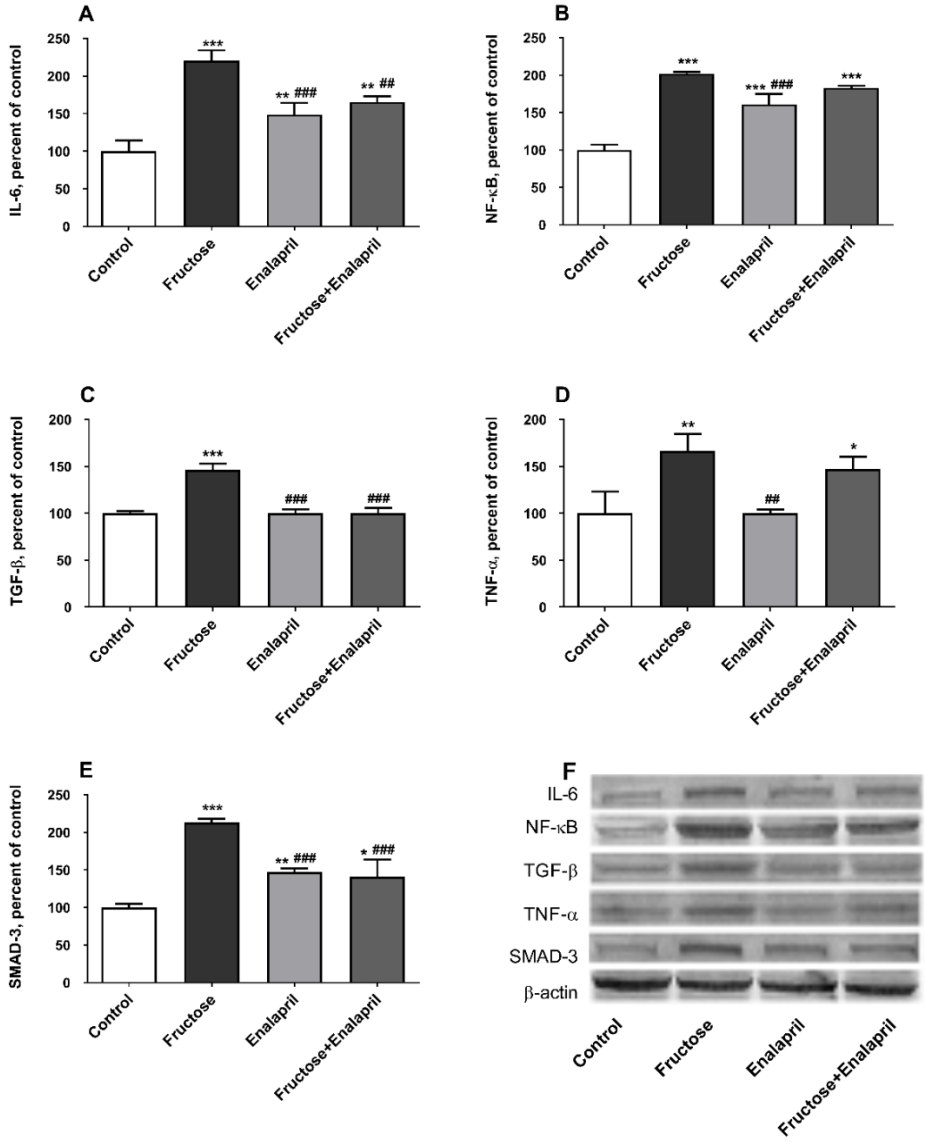

Figure 2. The effect of Enalapril on protein levels of IL-6 (Panel A), NF-κB (Panel B), TGF-β (Panel C), TNF-α (Panel D) and SMAD-3 (Panel E) in rats fed with fructose. The intensity of the bands was quantified by densitometric analysis and β-actin was included to ensure equal protein loading (Panel F). Data are expressed as percent of control value. Each bar represents the mean and standard error. Blots were repeated at least 3 times * P<0.05, ** P<0.01, *** P<0.001 as compared to control group; # P<0.05, ## P<0.01, ### P<0.001 as compared to fructose group

Discussion

In human studies and animal models, consumption of a high fructose diet seemed to be related to obesity, higher triglyceride levels, and insulin resistance (15). It has been shown that Wistar rats fed with 60% fructose diet for 6-8 weeks is sufficient to cause MS (16-18). In the light of these findings and studies, the model created with high fructose (60%) diet was deemed appropriate for the research.

At the end of 8 weeks, an increase in the body weights of the rats was observed in all four groups. However, there was no significant difference among the four groups regarding body weight. In one of the two different metabolic syndrome models, no statistical difference were detected between the groups in the weights of rats fed with 60% fructose after 12 weeks, while in another study, rats fed with 35% fructose were found to be heavier than the control group at the end of 16 weeks (2, 18). Our study and other studies show that the duration of fructose feeding, rather than the fructose content, causes a significant increase in body weight.

In a metabolic syndrome model performed in Wistar albino rats containing a 60% fructose diet, after eight weeks, an ARB application of telmisartan showed a significant decrease in AST, ALT, T-K, LDL-C, VLDL-C levels and a significant increase in HDL-C levels [15]. In our study, obtaining similar results at the end of 8 weeks supports that ACEi and ARBs show the same effects on liver enzymes and serum lipids, albeit with a different mechanism. However, the fact that HDL-C levels did not change with enalapril administration in our study may be attributed to the small sample size in the study.

In another metabolic syndrome model created with a high-fat diet in 20 weeks in rats, losartan administration, an ARB, showed an increase in T-C levels, which was expected to decrease, and no significant changes were observed in creatinine clearance (19). The fact that the metabolic syndrome model was made with a high-fat diet and losartan, an ARB, in the mentioned study may explain the differences with our study. In our study, it is a surprising result that serum creatinine values were found at such high levels in the fructose group. Despite the elevated creatinine levels, BUN did not increase in our study. This might be hypothesized that the creatinine elevation might be related to the decrease in tubular secretion rather than renal failure. It is also an interesting result that creatinine values were lower in the fructose + enalapril group than the fructose group in our study. The fact that there was no difference in creatinine levels in the group given enalapril alone compared to the control group suggests that the creatinine change associated with enalapril in rats may not be related to changes in intraglomerular pressure, so called RAS activation. The variable levels of creatinine, which increased in fructose, and decreased in the enalapril group, may be related to fructose effects on creatinine by mechanisms that we cannot explain.

In a study conducted in ZSF1 rats, which are genetically suitable for the metabolic syndrome model, enalapril applied at a dose of 60 mg/kg/day into the drinking water of rats for 32 weeks showed significant improvement in serum cholesterol and TG levels but no effect on serum glucose concentration (20). In our study, even enalapril administration given at a dose of 10 mg/kg/day for only eight weeks caused an improvement in serum glucose concentrations. A high dose of enalapril in the previously mentioned study may suppress rats' energy consumption via ACE inhibition (21). This suppression is thought to be related to the inhibition of adipocyte growth via AT-2 (22).

The studied IL-6, NF-κB, TGF-β, TNF-α, and SMAD-3 protein levels of rat kidneys were higher in the fructose given group than the control group. While enalapril administration caused a significant decrease in TGF-β, SMAD-3, IL-6 levels in the high fructose given group compared to the group given only fructose. However, enalapril administration caused a decrease in TNF-α and NF-κB levels; this decrease was not found statistically significant. In a metabolic syndrome model created with 60% fructose-fed rats for 12 weeks, significant increases in TNF-α, TGF-β, and NF-κB levels were observed in Western blot analyzes of liver tissues, and the levels of these inflammatory markers were found to be low in the group has given telmisartan, an ARB (18). In our study, although significant increases were observed in the levels of TGF-β, IL-6, SMAD-3, NF-κB, TNF-α in kidney tissues in the fructose-administered group, NF-κB, and TNF-α levels were not significantly decreased with enalapril administration. This can be attributed to the relatively short duration of our experiment. In a study conducted with Sprague-Dawley rats, the administration of benazepril, an ACEi, significantly decreased TNF-α, TGF-β, NF-κB, and SMAD- 3/4 expressions in the heart tissue of rats compared to the control group (23). The similar results obtained in our study can be explained by the fact that ACE inhibition stops the inflammatory process by inhibiting the intracellular pathways with AT-2 blockade (24).

Metabolic syndrome is a chronic disease with various components and many complications caused by these components. MS causes severe morbidity and mortality all over the world. It harms the national economy by causing injuries and loss of workforce and the care and treatment expenditures created by complications. The positive steps to be taken in the treatment of metabolic syndrome will contribute to society and the state economy. Therefore, metabolic syndrome and related complications can be prevented with treatment modalities targeting inflammatory markers involved in pathogenesis. Our study showed that enalapril, an ACEi, shows promising results in treating complications such as diabetes, kidney failure, and obesity caused by metabolic syndrome. However, further experimental and clinical studies are needed.

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