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Effects of Exercise and *Ferula Gummosa* on Apelin of Cardiac and Kidney Tissues in L-NAME induced Hypertension in Rats

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Abstract

Purpose: The present paper studied the effects of 8 weeks of aerobic training and *Ferula gummosa* on apelin and stress markers in heart and kidney tissues of hypertensive rats. Fifty adult, male, Wistar rats were randomly classified into five groups: aerobic training (AT), *Ferula gummosa* (FG), combination of aerobic training and *Ferula gummosa* (TFG),nitro-L-arginine-methyl ester(L-NAME) and sham (SH).

Material and Methods: Training program included treadmill running, 25-64 min per session, 15-22 m/min, 5 sessions a week, for 8 weeks. The FG supplement group (90 mg/kg) was fed through gavage. Hypertension was induced by L-NAME solution, intraperitoneally (10 mg/kg), for 8 weeks, 6 sessions a weekone-way ANOVA and Welch test for repeated measurements with post hoc testing by Tukey were performed to identify differences between groups.

Results: AT, FG, and TFG protocols resulted in an increase in apelin, Nitric oxide (NO) in heart and kidney tissues and significantly decreased angiotensin converting enzyme (ACE) as compared to L-NAME group. TFG protocols resulted in an increase in apelin and NO in the heart tissue as compared to sham group. In addition FG resulted in an increase in NO and apelin levels in heart and kidney tissues as compared to L-NAME group.

Discussion and Conclusion: It seems that aerobic exercise with moderate intensity and herbal supplements of apiaceae increase apelin and reduce blood pressure in rats with hypertension. This study provides the experimental evidence showing that the apelin has hypotensive and cardio protective effects in the kidney and heart tissues of rats.

Keywords: Aerobic training, Oxidative stress, Hypertension, Apeline, Herbal therapy

Introduction

Apelin is a bioactive peptide originally identified from bovine stomach extracts as the endogenous ligand of the G protein coupled receptor APJ [1]. The studies have demonstrated that apelin is widely detected in various tissues including the heart, lung, testes, ovary, mammary glands, brain, liver, skeletal muscle, and kidney [2], and is involved in the regulation of cardiovascular function and hypotension [3] and central fluid homeostasis [4]. Although little is known about the physiological role of apelin so far, it has been hypothesized that it may exert vasodilation and hypotensive effects as opposed to the presser action of angiotensin II/angiotensin-type 1 receptor (AT1) signaling [5].

In the cardiovascular system, apelin-like immunoreactivity (apelin-ir) has been detected in rat and human vascular endothelial cells. It is well known that apelin is an autocrine/paracrine factor in cardiovascular tissues and is one of the most potent positive inotropic substances identified to date [6].In addition, apelin is a strong vasodilator. The injection of apelin significantly reduced arterial blood pressure in Wistar rats [7] and spontaneously

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hypertensive rats (SHRs) [8]. Furthermore, Zhong et al. [9] reported that the mRNA and protein expression of apelin and APJ in heart and vessels was markedly depressed in SHRs. These findings suggest that apelin could be a novel, endogenous, anti-hypertension factor and that it could be markedly depressed in hypertensive diseases [6].

Hypertension is the major treatable risk factor of these cardiovascular disorders[10]. Hypertension and its complications remain a major public health problem today. Researchers have reported hypertension to be a significant, independent, risk factor for cardiovascular disease, chronic kidney disease (CKD) and the most important modifiable cause of mortality [11].The combination of cardiovascular risk factors account for a large proportion of cardiovascular morbidity and mortality. Low-grade inflammation occurs in the vasculature in several conditions that predispose to cardiovascular diseases, like hypertension. In patients with hypertension, the pathophysiology of cardiovascular disease is multifactorial, but recent evidence points toward the presence of an important component dependent on a low-grade inflammatory process. Angiotensin II may be to a large degree responsible for triggering vascular inflammation by inducing oxidative stress, resulting in up-regulation of pro-inflammatory transcription factors[12]. Recent evidence suggests that apelin can act as a proinflammatory factor that participates in vascular wall inflammation [13] and well known inflammatory markers (e.g.C-reactive protein) are increased in patients with hypertension, and predict the development of cardiovascular disease [14]. management of hypertension appears to be one of the major therapeutic goals. For example, prevention of hypertension becomes an important goal in overall efforts to control blood pressure and reduce the incidence of hypertension-related cardiovascular and chronic kidney diseases [10].

As early as 1983, the World Health Organization recommended the use of non-pharmacological approaches such as physical activity and taking antioxidants in the primary and adjunctive treatment of hypertension. Free radicals and reactive oxygen species are well-known inducers of cellular and tissue pathogenesis, leading to several diseases such as cardiovascular and inflammatory disorders [15]. Antioxidants protect living organisms from damages caused by the uncontrolled production of ROS. Consequently, the need to identify alternative, natural, and safe sources of oral antioxidants arose and the search for natural antioxidants, especially with herbal origin, has notably increased in recent years [16]. *Ferula gummosa boiss* (apiaceae) is a perennial plant native to central Asia [17]. In recent years there are some reports regarding the main effects of this plant's antibacterial and anti-inflammatory activities [18]. On the other hand, exercise is recognized as a useful, non-pharmacological intervention to reduce blood pressure in hypertension. Wallace reported exercise to be the most promising non-pharmacological treatment of hypertension[19]. Positive cardiovascular effects of exercise are associated with beneficial changes in antioxidant systems, blood pressure, adipogenesis, and inflammation. Myriad factors have been implicated, whereby exercise induces protective cardiovascular actions, including decreased sympathetic activity, reduced angiotensin II levels, increased Nitric oxide (NO) bioavailability, increased antioxidant capacity and expression of cardio protective factors, such as apelin [20]. In addition, growing evidence indicates that exercise prevents oxidative damage by reducing oxidative stress, an important factor in inflammation and hypertension [20]. Results from several studies demonstrated that training caused an average systolic blood pressure reduction of 11 mmHg (range 5–25 mmHg), and diastolic pressure of 8 mmHg (range 3–15 mmHg) [21]. Furthermore, training does not render an individual normotensive, and a reduction in blood pressure was not consistently observed in every hypertensive subjects. However, changes in apelin and biomarkers related to oxidant/antioxidant homeostasis and their relationship with physical activity and antioxidant turmeric supplement are poorly understood, particularly, during chronic hypertension. Despite the knowledge that hypertension can induce oxidative stress [12,14] on one hand, and taking into account , the effects of these peptides on blood pressure in different tissues on the other, little evidence is available with respect to individual and concomitant effects of regular aerobic training and turmeric antioxidant supplement on cardiac apelin and the oxidant/antioxidant and inflammatory processes particularly during chronic exposure to L-NAME-induced hypertension.

The purpose of the current study was to

determine the effects of aerobic exercise, and *Ferula gummosa* supplementation on cardiac and renal apelin in rats that have been chronically exposed to L-NAME-induced hypertension. In addition, given the relationship between hypertension and oxidative stress, levels of the angiotensin converting enzyme (ACE) and Nitric oxide (NO) were also assessed. It is hypothesized that the results of this study provide a novel insight into renal and cardiac ameliorative potential of antioxidant and exercise training.

Material and Methods

All experiments were performed in accordance with the guidelines outlined by the Experimental Animal Laboratory, were approved by the Department of Physiology, University of Mazandaran and were performed according to guiding procedures in the Care and Use of Animals, prepared by the Council of the American Physiological Society. The experiments were carried out on fifty, male, Wistar rats, (-weeks old, initially weighing 240 ± 20 g), which were obtained from the Pasture Institute of Iran. Rats were housed in standard cages of polycarbonate (20×15×15), in a large, air-conditioned room, with a controlled temperature of 22±2 °C, light- dark cycles of 12:12 hours, and humidity of 50±5%. The pollutant standard index (PSI) was in the acceptable range as determined by the Iranian Meteorological Organization. Rats were fed with standard rat chow provided by Pars Institute for animals and poultry with a daily regimen of 10 /100 gr body weight for each rat. Water was available ad libitum.

Rats in all groups were adapted to the treadmill running for 5 days. The familiarization protocol was designed as once a day, 10 min/session, at a speed of 10 m/min, and a slope of 0◦. An electric stimulus (30 V, 0.5 A) was manually turned on for less than 2 s when the animals stayed on the electric grid for longer than 10 s [22]. Following this familiarization period, they were randomly assigned into five experimental groups, 8 rats each. The groups were defined as follows:

Group1:the animals were intraperitoneally exposed to L-NAME, at a concentration of 10 mg/kg,. in the form of a solution, , 6 days a week, and for 8 weeks, in order to induce the hypertension [16]; Group 2–*Ferula gummosa* similarly received L-NAME, and *Ferula gummosa* federally and through a gavage, with 90 mg/kg dosage, 6 days a week, and for 8 weeks [23]; Group 3 – aerobic exercise - the rats in this group similarly received L-NAME, and performed a progressive running exercise of 15 to 22 m/min, 25 to 64 min, 5 times a week; The running speed and duration of exercise were progressively increased during a graded treadmill exercise protocol [24]. Group 4–aerobic exercise and *Ferula gummosa*: the rats in this group performed an aerobic training protocol similar to that of group 3, and received L-NAME and *Ferula gummosa* supplement; Group 5-the sham (control) group: these rats received 0.1 mg/kg of NACL solution, injected intraperitoneally, in the same manner and for the same duration of time as the other groups.

We have replicated a previously extract preparation, described by Mandegary et al.[18]. In summary, seeds and root of *Ferula gummosa* were dried at the lab temperature for a week. Aqueous and methanolic extracts were obtained through decoction. For the preparation of acetone extract, the seeds and root were macerated in acetone (2L), in portions of 200 and 300 g, respectively, for 3 days. After filtration of the mixtures, filtrates were concentrated by a rotary evaporator apparatus. The residues were then dried at room temperature. The final weight of crude acetone extracts was approximately 13.5 g which maintained at 4º C throughout the experiments.

All groups were anesthetized with ketamine (100mg/kg) and Xylozine (10mg/kg) and decapitated after 10 to 12 hours of overnight fasting. Blood samples were collected 24 h after the last dose of treatment. These blood samples were initially centrifuged by a refrigerated centrifuge at 3000 rpm, for 15 minutes, within 30 minutes of collection and then were stored at -80ºC for subsequent assay of ACE and NO.

The thoracic cavity was then opened and the kidney and heart tissues were quickly excised. kidney and heart tissues were weighed and the left ventricle and section of kidney tissue were placed into Petri dishes containing cold isolation medium (0.1 moll/L K_2HPO_4, 0.15 mol/L NaCl, pH 7.4) to remove the blood and were frozen immediately in liquid nitrogen and stored at -80ºC for subsequent analysis of apelin. Left ventricular and kidney tissues were squashed in liquid nitrogen, homogenized in a lysis buffer (5 ml/g of tissue) with a protease inhibitor cocktail for mammalian cell and tissue extracts (Sigma Aldrich, St. Louis,

U.S.A) 100 ul/1 ml, and 10 mMtris base (Sigma-Aldrich, St. Louis, U.S.A), pH 7.4 and were centrifuged at 1600 g, for 15 minutes, and at 4 ºC,. Left ventricular and kidney tissues supernatant were diluted 1:30. Plasma was diluted 1:10 and the fluids were used in apelin-13 ELISA kits (Phoenix peptides, Burlingame, California, USA), following the manufacturer's instructions. The assay kit was very specific and detected apelin-13 with 100% cross reactivity. It had an inter-assay variation less than 14% and an intra-assay coefficient of variation less than 10%. Apelin-13 in the mentioned sample was measured using ELISA kits, too (Rat Apelin, ELISA, USCN LIFE Science Inc, Wuhan, P. R. China, Life Science Inc, Sensitivity 0.128 ng/ml. IntraCV: 5% and Inter CV:14%).

The serum NO concentration was determined by first reducing the nitrate to nitrite, using nitrate reductase (Sigma). Plasma levels of ACE were measured using a sandwich enzyme-linked immunosorbant assay (ELISA). All data have been expressed as mean ± standard deviation (SD). Statistical analysis was performed using a commercial software package (SPSS version 16.0 for Windows). one-way ANOVA and Welch test for repeated measurements with post hoc Tukey test (Statistical software, StatSoft, Inc, Tulsa,OK,) were performed in order to identify differences between the groups. Differences were considered statistically significant at p-values<0.05.

Results

Levels of the kidney apelinergic system

Table 1 shows changes in kidney apelin, in the rats exposed to L-NAME and rats in the control (saline) group. Administration of L-NAME (10mg/kg) caused a markedly decrease in apelin (46%), as compared to saline group. In contrast, aerobic training protocols resulted in a significant increase in apelin (53%), as compared to control and L-NAME groups. The aerobic training+*Ferula gummosa* protocols resulted in a markedly increase in apelin(85%) as compared to L-NAME group (Figure.1). Also, significant differences were detected in the apelin level between rats in the aerobic training and aerobic training+*Ferula gummosa* groups, as compared to *Ferula gummosa* group (Figure.1). Moreover, insignificant increases were detected in the apelin levels of the aerobic training+*Ferula gummosa* group, as compared to *Ferula gummosa* group (Figure.1).

Table 1: Effect of aerobic training and *Ferula gummosa* supplement on apelin level in rats during chronic exposure to L-NAME

groups and markers	Sham	*Ferula gummosa*	Training	Training + *Ferula gummosa*	L-NAME
Heart Apelin	2.6750±0.471	2.0750±.79237	3.5000±2.600	4.3125±1.20764	.5625±.21339
Kidney Apelin	4.2675±1.522	3.0412±1.10849	6.5425±1.743	4.3000±1.27252	2.3200±.9374

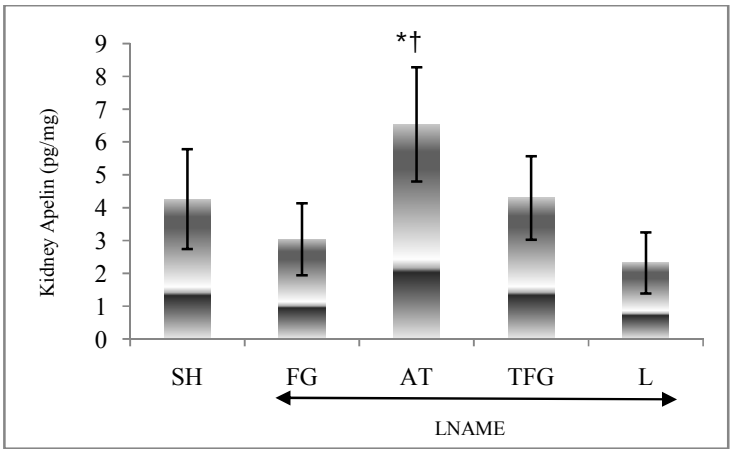

Figure1: .Kidney Apelin level during chronic exposure to nitro-L-arginine-methyl ester (L-NAME) and aerobic training and/or *Ferula gummosa* supplement. Abbreviation; L(L-NAME), FG(*Ferula gummosa*),AT(Aerobic Training), TFG (Training+ *Ferula gummosa*), SH(Sham). Data are presented as mean±SD for 8 Rats; †significantly different from sham group (P < 0.001), * significantly different from L-NAME group (P < 0.001), (P < 0.05).

Levels of the cardiac apelin

Data in table 1 shows changes in heart tissue apelin level, in the rats exposed to L-NAME solution and rats in the control (saline) group. Intra-peritoneal administration of L-NAME (10mg/kg) caused a significant decrease in apelin (79%), as compared to control group but aerobic training *Ferula gummosa* protocols resulted in a significant increase in apelin (61%)as compared to the control group. The *Ferula gummosa* and aerobic training + *Ferula gummosa* protocols resulted in a significant increase in apelin(26% and 66% respectively). aerobic training also resulted in an insignificant increase (52%) in apelin as compared to L-NAME group (Figure.2). ad significant differences were also detected in the apelin levels between training + *Ferula gummosa* protocols and *Ferula gummosa* groups (Figure.2).

Levels of the endothelial dysfunction markers

Table 2 shows changes in biomarkers related to endothelial dysfunction consisting of angiotensin converting enzyme (ACE) and Nitric oxide (NO) in the rats exposed to L-NAME. A significant increase (38%) in ACE levels and a markedly decrease (47%) in NO levels were detected in the rats exposed to chronic L-NAME administration as compared to the control group. ACE levels decreased significantly after 8weeks of aerobic training+*Ferula gummosa* and *Ferula gummosa* protocols (46%,21%, respectively), as compared to L-NAME group. On the other hand, the individual aerobic training protocol and/or the concomitant aerobic training+*Ferula gummosa* significantly increased the NO levels (70%,64%, respectively), as compared to L-NAME group. However, no significant differences were observed in NO and ACE levels between the aerobic training and *Ferula gummosa* groups (Figure.3). But, a significant decrease was observed in ACE levels between the aerobic training+*Ferula gummosa* and *Ferula gummosa* groups (Figure .3).

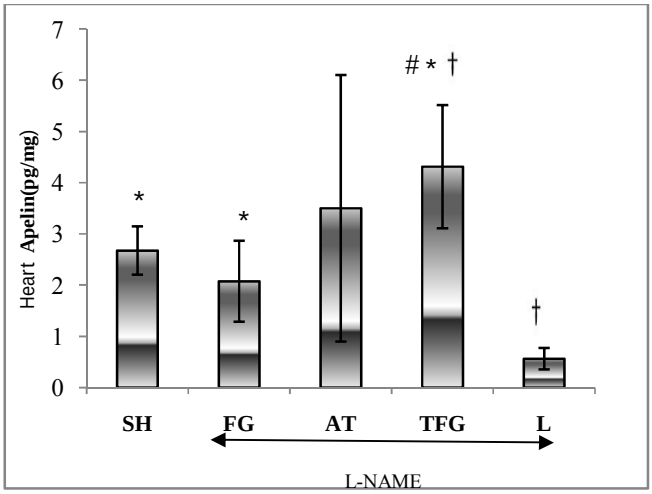

Figure 2: Heart Apelin level during chronic exposure to nitro-L-arginine-methyl ester (L-NAME) and aerobic training /or *Ferula gummosa* supplement. Abbreviation; L(L-NAME), FG(*Ferula gummosa*),AT(Aerobic Training), TFG (Trining+*Ferulagummosa*), SH(Sham). Data are presented as the mean±SD for 8 Rats; †significantly different from sham group ($P < 0.001$), * significantly different from L-NAME group ($P < 0.001$), # significantly different between combination (training + L-NAME+*Ferulagummosa*) and *Ferula gummosa* groups ($P < 0.05$).

Table 2: Effect of aerobic training and *Ferula gummosa* supplement on endothelial dysfunction levels in rats during chronic exposure to L-NAME

groups and markers	Sham	*Ferula gummosa*	Training	Training +*Ferula gummosa*	L-NAME
ACE (pg/ml)	178±19.22	197.25±32.25	204.83±34.27	134.43±22.67	247.25±36.14
NO(µmol/l)	32.1125±2.650	25.7125±3.152	46.1500±28/806	45.1875±29.426	17.5286±7.729

Abbreviation; angiotensin converting enzyme (ACE) and nitric oxide (NO) . Data are presented as the mean±SD for 8 Rats.

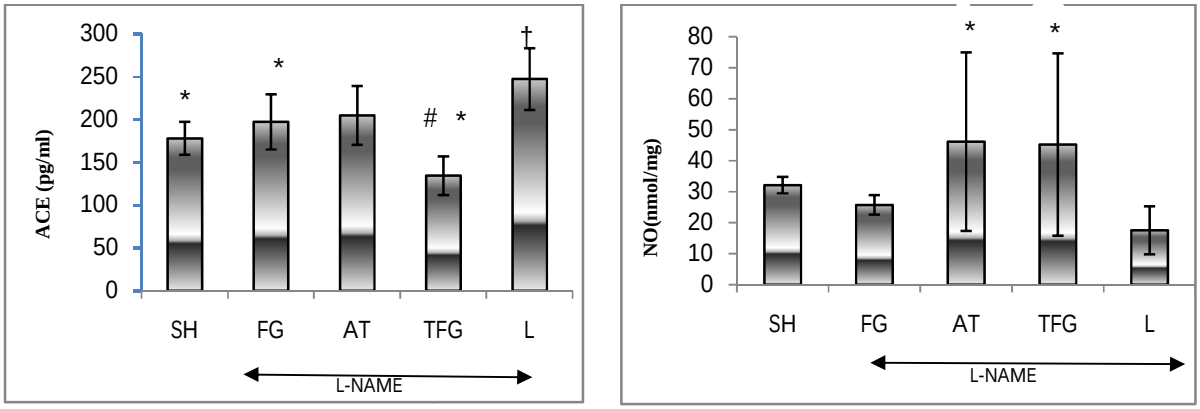

Figure 3: Markers of endothelial dysfunction during chronic exposure to nitro-L-arginine-methyl ester (L-NAME) and aerobic training /or *Ferula gummosa* supplement. Abbreviation; angiotensin converting enzyme (ACE and nitric oxide (NO).L(L-NAME), FG(*Ferula gummosa*), AT(Aerobic Training), TFG (Trining+ *Ferula gummosa*), SH(Sham). Data are presented as the mean±SD for 8 Rats; † significantly different from sham group ($P < 0.001$),*significantly different from L-NAMEgroup ($P<0.001$),#significantly different between combination (training + L-NAME+ *Ferula gummosa*) and training with *Ferula gummosa* groups ($P < 0.05$).

Discussion and Conclusion

The discovery of the apelin was an exciting development in cardiovascular research. Although the exact mechanisms of how this molecular pathway interacts with the Ang II-AT1 pathway are still to be fully elucidated, there is growing evidence that apelin may be involved in the hypotension to clinically significant heart failure. Apelin and its receptor (APJ receptor) as endogenous peptides have important roles in lowering blood pressure in various tissues, through increasing nitric oxide and reducing inflammation. Despite the effects of these peptides on blood pressure in different tissues, the functional significance of apelin and its relationship with physical activity and herbal Plants have gained little attention.

The main finding of this study was that chronically administered L-NAME resulted in an increased level of ACE and reduced the levels of apelin and NO as compared to the control group. the combination of aerobic exercise and *Ferula gummosa* significantly increased cardiac apelin and NO as compared to the L-NAME groups. Animal and human studies suggest that apelin ,a natural ligand to APJ, is a cardio protective peptide with hypotensive effects, both in vivo and in vitro, and that it acts through accepted cardio protective mechanisms and may play a role in the pathogenesis of renal and heart failure and hypertension [25].Studies have shown that apelin-13, injected intravenously into anesthetized rats, significantly decreased mean arterial blood pressure [26,27].Another study found that apelin-12, apelin-13, and apelin-36 decreased mean arterial pressure by 26, 11, and 5 mm Hg, respectively, when administered to anesthetized rats [27]. One set of experiments with apelin administered to conscious unrestrained rats showed that apelin could function as both an arterial and venous dilator in vivo [25,26,6].

Apelin is a multifunctional peptide that regulates cardiovascular homeostasis, immunity reaction and renal function [28]. In cardiovascular tissue, apelin has direct vasodilator effects and is a novel anti-hypertension factor. Recently, Tatemoto et al, reported that the hemodynamic mechanism of apelin might involve NO production [27]. Apelin-induced hypotensive response in rats was antagonized in vivo by pre-treatment with an intraperitoneal injection of an NO synthase inhibitor, L-NAME.In addition, APJ-deficient mice showed an increased vasopressor response to angiotensin II, and the baseline blood pressure of double mutant mice homozygous for both APJ and angiotensin-type Ia receptor was significantly elevated compared with that of angiotensin type Ia receptor-deficient mice [29]. These results demonstrate that apelin plays a counter-regulatory role against the pressor action of angiotensin II.

Regular exercise favorably changes established cardiovascular and renal risk factors such as hyperlipidemia, hypertension and diabetes mellitus [30]. Zhang et al, revealed that long-term swim

training reduced pathogenesis related to hypertension and reversed the down-regulation of the cardiovascular apelin induced by hypertension [6]. This suggests that the effects of exercise training on hypertension could be mediated by up-regulating cardiovascular apelin [6]. the current study, showed that AT protocols increased apelin levels in heart and kidney tissues, as compared to L-NAME group. It also demonstrated that AT protocols resulted in an increase in apelin levels in the heart and kidney tissue, ACE and NO as compared to the control group.

The specific mechanisms by which physical activity ameliorates hypertension have not been well elucidated. Physical activity has been associated with favorable modifications of blood pressure through a reduction in sympathetic activity, improved organ perfusion, regulated energy metabolism and adjusted mood. Furthermore, exercise affects the expression and activities of cardio vasoactive substances. It is well-known that appropriate exercise inhibits the pathological overexpression of angiotensin II and endothelin[31], while simultaneously reinforcing the activities of endogenous cardiovascular defensive systems such as adrenomedullin and nitric oxide/nitric oxide synthase (NO/NOS)[32],to maintain and reinstate cardiovascular homeostasis. Our results also corroborate these findings.

Recent investigations have led to the discovery of some new biological activities of the herbal plants. Together with the activities of the herbs, few activities have also been reported of *Ferula gummosa* species. These include anti-microbial, anti-inflammatory, anti-convulsing, anti-oxidant, and hypotensive activities [33]. In this study, we observed that exposure to *Ferula gummosa* and training, alone or together caused an increase in apelin levels. Studies demonstrate that apelin can exert many kinds of physiological effects through paracrine and autocrine modes [e.g. activate phospholipase C (PLC)] via APJ receptor, increases intracellular Ca2+ level through PLC inositol triphosphate (IP3), activates Ca2+/CaM-dependent nNOS, induces NO production, and exerts powerful physiological effects through NO-cGMP pathway, and that these effects can be greatly inhibited by NOS inhibitor [13,34]. Several Studies have shown that the hemodynamic effect of apelin is abrogated in the presence of a nitric oxide (NO) synthase inhibitor, suggesting that apelin may lower blood pressure via a nitric oxide-dependent mechanism. In rodent models, exogenous apelin administration caused a rapid nitric oxide (NO)-dependent fall in blood pressure and mean capillary filling pressure, indicating its powerful vasodilator and venodilator effects [35]. In ex vivo myography studies, apelin caused NO-dependent vasorelaxation in human mesenteric arteries [36] and venoconstriction in endothelium-denuded human saphenous veins [26].Our results also corroborate these findings. We observed that exposure to L-NAME caused a decrease in NO levels, whereas, *Ferula gummosa* and/or running on treadmill , increased NO levels, as compared to the saline group. The hypotensive effect of apelin is mediated by endothelium-derived NO, since the NO synthase inhibitor L-NAME abolished this effect both in rats [27] and in mice [29]. In addition, apelin increases plasma concentration of NO metabolites(nitrites+nitrates). In cultured mice endothelial cells, apelin stimulated the phosphorylation of endothelial NO synthase (eNOS) at Ser1176 by protein kinase B/Akt. Our data revealed a protective effect of aerobic training or *Ferula gummosa,* against hypertension.

In conclusion, it seems that aerobic exercise with moderate intensity and apiaceae herbal supplementation increased apelin and reduced blood pressure in rats with hypertension, which along with other treatment methods, may suggest a new approach to heart and renal protection against hypertension-induced chronic stress . The present study demonstrated that L-NAME administration caused a down-regulation of apeline, an imbalance in oxidant/antioxidant process, endothelial dysfunction and induced inflammation. Furthermore, *Ferula gummosa* supplementation and/or aerobic training had useful effects on reducing the L-NAME-induced hypertension, probably through increasing apelin effects and decreasing the oxidative, inflammatory and endothelial dysfunction biomarkers related to hypertension. Finally, simultaneous application of *Ferula gummosa* supplementation and aerobic training seems to exert more positive effects as compared to applying either of them alone.

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