Effect of Bosentan and Losartan on Oxidative Stress and Cortisol level in Endothelin-1 and Angiotensin II Treated Rats

Almas M.R Mahmud1  Ismail Mustafa Maulood1*  Sarkawt Hamad Ameen Hamad2

1 Biology Department, College of Science, Salahaddin University-Erbil, Kurdistan, Iraq
2 Biology Department, Faculty of Science, Soran University, Soran, Iraq;
*Corresponding Author; E-mail: Ismail.Maulood@su.eedu.krd

ARTICLE INFO

Article History:
Received: 03 /01/2016
Accepted: 04 /04/2016
Published:10/10/2016

Keywords:
ET-1;
Ang II;
Cortisol;
Magnesium,
Oxidative Stress.

ABSTRACT

The exact mechanism by which Endothelin-1 (ET-1), Angiotensin II (Ang II), and their antagonists act in physiology are controversial subjects among researchers, therefore the present work aimed to investigate the effects of bosentan and losartan on oxidative stress and serum cortisol level in rats. This study includes two experiments. The first one included four groups: The first group treated with saline, the second group treated with ET-1, the third group treated with Bosentan + ET-1, and the fourth group treated with Losartan + ET-1. The second experiment also included four groups: The first group treated with saline, the second group treated with Ang II, the third group treated with Losartan + Ang II, and the fourth group treated with Bosentan + Ang II. The results demonstrate that, bolus infusion of losartan significantly decreased serum cortisol level versus ET-1. Bosentan significantly decreased cortisol level compared with Ang II infusion. Neither losartan nor Ang II changed serum cortisol significantly versus Ang II and saline groups. Furthermore, bosentan caused rising in malondialdehyde (MDA) concentration compared to ET-1 infusion, but losartan slightly decreased it. MDA in Ang II infusion dramatically became high in comparison with saline infusion, and both losartan. Serum glucose concentration clearly rose in losartan infusion, while bosentan did affect it significantly. Serum chloride in both bosentan and losartan significantly increased compared to ET-1. Both ET-1 and Ang II infusions for one hour led to increasing Mg++ concentration versus saline infusion. In conclusion, both ET-1 and Ang II antagonists reduced cortisol level, but they did not change lipid peroxidation marker as elevated by Ang II infusion. Interestingly, ET-1 and Ang II markedly could increase serum Mg++ levels, but their antagonists did not return it to the normal levels.

1. INTRODUCTION

Steroids among them glucocorticoids contribute to many physiological changes such as disease, trauma, and toxins which are well responded by corticoids (Fernandes et al. 2008). The cortisol hormone which secretes in adrenal cortex play important roles in developing oxidative stress and increases free radicals level (Mercanoglu et al. 2008). ET-1 and Ang II stimulate adrenocorticioid secretion (Vierhapper et al. 1995; Rabano et al. 2014 ). On the other hand, ET-1 is widely synthesized in the endothelialial cell, it is well known as a potent vasoconstrictor by constricting blood vessels strongly (Lin et al. 2014). ET-1 has two receptor sub types, ET-1A which performs cell proliferation, vasoconstriction and many other physiological actions, thus through ET-1B facilitates vasodilation (Maguire and Davenport 2014). For antagonizing ET-1 many synthetic antagonists have been produced to block single or dual ET-1 receptors, such as BQ123, BQ 788 and Bosentan, but only bosentan reaches final step in the pharmaceutical process, and used as a drug (Busnadiego et al. 2014). Bosentan is ET-1A/B
dual receptor antagonist which is used to treat pulmonary hypertension (Markova et al. 2013).

Angiotensin II secrets in many tissues, but it produces from a precursor of angiotensin I by action of angiotensin converting enzyme. It has a variety of physiological functions through binding with two different types of Ang II receptors (Barrett et al. 2010). Ang II subtype one receptor (AT$_{1}$) is responsible for enhancing cell proliferation, vasoconstriction, oxidative stress, production of other endocrine hormones, and increase Na$^{+}$ / K$^{+}$ pump activity (Lottermoser et al. 2003; Rabano et al. 2004; Seifi et al. 2014). While, stimulation of Ang II subtype two receptor (AT$_{2}$) leads to vasodilation, pro-apoptotic, anti-inflammation and anti-growth (Savoia et al. 2011). Thus, one of the most anti-hypertension drug is losartan, it could attenuate Ang II hypertensive effects through an antagonizing AT$_{1}$ receptor (Shiga et al. 2014). Because of the interactions between ET-1 and Ang II, and their antagonist actions on the body organs are the most controversial subjects among researchers, therefore the present work aimed to investigate the effects of bosentan and losartan on oxidative stress and serum cortisol levels in rats.

MATERIALS AND METHODS

ANIMALS

Albino rats (Rattus norvegicus) were bred in the animal house belongs to Biology department, College of Science, Salahaddin University-Erbil. Forty-two male rats weigh between 300 – 400 grams have been used (six rats were kept in each plastic cage), overnight fasted (8-12) hours. They were allowed to free access of tap water ad libitum.

ANIMAL ANESTHESIA AND CONTROL OF THE BODY TEMPERATURE

The animals were anesthetized by intraperitoneal injection of a mixture of Ketamine hydrochloride 80 mg / Kg (Trittau, Germany) and Xylazin 12 mg / Kg (Interchem, Holland). The depth of anesthesia was monitored by loosing reflexes. The anesthesia remained for 1.5 – 2 hours and a supplement dose was used if necessary. The maximum volume anesthesia solution was 1ml / Kg; Rat’s body temperature was controlled by placing on an electrical heating pad between 35 - 37 ºC.

PROCEDURE FOR TRACHEOSTOMY

Tracheostomy was performed to achieve good ventilation and avoiding bronchial obstruction. Sterile forceps were used to pull up neck skin in the middle, and about 2 -3 cm incision was made longitudinally; layers of the neck skin were removed and cleaned from the connective tissue, the platysma muscle was dissected to observe trachea. Fine iris scissors were used to slit incision of the ventral part of trachea. It must be managed as fast as possible to prevent rat from bleeding and hypoxia, then polythene tube 2 mm OD, 4-5 cm was inserted and was tightened by a ligature and for prevention of clot inside the tube it was wetted by heparinized normal saline 10 IU/ml. A syringe connected to a PE tube (ID 0.58 mm, OD 0.96 mm. England) had been used to control bronchial secretion, so any solutions present in the trachea removed by it.

CANNULATION OF FEMORAL VEIN FOR INTRAVENOUS INFUSION

Concave sterile scissors were used for making a small incision 2 - 3 cm on the right thigh. The outer layer of skin and matrix of collagen fibers had been removed, and cleaned carefully; a transient obstruction of blood back flow to the heart, then a 27 G ½ needle filled with heparinized normal saline 10 IU / ml was inserted into the vessel which was attached to the polythene tube (ID 0.58 mm, OD 0.96 mm. England), that was connected to infusion pump (Advance series 1200 infusion system, USA). Through a 10 ml sized syringe, immediately after insertion of cannula normal saline was infused (15 ml / h /Kg). The administration of normal saline continued for an hour and it called equilibration period, then the experimental design conducted. The wound was kept moisture through covering by a gauze with heparinized normal saline.

Design of the experiments
The experimental design includes two sub-experiments: Experiment A involved of four groups, each of six rats. Group 1, Saline: Animals were infused with normal saline (15 ml / h/ Kg) after equilibration period, Group 2, Endothelin-1. Animals were infused with ET-1 (520 ng / min / Kg), Group 3, Bosentan + Endothelin-1: The rats were bolus infused with bosentan 10 mg/ 0.5ml / kg then continuously animals infused ET-1 (520 ng / min / Kg), and Group 4, Losartan + Endothelin-1: The rats were bolus infused with losartan 10 mg/ 0.5 ml / kg then continuously animals infused ET-1 (520 ng / min/ Kg). Experiment B also consisted of four groups each of six rats, Group 1, saline and treated as in experiment A, Group 2, Angiotensin II (n = 6) animals were infused Ang II (320 ng / min / Kg), Group 3, Losartan + Angiotensin II. The rats were infused bolus infusion of losartan 10 mg/ 0.5 ml / kg then the animals continuously infused Ang II (320 ng / min / Kg); and Group 4, Bosentan + Angiotensin II: The rats were bolus infused with bosentan 10 mg/ 0.5 ml / kg then continuously animals infused with Ang II (320 ng / min / Kg).

**Collection of blood samples**

At the end of the sixty minutes of infusion, about 7 ml of blood were obtained through puncturing of heart, then standing in a clean clot activator gel test tube for 30 minutes. The blood centrifuged at 1000 g for 15 minutes (Centromix-Mod. S-549), then serum was transferred into four clean eppendorf tubes and they were stored at -80 °C until assay (Sanyo – Ultra – Low Temperature, Japan).

**Determination of serum cortisol**

The enzyme-linked immunosorbent assay (ELISA) method was used for determination of serum cortisol. The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody sites.

**Determination of serum MDA**

Thiobarbituric acid (TBA) was used to determine serum MDA, serum sample 150 µL and 1ml of 17.5% Trichloroacetic acid (TCA) were added into clean centrifuge test tube (Supe-Rior, Germany), TCA was allowed to deproteinize specimen. One ml of 0.66% TBA added into same tube and it was mixed well by vortex (Vortex-Genie, Model K-550-Ge, USA). The sample was allowed for boiling at 95 °C in the water bath (Memmert, Gmbh+Co.KG, Germany) for a period of 45 minutes and left at room temperature 25 °C to cool. One ml of 70% TCA added to precipitate the ruminant serum protein, centrifuged (Centromix-Mod. S-549) at 1000 g for 15 minutes. The pink colour indicate reaction occurred, then read at 532 nm by spectrophotometer (Apel-PD303-Japan).

**Determination of serum glucose**

Glucose was determined by enzymatic reactions using (Randox) kit. It produced a violet quinoneimine color absorbed by spectrophotometer (500 nm) which was proportioned to glucose concentration.

**Determination of serum chloride**

BioLabo (France) kit method was used to determine serum chloride by using spectrophotometer (Apel-PD303-Japab).

**Determination of serum magnesium**

Gindler, Heth and Khayam-Bashi method was used for determination of magnesium. The BioLabo (France) kit method was used to determine serum magnesium by using spectrophotometer (Apel-PD303-Japab).

**Data analysis**

The present results are expressed as means ± standard error (SE) and data analysis was performed using available statistical software (Statistical package for social science (SPSS) version 11.5). Statistical analysis was made using one-way analysis of variance (ANOVA). The comparisons among groups were done using Duncan post hoc test. P values <0.05 were considered as significant.
RESULTS

In the experiment A, bolus infusion of losartan significantly (P < 0.05) decreased serum cortisol level as compared with an ET-1 group, while bosentan non-significantly reduced cortisol level. There was no statistical difference between ET-1 and saline group (Figure 1, A). Beside that, in the experiment B, bosentan could significantly decrease cortisol level versus Ang II. Neither losartan nor Ang II changed serum cortisol significantly in comparison with Ang II and saline groups, respectively (Figure 1, B).

On the other hand, bosentan raised MDA concentration after ET-1 infusion (P < 0.05), but losartan slightly decreased it (Figure 2, A). MDA in Ang II infusion dramatically became high in comparison with saline infusion while, both losartan and bosentan non-significantly returned it to the base line levels (Figure 2, B).

Furthermore, serum glucose concentration significantly raised (P < 0.05) in losartan infusion and bosentan did that but not statistically (P > 0.05) (Table 1). While, serum chloride in both bosentan (111.2 ± 3.590) and losartan (114.1 ± 2.869) significantly increased it compared to ET-1 infusion (104.7 ± 2.990) (Table 1). Also, ET-1 infusion for one hour led to rising Mg²⁺ concentration in concomitant with saline infusion. (Table 1).

Table 1: Effect of ET-1 infusion on serum glucose, chloride and magnesium.

| Parameters | Glucose * (mg/dL) | Chloride * (mg/dL) | Magnesium* (mg/dL) |
|------------|-------------------|--------------------|--------------------|
| Saline     | 154.9 ± 15.00ab   | 97.41 ± 2.926a     | 0.154 ± 0.0180a    |
| ET-1       | 123.2 ± 8.087a    | 104.7 ± 2.990ab    | 0.242 ± 0.016b     |
| ET-1 + Bosentan | 185.3 ± 25.35bc | 111.2 ± 3.590ab    | 0.264 ± 0.047b     |
| ET-1 + Losartan | 212.9 ± 13.56c  | 114.1 ± 2.869ab    | 0.227 ± 0.014ab    |

The different letters mean significant and the same letters mean no significant differences. The data mean ± SEM * P < 0.05 considered a significant difference according to 1-way ANOVA followed by Duncan post hoc test.

Table 2 shows that, Mg²⁺ was increased in bosentan (0.308 ± 0.040) and it was decreased in losartan (0.261 ± 0.044) as compared with Ang II (0.296 ± 0.025), but only bosentan could increase chloride (P < 0.05).
There was no statistical change in glucose concentration in experiment B.

| Parameters | Glucose (mg/dL) | Chloride * (mg/dL) | Magnesium * (mg/dL) |
|------------|-----------------|--------------------|--------------------|
| Saline     | 154.9 ± 15.00a  | 97.41 ± 2.926a     | 0.154 ± 0.0180a    |
| Ang II     | 161.2 ± 29.25a  | 100.9 ± 0.777ab    | 0.296 ± 0.025b     |
| Ang II + Losartan | 143.8 ± 11.04a | 103.5 ± 3.120ab    | 0.308 ± 0.040b     |
| Ang II + Bosentan | 156.8 ± 20.64a | 106.0 ± 2.259b     | 0.261 ± 0.044b     |

The different letters mean significant and the same letters mean no significant differences. The data mean ± SEM * P < 0.05 considered a significant difference according to 1-way ANOVA followed by Duncan post hoc test.

**DISCUSSION**

The present study demonstrates that both ET-1_{A/B} dual (bosentan) and Ang II AT_{1} (losartan) receptors antagonists attenuate cortisol concentration, oxidative stress and magnesium regulation, also bolus infusion of them affected chloride ion and glucose homeostasis. Losartan bolus infusion could reduce serum cortisol significantly as compared with ET-1 infusion for one hour (Figure 1, A). Additionally, bosentan markedly decreased the elevated cortisol concentration caused by Ang II infusion (Figure 1, B). The exact mechanisms by which losartan and bosentan caused cortisol reduction is not fully understood yet. However, the current result agree with (Ansurudeen et al. 2014) demonstrated that Ang II causes an increase in cortisol levels. (Paramonova et al. 2010) reported that ET-1 causes proliferation of adrenocortical cells and promotes cortisol secretion. Furthermore, hypercortisolemia has related with elevated ET-1 levels (Lederbogen et al. 1999). The current data disagree with (le Mevel et al. 1999) demonstrated that intra-arterial injection of ET-1 did not markedly modify cortisol levels. Also, (Vierhapper et al. 1995) concluded that cortisol concentration was unchanged by infusion of ET-1. Experimental data for reducing cortisol levels by bolus infusion of the losartan are limited, while a study observed that Ang II modulated transcription regulatory genes of cortisol secretion and expression levels of unique enzymes of the glucocorticoid biosynthesis pathways (Rondon et al. 2014). Also, Ang II directly stimulates adrenal cortisol production through releasing nitric oxide (Gauthier et al. 2005).

Although, ET-1 slightly elevated lipid peroxidation through serum MDA level (Figure 2, A and B), while, Ang II increased it significantly as compared with the saline infusion. Interestingly, Bosentan infusion markedly increased MDA levels, whereas Losartan infusion did not change it significantly. It has been reported that the Ang II infusion (Bild et al. 2013; Dianat et al. 2014) and ET-1 infusion would increase oxidative stress and alters the balance between oxidant and antioxidant enzymes (Fiore et al. 2005). More recently (Lankhorst et al. 2014) demonstrated that activation of the ET-1 axis induces oxidative stress. However, at present there is no exact explanation for MDA elevation by bosentan administration, but a report indicated that, bosentan enhances hepatic toxicity and liver damage (Eriksson et al. 2011). Additionally, free radical production is strongly related with glucose metabolism, hence there is a report demonstrated that bosentan affects liver glycogen content and serum glucose (Said et al. 2005), however, the current data is in contrast to (Demirci et al. 2015) concluded that bosentan treatment
improves diabetes – induces liver damage via oxidative stress reduction. Besides that, ET-1 infu- 
sion did not change serum glucose (Table 1), and the result may be due to ET-1 induces 
glucose uptake (Wu-Wong et al. 2002) and bosentan decreases serum glucose (Said et al. 
2005), also, (Strawbridge and Elmendorf 2005) concluded that ET-1 induced insulin resistance 
and impaired glucose transport. While, Telmisartan can improve diabetic rats and 
inulin resistance (Younis et al. 2012), but losartan increases serum glucose. Bolus 
infusion of both losartan and bosentan slightly increased serum chloride as compared with ET-
1 infusion (Table 1). The possible mechanisms may be returned to inhibition of Na+ / K+ - 2Cl-
co-transporter and Cl-/ HCO3- exchanger (Dai and Zhang 2004). On the other hand, ET-1 
inhibits thick ascending limb chloride flux via ETA receptor-mediated NO release (Plato et al. 
2000), and ET-1 can potently stimulate chloride secretion (Kuhn et al. 1997).

Magnesium is an important physiological 
intracellular ion, which has roles in 
cardiovascular regulation, and it relaxes vessels 
and hence reduces blood pressure (Finckenberg 
et al. 2005; Rondon et al. 2014). The present 
study showed that both ET-1 and Ang II 
infusion for one hour significantly increased 
sodium homeostasis. However, there is no 
previous report indicating such relation 
between magnesium ions and ET-1 and Ang II 
actions. However, many studies have been 
reported that magnesium ameliorates Ang II 
and ET-1 production (Berthon et al. 2003; 
Berthon et al. 2002; Ozturk et al. 2012). 
Although, Ang II infusion induced hypermagnesiuria, and hence reduces magnesium ions (Wu and Sonnenberg 1995), 
both ET-1 and Ang II increased magnesium. It is 
believed that this due to glomerular filtration 
rat (GFR) reduction and no more magnesium 
could be excreted through the urine but the 
exact explanation for this result needs further 
confirmation. In conclusions, both losartan and 
bosentan could reduce cortisol levels, and 
bosentan rather than losartan can elevate 
odative stress. Also, Ang II infusion could 
raise MDA levels more than ET-1 infusion. 
Interestingly, both ET-1 and Ang II can 
markedly elevate serum magnesium levels.

REFERENCES

Ansurudeen, I., Kopf, P. G., Gauthier, K. M., Bornstein, 
S. R., Cowley, A. W., Jr., and Campbell, W. B. 
(2014). "Aldosterone secretagogues increase 
adrenal blood flow in male rats." 
Endocrinology, 155(1), 127-32.

Barrett, K. E., Barman, S. M., Boitano, S., and Brooks, 
H. L. (2010). " Ganong’s review of medical 
physiology " McGraw-Hill, United States of 
America., Twenty third edition.

Berthon, N., Laurant, P., Fellmann, D., and Berthelot, A. 
(2003). "Effect of magnesium on mRNA 
expression and production of endothelin-1 in 
DOCA-salt hypertensive rats." J Cardiovasc 
Pharmacol, 42(1), 24-31.

Berthon, N., Laurant, P., Hayoz, D., Fellmann, D., 
Brunner, H. R., and Berthelot, A. (2002). 
"Magnesium supplementation and 
deoxycorticosterone acetate–salt hypertension: 
effect on arterial mechanical properties and on 
activity of endothelin-1." Can J Physiol 
Pharmacol, 80(6), 553-61.

Bild, W., Hritcu, L., Stefanescu, C., and Ciobica, A. 
(2013). "Inhibition of central angiotensin II 
enhances memory function and reduces 
odative stress status in rat hippocampus." 
Prog Neuropsychopharmacol Biol Psychiatry, 
43, 79-88.

Busnadeiego, O., Loureiro-Alvarez, J., Sandoval, P., 
Lagares, D., Dotor, J., Perez-Lozano, M. L., 
Lopez-Armada, M. J., Lamas, S., Lopez-
Cabrera, M., and Rodriguez-Pascual, F. (2014). 
"A Pathogenic Role for Endothelin-1 in 
Peritoneal Dialysis-Associated Fibrosis." J Am Soc Nephrol, 10, 2013070799.

Dai, Y., and Zhang, J. (2004). "Chloride efflux is 
inolved in ET-1 and 5-HT-induced contraction 
in rabbit basilar artery." J Cardiovasc 
Pharmacol, 44(1), S125-8.

Demirci, E., Ferah, I., Gundogdu, C., Ozkanlar, S., 
Baygutalp, N. K., Bayir, Y., Calik, M., and 
Ayaz, G. (2015). "Endothelin receptor 
inhibition with bosentan delays onset of liver 
jury in streptozotocin-induced diabetic 
condition." Drug Res, 65(5), 272-80.

Dianat, M., Hamzavi, G. R., Badavi, M., and 
Samarbafzadeh, A. (2014). "Effects of losartan 
and vanillnic Acid co-administration on 
ischemia-reperfusion-induced oxidative stress 
in isolated rat heart." Iran Red Crescent Med J, 16(7), 5.

Eriksson, C., Gustavsson, A., Kronvall, T., and Tysk, C. 
(2011). "Hepatotoxicity by bosentan in a patient 
with portopulmonary hypertension: a case-
report and review of the literature." J Gastrointestin Liver Dis, 20(1), 77-80.

Fernandes, D., Duma, D., and Assreuy, J. (2008). 
"Steroids and nitric oxide in sepsis." Front 
Biosci, 13, 1698-710.

Finckenberg, P., Merasto, S., Louhelainen, M., Lindgren, 
L., Vapaatalo, H., Muller, D. N., Luft, F. C., 
and Mervaala, E. M. (2005). "Magnesium
supplementation prevents angiotensin II-induced myocardial damage and CTGF overexpression." J Hypertens, 23(2), 375-80.

Fiore, G., Florio, F., Micheli, L., Nencini, C., Rossi, M., Cerretani, D., Ambrosini, G., Giorgi, G., and Petragni, F. (2005). "Endothelin-1 triggers placental oxidative stress pathways: putative role in preeclampsia." J Clin Endocrinol Metab, 90(7), 4205-10.

Gauthier, K. M., Zhang, D. X., Edwards, E. M., Holmes, B., and Campbell, W. B. (2005). "Angiotensin II dilates bovine adrenal cortical arterioles: role of endothelial nitric oxide." Endocrinology, 146(8), 3319-24.

Kuhn, M., Fuchs, M., Beck, F. X., Martin, S., Jahe, J., Klempnauer, J., Kaever, V., Reckhemmer, G., and Forssmann, W. G. (1997). "Endothelin-1 potently stimulates chloride secretion and inhibits Na(+)-glucose absorption in human intestine in vitro." J Physiol, 499(Pt 2), 391-402.

Lankhorst, S., Kappers, M. H., van Esch, J. H., Danser, A. H., and van den Meiracker, A. H. (2014). "Hypertension during vascular endothelial growth factor inhibition: focus on nitric oxide, endothelin-1, and oxidative stress." Antioxid Redox Signal, 20(1), 135-45.

Le Mevel, J. C., Delarue, C., Mabin, D., and Vaudry, H. (1999). "Central and peripheral administration of endothelin-1 induces an increase in blood pressure in conscious trout." Am J Physiol, 276(4 Pt 2), R1010-7.

Lederbogen, F., Weber, B., Colla, M., Heuser, I., and Deuschle, M. (1999). "Endothelin-1 plasma concentrations in depressed patients and healthy controls." Neuropsychobiology, 40(3), 121-3.

Lin, Y. J., Kwok, C. F., Juan, C. C., Hsu, Y. P., Shih, K. C., Chen, C. C., and Ho, L. T. (2014). "Angiotensin II enhances endothelin-1-induced vasoconstriction through upregulating endothelin type A receptor." Biochem Biophys Res Commun, 451(2), 263-9.

Lottermoser, K., Unger, T., Gohlke, P., Vetter, H., and Dusing, R. (2003). "Differential effect of acute angiotensin II type 1 receptor blockade on the vascular and adrenal response to exogenous angiotensin II in humans." Am J Hypertens, 16(6), 445-52.

Maguire, J. J., and Davenport, A. P. (2014). "Endothelin@25 - new agonists, antagonists, inhibitors and emerging research frontiers: IUPHAR Review 12." Br J Pharmacol, 180(10), 12874.

Markova, S. M., De Marco, T., Bendjilali, N., Kobashigawa, A. E., Mefford, J., Sodhi, J., Le, H., Zhang, C., Halladay, J., Rettie, A. E., Khojasteh, C., McGlothlin, D., Wu, A. H., Hsueh, W. C., Witte, J. S., Schwartz, J. B., and Kroetz, D. L. (2013). "Association of CYP2C9*2 with bosentan-induced liver injury." Clin Pharmacol Ther, 94(6), 678-86.

Mercanoğlu, G., Safran, N., Uzun, H., and Eroğlu, L. (2008). "Chronic emotional stress exposure increases infarct size in rats: the role of oxidative and nitrosative damage in response to sympathetic hyperactivity." Methods Find Exp Clin Pharmacol, 30(10), 745-52.

Ozturk, C. F., Karakelleoglu, C., Orbak, Z., and Yildiz, L. (2012). "The effect of serum magnesium levels and serum endothelin-1 levels on bone mineral density in protein energy malnutrition." West Indian Med J, 61(3), 213-8.

Paramonova, I., Haase, M., Mulders-Opgenoorth, B., Ansurudeen-Rafi, I., Bornstein, S. R., Papewalis, C., Schinner, S., Schott, M., Scherbaum, W. A., and Willenberg, H. S. (2010). "The effects of the endothelium on adrenal steroidogenesis and growth are mainly mediated by proteins other than endothelin-1." Horm Metab Res, 42(12), 840-5.

Plato, C. F., Pollock, D. M., and Garvin, J. L. (2000). "Endothelin inhibits thick ascending limb chloride flux via ET(B) receptor-mediated NO release." Am J Physiol Renal Physiol, 279(2), F326-33.

Raban, M., Pena, A., Brizuela, L., Macarulla, J. M., Gomez-Munoz, A., and Trueba, M. (2004). "Angiotensin II-stimulated cortisol secretion is mediated by phospholipase D." Mol Cell Endocrinol, 222(1-2), 9-20.

Rondon, L. J., Marccano, E., Rodriguez, F., and del Castillo, J. R. (2014). "Blood pressure, magnesium and other mineral balance in two rat models of salt-sensitive, induced hypertension: effects of a non-peptide angiotensin II receptor type 1 antagonist." Magnes Res, 27(3), 113-30.

Said, S. A., Ammar el, S. M., and Suddek, G. M. (2005). "Effect of bosentan (ETA/ETB receptor antagonist) on metabolic changes during stress and diabetes." Pharmacol Res, 51(2), 107-15.

Savoia, C., D’Agostino, M., Lauri, F., and Volpe, M. (2011). "Angiotensin type 2 receptor in hypertensive cardiovascular disease." Curr Opin Nephrol Hypertens, 20(2), 125-32.

Seifi, B., Kadkhodaei, M., Bakhshi, E., Ranjbaran, M., Zahmatakes, M., Sedaghat, Z., Aghhari, P., and Esmaeili, P. (2014). "Angiotensin II in paraventricular nucleus contributes to sympathoexcitation in renal ischemia-reperfusion injury by AT1 receptor and oxidative stress." J Surg Res, 5(14), 00620-9.

Shiga, Y., Miura, S. I., Norimatsu, K., Hitaka, Y., Nagata, I., Koyoshi, R., Morii, J., Kuwano, T., Uehara, Y., Inoue, A., Shirotani, T., Fujisawa, K., Matsunaga, E., and Saku, K. (2014). "Efficacy and safety of combination therapy of high-dose losartan and hydrochlorothiazide in patients with hypertension." J Renin Angiotensin Aldosterone Syst, 20, 1470320314529358.

Strawbridge, A. B., and Eldendorf, J. S. (2005). "Phosphatidylinositol 4,5-bisphosphate reverses endothelin-1-induced insulin resistance via an
actin-dependent mechanism." Diabetes, 54(6), 1698-705.

Vierhapper, H., Nowotny, P., and Waldhausl, W. (1995). "Effect of endothelin-1 in man: impact on basal and adrenocorticotropic-stimulated concentrations of aldosterone." J Clin Endocrinol Metab, 80(3), 948-51.

Wu, X., and Sonnenberg, H. (1995). "Effect of renal perfusion pressure on excretion of calcium, magnesium, and phosphate in the rat." Clin Exp Hypertens, 17(8), 1269-85.

Wu-Wong, J. R., Berg, C. E., and Dayton, B. D. (2002). "Endothelin-stimulated glucose uptake: effects of intracellular Ca(2+), cAMP and glucosamine." Clin Sci, 103(48), 418S-423S.

Younis, F., Oron, Y., Limor, R., Stern, N., and Rosenthal, T. (2012). "Prophylactic treatment with telmisartan induces tissue-specific gene modulation favoring normal glucose homeostasis in Cohen-Rosenthal diabetic hypertensive rats." Metabolism, 61(2), 164-74.