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PHARMACOLOGY

PHARMACODYNAMICAL EVALUATION OF MATRIX TYPE TRANSDERMAL THERAPEUTIC SYSTEMS CONTAINING CAPTOPRIL

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Abstract: The objective of this study was to evaluate pharmacodynamical properties of transdermal therapeutic systems (TTS) containing captopril together with synthetic and pH independent polymers, Eudragit RL 100 and RS 100. Optimum formulation was chosen according to the results of our previous study regarding *in vitro* dissolution and *ex vivo* diffusion rate studies through excised human skin by using Franz Diffusion Cell. Control group, hypertension group (HT) and TTS containing captopril hypertension group (HT-CAP) were assessed for the pharmacodynamic activity of the study. Pharmacodynamic activity of transdermal patches containing captopril was evaluated in rats by the measurement of systolic blood pressure for 24 h with the use of the tail cuff method**.** Blood pressure**,** heart rate, body and heart weight, heart and body weight ratio were determined. Lactate dehydrogenase (LDH), creatinine phosphokinase (CPK), glutathione (GSH), malondialdehyde (MDA), myeloperoxidase (MPO) and Na^+, K^+-ATPase were measured in the serum of rats. Histopathological evaluation of the heart tissue was conducted in order to determine any tissue damage. Blood pressure values of the TTS containing captopril hypertension group were decreased significantly and became almost similar with the blood pressure values of the control group. These results indicated that matrix type transdermal patches prepared with Eudragit RL 100 and RS 100 polymers containing captopril can be considered as transdermal therapeutic systems for chronical treatment of hypertension and congestive heart failure. However, further *in vivo* pharmacokinetic studies should be performed in order to determine the blood level of the drug.

Keywords: captopril, transdermal therapeutic system, blood pressure, heart rate, tail cuff

Captopril, an orally effective angiotensin I converting enzyme inhibitor, is used in chronical treatment of hypertension and congestive heart failure as first agent, because of the absence of side effects in the majority of patients. It has a relatively short elimination half life in plasma (2 h) and low oral bioavailability (60-75%) (1). For these reasons, by applying this drug as a transdermal therapeutic system, dosing time intervals will be expanded so that patient compliance will be arised and side effects will be minimized. Appropriate physicochemical properties for potential transdermal delivery are: low molecular weight, [217.29 Da], low polarity, low melting point (105-108^{o}C) and low daily therapeutic dose (50-75 mg) (2). Captopril possesses all these properties except of low polarity.

Transdermal patches are flexible pharmaceutical preparations of varying sizes, containing one or more active ingredients. They are designed to support the passage of drug substances from the surface of the skin, through its various layers and into the systemic circulation (3). They have been developed with the objective of overcoming the hepatogastrointestinal first pass metabolism, duplicating the benefits of intravenous drug infusion and achieving systemic rate controlled drug delivery (4). Drug levels can be maintained in the systemic circulation, within the therapeutic window for prolonged periods. Thus, duration of drug action following a single administration of the drug can be extended and the frequency of dosing is reduced. Patient compliance and acceptability of the drug therapy can be improved. Another advantage is that the drug therapy can be terminated by simply removing the patch from the skin. Also, in the cases where oral delivery is contraindicated or when the drug is poorly

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absorbed from the gastrointestinal tract, transdermal route of drug administration may be used (5).

The aim of this study was to evaluate the pharmacodynamical properties of transdermal therapeutic systems (TTS) containing captopril. TTS formulations were developed by using synthetic and pH independent polymers, Eudragit RL 100 and RS 100. Optimum formulation was chosen according to the results of our previous study regarding *in vitro* dissolution and *ex vivo* diffusion rate studies through excised human skin by using Franz Diffusion Cell. Matrix type of transdermal formulations containing captopril were evaluated by pharmacodynamic studies in rats based on the measurement of changes in arterial blood pressure. Blood pressure**,** heart rate, body and heart weight, heart and body weight ratio were determined. Lactate dehydrogenase (LDH), creatinine phosphokinase (CPK), glutathione (GSH), malondialdehyde (MDA), myeloperoxidase (MPO) and Na^+, K^+-ATPase were measured in serum of the rats. Histopathological evaluation of the heart tissue was conducted in order to determine any tissue damage.

MATERIALS

Captopril (Mustafa Nevzat Pharmaceuticals, Turkey), Eudragit RL 100 and Eudragit RS 100 (Evonik Röhm Pharma, Germany), polyethylene glycol 400 (Merck, Germany), acetone (Merck, Germany), polyisobutylene (BASF), hexane (Merck, Germany), N^{ω}-nitro-L-arginine methyl ester (L-NAME) (Sigma-Aldrich, USA) and other materials were all of analytical grade.

METHODS

Preparation of matrix type transdermal therapeutic systems containing captopril

Plasticizer 0.4 g PEG 400, polymers 1.3 g Eudragit RL 100 and 0.7 g Eudragit RS 100 were dissolved in 7 mL of acetone, then 0.4 g captopril was dissolved in 5 mL of acetone. Captopril solution was added to polymer solution and stirred by using a magnetic stirrer (RO 5 Power IKA Labortechnik). A glass mould of 5 cm diameter was coated with aluminium foil as impermeable backing layer. The solution prepared was poured into this mould and was allowed to dry at room temperature. Acetone was used in the minimum amount enough to solve the polymer and the drug. The formulations containing polyisobutylene (PIB) adhesive layer were prepared by adding the solution of 0.2 g PIB in 10 mL of hexane onto the dry transdermal film prepared and was allowed to dry at room temperature (6).

Animals

All experimental protocols were approved by the Marmara University (MU) Animal Care and Use Committee. Approval date and number: 26.03.2010-14.2010.mar. Male or female Wistar albino rats (250-300 g), supplied by the MU Animal Center (DEHAMER), were kept at a constant temperature (22 ± 1°C) with 12 h light and dark cycles. All animals in the study were nourished with pellet diet and water *ad libitum*.

Experimental design

The rats (n = 24) were randomly divided into three groups as: control, hypertension group (HT) and TTS containing captopril hypertension group (HT-CAP). In the HT group, Nω-nitro-L-arginine methyl ester (L-NAME) (40 mg/kg) was administered orally for a total of 4 weeks and hypertension was maintained. In the HT-CAP group, following a 4 week L-NAME (40 mg/kg) administration orally and confirming that they became hypertensive, hairs of the abdominal region of the rats were removed with a clipper and then, the animals were administered TTS containing captopril (5 mg/cm^2) upon abdominal region. Body weight, systolic blood pressure (BP), heart rate (HR) measurements were recorded at the beginning of the study to obtain the basal levels. Body weight, systolic blood pressure (BP), heart rate (HR), heart weight, heart/body weight ratio measurements were done at the end of the study to compare with basal levels. Then, the animals were decapitated, trunk blood was collected and immediately centrifuged at 3000 × g for 10 min to assay lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) levels in the plasma. In order to evaluate the presence of oxidative tissue injury, cardiac samples were taken and stored at -80°C for the determination of MDA and GSH levels, MPO and Na^+,K^+-ATPase activities. Additional tissue samples were taken for histological analyses.

Measurement of blood pressure

Indirect blood pressure measurement was made by the tail cuff method (Rhma-Labor Technique, 2 channel blood pressure monitor 8002). Initially, the rats were placed for 10 min in a chamber heated to 35°C. Then, the rats were placed in individual plastic restrainers and a cuff with a pneumatic pulse sensor was wrapped around their tails. Blood pressure and heart rate values recorded during each measurement period were averaged from at least three consecutive readings on that occasion obtained from each rat (7, 8).

Plasma assays

Plasma levels of LDH (9) and CPK (10) were determined spectrophotometrically using an automated analyzer (Bayer Opera biochemical analyzer, Germany).

Measurement of tissue malondialdehyde and glutathione levels

Heart samples were homogenized with icecold 150 mM KCl for the determination of MDA and GSH levels. MDA measurements were performed according to Beuge method (11). The MDA levels were assayed for products of lipid peroxidation by monitoring thiobarbituric acid reactive substance formation. Lipid peroxidation was expressed in terms of MDA equivalents using an extinction coefficient of $1.56 \times 105\ M^{-1}\ cm^{-1}$ and results are expressed as nmol MDA/g tissue. GSH measurements were performed using Beutler method, a modification of the Ellman procedure (12). After centrifugation at 3000 rpm for 10 min, 0.5 mL of supernatant was added to 2 mL of 0.3 mol/L $Na_2HPO_4 \times 2H_2O$ solution. A 0.2 mL solution of dithiobisnitrobenzoate (0.4 mg/mL 1% sodium citrate) was added and the absorbance at 412 nm was measured immediately after mixing. GSH levels were calculated using an extinction coefficient of $1.36 \times 104\ M^{-1}\ cm^{-1}$. Results are expressed in µmol GSH/g tissue.

Myeloperoxidase activity

MPO activity was measured in tissues in a procedure similar to that documented by Hillegas et al. (13). Tissue samples were homogenized in 50 mM potassium phosphate buffer (PB, pH 6.0) and centrifuged at 41,400 × g (10 min); pellets were suspended in 50 mM PB containing 0.5% hexadecyltrimethylammonium bromide (HETAB). After three freeze and thaw cycles, with sonication between cycles, the samples were centrifuged at 41,400 × g for 10 min. Aliquots (0.3 mL) were added to 2.3 mL of reaction mixture containing 50 mM PB, o-dianisidine, and 20 mM H_2O_2 solution. One unit of enzyme activity was defined as the amount of the MPO present that caused a change in absorbance measured at 460 nm for 3 min. MPO activity was expressed as U/g tissue.

Measurement of Na^+, K^+-ATPase activities in the cardiac tissue

Measurement of Na^+, K^+-ATPase activity is based on the measurement of inorganic phosphate released by ATP hydrolysis during incubation of homogenates with an appropriate medium. Since the activity of Na^+, K^+-ATPase, a membrane-bound enzyme required for cellular transport, is very sensitive to free radical reactions and lipid peroxidation, reductions in this activity can indicate membrane damage indirectly. The total ATPase activity was determined in the presence of 100 mM NaCl, 5 mM KCl, 6 mM $MgCl_2$, 0.1 mM EDTA, 30 mM Tris HCl (pH 7.4), while the Mg^{2+}-ATPase activity was determined in the presence of 1 mM ouabain. The difference between the total and the Mg^{2+}-ATPase activities was taken as a measure of the Na^+, K^+-ATPase activity (14, 15). The reaction was initiated with the addition of the homogenate (0.1 mL) and a 5-min pre-incubation period at 37°C was allowed. Following the addition of 3 mM Na_2ATP and a 10 min re-incubation period, the reaction was terminated by the addition of icecold 6% perchloric acid. The mixture was then centrifuged at 3500 × g and the supernatant fraction was determined by the method of Fiske and Subarrow (16). The specific activity of the enzyme was expressed as µmol mg^{-1} protein h^{-1}. The protein concentration of the supernatant was measured by the Lowry et al. (17) method.

Histopathological analysis

For light microscopic investigations, cardiac tissue specimens were fixed in 10% formaldehyde, dehydrated in alcohol series, cleared in toluene and embedded in paraffin. Paraffin sections (5 µm) were stained with hematoxylin and eosin (H&E) and examined under a photomicroscope (Olympus BH 2, Tokyo, Japan). All tissue sections were examined microscopically for the characterization of histopathological changes by an experienced histologist in blind fashion.

Statistical analysis

The results were expressed as the means ± standard deviations. Unpaired, two-tailed *t*-tests were performed at each time point. The threshold for statistical significance was at $p < 0.05$.

RESULTS AND DISCUSSION

Preparation and optimization of matrix type transdermal therapeutic systems containing captopril

TTS formulations were developed by using synthetic and pH independent polymers, Eudragit RL 100 and RS 100. All the formulations that are mentioned in our previous study (6) were evaluated for their macroscopic properties (general appearance, transparency, color, softness, homogeneity

and flexibility), thickness and captopril content. Optimum formulation was chosen according to the results of our previous study regarding *in vitro* dissolution and *ex vivo* diffusion rate studies through excised human skin by using Franz Diffusion Cell.

Measurement of systolic blood pressure (BP), heart rate (HR), body weight, heart weight and heart/body weight ratio

According to the systolic blood pressure results, blood pressure values of t_2 (24 h after the time period of t_1 values) were increased significantly compared with t_1 values [following a 4 week L-NAME (40 mg/kg) administration orally and confirming that rats became hypertensive] while HT-CAP group were decreased significantly compared with HT group t_2 values and became almost similar with control group ($p < 0.05$). This result showed that our newly developed matrix type transdermal therapeutic system containing captopril was effective for 24 h for decreasing blood pressure. When the heart rates of t_1 and t_2 were compared, there was no significant dif-

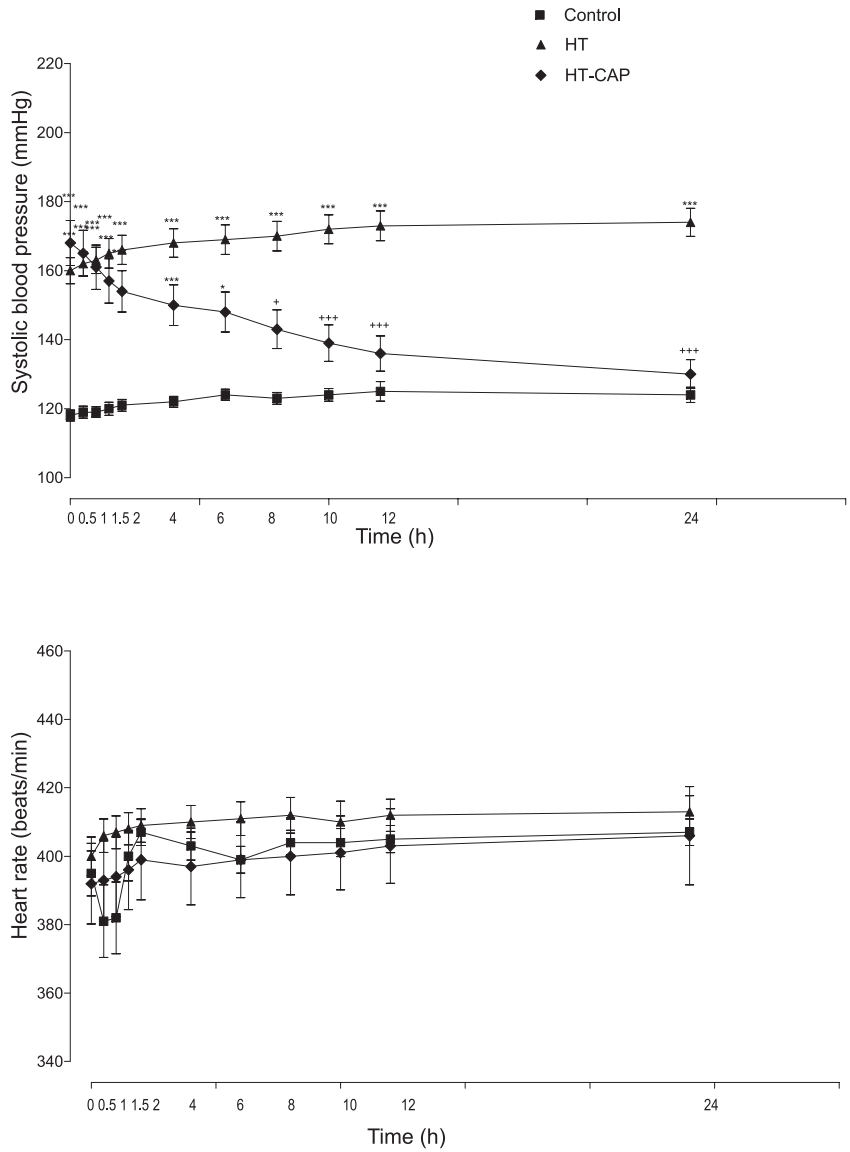

Figure 1. Blood pressure and heart rate values of the control, HT and HT-CAP groups in which hypertension was induced experimentally in rats by applying L-NAME. * $p < 0.05$, *** $p < 0.001$ according to control group, $^{+}$ $p < 0.05$, $^{+++}$ $p < 0.001$ comparisons according to HT group

Table 1. Blood pressure, heart rate, body weight, heart weight, heart/body weight ratio values of the control, HT and HT-captopril groups in which hypertension was induced experimentally in rats by applying L-NAME.

	Control	HT	HT-CAP
Blood pressure (mmHg)			
t_1	119 ± 1.5	159 ± 3.8 $^{\&\&\&}$	168 ± 6.6 $^{\&\&\&}$
t_2	124 ± 2.2	174 ± 4.1 $^{\&\&\&}$	131 ± 4.2 $^{+++}$
Heart rate (beat/min)			
t_1	395 ± 6.6	400 ± 5,6	392 ± 11.8
t_2	407 ± 3.8	413 ± 4.7	406 ± 14.4
Body weight (g)			
t_3	212 ± 8.3	227 ± 12.7	212 ± 10.3
t_4	240 ± 8.2	250 ± 14.5	242 ± 10.1
Heart weight (g)	473 ± 35	721 ± 50 $^{\&\&}$	534 ± 47 $^{+}$
Heart/body weight ratio (mg/g)	1.96 ± 0.08	2.78 ± 0.20 $^{\&\&}$	2.01 ± 0.06 $^{++}$

t_1: following a 4 week N^{ω}-nitro-L-arginine methyl ester (L-NAME) (40 mg/kg) orally and confirming that they became hypertensive; t_2: 24 h after the time period of t_1; t_3: before L-NAME administration; t_4: 24 h after the time period of t_1: $^{+}$ $p < 0.05$, $^{++}$ $p < 0.01$,$^{+++}$ $p < 0.001$ according to HT group; $^{\&\&}$ $p < 0.01$, $^{\&\&\&}$ $p < 0.001$ comparison to control group.

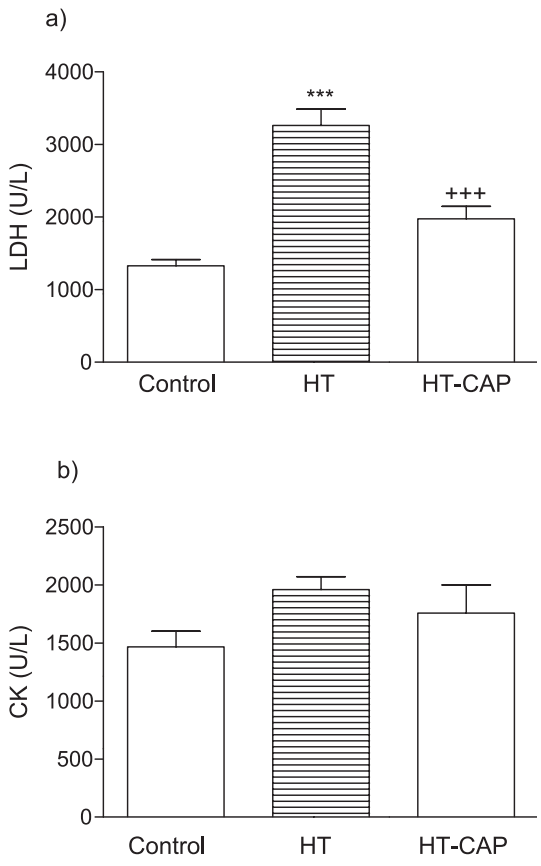

Figure 2. Lactate dehydrogenase (LDH), creatinine phosphokinase (CPK) values of the control, HT and HT-CAP groups in which hypertension was induced experimentally in rats by applying L-NAME. *** $p < 0.001$ according to control group, $^{+++}$ $p < 0.001$ HT comparisons with HT-CAP group

ference ($p > 0.05$) (Table 1, Fig. 1). There was an increase of body weight of control, HT and HT-CAP group at the end of the experiment but this increase was not significant ($p > 0.05$) (Table 1). Heart weight of HT group values increased significantly compared with the control group, however HT-CAP group heart weight values were found to be significantly lower than HT group ($p < 0.05$). When heart/body weight ratio values were evaluated, the values of HT group were found to be higher compared with the control group ($p < 0.05$). However, heart/body weight ratios of HT-CAP group were found to be significantly low compared with HT group ($p < 0.05$) (Table 1).

Plasma assays

Generally, the cytosolic enzymes lactate dehydrogenase (LDH) and creatine phosphate kinase (CPK) as well as lactate in the coronary effluent are important biomarkers for myocardial ischemia in hypertension (18). Plasma levels of LDH and CPK of HT group were found to be significantly higher than control group ($p < 0.05$). However, this increase of HT group became significantly lower in HT-CAP group and became almost similar with control group ($p < 0.05$) (Fig. 2). This decrease of LDH and CPK level in HT-CAP group showed the antihypertensive effect of our formulation.

Measurement of tissue glutathione and malondialdehyde levels

GSH levels measured from the cardiac tissue were found out to be lower in the group of HT com-

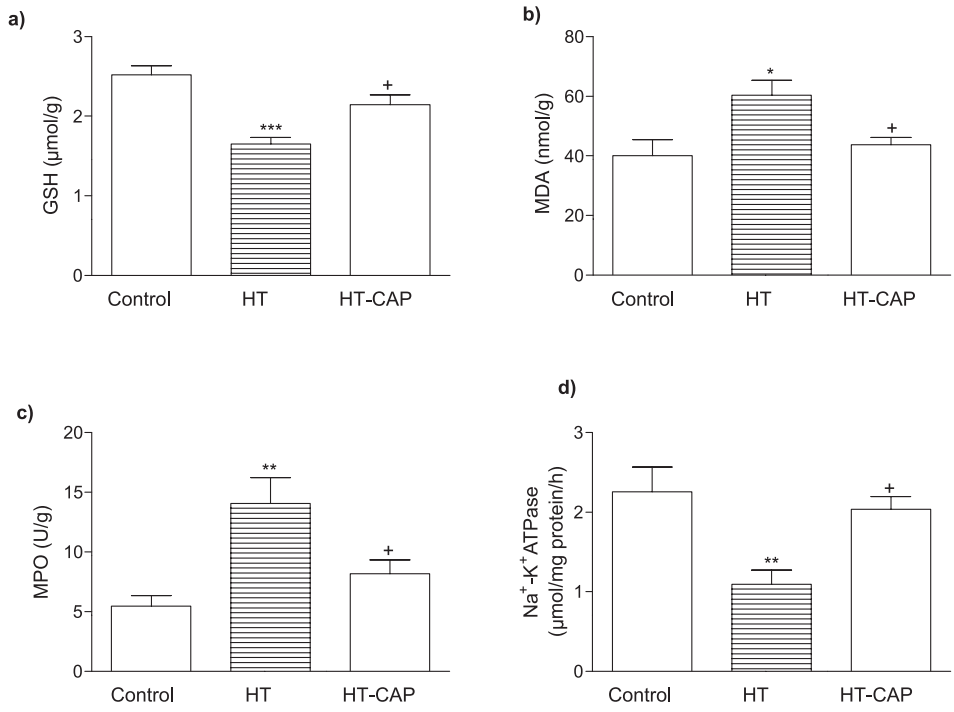

Figure 3. Glutathione (GSH), malondialdehyde (MDA), myeloperoxidase (MPO) and Na^+,K^+-ATPase values of the control, HT and HT-captopril groups in which hypertension was induced experimentally in rats by applying L-NAME. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ according to control group; $^+$ $p < 0.05$ comparison with HT-CAP group

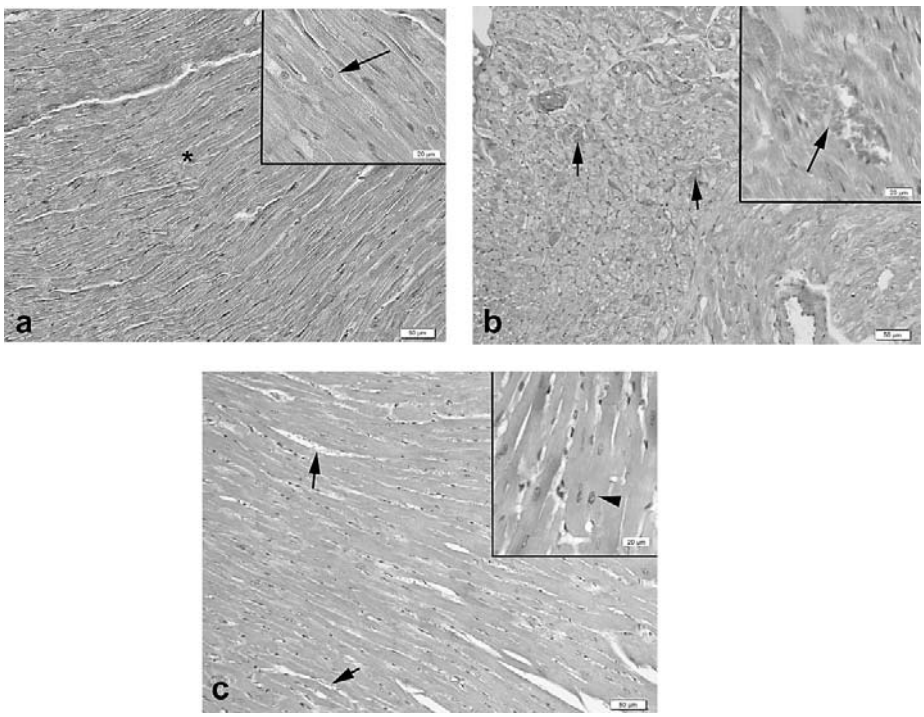

Figure 4. a) control group, regular morphology of cardiac muscle bundles (*) with nuclei (arrow); b) hypertension group, widespread congestion among the cardiac muscle bundles (arrows); c) treatment with captopril group, note the reduced congestion in the capillaries among the cardiac muscle bundles

pared with control group ($p < 0.05$). This decrease was prevented in HT-CAP group significantly ($p < 0.05$) (Fig. 3a). MDA levels measured from the cardiac tissue were found out to be higher in HT group compared with control group ($p < 0.05$). Application of TTS containing captopril (HT-CAP) decreased this increase of MDA levels significantly ($p < 0.05$). (Fig. 3b). Similar results were found with Zuhairu et al., hypertension produced significant increases of heart and kidney MDA indicating oxidative stress damage without any detected alteration in plasma (19).

Myeloperoxidase activity

One of the principal mediators secreted on polymorphonuclear neutrophils (PMN) activation is MPO, a hemoprotein traditionally viewed as a microbicidal enzyme (20). However, there is accumulating evidence that MPO also displays potent proatherogenic properties. For example, MPO can oxidize LDL cholesterol, thereby propagating uptake by macrophages and perpetuating foam cell formation (21). Furthermore, MPO has been shown to activate metalloproteinases and promote destabilization and rupture of the atherosclerotic plaque surface (22). Also, MPO catalytically consumes endothelium-derived nitric oxide, thereby reducing nitric oxide bioavailability and impairing its vasodilatory and anti-inflammatory functions (23, 24). In the hypertension group, neutrophil infiltration and related MPO levels increased significantly in cardiac tissue compared with the control group ($p < 0.05$). MPO activity decreased significantly in captopril applied HT group ($p < 0.05$) and the results were similar with the MPO levels measured in control group. In patients with acute coronary syndromes, MPO serum levels powerfully predict an increased risk for subsequent cardiovascular events and extend the prognostic information gained from traditional biochemical markers (25). In our formulation, as seen in Figure 3c, MPO activity decreased significantly in captopril applied HT group ($p < 0.05$) and the results were similar with the MPO levels measured in control group.

Measurement of Na^+, K^+-ATPase activities in the cardiac tissue

Decreased arterial Na^+-K^+ pump and cardiac Na^+, K^+-ATPase activities have been demonstrated in several types of experimental volume expanded hypertension (26). Na^+, K^+-ATPase activity in cardiac tissue were found out to be lower in HT group compared with the control group. However, this decrease was prevented with captopril applied HT group significantly ($p < 0.05$) (Fig. 3d).

Histopathological analysis

The results of the control groups were observed as regular alignment of cardiac muscle bundles with capillaries (Fig. 4a) whereas the hypertension group demonstrated congestion of capillaries (Fig. 4b). The treatment with matrix type transdermal therapeutic systems containing captopril reduced the capillary congestion among the muscle bundles (Fig. 4c).

CONCLUSION

Matrix type transdermal patches prepared with Eudragit RL 100 and RS 100 polymers containing captopril can be considered as transdermal therapeutic systems for chronical treatment of hypertension and congestive heart failure. However, further *in vivo* pharmacokinetic studies should be performed in order to determine the blood level of the drug.

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