

ORIGINAL ARTICLE

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Effect of *myrtus communis* extract on serum cytokines in angiotensin dependent hypertensive rats

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Abstract

In angiotensin dependent hypertension, high blood pressure and elevated angiotensin 2 levels lead to inflammation by increasing the pro-inflammatory cytokine levels. Since *Myrtus communis* have showed anti-inflammatory activity in ethnobotanical researches and experimental studies, we investigated the effect of *Myrtus communis* extract against inflammation in a rat model of angiotensin dependent hypertension. Wistar albino rats were divided as sham-operated control, RVH and *Myrtus communis* extract-treated RVH groups. Left renal arteries of the rats were implanted with silver clip. Indirect blood pressure measurement of rats was provided with tail-cuff method before the surgery, 3 and 9 weeks after the surgery. *Myrtus communis* extract (100 mg/kg, orally) or vehicle was administered for 6 weeks. At the end of the study, serum samples were collected to investigate tumor necrosis alpha (TNF- α), interleukin-1 beta (IL-1 β) and IL-6 levels. RVH resulted in significant increases in these proinflammatory cytokine levels. In the *Myrtus communis* extract treatment group, this increase was abolished. The present data demonstrated that *Myrtus communis* attenuates RVH-induced inflammation.

Keywords: Renovascular hypertension, angiotensin 2, inflammation, cytokines, *Myrtus communis*

Introduction

Renovascular hypertension (RVH) which is a common secondary hypertension arises from renal hypoperfusion due to anatomical stenosis of the renal artery. Renal hypoperfusion excessively activates the renin angiotensin aldosterone system (RAAS) [1]. RVH generates many complications especially in the vital organs such as heart, kidney and brain. One of the important mechanisms underlying the complications is that RVH promotes inflammation. The stress that high blood pressure exerts force on the artery wall attracts the inflammatory cells to the arteries [2-4] which produce pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF- α), Interleukin (IL)-6 [5, 6]. Moreover angiotensin 2 which is present in high blood levels in the RVH increases nuclear factor kappa β (NF-K β) activation, expression of cytokines as IL-1, IL-6, TNF- α , adhesion molecules and chemokines [7, 8]. Additionally mechanical stress on the vessel wall due to vasoconstrictive effect of angiotensin promotes local angiotensin 2 production that leads to further inflammation. Treatment of hypertension in animal models with angiotensin 2 receptor blockers reduced the

level of inflammatory cytokines in the systemic circulation [7, 9]. Moreover the results of the animal studies were also supported by clinical studies where angiotensin receptor blockers (ARBs) have reduced the levels of inflammatory mediators such as IL-6, TNF- α , C-reactive protein (CRP) [2, 3]. In vitro and in vivo studies have demonstrated that angiotensin converting enzyme inhibitors (ACEIs) also suppress the generation of inflammatory cytokines [10].

Myrtus communis' leaves, fruits and branches have been utilized by the public for many years in the treatment of numerous diseases. In reference to ethnobotanical studies, *Myrtus communis* has been used traditionally in the treatment of inflammation and hypertension [11]. According to the literature, myrtle exerts antiinflammatory effect. Hosseinzadeh et al. demonstrated that water and ethanol extract of aerial parts of *Myrtus communis* supplies antiinflammatory activity on the chemical induced ear edema. On the carrageenan-induced foot edema and pleural inflammation models, antiinflammatory effects of *Myrtus communis* leaves were investigated by Rossi et al. (2011) [12]. They have demonstrated that myrtucommulone content provides antiinflammatory effect. Myrtucommulone and semimyrtucommulone which are the important constituents of myrtle occur at the leaves of the plant. They provide antiinflammatory action of the plant via direct inhibition of cyclooxygenase 1 and lipoxygenase 5. Besides in polymorphonuclear leukocytes they inhibit calcium mobilization

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through G protein signal. Moreover they suppress the formation of reactive oxygen species [13]. The studies evaluating the effect of *Myrtus communis* extract on inflammation and its effect mechanisms are restricted. In this study, we aimed to investigate the possible positive effect of *Myrtus communis* extract on serum TNF- α , IL-1 β and IL-6 levels in the renovascular hypertensive rats.

Materials and Methods

Plant material and preparation of MC extract

From the Turgutlu region of Manisa, *Myrtus communis* samples were collected. Botanist in the School of Pharmacy, Marmara University identified the samples. Dried *Myrtus communis* samples were deposited in the Herbarium in the School of Pharmacy, MU (Herbarium protocol no: 13,006). Dried and powdered leaves were extracted with 96% EtOH with the help of Soxhlet apparatus. The extract evaporated at +4 °C until dry in vacuum and stored in a dark container in refrigerator (+4 °C).

Animals

Marmara University (MU) Animal Care and Use Committee approved all experimental protocols (#51.2019.mar). The institutional and national guide for the care and use of laboratory animals was followed. MU Animal Center (DEHAMER) supplied female and male Wistar albino rats (200-300 g). Animals were kept at a constant temperature 22 \pm 1 °C with 12 h light and dark cycles. Animals were fed with standard rat chow.

ELISA Kits

IL-1 β , IL-6, TNF- α ELISA kits (YL Biotech) were used.

Methods

Blood Pressure Measurement

Tail cuff method was used to measure the blood pressure. Indirect blood pressure measurement was performed before the surgery (t₁) and 3 weeks (t₂) and 9 weeks after the surgery (t₃). First of all, the rats were placed in a chamber heated to 35°C for 10 min. The rats were placed in individual plastic restrainers. Tails of the rats were wrapped with cuff with a pneumatic pulse sensor. At least three consecutive readings provided from each rat and averaged during each measurement period [14].

Two Kidney One Clip (2K 1C) Renovascular Hypertension Model

Left renal artery of the rats was implanted with a silver clip which has 0.25 mm internal diameter [14, 15]. In the control group, similar surgical procedure without clip insertion was performed. For anaesthesia, intraperitoneally 100 mg/kg ketamine and 0.75 mg/kg chlorpromazine were injected to the rats.

Treatment

Animals were divided into 3 groups; sham operated control, RVH and RVH+MC. Each group had 7 animals. Sham and RVH groups received SF while RVH+MC group received *Myrtus communis*

L. (Myrtaceae) (100 mg/ kg) extract by oral gavage. Treatment started by 3 weeks after clip placement surgery and continued for the remaining 6 weeks.

Measurement of Proinflammatory Cytokine (IL-1 β , IL-6, TNF- α) Levels

The commercial ELISA kits were used and the standard procedure was followed.

Results

Blood Pressure Measurement

Before the surgery, the basal blood pressure levels were not statistically different between the groups (Figure 1). In RVH and RVH+MC groups, the mean systolic blood pressure levels were high significantly at week 3 as compared with the control group (212,2 \pm 12,1 mmHg and 191,7 \pm 8,5 mmHg, respectively). In the RVH group this increases were continued. The week 9 value elevated (234,2 \pm 11,9 mmHg; p<0.001) with respect to control group. However, systolic blood pressure was reduced after 6 weeks of *Myrtus communis* treatment as compared with the week 3 recording (130,8 \pm 3,7 mmHg; p<0.001).

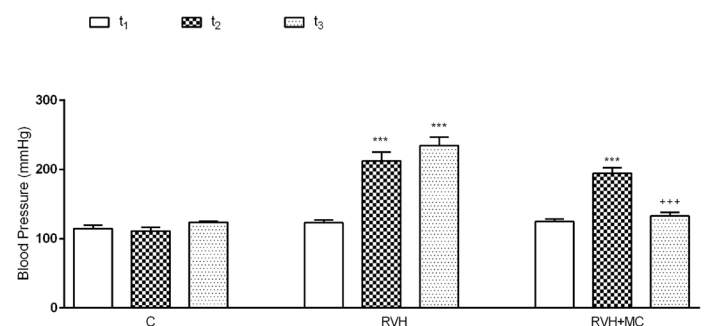


Figure 1. Systolic blood pressure values at baseline and at third and ninth weeks of the experiment. ***p<0.001: vs C group; +++p<0.001: vs 3th week values

Proinflammatory Cytokine Levels

TNF- α

In the RVH group, serum TNF- α levels were significantly increased as compared with control group (p<0.001) (Figure 2). *Myrtus communis* treatment caused decrease in serum TNF- α levels compared to RVH group (p<0.001).

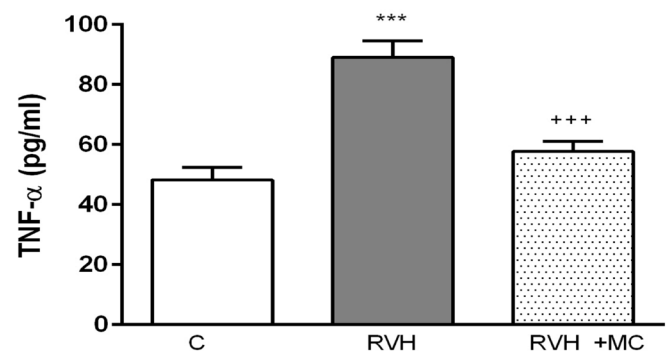


Figure 2. Serum TNF- α values of all groups. ***p<0.001: vs control group; +++<0.001: vs RVH group

IL-1 β

In the RVH group, serum IL-1 β levels were higher than control group ($p<0.001$) (Figure 3). In the *Myrtus communis* treatment group, increases in serum IL-1 β levels were abolished ($p<0.001$).

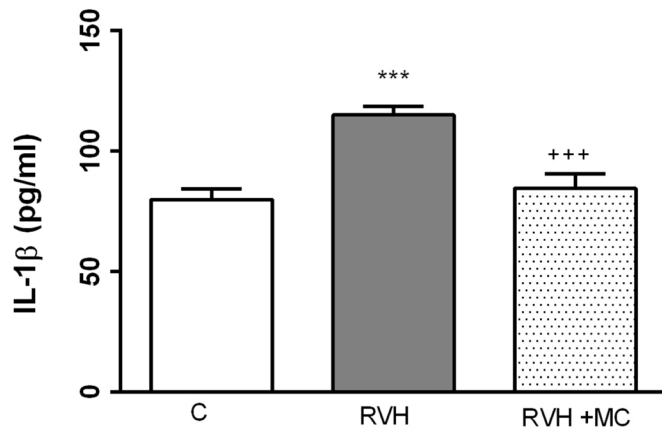


Figure 3. Serum IL-1 β values of all groups. *** $p<0.001$: vs control group; +++ $p<0.001$: vs RVH group

IL-6

In the RVH group, serum IL-6 levels were significantly found to be increased when compared with control group ($p<0.001$) (Figure 4). In the *Myrtus communis* treatment group, serum IL-6 levels were lower when compared with RVH group ($p<0.001$).

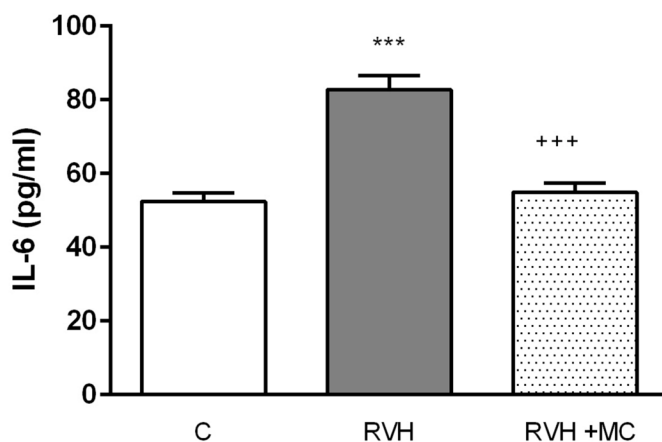


Figure 4. Serum IL-6 values of all groups. *** $p<0.001$: vs control group; +++ $p<0.001$: vs RVH group

Discussion

In the present study, RVH resulted in increased blood pressure. The serum levels of proinflammatory cytokines TNF- α , IL-1 β and IL-6 were found to be increased in the RVH rats. Furthermore, in the 2K1C rats, *Myrtus communis* treatment abolished the increases in both blood pressure and inflammatory cytokines.

RVH-caused increase in BP and angiotensin 2 levels [1], through increasing the inflammatory response contribute many organ complications such as heart, kidney and brain, increase the

morbidity and mortality [7, 8]. Thus, the aim of the treatment of hypertension is to reduce both blood pressure and to prevent complications caused by high pressure.

In this study, treatment with *Myrtus communis* extract, through decreasing the blood pressure, reduced cytokines, which suggest that the *Myrtus communis* extract could prevent the complications seen in RVH.

Conclusion

Myrtus communis extract showed beneficial effects in RVH, through reduction of blood pressure and proinflammatory cytokines TNF- α , IL-1 β and IL-6 serum levels. Further investigation is needed regarding the protective effects of *Myrtus communis* extract against tissue injury caused by inflammatory cytokines which generated in hypertension.

Conflict of interest

The authors declare that they have no competing interest.

Financial Disclosure

All authors declare no financial support.

Ethical approval

Ethics committee approval was obtained.

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