

Effects of Atenolol, Captopril, and Their Combination on Oxidative Stress in Hypertensive Patients

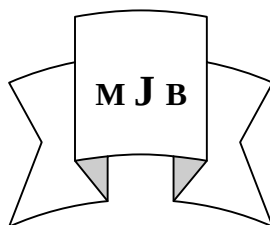
Haidar A. N. Abood

Riyadh H. Hashim

Majid K. Abbass

Abdul Razzak A. Latif

Dept. of Pharmacology, College of Medicine, Babylon University, Hilla, Iraq
P.O. Box (473), Hilla, Iraq.



Abstract

Oxidative stress plays an important role in the pathogenesis of hypertension, and oxidative imbalance involved in the development of endothelial dysfunction and vascular complications like atherosclerosis. It is important nowadays to evaluate the antioxidant effects of antihypertensive drugs in addition to blood pressure lowering effects, since this may provide a better treatment approach and participate in the prevention of complications of hypertension. We studied the antioxidant effects of atenolol, captopril, and their combination on 60 hypertensive patients aged from 45 – 60 years, treated for one year with different treatment lines and compared the results with healthy controls. Serum reduced glutathione (GSH) levels were estimated as a marker of oxidative status in the blood and lipid peroxidation at endothelial cell membrane was evaluated by measuring its byproduct, malondialdehyde (MDA) in the blood utilizing spectrophotometric techniques. Oxidative imbalance was present in all hypertensive patients in comparison with controls. Serum GSH levels significantly reduced and serum MDA levels significantly elevated in patients using atenolol and captopril alone, while patients who used atenolol/captopril combination significantly maintained blood GSH and MDA at levels closer to that of controls. Combination therapy was found to reduce GSH depletion and lipid peroxidation more than captopril, and captopril more than atenolol. In conclusion, combination therapy provides better protection against oxidative stress associated with hypertension, and hence better prevention of complications like atherosclerosis than both atenolol and captopril used alone.

تأثير الأتينولول و الكابتوبريل و اجتماعهما معا على الإجهاد التأكسدي في المرضى المصابين بارتفاع ضغط الدم

الخلاصة

يلعب الإجهاد التأكسدي دوراً مهماً في النشوء المرضي لارتفاع ضغط الدم، ويشترك عدم التوازن التأكسدي في تطوير عطل خلايا بطانة الأوعية الدموية وتعقيدات وعائية مثل تصلب الشرايين. من المهم في الوقت الحاضر تقييم التأثير المانع للتأكسد للأدوية المستخدمة في علاج ارتفاع ضغط الدم، حيث قد يساعد ذلك على إظهار فوائد علاجية جديدة لهذه الأدوية ويشارك في منع التعقيدات التي تصاحب ارتفاع ضغط الدم. لقد تمت دراسة التأثير المانع للتأكسد لعقار الأتينولول و عقار الكابتوبريل و

اجتماعهما معاً على ٦٠ مريض مصاب بارتفاع ضغط الدم معدل أعمارهم من ٤٥ إلى ٦٠ سنة، أعطي المرضى خطوط علاجية مختلفة خلال عام كامل وتمت مقارنة النتائج مع أشخاص أصحاء. تم قياس مستوى الجلوتاثيون المختزل (GSH) كمعيار لحالة الأكسدة في الدم وتم تقييم بيروكسيدية الدهون في أغشية خلايا بطانة الأوعية الدموية عن طريق قياس مستوى الناتج العرضي (MDA) في الدم لجميع المرضى ولأصحاء باستعمال تقنيات طيفية. أظهرت الدراسة وجود عدم توازن تأكسدي لدى جميع المرضى المصابين بارتفاع ضغط الدم مقارنة بالأصحاء. كان هنالك انخفاض ملحوظ في مستويات GSH وارتفاع ملحوظ في مستويات MDA في مصل الدم لدى المرضى الذين استعملوا عقار الأتينولول و عقار الكابتوبريل بصورة منفردة بينما بقي مستواه لدى المرضى الذين استعملوا العقارين معاً أقرب بشكل ملحوظ من تلك التي وجدت عند الأصحاء. إن استخدام الدوائين معاً خفض من نضوب الجلوتاثيون المختزل وقلل من معدل بيروكسيدية الدهون أكثر من استخدام الكابتوبريل لوحده والذي بدوره كان أدأؤه أفضل من استخدام الأتينولول لوحده. يستنتج من ذلك إن استعمال الأتينولول و الكابتوبريل بصورة مجتمعة في علاج ارتفاع ضغط الدم يعطي حماية أفضل ضد الإجهاد التأكسدي و وقاية أفضل من التعقيدات المحتملة مثل تصلب الشرايين من استعمالهما بصورة منفردة.

Introduction

Blood pressure control is the most important component in the management of patients with hypertension. In particular, β -blockers and angiotensin converting enzyme inhibitors (ACEIs) are widely used for the treatment of hypertension [1,2]. In addition to blood pressure control, prevention of complications represents a great challenge in the managements of hypertension, since hypertension is considered as cardiovascular risk factor that increases the risk of development of atherosclerosis through induction of oxidative stress on the arterial wall [3]. Regardless of the underlying pathological mechanisms, oxidative stress seems to be present in all forms of hypertension [4]. Oxidative stress is defined as an imbalance between oxidants and antioxidants in favor of oxidation that plays a key role in the pathophysiology of several major cardiovascular diseases [5]. There are evidences suggesting that angiotensin II plays an important role in stimulating the production of reactive oxygen species (ROS) and the activation of ancient inflammatory mechanism [6,7]. ROS are highly

reactive biomolecules with an unpaired electron in their outer orbital that can cause damage to proteins, lipids, membrane, DNA and alteration in the intracellular environment [8]. Antioxidants are molecules acts as free radical scavengers. Most antioxidants are electron donors and react with the free radicals to form innocuous end products such as water. Thus, they protect against oxidative stress and prevent damage to cells [9]. In hypertension the deficiencies of antioxidant molecules and enzymes can increase the risk of atherothrombotic cardiovascular disease [10]. Some antihypertensive drugs may also exert antioxidant and cytoprotective effect against free radical mediated vascular injury [11,12]. Atenolol and captopril are widely used as antihypertensive drugs in Iraq, and many previous clinical and animal studies observed a possible antioxidant role for ACEIs and β -blockers [13-16]. The aim of this study is to evaluate the effects of atenolol, captopril, and their combination on oxidative stress through estimation of the level of reduced glutathione (GSH) that represents the most important

antioxidant substance in the body [17]. and by studying their effects on lipid peroxidation that represents an important oxidative process in the endothelial cell membranes involved in the development of atherosclerosis and hypertension [3]. This study will provide information about the cytoprotective ability of atenolol and captopril in reducing vascular damage and hence reducing complications, frequently associated with hypertension in addition to their well known ability in lowering blood pressure.

Patients and Methods

1. Patients

The subjects (patients and control) were studied at Al-Mustafa Public Medical Clinic (MPC) in Kerbala city, 80 individuals were included in this study aged from 45 – 60 years, weighted from 72 – 82 kg, 47 were men and 33 were women. None of them refused to participate. They were divided into 4 groups:-

Group A : 20 healthy age-matched individuals (control group).

Group B : 20 hypertensive patients on atenolol (100mg / day) for 1 year.

Group C : 20 hypertensive patients on captopril (100 mg / day) for 1 year.

Group D : 20 hypertensive patients on combination therapy (atenolol 100 mg / day + captopril 50 mg / day) for 1 year.

The doses of the drugs were adjusted to maintain systolic blood pressure between 120 and 140 mmHg, and diastolic blood pressure between 80 and 90 mmHg. Hypertensive patients were regularly followed up at MPC, and those who exceeded one year of

therapy were prepared for sample collection.

2. Samples Collection:

Blood samples (5 ml) were collected from all subjects in this study during the period from March 2007 to September 2007, and serum was obtained after centrifugation at 3000 rpm . The serum samples were stored at (-20 C^o) during the period of collection. At the end of the period, serum samples were transferred to the Pharmacology Laboratory, at College of Medicine - University of Babylon where the biochemical analysis performed.

3. Biochemical Analysis

A- Estimation of GSH:

Serum GSH level was estimated according to the method of Ellman [18]. The used reagents were supplied by Biochemical's Co. L.t.d. for EDTA (Ethylene diamine tetracetic acid) and Sigma Co. L.t.d. for DTNB (Dithiobis nitro benzoate). The assay mixture contained supernatant and DTNB (0.01 M), trichloroacetic acid (TCA) 50 % , EDTA buffer (0.4 M) PH =9. (Unico Spect. 2100 IRC) spectrophotometer was used to read the final results at 412 nm. GSH was expressed as $\mu\text{mol/ml}$ using Molecular Extinction Coefficient of Chromophore ($1.74 \times 10^4 \times \text{M}^{-1}\text{cm}^{-1}$) [19].

B- Lipid peroxidation assay:

The quantitative measurement of lipid peroxidation was performed by measuring the amount of malondialdehyde (MDA) in the blood. Serum MDA level was determined according to the method of Tomotsu [20]. All chemicals were supplied by Merck Co. L.t.d. The assay mixture contained serum (1 ml), 20 % TCA, 0.6 % Thiobarbituric

acid (TBA), and N-Butyl alcohol. Same spectrophotometer was used to read the final results at 530 nm. **MDA** was expressed as nmol/ml using Molecular Extinction Coefficient of Chromophore ($1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$) [20].

4. Statistical analysis:

The data was expressed as mean $\pm$ SEM unless otherwise stated. Statistical analysis had been done by using paired t-test and ANOVA utilizing SPSS version 11. Significant difference was set at $\alpha = 0.05$.

Results

Serum glutathione levels were reduced in hypertensive patients taking atenolol (mean = $0.507 \pm 0.032 \mu\text{mol/ml}$) as compared with controls (mean $\pm$ SEM = $0.88 \pm 0.0401 \mu\text{mol/ml}$). Captopril was found to keep serum GSH at higher levels (mean $\pm$ SEM = $0.654 \pm 0.071 \mu\text{mol/ml}$) than that observed with atenolol. In combination therapy serum glutathione level (mean = $0.669 \pm 0.012 \mu\text{mol/ml}$) was higher than both atenolol and captopril used alone Figure 1. , Table 1.

Regarding serum MDA, there was clear elevation in their levels in hypertensive patients taking atenolol (mean $\pm$ SEM = $0.791 \pm 0.061 \text{ nmol/ml}$) as compared with controls (mean $\pm$ SEM = $0.491 \pm 0.027 \text{ nmol/ml}$). In patients taking captopril, although MDA serum levels were higher than control ($0.664 \pm 0.019 \text{ nmol/ml}$), but were less than those observed with atenolol. The use of combination therapy was shown to reduce the levels (mean $\pm$ SEM = $0.607 \pm 0.0201 \text{ nmol / ml}$) of serum

MDA more than both atenolol and captopril used alone, Figure 2, Table 1.

Comparison of mean differences among groups showed that, there was significant ($p < 0.05$) reduction in GSH levels in both atenolol and captopril groups but not with combination group in comparison with control group, also combination therapy significantly ($p < 0.05$) maintained GSH levels higher than atenolol group. Table 2. Regarding lipid peroxidation, there was significant ($p < 0.05$) increment in MDA levels in both atenolol and captopril groups but not with combination group in comparison with control group, also in combination group MDA levels were significantly ($P < 0.05$) lower than that of atenolol group. Table 3.

Regarding captopril group although serum GSH levels were higher and serum MDA levels were lower than that observed in atenolol group, but the mean difference was statistically non significant ($p > 0.05$) Also the mean difference was statistically non significant ($p > 0.05$) between combination and captopril groups for both GSH and MDA levels. Table 2. , Table 3.

Discussion

In this study, there was clear oxidative imbalance present in all hypertensive patients (group; B, C, and D) in comparison with non-hypertensive controls (group; A), regardless the type of antihypertensive drug used in the treatment. The same findings were observed by (Baykal, ...et al) in a previous study [14]. This is clarified by reduced serum GSH levels (Figure

1) and increased serum MDA levels (Figure 2) in all hypertensive patients as compared with controls, this is agrees with studies suggesting that oxidative stress plays an important role and present in all forms of hypertension [21, 4]. Although the oxidative imbalance in patients taking atenolol and patients taking captopril was significantly differ from control, the reduction in serum glutathione and elevation in serum MDA in patient taking combination therapy (atenolol + captopril) was statistically not significant from controls. i.e. the oxidative imbalance was minimum.

Glutathione is a potentially powerful and reliable biomarker of oxidative stress status and frequently used to evaluate whether, and to what extent, oxidative stress may be involved in various diseases [22]. lipid peroxidation byproduct (MDA) is also used as a marker for oxidation of lipoproteins in the wall of blood vessels that participates in the development of atherosclerosis and hypertension [23].

In comparison between groups, Figure 1 and Figure 2 showed that, the reduction in serum GSH levels and elevation in serum MDA levels in patients taking atenolol 100 mg/day was much greater than those taking captopril 100 mg/day. This is because captopril is an ACEI that prevents the production of angiotensin II, which plays an important role in the development of oxidative stress (reduction of GSH and elevation of MDA level in blood) in hypertensive patients [7]. While atenolol has minimal effects on angiotensin II production in the body through the inhibition of rennin-angiotensin system. This antioxidant

property of captopril was observed in previous studies [24,25]. The molecular mechanism by which captopril increased the GSH levels and decreased MDA levels is that the induction of angiotensin II increases the generation of ROS through the membrane bound NADH / NADPH oxidase, so, ACEIs decrease the level of angiotensin II that leads to decrease the ROS production causing decrease GSH depletion, [24]. and decrease MDA production [25]. also angiotensin II is a potent stimulant of inflammatory mediators (prostaglandins, prostacyclines, thromboxane, and leukotrienes) which play an important role in ROS generation and oxidative stress, so, inhibition of ACE leads to inhibition of inflammatory mediators release and ROS production [26].

Regarding atenolol, many previous studies observed weak or insignificant antioxidant properties, in relation to other β -blockers like propranolol, pindolol, and metoprolol [13,27]. This weak effect was clear on lipid peroxidation probably because atenolol is a hydrophilic blocker and ineffective in reducing lipid peroxidation even at elevated concentrations, while lipophilic beta-blocking drugs apparently are capable of exerting an antioxidant effect in protecting membrane lipids against peroxidation [28].

Other studies compared the antioxidant effects of atenolol with losartan (an angiotensin receptor blocker), demonstrated that losartan significantly improved endothelial function in patients with hypertension compared with atenolol through an antioxidant effect, independent of the blood pressure-lowering effect [29].

Also losartan was found to reduce lipid peroxidation (reduce MDA levels in blood) more than atenolol in a comparative experimental animal study [30]. These findings are in agree with our findings, because angiotensin receptor blockers were found to have similar antioxidant properties of ACEI through prevention of angiotensin II effects on vascular endothelium [14].

The reduction in serum GSH levels and elevation in serum MDA levels were minimal, and significantly maintained at levels closer to that of controls in patients with combination therapy (atenolol 100 mg/day + captopril 50 mg/day). To our knowledge, there were no data about the relationship between this treatment line and oxidative stress, a possible explanation is that atenolol may enhanced the antioxidant effects of captopril by a synergistic mechanism. Similar findings were observed by (Ewis SA and Abdel-Rahman MS) in their study on the effects of atenolol and metformin on blood and liver GSH levels in rats. They showed that treatment with atenolol alone did not change GSH levels significantly, however administration of metformin or atenolol/metformin combination increased significantly the GSH levels in both liver and blood [31].

Conclusions

- The use of atenolol/captopril combination in the treatment of hypertension, significantly maintained blood GSH and MDA at levels closer to that of non-hypertensive controls.
- Atenolol/captopril combination therapy significantly increases GSH

and reduces MDA blood levels than the use of atenolol alone in the treatment of hypertension.

- The use of combination therapy provides better protection against oxidative stress associated with hypertension, and hence better prevention of complications like atherosclerosis than both atenolol and captopril used alone.

References

1. Sripal, B.; Franz, H. ; John, B. ; Kostis, and Carl J. Cardiovascular protection using Beta-blockers. Journal of the American College of Cardiology. 2007; 50: 563-572.
2. Böhm, M. ; Angiotensin receptor blockers versus angiotensin-converting enzyme inhibitors: where do we stand now? American Journal of Cardiology. 2007; 100,(3): 38-44.
3. Wayne, R. ; Hypertension and the pathogenesis of atherosclerosis. Hypertension. 1995; 25: 155-161.
4. Zoltan, U. ; Anna, C. ; Pawel M. ; Michael S. and Akos K. Chronic high pressure-induced arterial oxidative stress. American journal of pathology. 2004; 165: 219-226.
5. Donald, D. and Heistad ; Oxidative stress and vascular disease. Arteriosclerosis, Thrombosis, and Vascular Biology. 2006; 26: 689-694.
6. Friedrich, C.; Luft ; Mechanisms and cardiovascular damage in hypertension. Hypertension. 2001; 37: 594-598.
7. Carlos, J. ; Jane, F. ; Role of angiotensin and oxidative stress in essential hypertension. Hypertension. 1999; 34: 943-949.
8. Schnackenberg, C.G. ; Oxygen radicals in cardiovascular-renal disease. Curr Opin Pharmacol.2002; 2: 121-125.

9. Pratico, D. ; Clark, CM. ; and Liun, F.; Increase of brain oxidative stress in mild cognitive impairment: a possible predictor of Alzheimer's disease. Arch Neurol. 2002 59: 972-976.
10. Jane, A. ; Leopold, ; Joseph, L. ; Oxidative enzymopathies and vascular disease. Arteriosclerosis, Thrombosis, and Vascular Biology. 2005; 25: 1332-1340.
11. Aarti, S. ; Mahajan , Rashmi, B. ; Nisheeth, K. ; Satish, K. ; Agarwal , and Prakash, C. ; Antihypertensive and antioxidant Action of Amlodipine and vitamin C in patients of essential hypertension. J. Clin. Biochem. Nutr. 2007; 40: 141-147.
12. Pattipati, S. ; Amanpreet S. ; and Shrinivas, K. ; Carvedilol attenuates neuroleptic-induced orofacial dyskinesia: possible antioxidant mechanisms. British Journal of Pharmacology. 2002; 136: 193-200.
13. Silva, JM. ; Filipe, PM. ; Fernandes, AC. and Manso, CF. ; Antioxidant effect of drugs used in cardiovascular therapy. Rev Port Cardiol. 1998 ;17(6):495-503.
14. Baykal, Y ; Yilmaz, MI ; Celik, T ; Gok, F ; Rehber, H ; Akay, C and Kocar, IH. Effects of antihypertensive agents, alpha receptor blockers, beta blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers and calcium channel blocker, on oxidative stress. J. Hypertens. 2003 Jun;21(6):1207-11
15. Rodney, J ; Bolterman , Melissa, C ; Manriquez , M ; Luis, A ; Juncos , J and Carlos R ; Effects of Captopril on the Renin Angiotensin System, Oxidative Stress, and Endothelin in Normal and Hypertensive Rats. Hypertension. 2005; 46: 943-947.
16. Mak, I ; and Weglicki, WB ; Protection by beta-blocker agent against free radical mediated sarcolemmal lipid peroxidation . Circulation Research. 1988; 63: 262-266.
17. Hayes, JD ; and Mclellan, LI ; Glutathione and glutathione dependent enzymes represent a co-ordinately regulated defence against oxidative stress. Free Radic Res. 1999; 31: 273 – 300.
18. Ellman, G.L ; Tissue sulfhydryl groups. Arch. Biochem. Biophys.1959; 82: 70-77.
19. BIOXYTECH® GSH-400™. Catalog Number 21011, Copyright© 2001.
20. Tomutso N. ; Dib M. ; Carrel C. and Robin V. Desnuelle C. Can malondialdehyde be used as biological marker of progression in neurodegenerative diseases? J. Neurol. 2002; 249: 367-47.
21. Antonio C. ; Possible Role of Oxidative Stress in the Pathogenesis of Hypertension. Diabetes Care 2008; 31: 181-184.
22. Ranieri, R ; Isabella D. ; Aldo, M. ; and Daniela, G. ; Oxidized Forms of Glutathione in Peripheral Blood as Biomarkers of Oxidative Stress. Clinical Chemistry. 2006; 52: 1406-1414.
23. Marklund, s.l. ; Development of atherosclerosis in extracellular (EC) Knock-down mice. 2nd Int. Conference on SOD, Paris, May, 18-19, 2000, abstract book, p. 34.
24. Rabih, I. ; Bechara, ; Andres P. ; Andres, P. ; Pratibha, C. ; Joshi, C. ; Michael H. ; Ann, S. ; Brown, R ; and David, M. Guidot. Angiotensin II mediates glutathione depletion, transforming growth factor-β1 expression, and epithelial barrier

dysfunction in the alcoholic rat lung. *Am. J. Physiol. Lung Cell Mol. Physiol.* 2005; 289: 363-370.

25. Uzunova, VV. ; Tolekova AN. ; Ilieva GS. ; and Trifonova, AP. Renin-Angiotensin System and Lipid Peroxidation. *Bulgarian Journal of Veterinary Medicine.* 2005; 1: 69-75.

26. Ianopol, N. ; Free radicals and eye inflammations. *Clinica a 11-a Oftalmologie, Spitalul de neurochirurgie, Lasi.* 1998; 42: 5-9.

27. Reddy, DS. ; Singh, M. and Chopra, K. Comparative antioxidant effects of beta-adrenoceptor blockers, calcium antagonists and U-74500A against iron-dependent lipid peroxidation in murine ventricular microsomal membranes. *Methods Find Exp Clin Pharmacol.* 1996;18(9): 559-67.

28. Jenkins, RR. ; Del, CM. ; Sauer, P. and Skelly, C. The effect of beta blocking drugs on lipid peroxidation in rat heart in vitro. *Lipids.* 1992; 27(7): 539-42

29. Flammer, AJ. ; Hermann, F. ; Wiesli, P. ; Schwegler, B. ; Chenevard, R. ; Hürlimann, D. ; Sudano, I. ; Gay, S. ; Neidhart, M. ; Riesen, W. ; Ruschitzka, F. ; Lüscher, TF. ; Nollm, G. and Lehmann, R. Effect of losartan, compared with atenolol, on endothelial function and oxidative stress in patients with type 2 diabetes and hypertension. *J Hypertens.* 2007; 25(4): 785-91.

30. Pechter, U. ; Aunapuu, M. ; Riispere, Z. ; Vihalemm, T. ; Kullissaar, T. ; Zilmer, K. ; Zilmer, M. and Ots, M. Oxidative stress status in kidney tissue after losartan and atenolol treatment in experimental renal failure. *Nephron Exp Nephrol.* 2004; 97(2): e33-e37.

31. Ewis, SA. and Abdel-Rahman, MS. Influence of atenolol and/or metformin on glutathione and magnesium levels in diabetic rats. *J Appl Toxicol.* 1997; 17(6): 409-13.

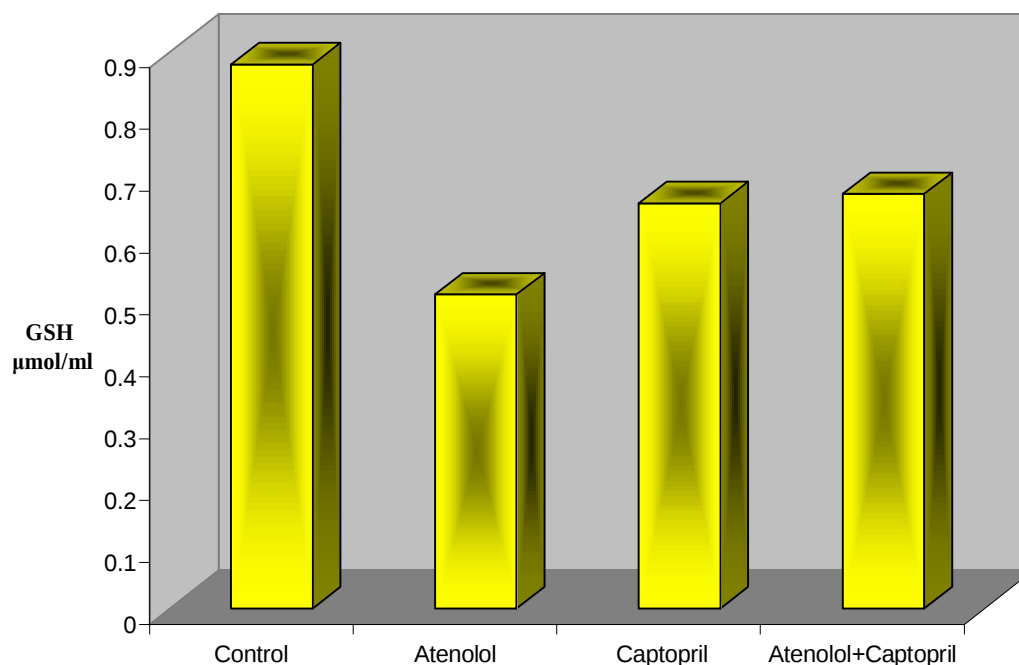
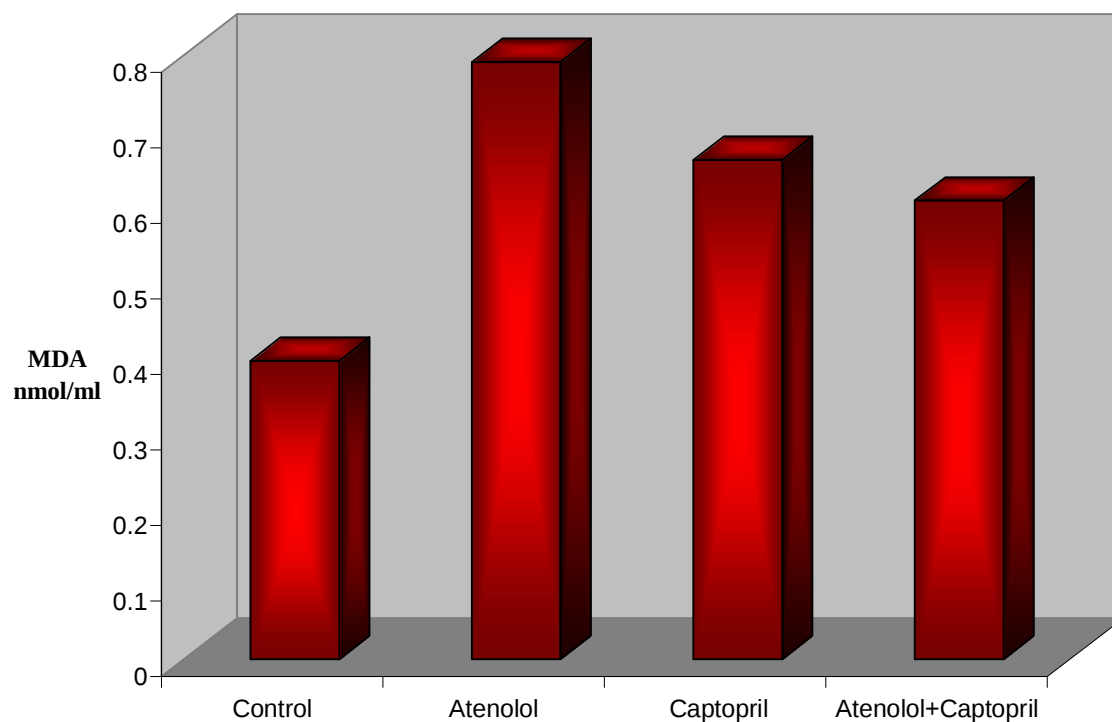


Figure 1 Glutathione levels in the study groups**Figure 2** MDA levels in the study groups**Table 1** GSH and MDA levels for all groups.

	Control Mean ± SEM	Atenolol Mean ± SEM	Captopril Mean ± SEM	Combination Mean ± SEM
GSH (μmol/ml)	0.88 ± 0.0401	0.507 ± 0.032	0.654 ± 0.071	0.669 ± 0.012
MDA (nmol/ml)	0.491 ± 0.027	0.791 ± 0.061	0.664 ± 0.019	0.607 ± 0.0201

Table 2 Comparisons between mean differences of serum GSH levels ($\mu\text{mol/ml}$) in different groups.

	Atenolol group	Captopril group	Combination group
Control group	0.395 ± 0.011 *	0.266 ± 0.02 *	0.212 ± 0.013
Combination group	0.183 ± 0.02 *	0.025 ± 0.04	
Captopril group	0.129 ± 0.101		

* P < 0.05 Significant

Table 3 Comparisons between mean differences of serum MDA levels (nmol/ml) in different groups.

	Atenolol group	Captopril group	Combination group
Control group	0.3 ± 0.013 *	0.173 ± 0.08 *	0.117 ± 0.031
Combination group	0.148 ± 0.07 *	0.025 ± 0.019	
Captopril group	0.127 ± 0.02		

* P < 0.05 Significant