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Effect of Valsartan and Folic Acid on Vascular Reactivity, Leptin Hormone and Some Biochemical Parameters in Fat Diet-Induced Obese Male Rats

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Abstract

Background: The development of a chronic low-grade inflammatory state has been shown to play a central role in the development of metabolic complications associated with obesity.

Aim of Study: The aim was to determine the impact of oral administration of valsartan and folic acid to fat diet-induced obese male rats on the vascular reactivity & on the serum levels of leptin and some inflammatory and oxidative stress markers.

Material and Methods: Fifty male albino rats of local strain were randomized into five equal groups: Control, obese, valsartan-treated obese, folic acid-treated obese and combined valsartan and folic acid-treated obese groups. Obesity was induced by feeding high fat diet and treatment was done for indicated groups for 16 weeks. Thereafter, weight and length were measured, and rats were subjected for rat tail-measurement of Systolic Blood Pressure (SBP). Then, fasting retro-orbital blood samples were collected for measuring serum Total Cholesterol (TC), Triglycerides (TGs), Low Density Lipoprotein-Cholesterol (LDL-C), High Density Lipoprotein-Cholesterol (HDL-C), Malondialdehyde (MDA), C-Reactive Protein (CRP) and leptin levels, as well as serum Glutathione Peroxidase (GPx) and Superoxide Dismutase (SOD) activities, andurinary nitrite/nitrate (NOx) level. Rats were tested for Vascular Reactivity to Acetylcholine (VRACh) and sodium nitroprusside (VRSNP) by Doppler flowmeter. Lastly, rats were sacrificed and abdominal visceral fats were resected and weighed.

Results: High fat diet resulted in marked impairment of all the measured parameters, except VRSNP. Treatment of obese rats with valsartan resulted in improvement of BMI, adipose tissue mass, TC, LDL, CRP, leptin, NOx, MDA, SBP and VRACh; however, total body weight, TGs, HDL-C and antioxidant enzyme activities were not affected. Folic acid administration to obese rats improves all serum lipid parameters, increased GPx and SOD activities, and showed a better effect than valsartan on NOx, leptin andVRACh, but less effect on CRP. On the other hand, folic acid had no effect on the anthropometric parameters and SBP of obese rats. Combined treatment showed a reduction in total body weight, and a significant better improvement of all the measured parameters, except MDA, than those of isolated valsartan or folic acid treatment, with normalization of MBI, TGs, LDL-C, NOx and VRACh.

Conclusion: Both valsartan and folic acid, when administered separately, ameliorate the detrimental effects of fat diet-induced obesity in rats. Combined valsartan and folic acid prophylactic treatment of fat diet-induced obese rats has an additive beneficial effect on the associated dyslipidemia, inflammatory state, oxidative stress, leptin hormone level, and endothelial dysfunction that is possibly achieved by different mechanisms of action.

Key Words: *Diet-induced obesity – Folic acid – Valsartan – Vascular reactivity – Leptin.*

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Introduction

OBESITY is a public health problem and can be classified as a world epidemic that leads to an elevation in medical costs. Over the last decade, we have realized the ramifications posed by obesity, yet the worldwide burden due to obesity is said to have doubled since the 1980s. Obesity is characterized by excess energy intake resulting in an expansion of adipose tissue depots, visceral adiposity, hypertrophy, hyperplasia, and adipocyte dysfunction [1].

In the obese state, the excess growth in adipose tissue has been shown to alter the adipokine profile, thereby initiating a detrimental cascade of metabolic disturbances. The development of a chronic low-grade inflammatory state has been shown to play a central role in the development of metabolic complications associated obesity, since it has been linked to the development of endothelial and microvascular dysfunctions, and insulin resistance [2].

Systemic oxidative stress is part of the numerous biological alterations reported during chronic obesity. An inverse relationship exists between body fat, visceral obesity, and antioxidant defence markers in obese individuals [3].

Adipokines exert their effects in a paracrine or endocrine manner. Leptin hormone plays important roles in the regulation of food intake, energy expenditure, metabolism, the neuroendocrine axis and immune function [1]. In addition, a significant positive correlation exists between leptin and Blood Pressure (BP), independent of body adiposity, in both normotensive and hypertensive individuals [4].

Impaired Nitric Oxide (NO)-mediated vasodilatation has been demonstrated in obesity, and functional leptin receptors are present on endothelial cells. Several human studies suggest that leptin contributes to endothelial dysfunction or damage in some pathological states; however, the actions of leptin to modulate endothelial function remain controversial [5]. The renin-angiotensin system (RAS), including angiotensinogen, renin, Angiotensin-Converting Enzyme (ACE), angiotensin II (Ang II), and its receptors, is involved in the maintenance of systemic blood pressure. In pathological state, Ang II also functions as a local biologically active mediator in the progression of cardiovascular remodeling and inflammation through Ang II type-1 receptors (AT 1R) [6].

The present study was conducted to investigate the effect of valsartan and folic acid administration to high energy fat diet-induced obese male rats on the deleterious effects of some associated metabolic errors and endothelial dysfunction.

Material and Methods

The study protocol was approved by the Ethical Committee of Faculty of Medicine, Menoufia University, and was carried out in strict accordance with the recommendations in the Guide for The Care and Use of Laboratory Animals of The Egyptian Universities. This study followed a randomized controlled animal experiment design, and was carried out at Medical Physiology Department, Faculty of Medicine, Menoufia University, Egyptfrom 8th of November 2016 till 1st of March 2017.

Experimental animals and groups:

Fifty male albino rats of local strain at 6 weeks of age, weighing 180-210gm each, were used in this study. Animals were housed in spacious wire mesh, fully ventilated cages (80 X 40 X 30cm), at room temperature with free access to water. Rats were randomly divided into five equal experimental groups, each of 10 animals:

- *Control (C) group:* Rats of this group were fed a standard laboratory chow diet, and were given 1ml of distilled water by gastric gavage, once daily for 16 weeks.
- *Diet-induced obese (DO) group:* Rats of this group were fed a highly palatable, high-energy fat diet for 16 weeks [7], and were given distilled water as in C group.
- *Valsartan-treated diet-induced obese (VDO) group:* Rats of this group were fed fat diet as in DO group, and were given valsartan, in a dose of 30mg/kg Body Weight (BW) dissolved in 1ml of distilled water, by gastric gavage, once daily for 16 weeks [8].
- *Folic acid-treated diet-induced obese (FDO) group:* Rats of this group were fed fat diet as in DO group, and were given folic acid, in a dose of 5mg/kg BW [9] dissolved in 1ml of distilled water, by gastric gavage, once daily for 16 weeks.
- *Combined valsartan- and folic acid-treated diet-induced obese (VFDO) group:* Rats were fed fat diet as in DO group, and both valsartan and folic acid were given as in VDO and FDO groups, respectively.

Dietary composition:

Regular rat chow diet was purchased from El-Wadi Company, Egypt. Highly palatable, high energy fat diet was formed by mixing the following constituents together: Sucrose of local source, lard of local source, cholesterol powder, extra pure, egg yolk of local source and regular chew (16.7%, 7%, 2%, 8.3% and 66% of total weight, respectively).

Anthropometric measurements and biochemical assays:

At the end of the experimental period (after 16 weeks), rats were weighed using Electronic sensitive balance (Oertling RC62, UK) with assessment of naso-anal length, and subjected for measurement of Systolic BP (SBP) by Pneumatic pulse transducer (Harvard apparatus Ltd, Aden Berge, England) [10]. Then, animals were fasted overnight and retro-orbital blood samples were collected and serum was separated to measure serum Total Cholesterol (TC), Triglycerides (TGs) and High Density Lipoproteins-Cholesterol (HDL-C). The serum glutathione peroxidase (GPx) and Superoxide Dismutase (SOD) activities, andthe serum Malondialdehyde (MDA) and urinary nitrite/nitrate (NOx) were measured by spectrophotometer using calorimetric

diagnostic kits purchased from Biodiagnostic Company (Cairo, Egypt), according to manufacturer's instructions. Low Density Lipoprotein-Cholesterol (LDL-C) was calculated according to the formula of LDL-C = TC – (HDL-C + TGs/5) [11].

Serum leptin and C-Reactive Protein (CRP) levels were determined with sandwich ELISA technique using their specific kits, purchased from DRG International Inc. (USA), according to the manufacturer's instructions.

Vascular reactivity to acetylcholine and sodium nitroprusside:

Thereafter, rats were subjected for testing the vascular reactivity to the endothelium-dependent vasodilator acetylcholine (VRACh), using a freshly prepared acetylcholine (ACh) in a dose of 10-10 Mole (El-Gomhoria Company, Egypt), and vascular reactivity to the endothelium-independent vasodilator sodium nitroprusside (VRSNP). sodium nitroprusside (SNP) was freshly prepared in a dose of 3 µg (El-Gomhoria Company, Egypt). Reactivity was measured in the rat abdominal aorta using Doppler flowmeter (HADECO, Japan). The technique for use the probe and application of the flowmeter was according to the method described by Ruan et al., [12]. Detection of the flow with the pulsed Doppler system is dependent on changes of the emitted ultrasound frequency produced by the reflection of the signal off moving blood cells. The change in frequency (Doppler shift) is proportional to the velocity of blood cells in a vessel. This velocity shift is directly proportional to volume flow in the vessels to which the probes are attached [12]. The vascular reactivity to the drug was calculated as the percent ratio of velocity of blood flow after its administration to the initial baseline velocity of blood flow.

Lastly, rats of all groups were sacrificed and the abdominal visceral fat was resected from each rat to estimate its weight.

Statistics analysis:

Data were collected, tabulated and statistically analysed using a personal computer with the "statistical package for social sciences" SPSS, Version 20, for windows (SPSS Inc., Chicago, Illinois, USA). The data were expressed as mean ± Standard Deviation (SD).Analytic statistics were done with the non-parametric test, Mann-Whitney U-test with Bonferroni's correction [13]. The level of statistical significance was taken at $p \leq 0.05$.

Results

Table (1) demonstrates the mean ± SD of thefinal body weight, BMI and adipose tissue mass, as well as serum CRP and leptin hormone levels in C, DO, VDO, FDO and VFDO groups. Table (2) demonstrates the mean ±SD of SBP, VRACh and VRSNP of the different experimental groups. Figure (1) illustrates the mean ± SD of the fasting serum lipid profile (TC, TGs, LDL-C and HDL-C) in the different groups. Fig. (2) illustrates the mean ± SD of the urinary NOx and serum MDA levels, as well as the antioxidant GPx and SOD enzyme activities in C and obese (DO, VDO, FDO and VFDO) groups.

Table (1): Final total body weight, body mass index (BMI), adipose tissue mass, and serum C-reactive protein (CRP) and leptin hormone levels in control (C), diet-induced obese (DO), valsartan-treated diet-induced obese (VDO), folic acid-treated diet-induced obese (FDO) and combined valsartan-and acid-treated diet-induced obese (VFDO) groups.

Parameter	C	DO	VDO	FDO	VFDO
Final body weight (g)	267.4± 10.3	351.5± 19.4*	341.2± 19.2*	353.8± 17.4*	312± 18.1 *#¥Ω
BMI (gm/cm^2)	0.61±0.055	0.81±0.080*	0.72±0.089*#	0.79±0.166*¥	0.66±0.049#¥Ω
Adipose tissue mass (g)	10.3± 1.35	18.7± 1.40*	15.3± 1.72*#	18.4± 1.7*¥	13.5± 1.72*#¥Ω
CRP (ng/ml)	0.69±0.09	4.30±0.53*	2.61±0.37*#	3.44±0.39*#¥	1.55±0.19*#¥Ω
Leptin (ng/ml)	0.19±0.02	1.72±0.20*	1.28±0.14*#	0.97±0.10*#¥	0.62±0.07*#¥Ω

- Results are expressed as mean ± SD (n=10). Significance was considered when p-value was ≤0.05. The marks *, #, ¥ and Ω beside values indicate that the values are significantly different, when compared with the corresponding values of C, DO, VDO and FDO groups, respectively.

Table (2): Systolic blood pressure (SBP) and vascular reactivity assessment by Doppler flowmeter in response to acetylcholione (VRACh) and sodium nitroprusside (VRSNP) in control (C), diet-induced obese (DO), valsartan-treated diet-induced obese (VDO), folic acid-treated diet-induced obese (FDO) and combined valsartan-and folic acid-treated diet-induced (VFDO) groups.

Parameter	C	DO	VDO	FDO	VFDO
SBP (mmHg)	108±8	138± 11*	106±9#	134±9*	105±8#Ω
VRACh (% of initial)	172.5± 18.74	96.7±7.89*	138.8± 14.12*#	121.8± 11.75*#	163± 18.34#¥Ω
VRSNP (% of initial)	279.17±41.20	265.56±37.25	263±42.79	258±38.81	271±47.44

- Results of vascular reactivity are represented as a percentage of the initial values before injection of the vasodilator agent. Results are expressed as mean ± SD (n=10). Significance was considered when p-value was ≤0.05. The marks *, #, ¥ and Ω beside values indicate that the values are significantly different, when compared with the corresponding values of C, DO, VDO and FDO groups, respectively.

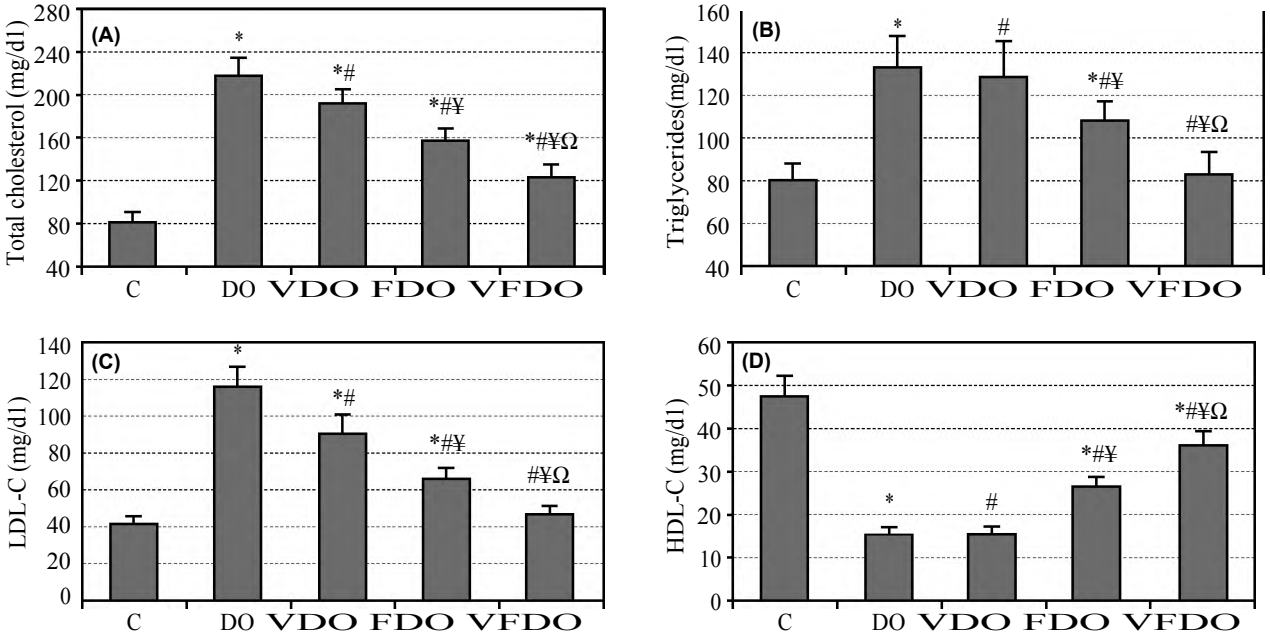

Fig. (1): Fasting serum total cholerterol (panel A), triglycerides (panel B), low density lipoprotein-cholesterol (LDL-C) (panel C) and high density lipoprotein cholesterol (HDL-C) (panel D) in control (C), diet-induced obese (DO), valsartan-treated diet-induced obese (VDO), folic acid-treated diet-induced obese (FDO) and combined valsartan-and folic acid-treated diet-induced obese (VFDO) groups.

Number of rats in each group were ten (n=10). Error bars represent standard deviation. Significance was considered when p-value was ≤0.05. The marks at top of columns *, #, ¥ and Ω indicate that values are significantly different when compared with the corresponding values of C, DO, VDO and FDO groups, respectively.

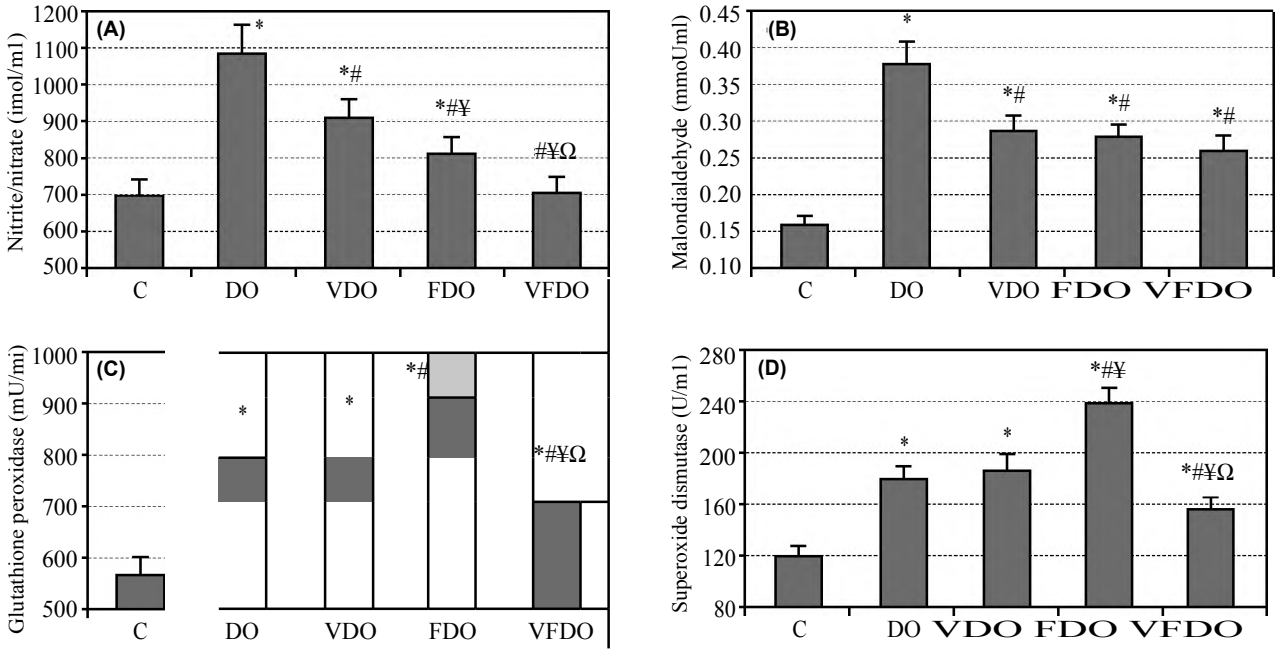

Fig. (2): Urinary nitrite/nitrate (panel A) and serum malondialdehyde (panel B) levels, and serum glutathione peroxidase (panel C) and superoxide dusmutase (panel D) activities in control (C), dirt-induced obese (DO), valsartan-treated diet-induced obese (VDO), folic acid-treated diet-induced obese (FDO) and combined valsartan-and folic acid-treated diet-induced obese (VFDO) groups.

Number of rats in each group were ten (n=10). Error bars represent standard deviation. Significance was considered when p-value was ≤0.05. The marks at top of columns *, #, ¥ and Ω indicate that values are significantly different when compared with the corresponding values of C, DO, VDO and FDO groups, respectively.

Discussion

The present study clearly demonstrated that feeding of male albino rats a high energy fat for 16 weeks caused significant increases in final body weight, BMI and adipose tissue mass, when compared with those of control group. Also, significant higher fasting serum TC, TGs and LDL-C, and a significant lower fasting serum HDL-C than normal were observed in obese animals.

This can be explained by inhibition of hormone-sensitive lipase-mediated lipolysis in adipose tissue that causes increased delivery of fatty acids to the liver and synthesis of very-low-density lipoprotein. In addition, increased levels of free fatty acids can decrease mRNA expression or activity of lipoprotein lipase in adipose tissue and skeletal muscle leading to decreased lipoprotein lipase-mediated lipolysis of chylomicron-TGs, which promotes hypertriglyceridemia **[14]**.

High fat diet obese rats showed a significant increase in the urinary NOx and serum MDA levels as well as in the serum GPx and SOD activities, when compared with those of the normal rats. In addition, serum CRP showed a significant increase in obese group when compared with those of normal animals.

Increased NOx level in serum has been reported in a group of diseases including cardiovascular diseases, hyperlipidemia, diabetes and metabolic syndrome; this transformation of eNOS from a protective enzyme to a contributor to oxidative stress has been observed in several in vitro models, in animal models of cardiovascular diseases, and in patients with cardiovascular risk factors **[15]**.

The increased activity of serum GPx and SOD observed in our study can be explained by the increase in biological availability of superoxide anion radicals and hydrogen peroxide in adipose tissue in obesity leading to an adaptive increase in the antioxidant enzymes activities **[16]**.

Systemic inflammation were evaluated with CRP, which is a marker that has role in vascular inflammation and is found associated with the prognosis of cardiovascular outcomes in many trials **[17]**. A significant positive association between adiposity indicators and CRP was reported in high fat diet fed rats in other studies as well **[18]**.

In the current study, as expected, serum leptin hormone level was significantly increased in the obese rats compared to normal ones. This can be explained by a feedback mechanism due to a reduced hypothalamic leptin receptor expression as seen in rodent models of diet-induced obesity **[19]**. In vitro investigation demonstrated the ability of human CRP to directly inhibit leptin binding to its receptor and related cell signaling in HEK293 cells or hypothalamic neurons **[20]**. Interestingly, chronic leptin-mediated central sympathetic activation, originating in the hypothalamus, was found to result in a systemic pressor effect that is believed to play a chief role in obesity-related hypertension **[21]**.

Regarding the effect of fat diet-induced obesity on vascular responses to vasoactive stimuli, the current study showed that high energy fat diet feeding for 16 weeks induced hypo-responsiveness to the endothelium-dependent vasodilator ACh, but not to the endothelium-independent vasodilator SNP. Therefore, this impairment could be explained in the light of obesity-induced endothelial dysfunction, possibly attributed by dyslipidemia, leptin resistance and oxidative stress. Mervaala et al., **[22]** data support the notion that endothelial dysfunction in double-transgenic rats is mediated, at least in part, by ROS generated by oxidative stress. In fact, FFAs can stimulate ROS production through activation of NAD(P)H oxidase in endothelial cells **[23]**. Although NO is a potent vasodilator that improves vascular health and function, elevated oxidative stress due to increased production of ROS contributes to NOS uncoupling **[24]**, quenches available NO, and depletes substrate and cofactor availability, resulting in the production of superoxide radicals rather than NO **[25]**, all of which have been implicated in the pathogenesis of vascular endothelial dysfunction.

The present experiment investigated the effect of valsartan treatment on the high fat diet-induced obesity in rats. The results indicate that valsartan prophylactic treatment effectively counteracts some of the disrupting effects seen in the rat obesity model. The BMI and adipose tissue mass of the valsartan-treated obese rats were significantly decreased compared to the obese animals that did not receive any treatment. In agreement, Fogari et al., **[26]** found that valsartan produced a significant reduction in BMI and HOMA-IR index. Angiotensin II receptor blockade by valsartan per se was suggested to affect adipocyte biology and promote the formation of small, metabolically efficient adipocytes **[27]**.

Our study demonstrates that fasting serum TC and LDL-C levels were significantly decreased in obese rats treated with valsartan compared to obese rats that were not treated; however, TGs and HDL-

C were not affected significantly. These findings were consistent with other study [28], who found that after a year of treatment with valsartan in normotensive type 2 diabetic patients there was a significant decrease in TC and LDL-C with a non-significant affection of TGs.

Our data shows that valsartan treatment of obese rats resulted in a significant reduction in urinary NOx and serum MDA levels, while the increased GPx and SOD enzyme activities observed in non-treated obese group were preserved, with insignificant difference between the two groups. Interestingly, a normalization of NOx excretion by valsartan was observed in rats by Mervaala et al., [22]. This could be explained by the enhanced antioxidant activity caused by valsartan that prevents NOS uncoupling [29]. Janic et al., [30] observed that low-dose valsartan alone, produced important anti-inflammatory and anti-oxidative effects in apparent healthy middle-aged male humans by increased antioxidant defenses, as measured by total antioxidant status and glutathione peroxidase. Valsartan pretreatment in a rat model of myocardial ischemia/reperfusion injury kept the SOD activity, but suppressed MDA content [31]. In contrast, treatment of hypertensive patients with valsartan but not placebo resulted in a progressive down-regulation of SOD-mRNA expression in polymorphonuclear leukocytes and a reduction in erythrocyte SOD activity [32].

The serum CRP of valsartan-treated obese group showed a significant decrease compared to that of obese animals that did not receive any treatment. This is consistent with prospective trials, in which valsartan reduced CRP levels in a manner independent of degree of blood pressure reduction [33]. This finding suggests a potential role for valsartan in decreasing the adipokine dysregulation associated with obesity. However, Unlu el al., [17] did not notice any significant decrease in the levels of CRP after treatment of newly diagnosed hypertensive patients with valsartan. Regarding serum leptin, our data have shown that its level is significantly lowered by treatment of obese rats with valsartan, when compared with that of non-treated obese group. This observation was also reported in obese hypertensive patients versus placebo [26].

As expected, SBP of valsartan-treated obese animals was significantly lower than that of non-treated obese group, and significantly indifferent from that of normal rats. This could be explained by the sympatho-inhibitory properties possessed by all angiotensin II receptor type 1 (AT 1) antagonists. Effects of different AT blockers on BP are close to each other, but some studies have shown that valsartan is the most specific, most effective, and the safest drug of all [34].

An improvement in the vascular reactivity to the endothelium-dependent ACh, assessed by Doppler flowmeter, was observed with valsartan-treated versus non-treated obese animals in this work. Mervaala et al., [22] also demonstrated in their study that chronic AT 1 receptor blockade effectively normalized endothelium-dependent vascular relaxation in double-transgenic rats. The improvement in endothelial function could be explained by activation of eNOS by valsartan [29] and decreasing oxidative stress [35], thus preventing uncoupling of NOS, and so restoring NO activity and playing a protective role in endothelial cells [29], together with the upregulation of the anti-oxidant enzymes GPx and SOD resulting in a decrease in the oxidative stress state, and the decreased serum TC, serum LDL-C, urinary NOxand serum leptin levels. The previously mentioned parameters are suggested to be atherogenic and their decrease can explain this detected improvement in endothelial-dependent dilatation upon valsartan treatment of obese animals.

Furthermore, blockade of the AT1 receptor by valsartan was found to enhance the alternative pathway of production of NO via the AT2 receptor in wild-type mice [36]. A complete correction of the endothelial dysfunction by valsartan with normalization of vascular reactivity was even reported by Mervaala et al., [22].

The impact of folic acid administration on the high fat diet-induced obese rats was also investigated in this work. Folic acid had no effect on the final body weight, BMI and adipose tissue mass of obese rats. On the other hand, serum TC, TGs and LDL-C showed a decrease to levels that were significantly lower than values of non-treated and valsartan-treated obese rats, but still significantly higher than those in normal rats. Serum HDL-C was significantly increased more than that of non-treated and valsartan-treated obese rats and less than that of control ones. The improvement in lipid parameters could be explained by the reduction of serum homocysteine level caused by folic acid supplementation [37].

A significant drop in urinary NOx level was observed in obese group treated with folic acid versus non-treated and valsartan-treated obese group, probably by preventing uncoupling of NOS by combating the associated oxidative stress with obesity. In consistency, it was found that 5-

methyltetrahydrofolate, the main form of folate in the circulation, can influence the enzymatic activity of uncoupled eNOS by reducing the superoxide generation more than can be explained by just a scavenging effect, and increasing NO synthesis [38].

A significant reduction in serum MDA was founded in our study after folic acid administration to obese animals, which indicates that folic acid minimizes lipid peroxidation in high fat diet-induced obesity. In agreement, folic acid pretreatment to humans exposed to acute oral fat load was associated with decreased production of plasma MDA [39]. Another study of Huang et al., [40] showed that decreasing dietary folate intake resulted in graded increases in plasma homocysteine concentrations that was correlated with increased liver lipid peroxidation. However, our findings are in contrast to the results of other study in which folic acid treatment alone had no effect on plasma MDA concentrations yet still improved vascular endothelial function in patients with coronary artery disease [41].

Surprisingly, folic acid-treated obese group showed a significant increase in GPx and SOD activities, when compared with obese animals that did not receive any treatment. This could be explained by the persistence of the overproduction of the antioxidant enzymes as a compensatory mechanism, but with a decrease in their utilization due to a relative reduction in ROS production compared to non-treated obese animals.

Our data shows that CRP of folic acid-treated obese group was significantly decreased compared to non-treated obese ones. The folic acid lowering effect of total homocysteine could explain this reduction in serum CRP. This finding suggests a potential therapeutic role for folic acid in the protection from atherogenesis and cardiovascular diseases as it reduces the circulating level of some inflammatory mediators independently of weight change.

Serum leptin hormone was significantly reduced in the present work when folic acid is administered to high fat diet-obese rats versus non-treated obese ones. The effect of folic acid dietary treatment on leptin receptor-L mRNA expression levels was seen in pigs, where the treatment lowered leptin receptor-L expression levels in both endometrial and embryonic tissues [42].

In our study, an improvement in the vascular reactivity to ACh in obese rats after folic acid treatment was observed. Endothelial dysfunction observed in healthy humans following an acute oral fat load was abolished by 2 weeks of folic acid pretreatment [39]. Overall, the available clinical data suggest that supplemental folic acid treatment is effective in improving NO-dependent vasodilation in patients with compromised endothelial function, with the exception of patients with chronic kidney disease. Coupled with the in vitro data, it is assumed that this improvement occurs through increased NOS coupling and subsequent increases in NO bioavailability, independent of decreases in plasma homocysteine. The improvement in lipid profile and the remarkable increase in activities of SOD and GPx with the significant decrease in CRP and serum leptin in folic acid-treated obese animals may help in improvement in vascular function and reactivity to ACh.

The major finding in our study is that the treatment of high fat diet-induced obese rats with a combination of valsartan and folic acid resulted in a significant decrease in the final body weight, BMI and adipose tissue mass compared to animals treated with either valsartan or folic acid alone, and to the extent that the BMI was showing a non-significant difference from that of regular chow diet fed rats, a result that was not achieved when each of them was administered separately.

Also, serum TGs and LDL-C in combined valsartan and folic acid treatment of obese rats were insignificantly different compared to normal control animals, but serum TC and HDL-C were still significantly higher and lower, respectively, than the control non-obese values; all lipid profile parameters were significantly improved in combined treatment versus treatment with valsartan or folic acid alone. The increase in HDL-C in combined treatment of obese rats is mostly due to folic acid effect, as its mean value is markedly more than that of valsartan-treated obese animals.

Upon valsartan and folic acid combination, urinary NOx level was significantly reduced compared to those of isolated valsartan and folic acid treatment of obese animals. Moreover, combined treatment resulted in normalization of the urinary NOx level in obese rats as it was statistically non-significant compared to normal chew diet fed rats. Serum MDA showed a nonsignificant variation between valsartan-treated, folic acid treated and combined treated groups, and remained significantly more than that in normal chew diet fed rats. Regarding anti-oxidant enzymes, the elevated GPx and SOD enzyme activities observed in folic acid-treated group were compromised in combined treatment as shown by significantly lower values

than those of non-treated and animals treated with either folic acid or valsartan. However, the antioxidant activities did not return to those of the normal chew diet fed rats. This could be explained by a probable minimization of the compensatory mechanisms associated with oxidative stress due to the observed marked relief of the latter by the combined treatment of folic acid and valsartan.

Combined valsartan and folic acid administration markedly lowered serum CRP level, and showed significant difference when compared with other obese groups whether non-treated or treated with valsartan or folic acid alone, however, level did not return to normal value. Regarding serum leptin level, a significant drop was observed in combined treatment of obese rats with valsartan and folic acid compared to treatment with either of them, but value was still significantly higher than normal.

SBP was significantly decreased compared to those of non-treated and folic acid-treated obese groups, but insignificantly different from that of valsartan-treated obese and control ones. Apparently, this decrease in BP is mostly due to the AT 1 receptor blocking effect of valsartan.

A better improvement of vascular reactivity to ACh, assessed by Doppler flowmeter, was significantly found with combined valsartan and folic acid administration to obese group than that of obese animals treated with either valsartan or folic acid alone. Interestingly, a non-significant variation in vascular reactivity to ACh was observed between combined valsartan and folic acid-treated obese and normal rats.

In our study, the reduction in NOx production together with correction of dyslipidemia and the significant decrease in CRP and serum leptin could explain this marked improvement in the endothelium-dependent vasodilatation induced by ACh after combination of valsartan and folic acid in obese rats.

Conclusion:

Our findings indicate that combination of valsartan and folic acid simultaneously improved metabolic errors and endothelial dysfunction associated with high fat diet-induced obesity to a greater extent than their isolated mono-treatment in male albino rats. This could be explained by additive beneficial effects on anthropometric parameters, lipid profile, NOx and MDA production, the antioxidant enzymes GPx and SOD activities, as well as the significant decrease in serum CRP and leptin levels, possibly through different mechanisms of action. Prolonged clinical trials of different doses of valsartan and folic acid are needed to determine if combined treatment regimens are effective in decreasing the incidence of the related cardiovascular diseases in obese patients with attenuated endothelial function.

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تأثير عقار فالسارتان وحمض الفوليك على تفاعلية الأوعية الدموية وهرمون الليبتن وبعض القياسات الكيميائية الحيوية فى ذكور الفئران التى تعانى من السمنة الناجمة عن النظام الغذائى الدهنى

مقدمة: قد يصاحب زيادة ترسب النسيج الدهنى إضطرابات فى التمثيل الغذائى، ويرجع هذا إلى وجود حالة من الإلتهاب المزمن ينتج عنها إضطراب فى وظيفة الغشاء المبطن للأوعية الدموية.

مواد وطرق البحث: تم إجراء هذه الدراسة على خمسون من ذكور الفئران البيضاء من السلالة المحلية وتتراوح أوزانهم بين ١٨٠–٢١٠ جرام لكل فأر، وتم تقسيمهم إلى خمس مجموعات متساوية (١٠ فئران لكل مجموعة): المجموعة الضابطة والمجموعة التى تعانى من السمنة الغذائية والمجموعة التى تعانى من السمنة الغذائية والمعالجة بعقار فالسارتان والمجموعة التى تعانى من السمنة الغذائية والمعالجة بحمض الفوليك والمجموعة التى تعانى من السمنة الغذائية والمعالجة بكل من عقار فالسارتان وحمض الفوليك معا. وفى نهاية التجربة–بعد ١٦ أسبوع– تم قياس أوزان الفئران وطولهم من الأنف إلى الشرج وقياس ضغط الدم الإنقباضى عن طريق الذيل. ثم تم تصوير الفئران طوال الليل وسحب عينات دم من خلف حجاج العين وفصل مصل الدم لقياس مستوى دهون الدم (الكوليسترول، الدهون الثلاثية، الدهون مرتفعة الكثافة) وحساب الدهون منخفضة الكثافة، وكذلك قياس البروتين التفاعلى ج وهرمون الليبتن والمالون ثنائى ألدهيد ونشاط الجلوتاثيون بيروكسيد ونشاط الفائق ديسموتاز. وتم جمع البول لقياس النتريت/نترات. ثم بعد ذلك تم دراسة تفاعلية الأوعية الدموية بالنسبة إلى كل من الأستيل كولين والصوديوم نيتروبروسيد بإستخدام دوبلر لقياس الجريان. وأخيرا تم التضحية بالفئران من جميع المجموعات وإستئصال الدهون الحشوية فى البطن وقياس وزنها.

النتائج: أظهرت نتائج هذه الدراسة أن إتباع نظام غذائى عالى الدهون لمدة ١٦ أسبوع فى ذكور الفئران البيضاء تسبب فى زيادة ذات دلالة إحصائية فى جميع الدلائل مع تغير غير معنوى فى تفاعل الأوعية الدموية لصوديوم نيتروبروسيد بالمقارنة مع القيم المقابلة للمجموعة الضابطة. تسبب تعاطى عقار فالسارتان فى تحسن ذو دلالة إحصائية فى مؤشر كتلة الجسم والكوليسترول الكلى والبروتين التفاعلى جوهرمون اللبتين فى مصل الدم، والنتريت/نترات فى البول. وقد إرتبط هذا مع إنخفاض فى ضغط الدم الإنقباضى وتحسين تفاعل الأوعية الدموية للأستيلكولين. تسبب تعاطى عقار حمض الفوليك فى إنخفاض ذو دلالة إحصائية فى الكوليسترول الكلى فى الدم والدهون الثلاثية والدهون منخفضة الكثافة والبروتين التفاعلى جوهرمون اللبتين فى مصل الدم، مع زيادة فى الدهون مرتفعة الكثافة ونشاط الجلوتاثيونبيروكسيد ونشاط الفائق ديسموتاز. إرتبط هذا مع إنخفاض فى النتريت/نترات فى البول وتحسين تفاعل الأوعية الدموية للأستيلكولين. تسبب الجمع بين فالسارتان مع حمض الفوليك فى إنخفاض ذو دلالة إحصائية فى الوزن الكلى مع تحسن أفضل فى جميع الدلالات التى تم قياسها ما عدا المالون ثنائى ألدهيد. إرتبط هذا مع معدلات طبيعية لمؤشر كتلة الجسم والدهون الثلاثية والدهون منخفضة الكثافة والنتريت/نترات وتفاعل الأوعية الدموية للأستيلكولين.

الإستنتاجات: تعاطى عقار فالسارتان وحمض الفوليك–كلا على حده–يؤدى إلى تحسين الإضطرابات الناجمة عن النظام الغذائى الدهنى. المزج بين عقار فالسارتان وحمض الفوليك يجمع الفائدة من كليهما فى تحسين مستوى الدهون فى الدم والإجهاد المؤكسد وهرمون الليبتن وتفاعلية الأوعية الدموية وذلك بآلية من كل منهما قد تكون مختلفة.