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Aqueous Extract of Thymus Vulgaris-induced Prevention of Kidney Damage in Hypertensive Adult Male Albino Rat: Biochemical and Ultrastructural Study

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Abstract: Different metabolic disorders including hypertension cause renal damage. The aim of this study was to study the effect of Thymus Vulgaris (TV) on kidneys of induced hypertensive rats through biochemical and ultrastructure examination. Thirty three adult male albino rats were divided into 3 groups 11 animals each (control, untreated and treated). The first (control) group was fed and followed up for 8 weeks by measuring the systolic BP. In the second group, hypertension was induced by ligation of left renal artery to have systolic BP >150 mmHg and was followed up till the end of study. The third group in which hypertension was induced were given TV as 100 mg/kg body weight daily. The surviving 11 rats per each group were weighed again and killed with an overdose of phenobarbital and blood was collected to measure the blood chemistry. Following laparotomy, right kidney was dissected and excised from each rat and divided into 2 parts: one part was immersed in 10% formal saline and prepared for light microscopic examination. The other parts was immersed into 2.5 % glutaraldehyde and prepared for electron microscopic examination using transmission microscopy in faculty of medicine Zagazig university Egypt. A Significant increased was noticed in creatinine and cholesterol in untreated in contrast to control and treated hypertensive rats. There was significant reduction of Blood Pressure (BP) on using TV in contrast to untreated group. In untreated hypertensive group some cortical fields showed scars surrounding obsolete shrinkage glomeruli with inflammatory infiltrates with reciprocal dilatation of their Bowman's capsules. Ultrastructure examination showed thick glomerular endothelium with obliterated endothelial fenestrations. In treated hypertensive group the rat kidneys showed preserved cortical appearance and normal trilaminar structure but with focal thickening. Induced hypertension in rats affected renal tissues with biochemical alterations which all can be improved and/or prevented by using Thymus vulgaris herbal extract.

Key words: Hypertensive, kidney damage, thymus vulgaris, biochemical - ultrastructural, creatinine - cholesterol

INTRODUCTION

Treatment of chronic diseases by traditional drugs had failed to have the expected and hoped influences on reducing cardiovascular morbidity and mortality due most likely to widespread structural changes at the level of hypertensive nephropathy (Xue *et al*., 2005).

Different metabolic disorders including hypertension, aging process of the renovascular system and the correlation between systolic blood pressure and glomerulosclerosis may illustrate the potential renal damage in hypertensive patients (Gonzalez-Albarran *et al*., 2003).

Thymus vulgaris extract is a complex mixture extracted from leaves and has different pharmacological activities not only as preventive but also as a treatment of some diseases with full relief of their symptoms (Burt, 2004).

The current research studies introduced many antihypertensive agents with limited adverse side effects, however the recognition of herbal remedies as another therapeutic alternatives (anti-thrombin activities) have been considered as an important goal for the pharmaceutical industries through their components (Goun *et al*., 2002).

Thymus vulgaris displayed anti-platelet properties in human, has a role in controlling blood sugar and also inhibits abnormal cell growth, it also helps in maintaining healthy cholesterol and blood pressure (ElKayam *et al*., 2003).

Thymus vulgaris is widely distributed through different regions as Morocco, Russia and Egypt (Chabarovs, 2007).

The main pharmacological effects of Thymus vulgaris are being anti-oxidant, anti-thrombin and potent anti-hypertensive through maintaining renal function, creatinine clearance and inhibiting mechanical forces acting on arterial wall so preventing excess hardening and thickening (Goun *et al*., 2002; Kulisic *et al*., 2004).

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Therefore, our research aimed to study the effect of Thymus vulgaris on kidneys of hypertensive rats through biochemical and ultrastructure examination and to compare the results with those of control rats in order to prevent parenchymatous changes that occur in early stages of hypertensive nephropathy.

MATERIALS AND METHODS

Thirty three adult male albino rats were used in this study which has been carried out in the animal house of the Faculty of Medicine, Umm Al Qura University, Makkah. The rats were randomly collected and divided into 3 groups (11 animals each).The first group was considered as a control, the second as hypertensive untreated while the third group as hypertensive treated with Thymus Vulgaris.

All 33 animals were weighed before starting the study and initial blood samples were collected from the vein of the eye lid for cholesterol and creatinine. They were maintained in cages less than 12 hr cycles of light and dark and the room temperature was kept at 20-25°C.

The first (control) group was fed with a standard diet and tap water *ad lipitum* plus citrate buffer and followed up through the period of study by measuring the systolic blood pressure every 2 weeks for 8 weeks (period of study). In the second group, hypertension was induced by ligation of left renal artery to raise systolic BP to more than 150 mmHg and they were randomly assigned on a daily placebo (water) during the 8 weeks, starting from the raised systolic BP point, via a stomach tube at 8.00 am. Systolic blood pressure was measured every 2 weeks till the end of study by a tail cuff plethysmographic noninvasive methods (Letica LE 5100, panlab, Barcelona, Spain). The third group in which hypertension was induced as mentioned above and systolic pressure was above 150 mmHg were treated with Thymus Vulgaris as 100 mg/kg body weight daily in doses 20 mg/block and given orally by gastric intubation for 8 weeks. The extract of Thymus Vulgaris was prepared by mixing 1 gm powdered leaves with 100 ml distilled water. The mixture was boiled for 10 minutes and left to cool for 15 min. The aqueous extract was filtered using filter paper to remove the particulate matter (0.2 mm) then the filtrate was freely dried (Lyophilized) and reconstituted in 1.5 ml of distilled water (100 mg/kg body weight). After 8 weeks of starting the study, the aorta was exposed, cannulated with polyethylene catheter and connected to a blood pressure monitor transducer 8/38 to monitor the mean arterial pressure and then blood samples were collected from the aortic cannula in heparinized tubes for final analytical assays for cholesterol and creatinine. The rats were finally weighed and killed with an overdose of phenobarbital (60 mg intraperitoneal). Following laparotomy, right kidney was dissected and excised from each rat and both were divided into 2 parts: one part was immersed in 10% formal saline. After fixation the tissues were embedded in paraffin blocks and 5 microns thick tissue sections were cut. The specimens were then stained with hematoxylin and eosin (H&E). The other parts of right kidney was immersed into 2.5% glutaraldehyde. After the process of chemical fixation, dehydration, drying and conductivity enhancement, all samples were observed and photographed using transmission microscopy (Lice Stereoscan 260 England) in Faculty of medicine Zagazig university Egypt. The findings in this study were recorded as mean, 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 Statistical analysis was performed with SPSS software version 17 (Budneck, 1987).

RESULTS

Biochemical findings: Table 1 and 2 showed that Initial and final body weight (gm), serum creatinine (mg/dl), cholesterol levels (mg/dl) and systolic blood pressure (mmHg) of control (-), untreated hypertensive and treated hypertensive rats. A highly significant decrease in body weight ($p<0.01$) was noticed in untreated group which accompanied by a highly significant increase in creatinine and cholesterol levels ($p<0.02$) in the same group in contrast to treated and control groups which showed insignificant increase. Also there was a significant increase in BP in untreated group ($p = 0.001$) and a highly significant reduction in BP in treated group ($p<0.001$) from initial to final which not followed by reduction in creatinine and cholesterol levels in the same group.

Histological findings

Right kidney

Control group

Light microscope study: Light microscopical study showed transverse and oblique sections of normal renal convoluted tubules with prominent healthy blood vessels (Fig. 1-2).

Table 1: Initial and final body weight (gm), serum creatinine (mg/dl), cholesterol levels (mg/dl) and systolic blood pressure (mmHg) of control (-), untreated hypertensive and treated hypertensive rats

	Control		Untreated		Treated	
	Initial	Final	Initial	Final	Initial	Final
Body weight (gm)	205.4±2.2	223.5±2.6***	214.6±3.2	197.6±4.2**	211.4±2.4	229.5±2.6**
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}	203.3±7.9	272.8±11.2***	200.7±9	214.8±11.8^{NS}
Blood pressure	130±3.02	136±1.67**	163±2.62	186±2.53***	166±1.43	138±3.12***

Table 2: Final body weight (gm), serum creatinine (mg/dl), cholesterol levels (mg/dl) and systolic blood pressure (mmHg) of control (-), untreated hypertensive and treated hypertensive rats

	Control	Untreated	Treated
	---------- Final ----------		
Body weight (gm)	223.5±2.6	197.6±4.2**	229.5±2.6^{NS}
Creatinine (mg/dl)	0.9±0.1	2.08±0.12^{NS}	0.90±0.07^{NS}
Cholesterol (mg/dl)	202±11.1	272.8±11.2^{NS}	214.8±11.8*
Blood pressure	136±1.67	186±2.53***	138±3.12^{NS}

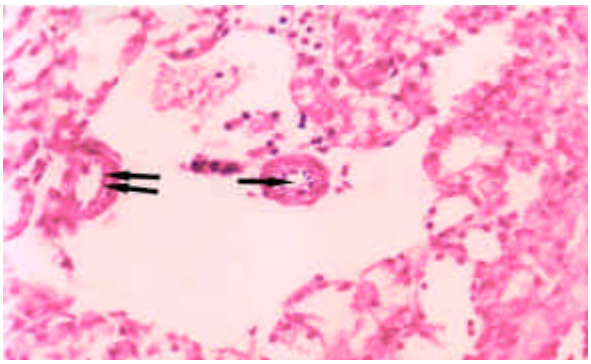

Fig. 1: A photomicrograph of right kidney of controlled adult male albino rat showing preserved tubular lumens (arrow) with normal blood vessels (double arrows) x400

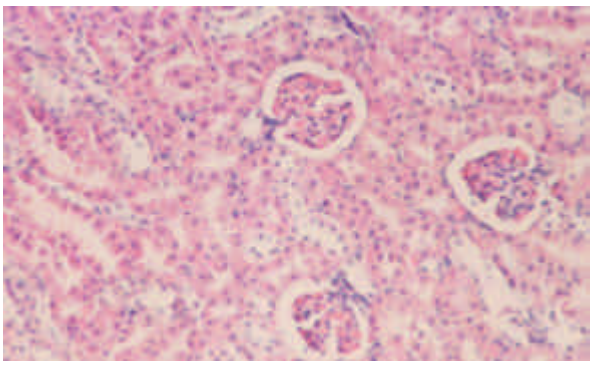

Fig. 2: A photomicrograph of right kidney of controlled adult male albino rat showing the normal histology of renal parenchyma (H&E x200)

Ultrastructure findings: The proximal convoluted tubules of renal cortex were resting on basement membranes and had apical profuse regular microvilli, elongated mitochondria with basal infoldings and spherical or oval euchromatic nuclei with prominent nucleoli.

The distal convoluted tubular cells had similar ultrastructure features to proximal convoluted tubules, in particular, the basal infoldings and elongated mitochondria. However they had few short irregular microvilli (Fig. 3).

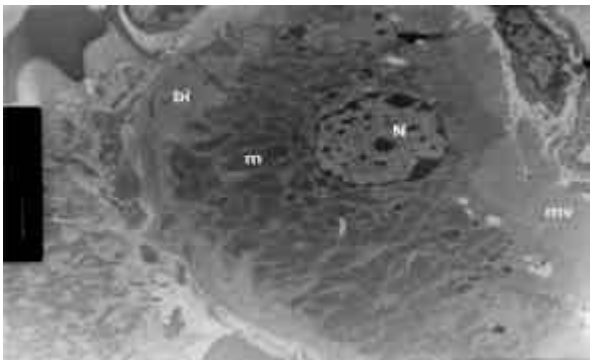

Fig. 3: An electron micrograph of right kidney of the control adult male albino rat showing proximal convoluted tubules with apical microvilli (mv) and euchromatic Nucleus (N) with normal basal infoldings (bi) and elongated mitochondria (m). x6500

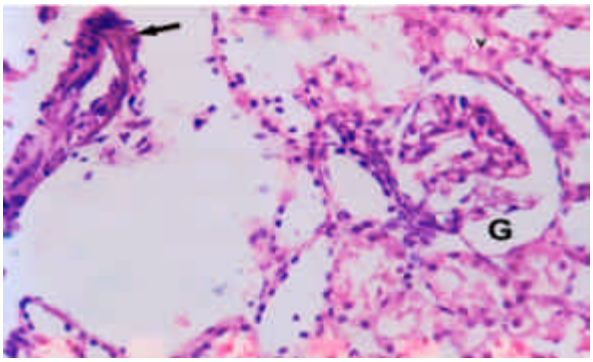

Fig. 4: A photomicrograph of right kidney of untreated hypertensive adult male albino rat showing thickened fibrosed blood vessels (thick arrow) and shrinkage Glomerulus (G) with inflammatory infiltrates (thin arrow). x400

Untreated hypertensive group

Light microscope study: Light microscopical study showed some cortical fields' scars surrounding obsolete glomeruli with different extension of inflammatory infiltrate. The glomerular tuft of most of renal corpuscles was shrinkage with reciprocal dilatation of their Bowman's capsules. The wall of the capsule was thickened with focal areas of hydropic degeneration in the tubules. The shape of the cortex was altered by appearance of necrosis and fragmentation with dissolution of the basement membrane. Both arterioles and small arteries among the cortical areas showed degenerative changes (Fig. 4-9).

Ultrastructure examination: Ultrastructure examination showed thick glomerular endothelium and basement membrane with loss of its normal trilaminar appearance. Broad foot processes were also observed. (Fig. 10).

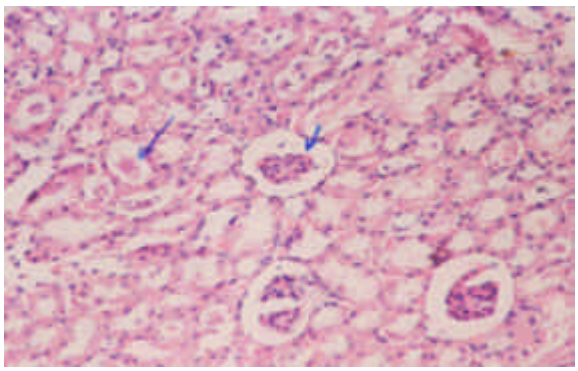

Fig. 5: Photomicrograph of right kidney of untreated hypertensive adult male albino rat showing eosinophilic proteinaceous casts in the lumen of renal tubules (H&E x200)

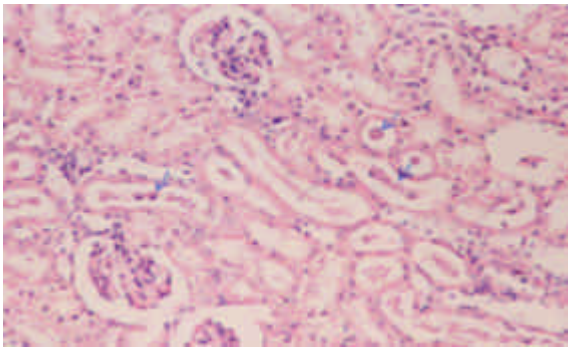

Fig. 6: Photomicrograph of right kidney of untreated hypertensive adult male albino rat showing proteinaceous cast in the lumen of some renal tubules (H&E x200)

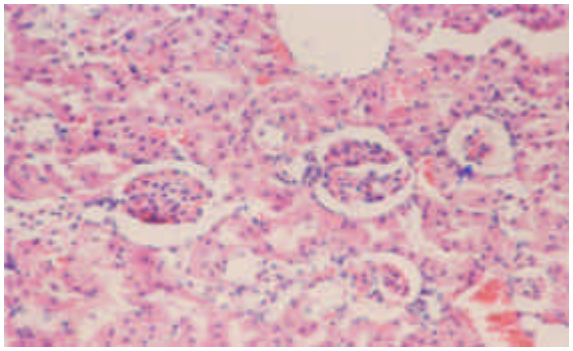

Fig. 7: Photomicrograph of right kidney of untreated hypertensive adult male albino rat showing atrophy of some glomerular tufts (H&E x200)

Some proximal tubular cells showed multiple, disarranged basal infoldings, as well as electron dense lysosomes-like structures and mesangial matrix with heterochromatic nuclei (Fig. 11).

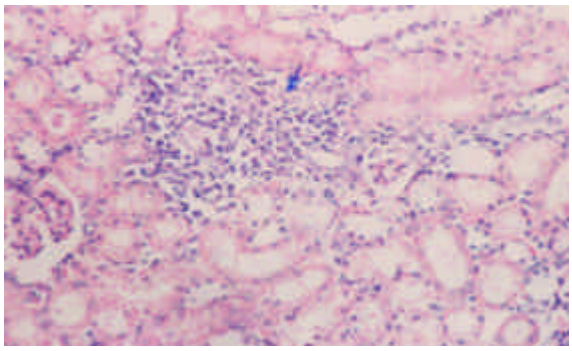

Fig. 8: Photomicrograph of right kidney of untreated hypertensive adult male albino rat showing focal area of tubular necrosis associated with mononuclear cells infiltration (H&E x200)

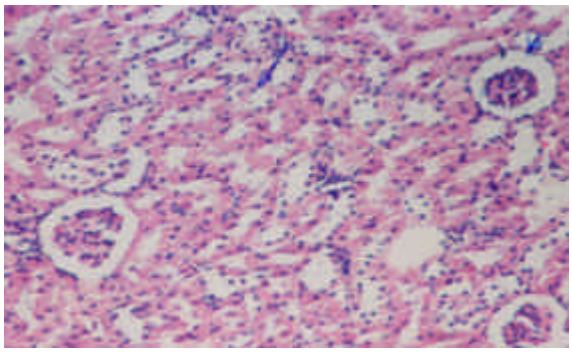

Fig. 9: A photomicrograph of right kidney of untreated hypertensive adult male albino rat showing atrophy of glomerular tufts and pyknosis of nuclei of renal tubular epithelium (H&E x200)

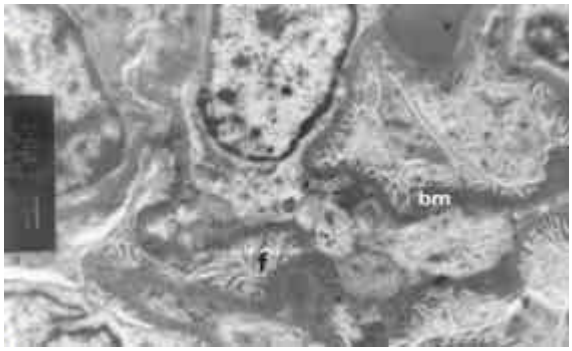

Fig. 10: An electron micrograph of right kidney in untreated hypertensive adult male albino rat showing thickened glomerular basement membrane (bm) and broad foot processes (f) x6500

Distal tubular cells appeared preserved with the exception of some small nuclei; in addition multiple

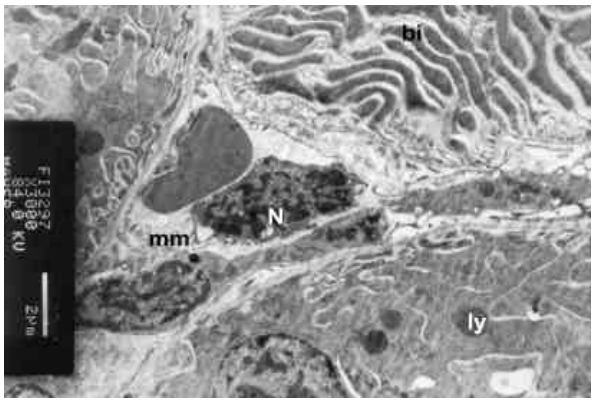

Fig. 11: An electron micrograph of right kidney in untreated hypertensive adult male albino rat showing disarranged basal infoldings (bi) and multiple lysosomes (ly) with heterochromatic Nucleus (N) and messangial matrix (mm). x10000

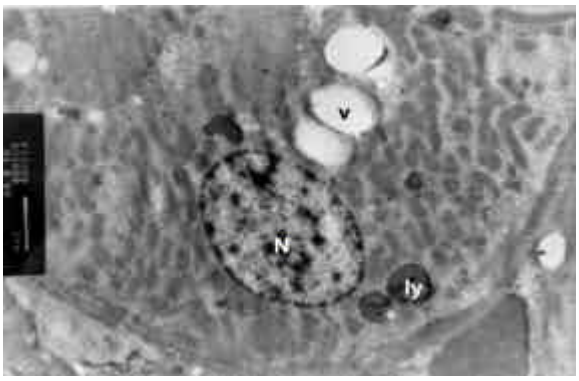

Fig. 12: An electron micrograph of right kidney in untreated hypertensive adult male albino rat showing few lysosomes (ly) and multiple cytoplasmic vacuoles (v) with euchromatic Nucleus (N). x10000

lysosomes and cytoplasmic vacuoles with euchromatic nuclei have been shown (Fig. 12).

Treated hypertensive group

Light microscopical findings: After 8 weeks of treatment of hypertension, the rat kidneys showed preserved cortical structure, less frequently appearance of inflammatory infiltrates. The renal vessel regained its normal configuration with intact and patent lumen with thick tunica media as well as preserved media/lumen ratio (Fig. 13-16).

Ultrastructure findings: Ultrastructure findings revealed that the glomerular basement membrane appeared as a normal trilaminar structure with primary and secondary foot processes with endothelial nuclei bulging into the lumen (Fig. 17). The proximal tubular cells showed arranged basal infoldings, rounded mitochondria and euchromatic nuclei (Fig. 18).

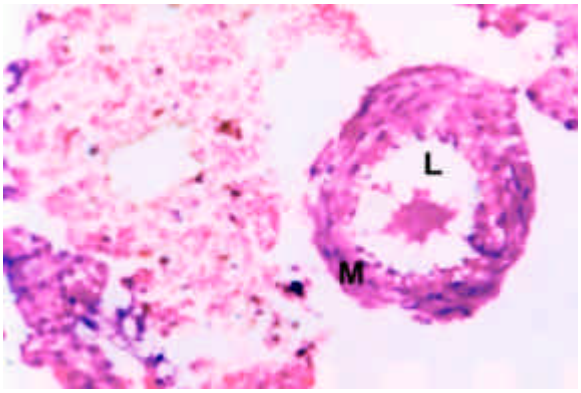

Fig. 13: A photomicrograph of right kidney of treated hypertensive adult male albino rat showing regaining of vascular Lumens (L) and slightly thickened blood vessels with tunica Media (M) and preserved media/ lumen ratio. x450

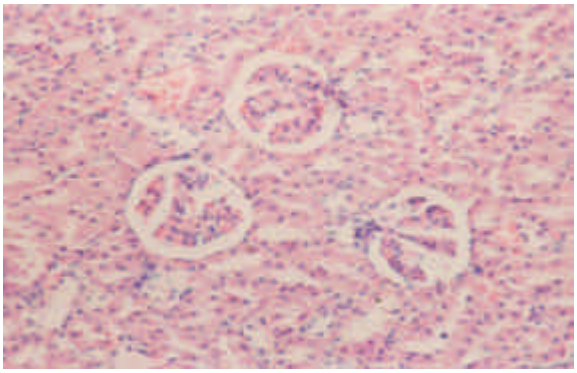

Fig. 14: A photomicrograph of right kidney of treated hypertensive adult male albino rat showing no histopathological abnormalities (H&E x200)

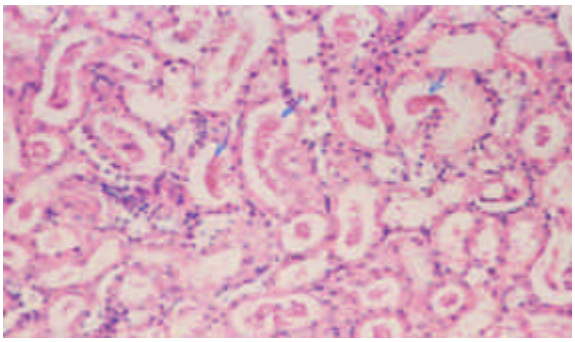

Fig. 15: A photomicrograph of right kidney of treated hypertensive adult male albino rat showing eosinophilic proteinaceous cast in the lumen of some renal tubules (H&E x200)

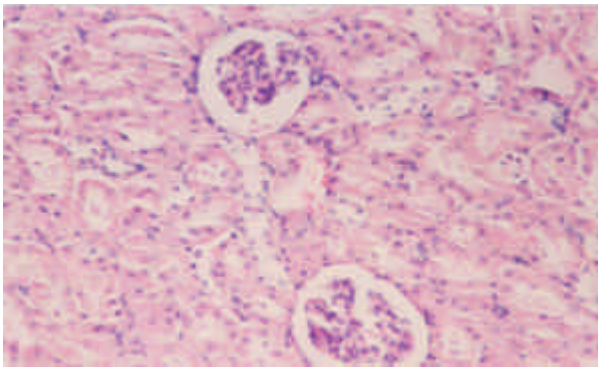

Fig. 16: A photomicrograph of right kidney of treated hypertensive adult male albino rat showing apparent normal renal parenchyma (H&E x200)

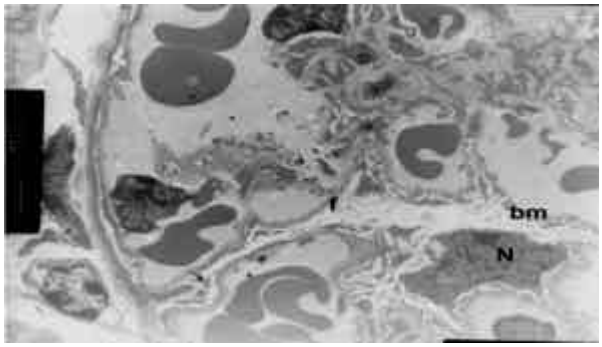

Fig. 17: An electron micrograph of right kidney of treated hypertensive adult male albino rat showing primary and secondary foot processes (f), basal membrane (bm) with endothelial nuclei bulging into the lumen (N) x5500

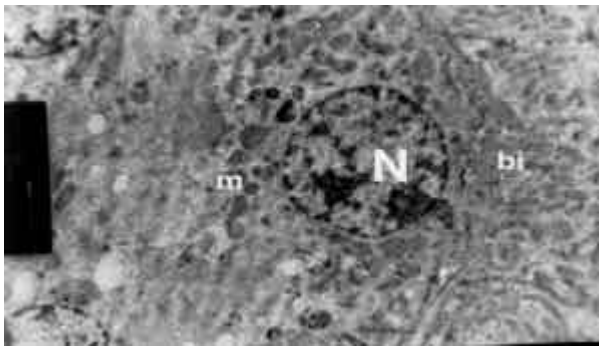

Fig. 18: An electron micrograph of right kidney of treated hypertensive adult male albino rat showing proximal convoluted tubules with arranged basal infoldings (bi) with rounded mitochondria (m) and euchromatic Nucleus (N) x10000

DISCUSSION

Various reports have suggested that damage to the vascular endothelial cells of the renal artery associated with hypertension may promote accumulation of macromolecules particularly lipoproteins in the intima (Xue *et al*., 2005). The apparent epidemic of hypertension is strongly related to life style and economic changes. The damage of vascular endothelial cells may be due to exposure to oxygen radical stress associated with aging and hypertension which cause vascular pathological changes like a lot of protruding villi and granular structures (Itoh *et al*., 2002). In this study a significant increase in total serum cholesterol was found in untreated hypertensive group in contrast to non-significant changes in treated and control groups which may accelerate the development of glomerulosclerosis and pathological changes in both hypertensive nephropathy and angiopathy. These results agreed with Grone *et al*. (1989) who reported that dietary lipids (fat and cholesterol) aggravate lesions and nephron loss in rats with chronic hypertension and antihypertension effects caused by TV suggested their use in the prevention of hypertension through reduction of cholesterol while our findings of raised cholesterol in untreated hypertensive rats didn't agree with Gonzalez-Albarran *et al*. (2003).

The present study supported the idea of beneficial effect of TV intake supplementation to hypertensive rats. The major effect of TV intake was observed to reduce both BP and glomerular hypertrophy and decreasing loss of glomeruli which coincided with Cullen-McEwen *et al*. (2003) who reported deleterious effects on kidney nephrons and functions as well as blood pressure in aged heterozygous rat.

In this work control of blood pressure by TV in treated hypertensive group has markedly delayed and slightly improved the histological changes that appeared in untreated hypertensive rats which coincided with Eddouks *et al*. (2002) who have demonstrated the importance of intensive BP control preferably systolic BP less than 130 mmHg to delay progression of renal disease in hypertension and diabetes.

The significant drop of systolic BP in treated hypertensive rats with TV as shown in this study agreed and fully explained by Englerm *et al*. (2003) and Aguila *et al*. (2004) who reported that TV reduced the BP by reduction of vascular reactivity to noradrenaline blunting the rennin-angiotensin-aldosterone system by decreasing adrenal synthesis of aldosterone.

In this study serum creatinine has shown a significant increase in untreated hypertensive group which was due most likely to a drop of number of functioning nephrons agreed with Cullen-McEwen *et al*. (2003) who reported that different aspects should be considered concerning loss of glomeruli in hypertension and estimation of glomeruli number and glomerular volume which all allowed to inform about the renal response to hypertension progression and/or antihypertensive treatment efficiency.

Other study reported that the common renal injury of hypertension is a complex dynamic process involving several players such as inflammatory agent, cytokines, vasoactive agents and enzymes participating in extracellular matrix assembly, anchoring or degradation (Gonzalez-Albarran *et al*., 2003).

The progression starts in glomeruli with loss of separation of tuft and Bowman's capsule by forming cell bridge passing on damage from lost and/or damaged nephrons to so far healthy nephrons (Kriz and Le-Hir, 2005; White *et al*., 2005) which agreed with our recorded results about the ultrastructure changes that developed in Bowman's capsule and glomerular tufts including shrinkage of glomeruli, glomerulosclerosis, widening of space beneath Bowman's capsule, decreased number of glomeruli and thickened glomerular basement membrane with prominent vesicular cytoplasm.

After treatment with TV in this work, the renal and vascular damage have been minimized and the pathological changes in renal and vascular architecture have been delayed and/or prevented which agreed with Medeiros *et al*. (2006) who documented renal cortex remodeling in streptozotocin-induced diabetic spontaneously hypertensive rats treated with olive oil, palm oil and fish oil and disagreed with Susic *et al*. (1990) who reported that spontaneous-induced diabetes mellitus lowered blood pressure in spontaneously hypertensive rats which had not been investigated in the present work.

Allicin from oil of oregano helps to maintain health, cholesterol and blood pressure levels and plays a role in controlling blood sugar and abnormal cell growth and contain carvocrol and thymol which have anti-inflammatory effect (Medeiros *et al*., 2006).

A significant weight gain was recorded in our study in all groups of experimental animals which didn't agree with Aguila *et al*. (2004) who didn't record any changes in body weight of experimental animals on long-term intake of edible oils.

Hypertension accelerated the aging process of both kidney and aorta like hypertrophic changes which led to structural destruction (Osicka *et al*., 2003) while in the present study TV has reduced and alleviated pathological damage in both kidney which coincided with Lermioglu *et al*. (1997) who related these effects due most likely to reduced toxins released in hypertensive rats treated by TV.

Different antihypertensive medications are now available in the market with different biological effects in hypertensive human subject. However very little data are published about the histopathological abnormalities resulted from using of such extracts and also no enough references were reported to deal with histological effect of Thymus vulgaris on kidney of the experimental animals.

Conclusion: In summary, induced hypertension in rats have had marked deleterious effects on body weight, creatinine and cholesterol as well as serious histological and parenchymatous changes of both kidney and vascular tissues. Intake of Thymus vulgaris as a herbal remedies has shown marked improvement and/or prevention of biochemical changes and both renal and vascular parenchymatous damage in experimental animals.

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