

Full Length Research Paper

***Thymus vulgaris* supplementation attenuates blood pressure and aorta damage in hypertensive rats**

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In the present study, we investigated the possible antihypertensive effect of *Thymus vulgaris* (*T. vulgaris*) and its possible protective role against hypertension-induced aorta damage in hypertensive rats. Hypertension was induced by ligation of left renal artery, and *T. vulgaris* aqueous extract was administered (100 mg/kg/day, orally) for 8 consecutive weeks. Systolic blood pressure, body weight, and the serum concentrations of creatinine and cholesterol were measured at the beginning and at the end of the study. Thoracic aorta was isolated at the end of the study for both light and electron microscopic examinations. The antihypertensive effect of *T. vulgaris* was clearly observed here, as well its significant reducing effect on hypertension-induced increases in serum levels of creatinine and cholesterol. The light microscopic findings indicated that the surface endothelium of thoracic aorta of hypertensive-untreated rats was coarse, wrinkled and protuberant, and its lumen adsorbed more debris and red blood cells; however, these phenomena were almost disappeared when these animals were treated with *T. vulgaris*. Similarly, the electron microscopic examinations showed a remarkable increase in aortic extracellular matrix with dispersion of their cell nuclei in hypertensive-untreated rats but not in rats treated with *T. vulgaris*. Therapy with *T. vulgaris* also reduced hypertension-induced aortic smooth muscle cell mass hypertrophy and normalized both aortic lumen diameter and media thickness. In conclusion, our results indicate that hypertension induced in rats was associated with injury of aortic tissue that may accelerate the arterial dysfunction in uncontrolled hypertensive conditions. More importantly, supplementation with *T. vulgaris* as herbal remedy has shown remarkable antihypertensive effect and marked improvement on hypertension-related biochemical changes and aortic vascular damage in rats.

Key words: Aorta, hypertensive, thymus vulgaris, rat.

INTRODUCTION

Until now, cardiovascular disease, especially the hypertension, is still the most important factor that affects people's life despite substantial advances in diagnostic and therapeutic tools (Vilela-Martin et al., 2011). In this concept, there were 1.5 billion hypertensive cases in the world accounting for 21.4% of world population, and causes approximately 7.1 million worldwide deaths per year (Zeng et al., 2011). Hypertension damages major organs, eventually leading to heart failure, vascular injury,

microvascular functional impairment, atherosclerosis and renal dysfunction (Chobanian et al., 2003). Accumulating evidence indicates that vascular remodeling, ultra-structural destruction and apoptosis in the aorta, heart and kidneys contribute to end-organ damage and failure in hypertensive patients. Numerous inflammatory modulators, including reactive oxygen species, are significantly expressed in hypertension associated vascular injury. Moreover, growth, inflammation and fibrosis all also contribute to arterial remodeling in hypertension, meaning prevention of this phenomenon should be a therapeutic aim of antihypertensive therapy (Intengan and Schiffrin, 2001; Hayden et al., 2012).

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The use of medicinal plants, or extracts from them, has been traditionally practiced worldwide in the prevention and treatment of several chronic diseases such as cardiovascular diseases, inflammatory diseases, arthritis, diabetes, and others (Juhás et al., 2008). *T. vulgaris* which is a perennial herb indigenous in central and southern Europe, Africa and Asia, has been frequently used for those purposes (Rustaiyan et al., 2000; Braga et al., 2006). Extracts from *T. vulgaris* have been used in traditional medicine for their anti-asthmatic, bronchodilator, antiseptic, antispasmodic, antitussive, antibacterial, antifungal and antiviral activities (Marino et al., 1999; Pina-Vaz et al., 2004). Also, these extracts have shown anticarcinogenic (Arcila-Lozano et al., 2004) and immunomodulating properties (Bukovska et al., 2007; Ocaña and Reglero, 2012). *T. vulgaris* is also quoted by various authors for its polyphenol and flavonoid contents and its potential antioxidant and free radical scavenging, anti-inflammatory, vasorelaxant, anti-platelet, anti-thrombin, anti-hyperlipidemic and anti-diabetic properties (Miura et al., 2002; Vigo et al., 2004; El-Nekeety et al., 2011).

To date, little evidence exists regarding the therapeutic benefit of *T. vulgaris* against hypertension and its associated serious organ and tissue complications. Therefore, this study was designed to investigate the possible antihypertensive effect of *T. vulgaris* aqueous extract and also to evaluate its possible protective effects against hypertension-induced aorta damage in hypertensive rats.

MATERIALS AND METHODS

Preparation of plant extracts

T. vulgaris leaves were bought from Sekem Company for medicinal plant (Cairo, Egypt). The aqueous extracts of *T. vulgaris* were prepared from its dried leaves as described previously (Ramchoun et al., 2009). In brief, the powdered leaves of *T. vulgaris* were mixed with distilled water (1 gm/100 ml), boiled for 30 min (100°C), and then left to cool for 30 min. The extracts were filtered and the obtained solutions were concentrated in rotatory evaporator under vacuum at 65°C. Finally, the dried extract was reconstituted in distilled water (100 mg/ml) to be used in the study.

Animals and experimental protocol

A total of forty five adult male Wistar rats were used in this study. Rats were cared for in accordance with the Guide for the Care and Use of Experimental Animals of Umm Al-Qura University, KSA, and the entire experiments were carried out in accordance with the International Principles of Laboratory Animal Research. All animals were weaned and housed at 22 to 25°C on a 12:12 h dark-light cycle (07.00 to 19.00 lights on) and maintained on a pellet diet and tap water *ad libitum* during the entire period of the study (8 weeks). The animals were randomly assigned into the following 3 groups (15 animals each): control normotensive group, hypertensive-untreated group, and group of hypertensive rats daily treated with *T. vulgaris* (100 mg/kg body weight; orally by gastric intubation for 8 consecutive weeks). In groups 2 and 3, hypertension was induced by ligation of left renal artery. Systolic blood pressure, body weight, and the plasma concentrations of creatinine and cholesterol were

measured in all groups at the beginning and at the end of the study.

Measurement of systolic blood pressure

At the beginning and at the end of the study, systolic blood pressure (mmHg) was recorded in pre-warming animals using the tail-cuff plethysmographic non-invasive method (Letica LE 5100, panlab, Barcelona, Spain). Several systolic blood pressure readings were recorded for each rat, and four median systolic blood pressure readings in which the differences were within 10 mmHg, were averaged. The average of four median readings was used as the mean systolic blood pressure.

Blood biochemistry assessments

The blood samples were collected at the beginning of the study (from tail veins) and at the end of the experiments after 8 weeks (from vena cava after anesthesia and scarification process) into non-heparinized microtubes. The samples were centrifuged to obtain their corresponding sera. The sera samples were individually separated into new microtubes and used for blood biochemistry analysis. Commercially available commercial test kits and an autoanalyzer system were employed in measurement of the serum levels of creatinine (mg/dl) and cholesterol (mg/dl).

Isolation of thoracic aorta for histological and ultra-structure examinations

The animals were sacrificed by an overdose of anesthesia (Phenobarbital 60 mg, intraperitoneally). Following thoracotomy and laparotomy, the thoracic aorta was dissected, cleaned of connective tissue excised and immediately immersed in heparinized media to make sure that there is no blood clot on collected area. Each isolated aorta was divided into 2 parts. The first part was immersed in 10% formal saline embedded in paraffin blocks, sectioned into 5 microns thick tissue sections, and stained with haematoxyline and eosin (H & E) for histopathological examination under a light microscopy. The other part was immersed into 2.5% glutaraldehyde and then underwent chemical fixation, dehydration, and drying, and photographed using transmission electron microscopy (Lice Stereoscan 260 England).

Statistical analysis

Data obtained were recorded as mean \pm standard error of mean (SEM). Student's t-test was used and both t-test and probability (p) values were estimated. The results were considered significant when the two tailed p value was less than 0.05.

RESULTS

Treatment with *T. vulgaris* improves body weight, blood pressure, renal function, and cholesterol level in hypertensive rats

As shown in Table 1, ligation of renal artery in rats resulted in significant decrease in body weight and significant increases in the systolic blood pressure, serum creatinine (as a biochemical index of renal function) and cholesterol levels, compared with controls. By contrast, these values were not significantly different among rats with ligated

Table 1. Initial and final body weight, systolic blood pressure, the serum levels of creatinine and cholesterol of normal controls and rats with induced hypertension and then treated or not treated with *Thymus vulgaris* for 8 consecutive days (100 mg/kg/day).

Parameter	Control		Hypertensive-untreated		Hypertensive-treated	
	Initial	Final	Initial	Final	Initial	Final
Body weight (g)	205.4±2.2	223.5±2.6*	214.6±3.2	197.6±4.2*	211.4±2.4	229.5±2.6*
Blood pressure (mmHg)	131± 3.02	133±1.67 ^{NS}	130±2.62	186±2.53*	133±1.43	138±3.12 ^{NS}
Creatinine (mg/dl)	1.0±0.15	0.9±0.1 ^{NS}	0.9±0.09	2.08±0.12*	0.8±0.06	0.90±0.0 ^{NS}
Cholesterol (mg/dl)	198.2±12.5	202±11.1 ^{NS}	201.3±7.9	272.8±9.2*	200.7±9	209.8±11.8 ^{NS}

The results are expressed as mean ± SE. * $P < 0.05$ and NS = non significant versus initial values according to Student *t*-test.

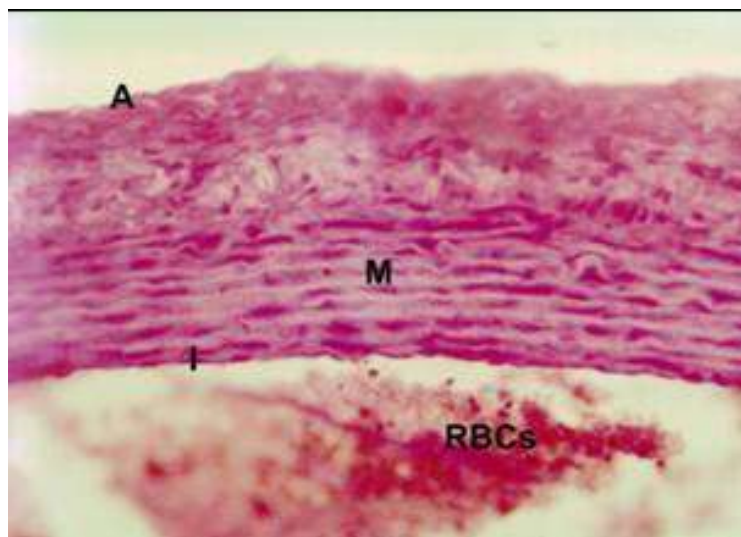


Figure 1. A photomicrograph of thoracic aorta of normotensive control rats showing tunica intima (I), thick tunica media (M) and thin adventitia (A) with red blood cells in lumen (RBCs) ×200.

renal artery and treated with *T. vulgaris* and controls (Table 1).

Effects of *T. vulgaris* on histology and ultra-structure of thoracic aorta in hypertensive rats

Light microscopic findings

As shown in Figure 1, the wall of thoracic aorta of normotensive control rats has tunica intima, thicker tunica media and thin adventitia with blood inside its lumen. The light microscopic findings indicated that the lumen of thoracic aorta of hypertensive-untreated rats adsorbed more debris and red blood cells (Figure 2) than that of controls (Figure 1); however, these observations almost disappeared when these animals were treated with *T. vulgaris* (Figure 3). Moreover, in these hypertensive-untreated rats, the aortic endothelium was coarse, wrinkled, and protuberant, with thicker tunica media and thicker-fibrosed tunica adventitia (Figure 2). In contrast,

treatment of these hypertensive rats with *T. vulgaris* (Figure 3) minimized smooth muscle cell mass, kept both lumen diameter and media thickness to near normal and prevented alterations in media/lumen ratio. As a result of treatment, part of the aortic wall was regular and well arranged and the other part was still disarranged and irregular (Figure 3).

Ultrastructural findings by transmission electron analysis

Ultrastructure examination of the tunica intima of thoracic aorta of normal control rats by transmission electron microscopy showed the presence of well differentiated internal elastic lamina, thick media with fenestrated elastic membrane enclosing in between smooth muscle, collagen and reticular fibres, and containing red blood cells in its lumen (Figure 4). On the other hand, in hypertensive-untreated rats, there was an increased extracellular matrix with dispersion of their cell nuclei.

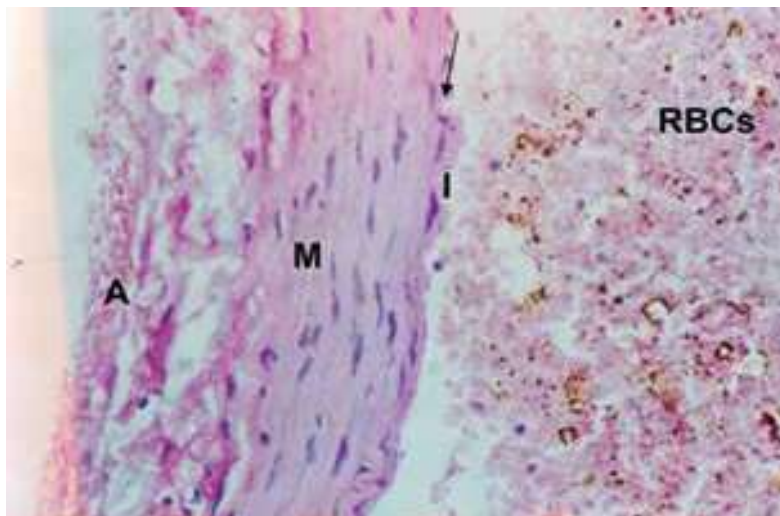


Figure 2. A photomicrograph of thoracic aorta of hypertensive-untreated rats showing aortic lumen containing debris and blood cells (RBCs), tunica intima (I), thick tunica media (M) and thick, fibrosed tunica adventitia (A) with interrupted, wrinkled endothelium (arrow) $\times 200$.

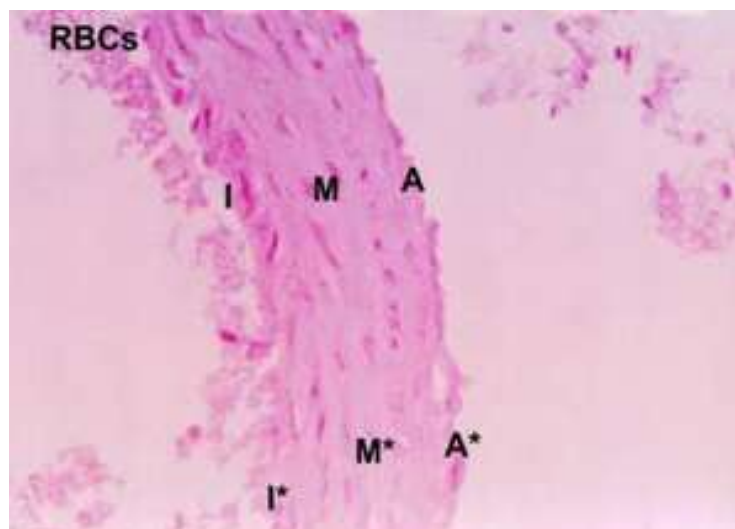


Figure 3. A photomicrograph of thoracic aorta of hypertensive rats treated with *T. vulgaris* and showing regular pattern of tunica intima (I), thick tunica media (M) and thin adventitia (A) with irregular pattern of tunica intima (I*), media (M*) and adventitia (A*) and RBCs in lumen $\times 200$.

Tight junctions were discontinuous and less well organized, and nuclei appeared round with pyknotic cells. Moreover, tunica media became thicker with marked cytoplasmic vacuulations (Figure 5). When these hypertensive rats were treated with *T. vulgaris*, these aortic ultrastructures showed the presence of thick, disarranged tunica media with irregular and euchromatic nuclei (Figure 6). Tunica intima limited externally by internal elastic lamina which was fused with tunica media and was also shown with red blood cells inside the lumen (Figure 7).

DISCUSSION

Hypertension is a major risk factor of cardiovascular diseases associated with vascular endothelial dysfunction and end-organ lethal damage (Vilela-Martin et al., 2011). Structural alterations of endothelium in vessels observed in hypertension indicated their potential contribution to the development or maintenance of the high blood pressure. The vascular endothelium is an active, dynamic tissue that controls many important functions, including regulation of vascular tone, maintenance of blood circu-

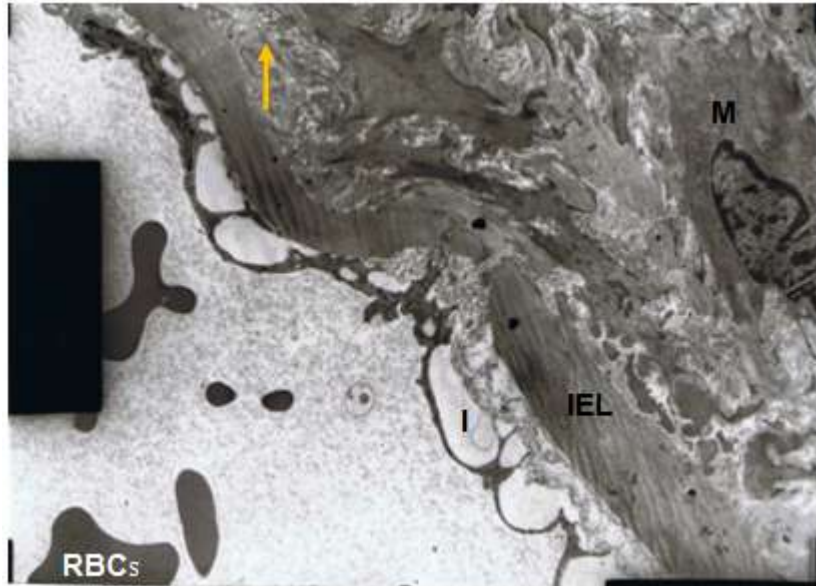


Figure 4. An electron micrograph of thoracic aorta of normotensive control rats showing tunica intima (I), thick tunica media (M), internal elastic lamina (IEL), collagen and reticular fibers (arrows) and red blood cells in lumen (RBCs) $\times 7000$.

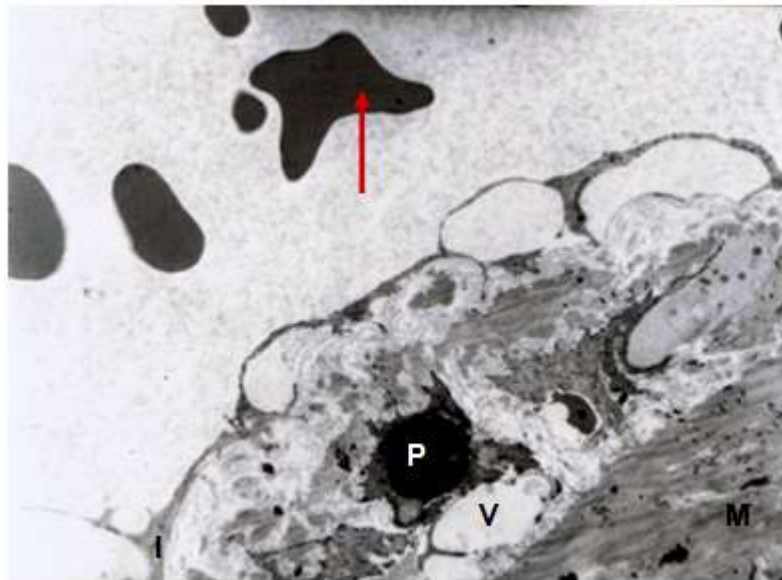


Figure 5. An electron micrograph of thoracic aorta of hypertensive-untreated rats showing tunica media (M), vacuolated cytoplasm (v), and pyknotic cells (p) with blood cells in its lumen (arrow) $\times 7000$.

lation, fluidity, coagulation, and inflammatory responses. Therefore, the impairment of endothelial structure may contribute to endothelial dysfunction that may initiate or contribute to changes in cell adhesion, lipid deposition, and other early steps leading to vascular diseases (Galley and Webster, 2004; Hayden et al., 2012). In the present study, we have examined the possible antihypertensive effect of the herbal plant, *T. vulgaris*, as

well as its possible protective effects against hypertension-induced aorta damage in hypertensive rats. The most important finding of this study was that therapy with aqueous extract of *T. vulgaris* produced antihypertensive effect and reduced the incidence and severity of hypertension-induced alterations in aortic structures of rats. The rennin-angiotensin system performs a basic function in regulating blood pressure,

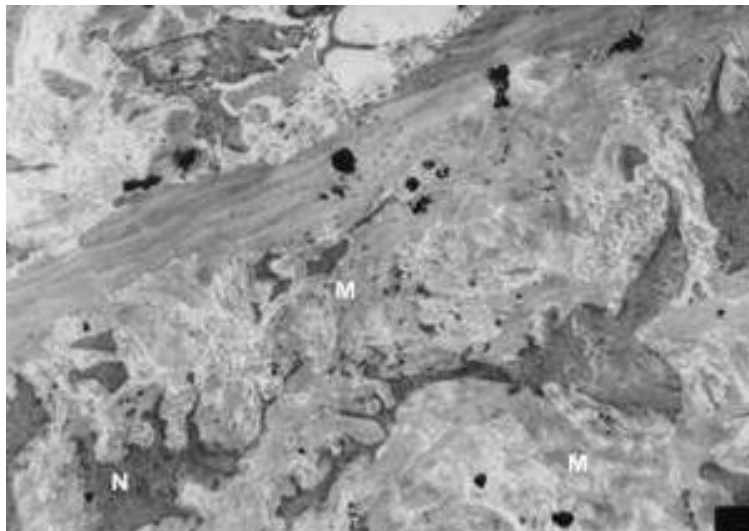


Figure 6. An electron micrograph of thoracic aorta of hypertensive rats treated with *T. vulgaris* and showing thick, disarranged tunica media (M) and irregular euchromatic nucleus (N) $\times 7000$.

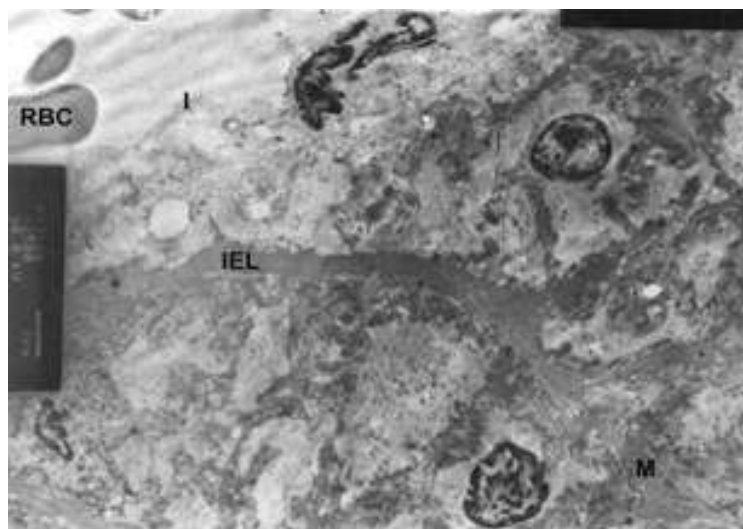


Figure 7. An electron micrograph of thoracic aorta of hypertensive rats treated with *T. vulgaris* showing tunica intima (I), limited externally by internal elastic lamina (IEL) fused with tunica media (M), with RBCs in lumen $\times 6000$.

and renal ischemia is an important cause of renovascular hypertension. In support, basal blood pressure of normal control rats in the present work was in the range 130 to 136 mm Hg and was comparable to that reported by Woodworth et al. (1990); however, it was significantly increased secondary to renal artery ligation.

Impaired endothelial-dependent vascular relaxation was observed in offspring of hypertensive patients (Esch et al., 2002) and animals as well (Liu et al., 2002). Our results showing local damage of endothelial cells of the aorta of hypertensive untreated rats indicated that it might affect endothelial integrity and permeability and activate

inflammatory processes, which can initiate atherogenesis in a vessel (Aguila et al., 2004). Since no such prominent structural alterations were demonstrated in the aortic endothelium of normotensive control rats, it is possible to attribute this vascular injury to the induced hypertension. Interestingly, blood pressure was shown to be significantly decreased and the levels of hypertension-related aortic injury were found to be significantly reduced when these animals were treated with *T. vulgaris*. Although, the underlying mechanisms of the observed antihypertensive effect of *T. vulgaris* are unclear, it might be attributed to its direct smooth muscle relaxant and

vasorelaxant properties. Moreover, the significant drop of systolic BP in *T. vulgaris*-treated hypertensive rats as shown in this study was previously reported and explained by Aguila et al. (2004) that *T. vulgaris* reduced the BP by suppressing the vasoconstrictor reactivity to noradrenalin and rennin-angiotensin-aldosterone system.

In this work, the light microscopic findings showed that the aortic lumen of hypertensive-untreated rats contained excess debris and red blood cells (RBCs) than either normotensive controls or hypertensive-*T. vulgaris* treated rats. It is well known that the vascular endothelium functions as a barrier between tissue and blood. Vascular endothelial dysfunction is thought to be critical in the development of vascular diseases such as inflammation, atherosclerosis, and thrombosis (Deanfield et al., 2007). Recently, it has been reported that the extent of RBCs adhesion to vascular endothelium is correlated with the incidence of vascular complications and the severity of the disease (Wautier and Wautier, 2011). In this concept, Kiefmann et al. (2008) reported that RBCs adhesion induced inflammation in hypoxic vascular endothelium by producing reactive oxygen species (ROS) that diffuse to endothelial cells of adjoining blood vessels. Furthermore, Huertas et al. (2012) showed that adhered RBCs are responsible for activation of multiple proinflammatory gene transcriptions in hypoxic vascular endothelium.

It has been demonstrated that hypertensive experimental rats are hyper-responsive to lipid peroxidation productions and to inflammatory stimuli (Török et al., 2006). Moreover, various reports have suggested that damage to the vascular endothelial cells with hypertension may promote accumulation of macromolecules particularly lipoproteins in the intima (Xue et al., 2005; Török et al., 2006). In support with these facts, we detected here a significant increase in serum cholesterol level in untreated hypertensive group which may accelerate the development of pathological changes in hypertensive angiopathy. This also coincided with ultra structure findings of the present study which showed various damaged sites in the endothelium of the aorta. Interestingly, both cholesterol level and aortic injury were significantly reduced in the hypertensive and *T. vulgaris* treated animals. The previously published facts that *T. vulgaris* has strong antioxidant and free radical scavenging, anti-inflammatory, anti-hyperlipidemic, anti-platelet, anti-thrombin properties (Goun et al., 2002; Arcila-Lozano et al., 2004; Braga et al., 2006; El-Nekeety et al., 2011) might also explaining its observed protective effects against hypertension-induced hypercholesterolemia and aortic injury. Accumulating evidence indicates that vascular remodeling and increased apoptosis and ultra-structural destruction contribute to end-organ damage in hypertension, including hypertension associated heart failure and renal dysfunction (Intengan and Schiffrin, 2001; Hayden et al., 2012). In agreement, we observed here a significant elevation in the serum creatinine level in untreated hypertensive rats but not in *T. vulgaris* treated animals. This elevation in serum

creatinine might be secondary to the drop of number of functioning nephrons as previously demonstrated by Cullen-McEwen et al. (2003).

Conclusion

Our results indicate that hypertension is associated with the sub-cellular injury of aortic endothelial cells that may accelerate the arterial dysfunction in uncontrolled hypertensive patients. More interestingly, supplementation with *T. vulgaris* as an herbal remedy has shown remarkable antihypertensive effect and marked improvement on hypertension-related biochemical changes and aortic vascular damage in rats.

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