

Adverse Effects of Bisoprolol in Rats

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Abstract

The present study was designed to investigate the adverse effect of Bisoprolol and protective effect of Vitamin E and To Show the result of the combination between Bisoprolol and Vit. E on hepatic and renal disturbance induced in rats by Bisoprolol. Experimental design: After one week of acclimatization, eighty rats randomly allocated into 4 equal groups, each of 20 rats. The 1st group: Rats in this group were not medicated and left as control and received Nacl as a vehicle. The 2nd group (VE): Rats in this group treated with vitamin E (1.8mg/kg b.wt. once daily) per os for successive 21 days. The 3rd group (B): Rats in this group treated orally with Bisoprolol (1.8mg/kg b.wt. once daily) for successive 21 days as standard. The 4th group (VE+B): Rats in this group received orally a combination of vitamin E (1.8mg/kg b.wt. once daily) with Bisoprolol (1.8mg/kg b.wt. once daily) for successive 21 day. At the end of the experiment, all rats were sacrificed, Liver and kidney samples were taken and blood was taken to centrifuge to gain serum for biochemical investigations. Effect of Bisoprolol, Vit.E and their combination on liver parameter (ALT, AST, ALP, Total serum proteins, Serum albumin, serum globulin). Oral administration of Bisoprolol (1.8mg/kg b.wt. once daily for 21 days) showed a significant increase in (ALT, AST, ALP) while total serum proteins, serum albumin, and serum globulin showed a significant decrease compared with control group. Administration of Vit.E (1.8mg/kg b.wt. once daily for 21 days) and its combination with Bisoprolol (1.8mg/kg b.wt. once daily for 21 days) showed a decrease in (ALT, SAT, ALP) while total serum proteins, serum albumin, and serum globulin showed an increase compared with Bisoprolol group. 2- Effect of Bisoprolol, Vit.E and their combination on kidney parameters (creatinine and uric acid). Oral administration of bisoprolol (1.8mg/kg b.wt. once daily for 21 days) showed a significant increase in (Creatinine and uric acid) compared with control group. Administration of Vit E (1.8mg/ kg b.wt. once daily for 21 days and its combination with bisoprolol (1.8mg /kg b.wt. once daily for 21 days) showed a decrease in (creatinine and uric acid) compared with bisoprolol group. 3- Effect of Bisoprolol and vit. E on antioxidant activities a- CAT, SOD and GPX activity Administration of Bisoprolol (1.8mg/kg b.wt. once daily for 21 days) showed a significant decrease in CAT, SOD and GPX activity compared with control group. Administration of Vit.E (1.8mg/kg b.wt. once daily for 21 days) and its combination with bisoprolol showed a significant increase in CAT, SOD and GPX activity compared with bisoprolol group. b- Lipid peroxidation (MDA) Oral administration of bisoprolol (1.8mg/kg b.wt. once daily for 21 days) showed a significant increase in MDA activity compared with control group.

Administration of Vit E (1.8mg/kg b.wt. once daily for 21 days) and its combination with bisoprolol Showed as insignificant decrease in MDA activity compared with bisoprolol group. 4- Histopathological finding. a- Histopathological finding in liver. Histopathological Results in 1st day after administration with bisoprolol showing mild to moderate congestion of hepatic blood vessels, portal biliary proliferation, round cells aggregations and fibroplasia in addition to minute interstitial aggregation of round cells. After administrated with bisoprolol and vit E showed apparently normal hepatic parenchyma, however the portal areas showed aggregations of round cells and mild biliary proliferation. The hepatic sinusoids revealed mild lymphocytosis and the kupffer cells were 7th day after administrations with bisoprolol showing sever congestion of hepatic blood vessels, portal biliary hyperplasia and round cells aggregations. After administrated with bisoprolol and vit E showed normal hepatic parenchyma. However, mild congestion of portal blood vessels with biliary proliferation and round cells aggregations were seen. The kupffur cells were prominantaly hypertrophied. b- Histopathological finding in kidney In 7th day after administration with bisoprolol showing congested renal blood vessels with peri vascular edema, multi focal necrosis of renal tubules. After administrated with bisoprolol and vit. E showed apparently normal renal parenchyma with focal interstitial round cells aggregation, mild degenerative changes in some renal tubular epithelium and intratubular hyaline casts in some tubules.

Keywords: Bisoprolol; Vitamin E; Antioxidant Activity; Histopathology

Abbreviations: HF: Heart Failure; SAN: Sinoatrial Node; HCN: Hyperpolarization-Activated Cyclic Nucleotide-Gated

Introduction

Bisoprolol, an antagonist of β 1-adrenergic receptors, is effective in reducing the morbidity and mortality in patients with heart failure (HF). It has been found that HF is accompanied with dysfunction of the sinoatrial node (SAN). However, whether bisoprolol reverses the decreased SAN function in HF and how the relevant ion channels in SAN change were relatively less studied. SAN function and messenger RNA (mRNA) expression of sodium channels and hyperpolarization-activated cyclic nucleotide-gated (HCN) channel subunits were assessed in shamoperated rats, abdominal arterio-venous shunt (volume overload)-induced HF rats, and bisoprolol-treated HF rats. SAN cells of rats were isolated by laser capture microdissection. Quantitative real-time PCR analysis was used to quantify mRNA expression of sodium channels and HCN channel subunits in SAN. Intrinsic heart rate declined and sinus node recovery time prolonged in HF rats, indicating the suppressed SAN function, which could be improved by bisoprolol treatment. Nav1.1, Nav1.6, and HCN4 mRNA expressions were reduced in SAN in HF rats compared with that in control

rats [1]. Treatment with bisoprolol could reverse both the SAN function and the Nav1.1, Nav1.6, and HCN4 mRNA expression partially. These data indicated that bisoprolol is effective in HF treatment partially due to improved SAN function by reversing the down-regulation of sodium channels (Nav1.1 and Nav1.6) and HCN channel (HCN₄) subunits in SAN in failing hearts [1]. Heart failure (HF) is a major public health issue, with a high prevalence, poor clinical outcomes, and huge health care costs. Sudden cardiac death accounts for 50 % of deaths in patients with HF [2]. Although most sudden deaths in chronic HF could be attributed to ventricular arrhythmias, bradyarrhythmias also accounted for up to 42 % of sudden deaths in hospital [3]. Clinically, patients with HF share slower intrinsic heart rates [4], increased corrected sinoatrial node (SAN) recovery time (SNRTc), and abnormal propagation of the action potentials from the SAN, suggesting that SAN function is affected by HF [5].

Aim of Work

The present study was designed to investigate effect of Bisoprolol on kidney and liver in rats. Determine the protective effect of vitamin E through measuring of: Some liver and kidney functions, antioxidant activity, as well as histopathological findings. 1) Biochemical constituents in sera such as: Serum alanine aminotransferase (ALT),

aspartate aminotransferase (AST), alkaline phosphate (ALP), total protein, albumin and globuline. a) Serum creatinine and uric acid, b) Catalase (CAT), d) Superoxide dismutase (SOD), e) Glutathione peroxidase (GPX). f) Malondialdehyde (MDA) (marker of lipid peroxidation). 2) Histopathology of liver and kidney.

Materials and Method

Material

• **Drugs:** Bisoprolol, marketed under the trade name (Zebeta) among others, is a medication most commonly used for heart diseases. This specifically includes high blood pressure, chest pain from not enough blood flow to the heart, and heart failure. It is taken by mouth.

• Structural formula:

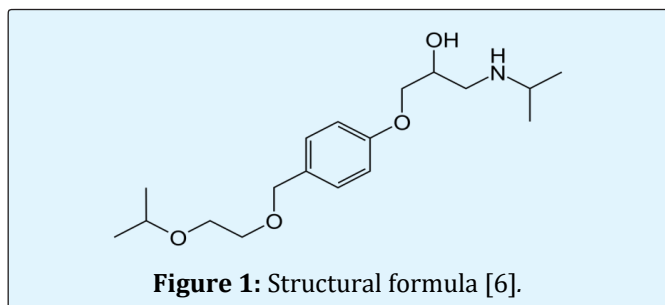


Figure 1: Structural formula [6].

- **Dose:** 10 mg/ day recommended dose for humans per os.
- **Absorption:** Well absorbed. Bioavailability > 80%. Absorption is not affected by food. Peak plasma concentrations occur within 2-4 hours.
- The recommended dose for rats is 1.8 mg according to Paget and Barnes.

Vitamin E (Vitamin E capsules ®):

Structural formula

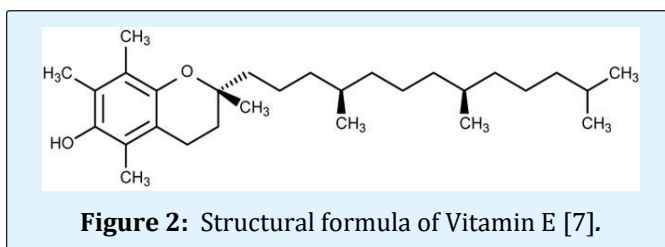


Figure 2: Structural formula of Vitamin E [7].

- **Molecular Formula:** C₂₉H₅₀O₂.
- **Molecular weight:** 430.717 g/mol.
- **Dose:** oral intake of (100 mg/kg b.wt.) for human dissolved in corn oil once daily for 21 successive days [8].

Experimental Animals

Eighty rats weighed (180 ± 20 gm.) were used in this study were obtained from the laboratory farm of Faculty of Vet. Medicine and allowed to acclimatize for one week at the animal house before starting the' experiments. the rats were randomly assigned into four equal groups, twenty rats each and kept in a cage for each group, maintained at a 12 hour light dark cycle and of a constant temperature of Rats received regular basic ration and water was provided ad.lib, the experimental protocols were approved by the ethics of Faculty of Veterinary Medicine, Zagazig University, Egypt.

Methods

Experimental Design: Eighty rats, weighed (180 ± 20 gm) were used in this study, the rats were divided into four equal groups each of 20 rats, they were kept under hygienic conditions and feed on basic ration free from any medications or chemicals *ad.lib* and water was provided. Groups divided as follow: Group one (Control group): Non treated rats in the first group received no drugs and remained as control group. Group two: Treated rats with 1.8 mg/kg b.wt. of Vitamin E orally which is the calculated therapeutic dose of the drug. Group three: Treated rats with 1.8 mg/kg b.wt. of Bisoprolol per os. Group four (Combination group): Treated rats with 1.8 mg/kg b.wt. of Vitamin E and 1.8 mg/kg b.wt. of Bisoprolol as a combination therapy per os. Drugs were given to rats early in the morning orally once daily for a successive 21 days.

Collection of Samples: A) Blood Samples Collection: Blood was collected at 1st, 7th and 14th day of the experimental period. Five rats from each group were used for collection of blood samples in clean and dry bottles. The blood samples were taken from each group, for biochemical studies. The sample was collected without anticoagulant for separation of clear serum for biochemical analysis, each blood sample was collected in centrifuge tubes and left in slope position to clot at room temperature. Serum was centrifuged at 3000 rpm for 10 min for separation of clear serum, serum samples were put in clean dry vials and used for determination of Liver and Kidney function tests. Anti oxidant enzymes and MDA. B) Histopathological samples collection: Liver and Kidney samples from all groups were immediately removed and kept in 10% neutral formalin and processed in paraffin wax. Sections of 5 microns thickness were stained with Hematoxylin and Eosin and examined microscopically according to Bancroft & Gamble [9].

Laboratory assay:**1) Serum biochemical analysis:****a) Liver function tests:****a. Estimation of alanine transaminase activity of (ALT):**

b) ALT was estimated according to Reitman & Frankel [10]

a. Estimation of Aspartate Transaminase activity of (AST):

c) AST was estimated according to Reitman & Frankel [10]

a. Estimation of Alkaline phosphatase (ALP):

d) Colorimetric determination of ALP was performed according to the method of Kind & King [11] which is based on the following reaction.

a. Estimation of serum total protein:

e) Total protein was estimated according to Grant, et al. [12].

a. Estimation of serum albumin :

f) Albumin was determined according to Doumas, et al. [13].

$$g) = \frac{A_{sample}}{A_{standard}} \times 4 = g/dl$$

2) Kidney function tests**a. Estimation of serum creatinine:**

Estimation of serum creatinine is accomplished by photometric colorimetric test for kinetic measurements, without deproteinization according to Henry [14].

b. Determination of serum uric acid :

Colorimetric determination of serum uric acid was performed according to the method of Artiss [15].

3) Antioxidant enzyme activities:

a. Estimation of Catalase activity: Catalase activity was determined colorimetrically in tissue homogenate

according to the method described by Aebi [16].

b. Estimation of Superoxide dismutase activity:

Superoxide dismutase activity was determined in tissue homogenate according to the method described by Nishikimi, et al. [17].

c. Estimation of glutathione peroxidase activity:

Glutathione peroxidase activity in tissue homogenate was determined according to the method adapted by Paglia & Valentine [18].

d. Estimation of Malondialdehyde (MDA) activity:

Malondialdehyde activities were determined according to the method adapted by Ohkawa, et al. [19].

e. Histopathology: Bancroft & Gambl [9].

Statistical analysis: In order to assess the influence of Vitamin E, Bisoprolol and their combination on some biochemical parameters at 1st, 7th and 14th day post treatment, one-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (Tukey's HSD) test as post hoc test was used. Analysis was done using Statistical Package for Social Sciences version 22.0 (IBM Corp., Armonk, NY, USA). Results were reported in means \pm SEM (Standard Error of Mean). The value of $P < 0.05$ was used to indicate statistical significance.

Results**Biochemical studies - Liver function test Effect on serum ALT**

It was clearly evident from (Table 1) that the administration of Bisoprolol in therapeutic dose for 21 successive days to rats resulting in a significant increase in ALT level after one day post-administration when compared with control group but this elevation decrease gradually by time.

	ALT (U/L)		
	1 st day	7 th day	14 th day
Control	14.66 c ± 0.881	13.66 c ± 0.881	16.00 c ± 0.577
Vit. E	14.33 c ± 1.45	13.33 c ± 1.45	15.00 c ± 0.577
Bisoprolol	56.33 a ± 1.20	43.00 a ± 1.15	34.66 a ± 1.45
Bisoprolol + Vitamin E	43.00 b ± 1.15	34.33 b ± 1.45	24.00 b ± 2.08

One way ANOVA results expressed as means \pm SD. The different letters at the same column mean that there was a significant change at $p < 0.05$.

Table 1: Effects of Bisoprolol, Vitamin E and their combination on ALT (U/L) at 1st, 7th and 14th day post-administration, (n=5) in rats.

The administration of Bisoprolol and Vit. E combination in their therapeutic dose for 21 successive days to female rat resulting in decrease in ALT level after one day post-administration compared with Bisoprolol group.

Effect on Serum AST

It was clearly evident from (Table 2) that the administration of Bisoprolol in therapeutic dose for 21

successive days to rats resulting in a significant increase in AST level after one day post-administration when compared with control group but this elevation decrease gradually by time. The administration of Bisoprolol and Vit. E combination in their therapeutic dose for 21 successive days to female rat resulting in decrease in AST level after one day post-administration compared with Bisoprolol group.

	AST (U/L)		
	1 st day	7 th day	14 th day
Control	21.33 c ±.881	22.66 c ±.666	24.00 b ±1.52
Vit. E	21.33 c ±1.76	23.33 c ±1.45	22.66 b ±2.18
Bisoprolol	46.00 a ±1.52	37.66 a ±.881	30.66 a ±.333
Bisoprolol + Vitamin E	34.66 b ±1.76	29.66 b ±1.20	24.33 b ±1.20

One way ANOVA results expressed as means ±SD. The different letters at the same column mean that there was a significant change at $p < 0.05$.

Table 2: Effects of Bisoprolol, Vitamin E and their combination on AST (U/L) at 1st, 7th and 14th day post-administration, (n=5) in rats.

Effect on serum ALP: It was clearly evident from (Table 3) that the administration of Bisoprolol in therapeutic dose for 21 successive days to rats resulting in a

significant increase in ALP level after one day post-administration when compared with control group but this elevation decrease gradually by time.

	ALP (U/L)		
	1 st day	7 th day	14 th day
Control	123.33 c ±1.20	124.33 c ±2.40	123.66 b ±.881
Vit. E	123.00 c ±1.73	124.66 c ±2.027	123.66 b ±1.20
Bisoprolol	153.66 a ±1.76	145.33 a ±2.60	138.00 a ±2.30
Bisoprolol + Vitamin E	146.66 b ±1.76	133.66 b ±2.72	128.66 b ±1.20

One way ANOVA results expressed as means ±SD. The different letters at the same column mean that there was a significant change at $p < 0.05$

Table 3: Effects of Bisoprolol, Vitamin E and their combination on ALP (U/L) at 1st, 7th and 14th day post-administration, (n=5) in rats.

The administration of Bisoprolol and Vit. E combination in their therapeutic dose for 21 successive days to rat resulting in decrease in ALP level after one day post-administration compared with Bisoprolol group.

therapeutic dose for 21 successive days to rats resulting in a significant decrease in total protein level after one day post-administration when compared with control group but this decrease elevate gradually by time. The administration of Bisoprolol and Vit. E combination in their therapeutic dose for 21 successive days to female rat

Effect on Total Protein: It was clearly evident from (Table 4) that the administration of Bisoprolol in

resulting in increase in total protein level after one day post-administration compared with Bisoprolol group.

	Total protein (g/dl)		
	1 st day	7 th day	14 th day
Control	7.56 a ±.04	7.80 a ±.034	7.84 a ±.029
Vit. E	7.49 a ±.104	7.8367 a ±.05364	7.83 a ±.040
Bisoprolol	5.25 c ±.086	6.02 c ±.040	6.68 c ±.063
Bisoprolol + Vitamin E	6.33 b ±.073	6.89 b ±.097	7.11 b ±.106

One way ANOVA results expressed as means ± SD. The different letters at the same column mean that there was a significant change at $p < 0.05$

Table 4: Effects of Bisoprolol, Vitamin E and their combination on Total Protein (g/dl) at 1st, 7th and 14th day post-administration, (n=5) in rats.

Effect on Albumin: It was clearly evident from (Table 5) that: The administration of Bisoprolol in therapeutic dose for 21 successive days to rats resulting in a significant decrease in albumin level after one day post-administration when compared with control group but

this decrease elevate gradually by time. The administration of Bisoprolol and Vit. E combination in their therapeutic dose for 21 successive days to rat resulting in increase in albumin level after one day post-administration compared with Bisoprolol group (Table 5).

	Albumin (g/dl)		
	1 st day	7 th day	14 th day
Control	5.00 a ±.057	4.94 a ±.041	4.98 a ±.026
Vit. E	5.01 a ±.043	4.94 a ±.094	5.02 a ±.020
Bisoprolol	3.11 c ±.031	3.86 c ±.040	4.12 c ±.070
Bisoprolol + Vitamin E	3.90 b ±.023	4.14 b ±.078	4.50 b ±.040

One way ANOVA results expressed as means ±SD. The different letters at the same column mean that there was a significant change at $p < 0.05$

Table 5: Effects of Bisoprolol, Vitamin E and their combination on Albumin (g/dl) at 1st, 7th and 14th day post-administration, (n=5) in rats.

Effect on Globulin: It was clearly evident from (Table 6) that the administration of Bisoprolol in therapeutic dose for 21 successive days to rats resulting in a significant decrease in globulin level after one day post-administration when compared with control group but

this decrease elevate gradually by time. The administration of Bisoprolol and Vit. E combination in their therapeutic dose for 21 successive days to rat resulting in increase in globulin level after one day post-administration compared with Bisoprolol group.

	Globulin (g/dl)		
	1 st day	7 th day	14 th day
Control	2.56 a ±.090	2.86 a ±.070	2.86 a ±.034
Vit. E	2.48 a ±.101	2.89 a ±.145	2.81 a ±.055
Bisoprolol	2.13 b	2.16 b	2.56 a

	$\pm.054$	$\pm.070$	$\pm.011$
Bisoprolol + Vitamin E	2.42 a $\pm.073$	2.75 a $\pm.147$	2.60 a $\pm.068$

One way ANOVA results expressed as means \pm SD. The different letters at the same column mean that there was a significant change at $p < 0.05$

Table 6: Effects of Bisoprolol, Vitamin E and their combination on Globulin (g/dl) at 1st, 7th and 14th day post-administration, (n=5) in rats.

Kidney Function Test

Effect on Serum Creatinine: It was clearly evident from (Tables 7) that the administration of Bisoprolol in therapeutic dose for 21 successive days to rats resulting in a significant increase in serum creatinine level after one day post-administration when compared with control group but this decrease elevate gradually by time. The administration of Bisoprolol and Vit. E combination in their therapeutic dose for 21 successive days to rat resulting in decrease in serum creatinine level after one day post-administration compared with Bisoprolol group.

Effect on Serum Uric Acid: It was clearly evident from (Table 8) that the administration of Bisoprolol in therapeutic dose for 21 successive days to rats resulting in a significant increase in serum uric acid level after one day post-administration when compared with control group but this decrease elevate gradually by time. The administration of Bisoprolol and Vit. E combination in their therapeutic dose for 21 successive days to rat resulting in decrease in serum uric acid level after one day post-administration compared with Bisoprolol group.

	Creatinine (mg/dl)		
	1 st day	7 th day	14 th day
Control	0.633 c $\pm.020$	0.710 b $\pm.011$	0.756 b $\pm.012$
Vit. E	0.646 c ± 0.017	0.723 b ± 0.008	0.756 b ± 0.017
Bisoprolol	1.39 a ± 0.094	1.14 a ± 0.037	1.01 a ± 0.032
Bisoprolol + Vitamin E	1.05 b ± 0.029	0.863 b ± 0.024	0.800 b ± 0.020

One way ANOVA results expressed as means \pm SD. The different letters at the same column mean that there was a significant change at $p < 0.05$.

Table 7: Effects of Bisoprolol, Vitamin E and their combination on creatinine (mg/dl) at 1st, 7th and 14th day post-administration, (n=5) in rats.

	Uric acid (mg/dl)		
	1 st day	7 th day	14 th day
Control	6.13 c ± 0.228	5.18 c ± 0.044	5.51 b ± 0.081
Vit. E	5.91 c ± 0.610	5.12 c ± 0.064	5.66 b ± 0.059
Bisoprolol	18.48 a ± 0.379	12.95 a ± 0.439	9.20 a ± 0.430
Bisoprolol + Vitamin E	12.57 b ± 0.700	8.96 b ± 0.424	6.90 b ± 0.290

One way ANOVA results expressed as means \pm SD. The different letters at the same column mean that there was a significant change at $p < 0.05$

Table 8: Effects of Bisoprolol, Vitamin E and their combination on uric acid (mg/dl) at 1st, 7th and 14th day post-administration, (n=5) in rats.

Antioxidant Enzymes Activity

Effect on Catalase Activity (CAT): It was clearly evident from (Table 9) that the administration of Bisoprolol in therapeutic dose for 21 successive days to rats resulting in a significant decrease in catalase activity after one day post-administration when compared with control group but this decrease elevate gradually by time. The administration of Bisoprolol and Vit. E combination in their therapeutic dose for 21 successive days to rat resulting in increase in catalase activity after one day post-administration compared with Bisoprolol group.

Effect on Superoxide Dismutase Activity (SOD): It was clearly evident from (Table 10) that the administration of Bisoprolol in therapeutic dose for 21 successive days to rats resulting in a significant decrease in superoxide

dismutase activity after one day post-administration when compared with control group but this decrease elevate gradually by time. The administration of Bisoprolol and Vit. E combination in their therapeutic dose for 21 successive days to rat resulting in decrease in superoxide dismutase activity after one day post-administration compared with Bisoprolol group.

Effect on Glutathione peroxidase activity (GPX): It was clearly evident from (Table 11) that the administration of Bisoprolol in therapeutic dose for 21 successive days to rats resulting in a significant decrease in glutathione peroxidase activity after one day post-administration when compared with control group but this decrease elevate gradually by time.

	CAT (U/L)		
	1 st day	7 th day	14 th day
Control	206.66 a ±2.38	214.86 a ±1.22	216.42 a ±0.890
Vit. E	208.74 a ±2.58	216.97 a ±0.931	218.17 a ±1.16
Bisoprolol	171.51 c ±0.965	189.84 c ±.763	201.54 c ±2.77
Bisoprolol + Vitamin E	185.83 b ±0.534	201.77 b ±3.67	209.55 b ±1.23

One way ANOVA results expressed as means ±SD. The different letters at the same column mean that there was a significant change at $p < 0.05$.

Table 9: Effects of Bisoprolol, Vitamin E and their combination on catalase (U/L) at 1st, 7th and 14th day post-administration, (n=5) in rats.

	SOD (U/ml)		
	1 st day	7 th day	14 th day
Control	3.26 a ±.433	3.84 a ±.043	3.11 a ±.012
Vit. E	3.88 a ±.053	3.81 a ±.138	3.23 a ±.088
Bisoprolol	.95 b ±.035	1.65 c ±.076	2.05 c ±.081
Bisoprolol + Vitamin E	1.20 b ±.049	2.10 b ±.120	2.42 b ±.104

One way ANOVA results expressed as means ±SD. The different letters at the same column mean that there was a significant change at $p < 0.05$.

Table 10: Effects of Bisoprolol, Vitamin E and their combination on superoxide dismutase (U/ml) at 1st, 7th and 14th day post-administration, (n=5) in rats.

	GPX (U /ml)		
	1 st day	7 th day	14 th day
Control	134.31 a ±2.53	134.83 a ±1.81	134.83 a ±.815
Vit. E	134.96 a ±1.38	134.10 a ±1.81	133.88 a ±1.52
Bisoprolol	89.55 c ±1.52	101.54 c ±5.54	115.03 c ±4.41
Bisoprolol + Vitamin E	104.89 b ±3.29	113.69 b ±2.47	124.34 b ±1.67

One way ANOVA results expressed as means ±SD. The different letters at the same column mean that there was a significant change at p<0.05.

Table 11: Effects of Bisoprolol, Vitamin E and their combination on glutathione peroxidase (U /ml) at 1st, 7th and 14th day post-administration, (n=5) in rats.

The administration of Bisoprolol and Vit. E combination in their therapeutic dose for 21 successive days to rat resulting in decrease in glutathione peroxidase activity after one day post-administration compared with Bisoprolol group.

Effect on MDA Activity: It was clearly evident from (Table 12) that the administration of Bisoprolol in therapeutic dose for 21 successive days to rats resulting

in a significant increase in MDA activity after one day post-administration when compared with control group but this decrease elevate gradually by time. The administration of Bisoprolol and Vit. E combination in their therapeutic dose for 21 successive days to rat resulting in decrease in MDA activity after one day post-administration compared with Bisoprolol group (Table 12).

	MDA (nmol/ml)		
	1 st day	7 th day	14 th day
Control	10.77 c ±0.865	12.63 c ±0.538	13.52 c ±0.487
Vit. E	10.01 c ±0.661	11.71 c ±1.24	12.57 c ±0.786
Bisoprolol	33.92 ±2.38	26.04 a ±2.64	21.29 a ±0.705
Bisoprolol + Vitamin E	24.01 b ±1.74	16.77 bc ±2.10	15.97 bc ±0.482

One way ANOVA results expressed as means ±SD. The different letters at the same column mean that there was a significant change at p<0.05

Table 12: Effects of Bisoprolol, Vitamin E and their combination on MDA (nmol/ml) at 1st, 7th and 14th day post-administration, (n=5) in rats.

B- Histopathologic studies (7th day)

Rats Of Control Group (Not Treated):

1. Liver: Examined sections from liver showing apparently normal hepatic parenchyma with preserved lobular pattern, cords arrangement, central veins,

sinusoids, portal triads structures and stromal components.

2. Kidney: Sections from kidney revealed normal nephron units with preserved renal papillae, renal pelvis and stromal structures (Figure 3).

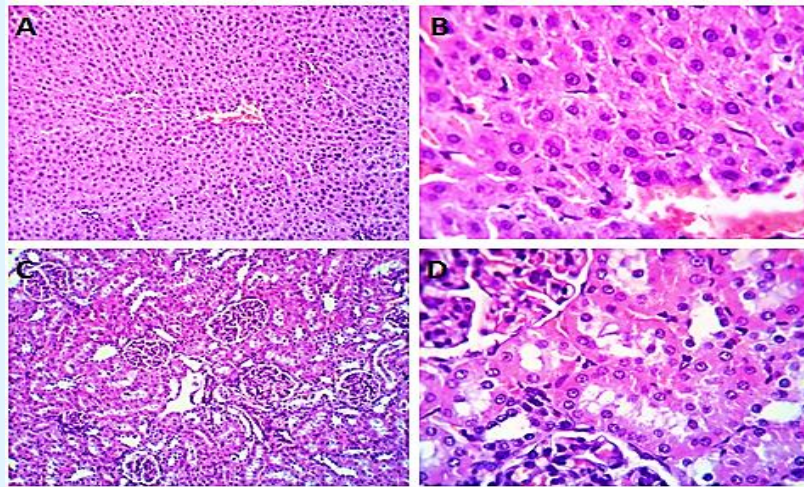


Figure 3: Photomicrograph of liver (A & B) showing apparently normal hepatic parenchyma with preserved cords arrangement, central veins, sinusoids, portal triads structures. Kidney (C & D) showing normal glomerular and renal tubular structures. H&E X 100(A,C), 400 (B,D).

Rats Treated with Vitamin E:

1. Liver: Examined sections from liver showing apparently normal hepatic parenchyma with preserved lobular pattern, cords arrangement, central veins,

sinusoids, portal triads structures and stromal components.

2. Kidney: Sections from kidney revealed normal nephron units with preserved renal papillae, renal pelvis and stromal structures (Figure 4).

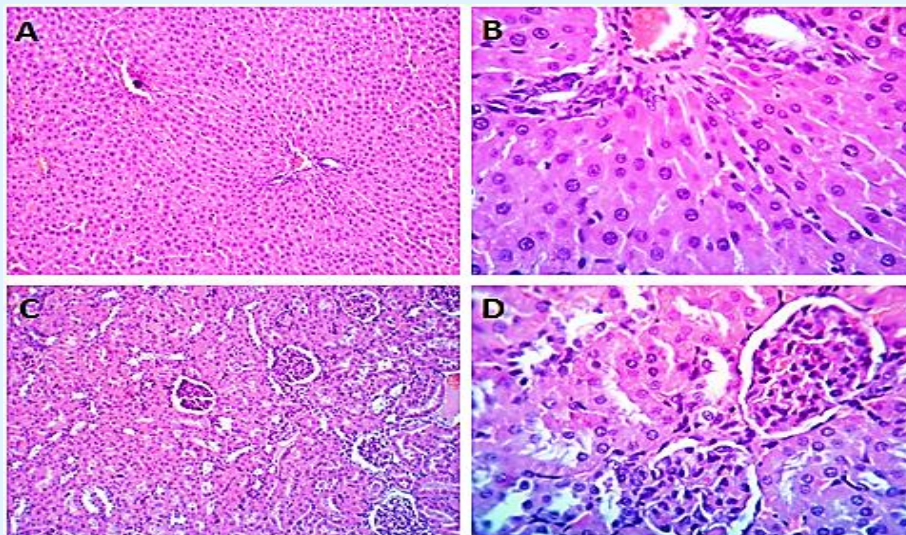


Figure 4: Photomicrograph of liver (A & B) showing apparently normal hepatic parenchyma with preserved cords arrangement, central veins, sinusoids, portal triads structures. Kidney (C & D) showing normal glomerular and renal tubular structures. H & E X 100 (A ,C), 400 (B,D).

Rats Treated with Bisoprolol

1. Liver: Liver sections revealed moderate to severe congestion of hepatic blood vessels, portal biliary hyperplasia and round cells aggregations, the latter were seen also in the interstitial tissue. The kupffer cells were prominently hypertrophied. Some hepatic cells showed cloudy swelling or hydropic degeneration.

2. Kidney: Characteristic renal lesions were seen in the form of congested renal blood vessels with perivascular

edema, multifocal interstitial round cells aggregations and focal co-agulative necrosis of the renal tubules.

Perivascular and periglomerular round cells aggregations were also detected. Some glomeruli were lobulated or shrunk. Some tubules particularly in the cortex were cystically dilated and others showed hyaline casts (Figure 5)

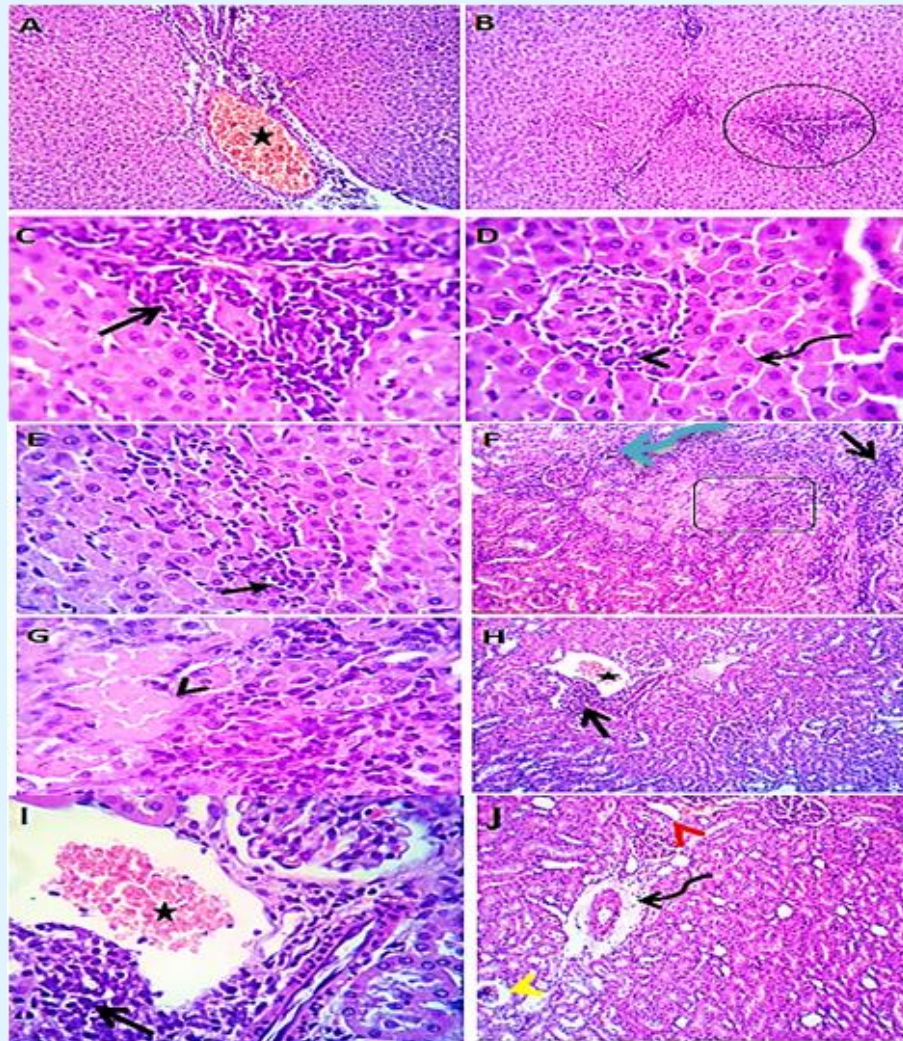


Figure 5: Photomicrograph of liver showing moderate to severe congestion (star) of hepatic blood vessels and round cells aggregations in portal areas (open arrow), interstitial tissue (arrow head & closed arrow). Some hepatic cells showing degenerative changes (curved blue arrow). Kidney showing congested renal blood vessels (star) with perivascular edema (curved arrow), focal coagulative necrosis (arrow head) of the renal tubules in addition to perivascular and periglomerular round cells aggregations (open arrows). Some tubules with cystically dilated lumen (red arrow head) and some glomeruli with lobulated or shrunk tufts (yellow arrow head). H&E X 100 (B, F, H), 200 (A, J), 400 (C, D, E, G, I).

Rats treated with Bisoprolol + vitamin E:

1. Liver: Liver sections revealed normal hepatic parenchyma. However, mild congestion of portal blood vessels with biliary proliferation and round cells aggregations were seen. The kupffur cells were prominentanly hypertrophied.

2. Kideny: Examined sections from kidney showed apparently normal renal parenchyma with focal interstitial round cells aggregation, mild degenerative changes in some renal tubular epithelium and intratubular hyaline casts in some tubules (Figure 6).

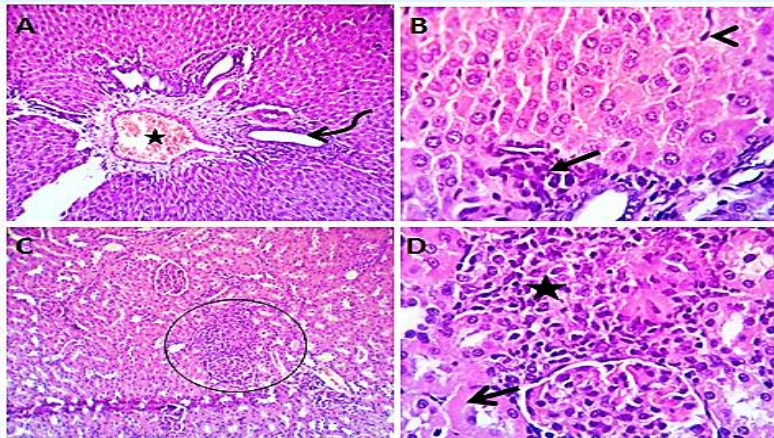


Figure 6: Photomicrograph of liver (A & B) showing normal hepatic parenchyma with mild congestion of portal blood vessels (star), biliary proliferation (curved arrow), round cells aggregations (closed arrow) and hypertrophied kupffur cells (arrow head). Kidney (C & D) showing apparently normal renal parenchyma with focal interstitial round cells aggregation (star), mild degenerative changes in some renal tubular epithelium (open arrows). H&E X 100 (A,C), 400 (B,D).

Discussion

The present study was designed to investigate the adverse effect of Bisoprolol and protective effect of vitamin E against hepatic and renal disturbance included in rats by Bisoprolol. In this study Bisoprolol is used in dose (1.8mg/kg b.wt.) once daily for successive 21 days. Liver enzymes such as ALT, AST and ALP are known marker enzymes for the assessment of the functional integrity of the liver cells. The results suggested that the administration of Bisoprolol in therapeutic dose for 21 successive days to rats resulting in a significant increase in ALT level after one day post-administration when compared with control group but this elevation decrease gradually by time. The administration of Bisoprolol in therapeutic dose for 21 successive days to rats resulting in a significant increase in AST level after one day post-administration when compared with control group. The administration of Bisoprolol in therapeutic dose for 21 successive days to rats resulting in a significant increase in ALP level after one day post-administration when compared with control group. The administration of bisoprolol in therapeutic dose for 21 successive days to

female rats resulting in a significant decrease in total protein level after one day post-administration when compared with control group. The administration of bisoprolol in therapeutic dose for 21 successive days to rats resulting in a significant decrease in albumin level after one day post-administration when compared with control group. The administration of bisoprolol in therapeutic dose for 21 successive days to rats induced a significant decrease in Globulin level after one day post-administration when compared with control group. Our results are in harmony with the results reported by Allen, et al. [20], who found that the effect of bisoprolol included decrease in albumin. Histopathological Results in 1st day after administration with bisoprolol showing mild to moderate congestion of hepatic blood vessels, portal biliary proliferation, round cells aggregations and fibroplasia in addition to minute interstitial aggregation of round cells. In 7th day after administration showing sever congestion of hepatic blood vessels, portal biliary/hyperplasia and round cells aggregations. In 14th day after administration showing multi focal interstitial round cells aggregations with replacement of hepatocyte. Multi focal necrosis of hepatic parenchyma. The results suggested

that administration of bisoprolol in the therapeutic dose for 21 successive days to rats resulting in a significant increase in serum creatinine level after one day post-administration when compared with control group. The administration of bisoprolol in therapeutic dose for 21 successive days to rats resulting in a significant increase in serum uric acid level after one day post-administration when compared with control group. Our results are in harmony with the results reported by Allen, et al. [20]. Who found that the effect of bisoprolol included increase creatinine and uric acid. Histopathological results in 1st day after administration with bisoprolol showing moderate congestion of the renal blood vessels, perivascular edema and degenerative changes in a moderate number of renal tubular. In 7th day after administration showing congested renal blood vessels with perivascular edema, multi focal necrosis of renal tubules. In 14th day week after administration showing moderate congestion of renal blood vessels and capillaries, perivascular edema, degenerative changes in the renal tubular epithelium mainly cloudy swelling and hydropic degeneration beside hyaline casts in some renal tubules. Our results are in harmony with the results reported by Reubi. Who found that the general mechanisms that cause renal disturbance included change in renal blood vessels, renal tubular epithelium capillaries. The results suggested that administration of bisoprolol in therapeutic dose for 21 successive days to rats resulting in a significant decrease in catalase activity after one day post-administration when compared with control group.

The administration of bisoprolol in therapeutic dose for 21 successive days to rats resulting in a significant decrease in superoxide dismutase activity after one day post-administration when compared with control group. The administration of bisoprolol in therapeutic dose for 21 successive days to rats resulting in a significant decrease in Glutathione peroxidase activity after one day post-administration when compared with control group. The administration of bisoprolol in therapeutic dose for 21 successive days to rats resulting in a significant increase in MDA activity after one day post-administration when compared with control group. Drugs and some chemical agents are known to induce hepatic and renal disturbance in human. Chemical agent are screened daily for their hepatoprotective and nephro-protective properties, for example Vit. E. It is one of the most important antioxidant drugs due to its hepatonephroprotective properties as reported by researches. This study demonstrated the hepato- nephroprotective effect of Vit. E. Vit. E has hepatoprotective effect as it expresses important functions in the membranes;

preventing ROS damage in polyunsaturated fatty acids a lipid soluble antioxidant and acting against damage caused to phospholipids as a membrane – stabilizing agent. Additionally, Vit. E is known to act by breaking the antioxidant chain that prevent ROS- produced cell membrane. It decreases lipid peroxidation and protects against liver injury. It decrease liver fibrosis, tumor necrosis factors, inflammation and hepatic porphyrin [21].

The previous observation explained the hepatoprotective effects of Vit. E. observed in the present study. Vit. E. normalized levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, serum bilirubin, glutathione peroxidase superoxide dismutase, malondialdehyde and improved histopathological changes in the liver induced by chemical agents in the control group. The possible pathway can be explained through the structure of Vit. E, the side chain in the 2-position facilitates the incorporation and retention of Vit. E in bio membranes so that the 6- position is optimal for scavenging free radicals and terminating lipid peroxidation. Furthermore, its antioxidant activity is exhibited through protection of poly unsaturated fatty acid from oxidation by reactive oxygen species, stabilization of membrane and breaking of antioxidant chains that prevent reactive oxygen species damage to membranes. In the present study, Vit. E used in a dose (1.8 mg/kg b.wt.) once daily for 21 days. The administration of bisoprolol and Vit. E combination in their therapeutic dose for 21 successive days to rat resulting in decrease in ALT level after one day post-administration compared with bisoprolol group. The administration of bisoprolol and Vit. E combination in their therapeutic dose for 21 successive days to rat resulting in decrease in AST level after one day post-administration compared with group. The administration of bisoprolol and Vit. E combination in their therapeutic dose for 21 successive days to rat resulting in decrease in ALP level after one day post-administration compared with bisoprolol. The administration of bisoprolol and Vit. E combination in their therapeutic dose for 21 successive days to rat resulting in increase in total protein level after one day post-administration compared with bisoprolol. The administration of bisoprolol and Vit. E combination in their therapeutic dose for 21 successive days to rat resulting in increase in albumin level after one day post-administration compared with bisoprolol. The administration of bisoprolol and Vit. E combination in their therapeutic dose for 21 successive days to rats induced a significant increase in globulin level after one week post-administration when compared with control and bisoprolol group. Sanchez-Valle, et al. [22] reported

the anti-oxidative therapy, mainly using natural and synthetic antioxidants, represents a reasonable therapeutic approach for the prevention and administration of liver diseases due to the role of oxidative stress in contributing to initiation and progression of hepatic damage.

Medina & Moreno Otera [23] reported that the antioxidant therapy has been considered to have the possibility of beneficial effects in the management of these liver diseases; antioxidant have produced mixed results in a number of clinical trials of efficacy. Parola, et al. [24] reported that hepatoprotective effects of Vit. E against malathion- induced mitochondrial dysfunction in rat liver. Meagher, et al. [25] reported that hepatoprotective effects of Vit. E against malathion-induced mitochondrial dysfunction in rat liver. The results suggested that the administration of bisoprolol and Vit. E Combination in their therapeutic dose for 21 successive days to rat resulting in decrease in serum creatinine level after one day post-administration compared with bisoprolol group. The administration of bisoprolol and Vit. E combination in their therapeutic dose for 21 successive days to rat resulting in decrease in serum uric acid level after one day administration compared with bisoprolol. Histopathological results in 1st day post-administration with bisoprolol showing moderate degenerative changes in renal tubular epithelium. In 7th day showing mild degenerative changes in renal tubular epithelium. In 14th day showing normal with mild congestion in renal bl.Vs.

The administration of bisoprolol and Vit. E Combination in their therapeutic dose for 21 successive days to female rat resulting in increase in glutathione peroxidase activity after one day post-administration compared with bisoprolol. The administration of bisoprolol and Vit. E Combination in their therapeutic dose for 21 successive days to rat resulting in decrease in MDA activity after one day administration compared with bisoprolol group. Beytut, et al. [26] reported that the increased level of antioxidant enzymes, CAT, SOD, GPX resulted from administration of Vit. E might normalized the lipid peroxidation reaction and related biochemical changes which in turn protects the cells from the increased risk of peroxidative damage as a result of administration of cytotoxic drugs. Kagan & Tyurins [27] reported that one of the ways in which α -tocopherol was believed to stabilize membranes to form a complex with the membrane lipids components that have tendency to destabilize the bilayer structure thereby countering their effects and rendering the membrane more stable as also supported by the observed reduction of MDA and nitric

oxide as well as increased glutathione. Another explanation of the action of Vit. E is the decrease of lipid peroxidation radical was suggested by Halliwell, et al. [28], [29] which suggested that administration of α -tocopherol averted oxidative damage, probably through its capacity to quickly and efficiently scavenge lipid peroxide radicals before they attack the membrane lipid. This ability might be related to the fact that lipid peroxy radicals react more rapidly (by four orders of magnitude) with α -tocopherol than with membrane lipids.

Conclusion

From the obtained results it could be concluded that bisoprolol has hepatic and renal disturbance effect in rats; Vit. E has a protective effect against hepatic and renal adverse effect of bisoprolol, which may attribute to decrease the harmful effects of bisoprolol inhibiting free radical formation and by restoration of the antioxidant systems. The combination of Vit.E and bisoprolol showed better results than Bisoprolol alone. So, bisoprolol should be used with caution in people with liver or kidney problems.

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