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EFFECTS OF THE AMOUNT ETHANOL DAILY INGESTED ON THE TIME APPEARANCE OF HYPERTENSION IN MALE WISTAR RATS

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ABSTRACT

Aims: Heavy alcohol consumption is known to induce hypertension and other issues. Therefore, the present work was designed to study relationship between the dose of ethanol ingested and the time induction of hypertension. **Methods:** Male albino Wistar rats aged 08 to 10 weeks were randomly divided into 5 groups. Each group was made up of control animals treated with distilled water (5 subgroups) and ethanol (5 subgroups).Treated subgroups daily received orally ethanol at the doses of 1.5 g/kg, 3 g/kg, 4.5 g/kg, 6g/kg and 9 g/kg, respectively. Arterial blood pressure of animals receiving ethanol was measured every week. The time of hypertension confirmation was considered as the time of induction. Some hemodynamic and biochemical parameters of the concern ethanol treated subgroup (5 rats) as well as the corresponding control (5 rats) were assessed. **Results**: The administration of ethanol induced hypertension respectively after 12 weeks (1.5 g/kg), 8 weeks (3 g/kg), 6 weeks (4.5 g/kg), 3 weeks (6g/kg) and 2 weeks (9 g/kg). Moreover, significant increase in serum triglycerides and LDL cholesterol levels as well as ALT, AST, ALP and GGT activities were observed in ethanol hypertensive rats. Also, ethanol lowered reduced glutathione level as well as SOD and catalase activities in various tissues; meanwhile, MDA level was significantly increased. **Conclusion**: The above results show that the dose of

ethanol is proportional to the time of hypertension induction. This animal model is associated with abnormalities such as hyperlipidemia, oxidative stress and endothelial impairment.

KEYWORDS: Ethanol, hypertension, oxidative stress, endothelial dysfunction.

INTRODUCTION

Cardiovascular diseases are the leading cause of early death and disability worldwide.[1] The increased incidence of risk factors for these diseases is due among other things to a sedentary lifestyle, the consumption of high calorie foods, salty foods, cigarettes and alcohol.[2,3] The effects of alcohol on the cardiovascular system have long been of interest to physicians, scientists and the public. Habitual light to moderate alcohol intake is associated with decreased risks for total mortality, coronary artery disease, diabetes mellitus, congestive heart failure, and stroke. However, chronic alcohol consumption is associated with increased cardiovascular risk such as coronary heart disease, stroke, peripheral arterial disease, cardiomyopathy and hypertension.[4,5] Hypertension is the main risk factor of cardiovascular diseases such as cerebrovascular and ischemic heart diseases, as well as premature death worldwide.[6] However, its pathogenesis is not fully understood and its treatment is not yet satisfying.[7] For these reasons, there is an ongoing search for animal models that better mimic changes resulting from this disease. Because of the complexity of hypertension, the use of animal models to elucidate the pathogenesis and to evaluate new therapeutic possibilities is an important tool. Those models are used to understand the disease since it enables consistent experimental strategies that are impractical in humans.[8] Many useful experimental animal models have been developed and based on non-genetic, secondary factors of hypertension. Alcohol-induced hypertension is already an established animal model, but not yet well characterized nor standardized. Therefore, the present work aimed to study the relationship between the dose of ethanol ingested and the time of induction of hypertension as well as some related injuries in rats.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents used in the experiment were purchased from Sigma Chemical, St Louis, MO, USA.

Experimental animals

Male albino Wistar rats aged 08 to 10 weeks and weighting 150-170 g were used in the present study. These animals were housed in plastic cages and allowed free access to water and standard rat chow. Animal studies were conducted according to Institute Animal Ethics Committee regulations approved by the Cameroon National Ethical Committee (Reg. N°FWAIRD 0001954).

Experimental design

Rats were randomly assigned into 5 groups of ten rats. Each group was made up of a control animals daily treated with distilled water (5 rats) and ethanol-treated (5 rats). The five ethanol-treated subgroups daily received via oral gavage ethanol 40°at the doses of 1.5 g/kg, 3 g/kg, 4.5 g/kg, 6g/kg and 9 g/kg respectively. Arterial blood pressure of animals receiving ethanol was measured every week. When hypertension was confirmed, the treatment was ended for the group as well as its corresponding control. Then, some hemodynamic and biochemical parameters of the concern ethanol treated subgroup (5 rats) as well as the corresponding control (5 rats) were measured.

Ethanol 95° was diluted with distilled water to get ethanol 40°. The dilution of ethanol was done according to Gay Lusac's table by adding to 100 mL of ethanol 95° 156.58 mL of distilled water.

Measurement of body weight and food intake

The body weight of each rat was recorded before starting the treatment, twice a week during the experimental period and the end of the study. The percentage of body weight change was calculated according to the following equation:

$$\frac{(Final\ body\ weight\ (g) - Initial\ body\ weight\ (g))x100}{Initial\ body\ weight\ (g)}$$

In addition, the food intake was daily performed. The consumed amount of food was measured before provide to each group, their remnants were calculated next day to get the differences. The mean food intake was calculated (g/rat/day).

Hemodynamic parameters recording

Hemodynamic parameters (systolic arterial blood pressure and heart rate) of rats in all groups were recorded at the end of the induction of hypertension by different doses of ethanol as

previously described by Bopda et al.[9] Briefly, rats were anaesthetized using urethane 15% (1.5g/kg, *i.p*). The trachea was exposed and cannulated to facilitate spontaneous respiration. Blood pressure and heart rate were measured by the direct method from right carotid artery, using a cannula connected to a pressure transducer (RX 104A, BIOPAC Systems Inc., California, USA) coupled to the Biopac acknowledge data acquisition analysis software (Biopac Student Lab, MP35 type) and a computer. Electrocardiogram (ECG) was recorded using high sensitivity needles (electrodes), connected to the same recorder and computer. Before recording the data, each animal was allowed to stabilize for at least 30 min.

Biochemical assays

At the end of hemodynamic parameters measurement, the free-running blood from the carotid was collected in unconscious rats which were sacrificed by haemorrhagic shock. Serum was separated by centrifugation and used for the determination of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransaminase (AST), gamma glutamyl transferase (GGT), creatinine, total-cholesterol (CHOL), triglycerides (TG), LDL-cholesterol and HDL-cholesterol (HDL) using commercial diagnostic kits Fortress, UK. The abdominal cavity was opened after blood collection; aorta, heart, liver and kidney were dissected out and homogenized in Mc Ewen solution for aorta and heart or Tris-HCl 50 mM buffer solution for liver and kidney to make a 20% homogenate. Tissue protein concentration was assayed according to Gornall et al.[10] Catalase was determined according to Sinha,[11] whereas glutathione reduced and superoxide dismutase were determined using the method of Ellman.[12] and Misra et Fridovish,[13] respectively. The end product of lipid peroxidation (malondylaldehyde, MDA) was determined using the procedure of Wilbur et al.[14] and the Nitrite concentration (an indirect measurement of NO synthesis) was assayed using Griess reagent in acidic medium.[15] All the above biochemical markers were measured using the spectrophotometer Genesys 20.

Statistical analysis

The data were expressed as mean ± standard deviation (SD). The SPSS (version 10.1) was used in data analysis. Data were analyzed with one-way analysis of variance (ANOVA) followed by the Duncan's post hoc test). A value of $p<0.05$ was considered to be statistically significant.

RESULTS

Effect of ethanol on body weight and food intake

Table 1 shows the level of body weight and food intake in control rats and ethanol treated rats. This table shows that ethanol consumption had a biphasic effect on body weight. The higher doses of ethanol (6 and 9g/kg) used in this study induced a decrease of body weight by 7.06% ($P>0.05$) and 13.46% ($p<0.05$), respectively as compared to their initial values. In rats fed with lower doses of ethanol (4.5, 3 and 1.5g/kg) however, the body weight increased ($p<0.01$) by 21.55%, 111.21% and 91.20%, respectively as compared to their initial values. In addition, rats treated with ethanol (9g/kg, 6g/kg, 4.5g/kg and 3g/kg) showed a significant reduction in food intake compared to their respective control groups. However, there was no significant variation in food intake in group receiving ethanol (1.5g/kg) when compared to its control group.

Table 1: Effects of various doses of ethanol on body weight and food intake.

Groups	Body weight gain (%)	Food intake (g/rat/day)
Distilled water (1mL/kg)	43.42	8.88±0.10
Ethanol (9g/kg)	**-07.06****	**4.45**±040**
Distilled water (1mL/kg)	70.56	8.12±0.17
Ethanol (6g/kg)	**-13.46****	**5.10±0.31****
Distilled water (1mL/kg)	77.31	8.12±0.17
Ethanol (4.5g/kg)	**21.55****	**4.23±0.72****
Distilled water (1mL/kg)	96.85	7.93±0.77
Ethanol (3g/kg)	**111.32**	**5.64±0.13****
Distilled water (1mL/kg)	138.77	8.01±0.44
Ethanol (1.5g/kg)	**91.20****	**8.20±0.58**

Each value represents means±S.E.M. of 5 rats. **$P<0.01$, significantly different compared to the corresponding control group.

Effect of ethanol on arterial blood pressure and heart rate

Table 2 summarizes respectively the effect of ethanol on systolic arterial blood pressure and heart rate. Compared with their corresponding controls, the level of systolic arterial blood pressure and heart rate increased ($p<0.01$) by 21.11% and 17.41%, 38.27 and 10.85%, 49.10% and 15.35%, 57.82% and 13.90%, 54.62 and 15.10% in rats treated with ethanol 1.5, 3, 4.5, 6 and 9g/kg/day, respectively. The table also indicates that the daily administration of higher doses of ethanol 40° (9 and 6g/kg/day) induced hypertension after 2 and 3 weeks, respectively. In the same condition, the lower doses of ethanol induced hypertension after 6 weeks at the dose of 4.5g/kg/day, 8 weeks at the dose 3g/kg/day of and 12 weeks at the dose of 1.5g/kg/day.

Table 2: Effects of various doses of ethanol on systolic arterial blood pressure and heart rate.

Groups	Systolic arterial blood pressure (mmHg)	Heart rate (beat/minute)	Experimental period (weeks)
Distilled water (1mL/kg)	119.10±1.67	347±6	**2**
Ethanol (9g/kg)	**184.16±1.62*****	**400±3***	
Distilled water (1mL/kg)	116.26±2.43	321±3	**3**
Ethanol (6g/kg)	**183.49±3.71*****	**365±3***	
Distilled water (1mL/kg)	117.38±2.01	300±6	**6**
Ethanol (4.5g/kg)	**175.02±2.64*****	**346±4***	
Distilled water (1mL/kg)	124.70±1.46	256±4	**8**
Ethanol (3g/kg)	**172.43±2.23*****	**284±3***	
Distilled water (1mL/kg)	127.40±0.97	305±3	**12**
Ethanol (1.5g/kg)	**148.67±5.38*****	**358±5***	

Each value represents means±S.E.M. of 5 rats. *P<0.05; ***P<0.001, significantly different compared to rats treated with distilled water.

Effect of ethanol on electrocardiogram (ECG)

As shown in table 3, the duration of P wave, PR segment, QRS complex and T wave was not significantly affected by ethanol administration as compared to each corresponding control group. Meanwhile, when compared to their respective control groups, ethanol significantly increased the duration of QT interval by 15.55% (P<0.05) at the dose of 4.5g/kg, 42.85% (P<0.01) at the dose of 6g/kg and 35.13% (P<0.01) at the dose of 9g/kg. In the same condition, the duration of ST segment significantly increased (P<0.01) by 55.55 and 66.66% in groups daily receiving ethanol 6 and 9g/kg, respectively compared to its control group.

Daily ethanol administration also induced significant increase of P wave and QRS complex amplitudes respectively by 50.00 and 45.71% at the dose of 4.5g/kg, 50.00 and 38.23% at the dose of 6g/kg and 100.00 and 57.14% at the dose of 9g/kg. The results also indicate significant increase of the T wave amplitude in all ethanol treated groups as compared to their respective control animals.

Table 3: Effect of various doses of ethanol on ECG.

	Duration (ms)						Amplitude (mV)		
Groups	**P Wave**	**PR Segment**	**QRS Complex**	**QT Interval**	**ST Segment**	**T wave**	**P Wave**	**QRS Complex**	**T Wave**
DH_2O (1mL/kg)	5±0	52±3	35±5	48±2	32±4	5±0	0.03±0.00	0.35±0.07	0.15± 0.00
EthOH (1.5g/kg)	**5±0**	**47±4**	**28±2**	**47±2**	**27±2**	**5±0**	**0.05±0.01****	**0.27±0.03***	**0.23±0.01***
DH_2O (1mL/kg)	5±0	38±2	23±2	45±3	25±0	5±0	0.05±0.01	0.48±0.05	0.14±0.01
EthOH (3 g/kg)	**5±0**	**42±3**	**27±2**	**43±2**	**23±2**	**5±0**	**0.04±0.00**	**0.45±0.03**	**0.17±0.01***
DH_2O (1mL/kg)	5±0	42±2	28±2	45±0	28±3	5±0	0.02±0.00	0.35±0.03	0.14±0.00
EthOH (4.5g/kg)	**5±0**	**47±2**	**25±3**	**52±2***	**32±2**	**5±0**	**0.03±0.00***	**0.51±0.00****	**0.26±0.00****
DH_2O (1mL/kg)	5±0	47±3	28±3	42±2	27±3	5±0	0.04±0.00	0.34±0.01	0.11±0.01
EthOH (6g/kg)	**5±0**	**45±0**	**23±2**	**60±3****	**42±2****	**5±0**	**0.06±0.00****	**0.47±0.02***	**0.19±0.01****
DH_2O (1mL/kg)	5±0	43±4	30±5	37±2	27±2	5±0	0.02±0.00	0.35±0.02	0.10±0.00
EthOH (9g/kg)	**5±0**	**47±2**	**28±2**	**50±0****	**45±3****	**5±0**	**0.04±0.00*****	**0.55±0.02***	**0.15±0.02****

Each value represents means±S.E.M. of 5 rats. DH_2O: distilled water, EthOH: Ethanol. *P<0.05; **P<0.01, ***P<0.001, significantly different compared to rats treated with distilled water.

Effects of ethanol on some serum markers of lipid profile

As shown in Table 4, the lipid profile parameters (triglycerides, total cholesterol, HDL-Cholesterol and LDL-Cholesterol) were affected by ethanol consumption in a dose and time-dependent manner. The level of triglycerides, total cholesterol and LDL-Cholesterol increased in ethanol treated rats by 56.59% (P<0.01), 8.93% (P>0.05) and 44.40% (P<0.01) at the dose of 9g/kg and 41.46% (P<0.01), 45.47% (P<0.01) and 160.38 (P<0.01), at the dose of 6g/kg respectively as compared to their control. The increase in the level of lipid markers evaluated in animals treated with low doses of ethanol (4.5, 3 and 1.5g/kg) was greater when compared to rats receiving high amount of ethanol (9 and 6g/kg). Indeed, the level of triglycerides, total cholesterol and LDL-Cholesterol increased (P<0.01) respectively by 173.51%, 33.40% and 21.22% for 4.5g/kg, 258.00%, 513.15% and 1521.45% for 3g/kg, 494.57%, 587.69% and 1627.30% for 1.5g/kg. The HDL-Cholesterol levels in ethanol hypertensive rats decreased (P<0.01) by 28.72% and 20.91%, respectively at the doses of 9

and 6g/kg compared to control rats. However, for the doses of 4.5, 3 and 1.5g/kg, ethanol induced the increase in HDL levels by 14.85% ($P<0.05$), 86.67% ($P<0.01$) and 149.19% ($P<0.01$), respectively as compared to their respective control groups.

Table 4: Effects of various doses of ethanol on some markers of lipid profile.

Groups	Triglycerides (mg/dL)	Total Cholesterol (mg/dL)	HDL-Cholesterol (mg/dL)	LDL-Cholesterol (mg/dL)
Distilled water (1mL/kg)	27.05±0.37	47.32±1.04	23.85±1.04	18.06±1.73
Ethanol (9g/kg)	**42.36±0.54****	**51.55±2.36**	**17.00±1.95****	**26.08±3.06***
Distilled water (1mL/kg)	28.53±0.16	48.88±0.84	27.25±0.71	15.93±0.89
Ethanol (6g/kg)	**40.36±1.32****	**71.11±1.19****	**21.55±0.82****	**41.48±1.34****
Distilled water (1mL/kg)	24.28±2.14	50.08±0.64	20.20±0.97	25.02±0.92
Ethanol (4.5g/kg)	**66.41±3.61****	**66.81±0.37****	**23.20±0.64***	**30.33±0.66****
Distilled water (1mL/kg)	28.34±0.60	44.99±0.75	26.55±0.39	12.77±1.14
Ethanol (3g/kg)	**101.46±6.14****	**275.86±6.85****	**48.50±0.28****	**207.06±7.42****
Distilled water (1mL/kg)	30.25±0.14	50.81±2.32	31.10±1.63	13.66±1.54
Ethanol (1.5g/kg)	**179.86±7.85****	**349.42±8.93****	**77.50±2.70****	**235.95±10.49****

Each value represents means ± S.E.M. of 5 rats. HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein. $^{*}P<0.05$, $^{**}P<0.01$, significantly different compared to rats treated with distilled water.

Effects of ethanol on some serum markers of renal and hepatic functions

The effect of various doses of ethanol on some serum markers of renal and hepatic functions is presented in Table 5. The results indicate that creatinine levels are significantly elevated after ethanol exposure only in groups receiving respectively 6 and 4.5g/kg as compared to their control groups. The activities of liver specific enzymes such as AST, ALT, ALP and GGT increased significantly in response to ethanol exposure (Table 5). The results indicate that globally these enzymes were remarkably elevated in rats treated with high doses of ethanol in comparison with normal group. As shown in Table 5, the oral administration of ethanol (9g/kg) for 2 weeks caused the increase ($P<0.01$) in the activities of AST by 141.44%, ALT by 180.18%, ALP by 65.97% and GGT by 311.36% as compared with the corresponding normal control group. However, the increase induced by the 1.5g/kg dose of ethanol during 12 weeks of treatment was by 250.78% ($P<0.01$) for ALT, 18.63% ($P<0.05$) for ALP and 97.88% ($P<0.01$) for GGT as compared to normotensive rats.

Table 5: Effects of ethanol on some serum markers of renal and hepatic functions.

Groups	Creatinine (mg/dL)	ALP (U/L)	GGT (U/L)	AST (U/L	ALT (U/L)
Distilled water (1mL/kg)	0.84±0.06	25.22±1.59	2.64±0.69	44.40±1.16	11.10±0.29
Ethanol (9g/kg)	**0.95±0.05**	**41.86±0.97****	**10.86±0.56****	**107.20±1.35****	**31.10±0.05****
Distilled water (1mL/kg)	0.92±0.16	28.51±0.58	3.57±0.17	36.00±0.89	11.30±0.25
Ethanol (6g/kg)	**1.08±0.02****	**45.88±0.53****	**10.71±0.67****	**131.60±0.74****	**27.30±0.68****
Distilled water (1mL/kg)	0.61±0.01	23.03±0.97	4.12±0.29	23.20±0.17	11.50±0.35
Ethanol (4.5g/kg)	**0.82±0.03****	**52.09±0.40****	**13.43±0.59****	**80.12±2.30****	**52.20±0.66****
Distilled water (1mL/kg)	0.91±0.09	23.58±0.67	3.37±0.04	42.48±0.41	14.20±0.20
Ethanol (3g/kg)	**0.88±0.09**	**31.99±0.80****	**13.80±0.19****	**59.16±3.92****	**91.50±1.70****
Distilled water (1mL/kg)	0.88±0.06	27.42±1.93	4.73±0.09	47.96±0.16	12.80±0.45
Ethanol (1.5g/kg)	**0.97±0.07**	**32.53±0.46***	**09.36±0.51****	**16.48±0.50****	**44.90±1.44****

Each value represents means ± S.E.M. of 5 rats. ALP: Alcaline Phosphatase, GGT: Gamma Glutamyl transferase, AST: Aspartate Aminotransaminase, ALT: Alanine Aminotransferase. $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different compared to rats treated with distilled water.

Effects of ethanol on some tissue markers of oxidative stress

The aorta, heart, liver and kidney tissues were used to determine the effect of various doses of ethanol on some markers of oxidative stress (mainly catalase, GSH, SOD and MDA) and the results are summarized in Figures 1-4.

As shown in Figure 1, the activity of catalase significantly decreased in investigated tissues of ethanol hypertensive rats at all the doses excepted for the 1.5 g/kg dose in aorta and heart, and the 9 g/kg dose in aorta as compared to respective control rats. The decrease of the level of catalase was by 25.05% (P>0.05) in aorta, 18.38% (P>0.05) in heart, 69.85% (P<0.01) in liver and 69.32% (P<0.01) in kidney at the 1.5g/kg dose of ethanol. The reduction of the level of catalase in homogenates tissues of rats treated with ethanol (9g/kg) was by 34.75% (P<0.01) in heart, 21.18% (P<0.05) in liver and 19.93% (P<0.01) in kidney compared to their control, respectively.

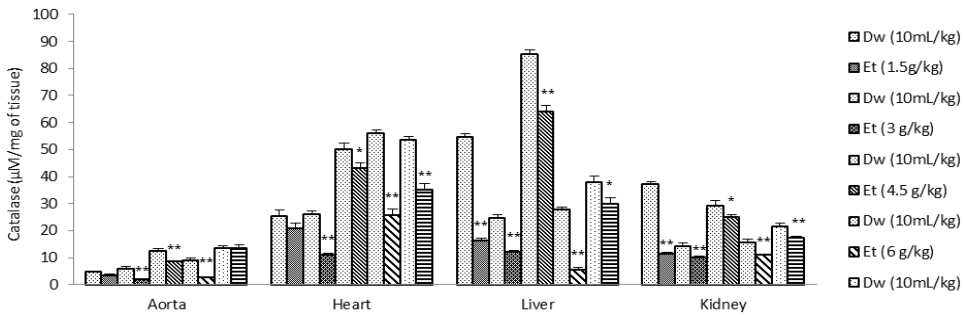

Fig. 1: Effects of various doses of ethanol on catalase activity.

Each bar represents means ± S.E.M. of 5 rats; $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different compared to rats treated with distilled water. Dw: Distilled water, Et: Ethanol.

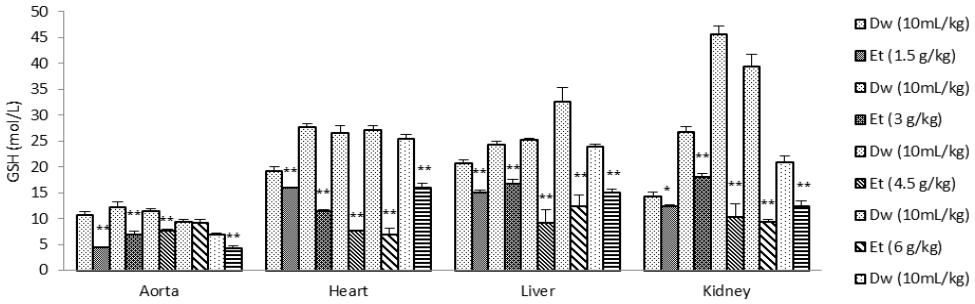

Fig. 2: Effects of various doses of ethanol on GSH levels.

Each bar represents means ± S.E.M. of 5 rats; $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different compared to rats treated with distilled water. Dw: Distilled water, Et: Ethanol.

The content of GSH (Figure 2) in ethanol treated rats was significantly lower as compared with the control apart from the heart of rats which received the 6g/kg dose of alcohol. Ethanol (1.5g/kg) feeding for 12 weeks provoked the decrease of the level of GSH by 58.81% ($P<0.01$) in aorta, 17.01% ($P<0.01$) in heart, 27.01% ($P<0.01$) in liver and 14.26% ($P<0.05$) as compared to normotensive rats. In the same condition, ethanol (9g/kg) caused the decline of GSH content by 38.84% ($P<0.01$) in aorta, 37.26% ($P<0.01$) in heart, 37.43% ($P<0.01$) in liver and 40.21% ($P<0.01$) as compared to normal rats.

As shown in Figure 3, the values of the activities of SOD in various tissues investigated decreased by 66.29% ($P<0.01$), 64.52% ($P<0.01$), 4.27% ($P>0.05$), 31.69% ($P>0.05$) and 34.57% ($P<0.01$) in aorta, 90.68% ($P<0.01$), 60.55% ($P<0.01$), 64.00% ($P<0.01$), 13.20% ($P>0.05$) and 57.57% ($P<0.01$) in heart, 69.76% ($P<0.01$), 59.75% ($P<0.01$), 67.50% ($P<0.01$), 21.95% ($P>0.05$) and 82.60% ($P<0.01$) in liver and 88.00% ($P<0.01$), 58.82% ($P<0.01$), 69.04% ($P<0.01$), 27.02% ($P>0.05$) and 66.37% ($P<0.01$) in kidney, respectively

for animals receiving the 1.5, 3, 4.5, 6 and 9 g/kg doses of ethanol as compared with their control.

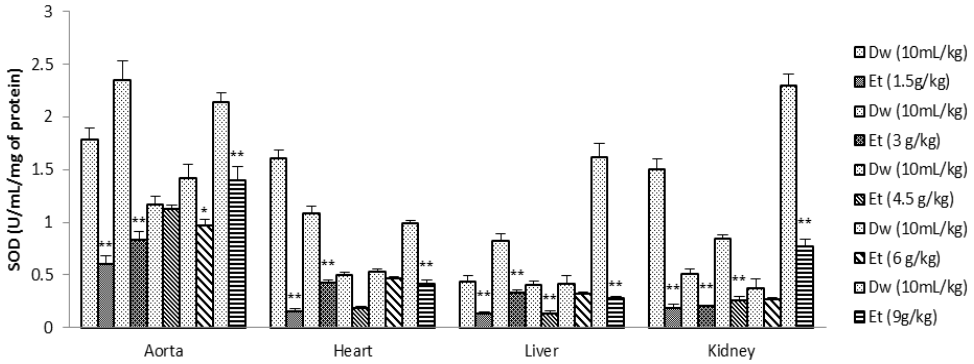

Fig. 3: Effects of various doses of ethanol on SOD.

Each bar represents means ± S.E.M. of 5 rats; $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different compared to rats treated with distilled water. Dw: Distilled water, Et: Ethanol.

The effects of ethanol consumption on the end product of lipid peroxidation were determined by measuring the level of MDA (Figure 4). Our results indicate that the various doses of ethanol daily administered induced significant increase ($P<0.01$) in level of MDA in aorta (excepted at the 6 g/kg dose), in the heart (excepted at the 3 g/kg dose), in the liver and in the kidney compared to rats treated with distilled water.

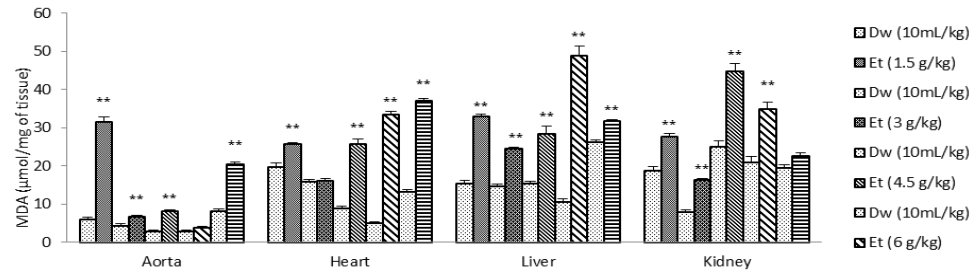

Fig. 4: Effects of various doses of ethanol on MDA levels.

Each bar represents means ± S.E.M. of 5 rats; $^{*}P < 0.05$, significantly different compared to rats treated with distilled water. Dw: Distilled water, Et: Ethanol.

Effects of ethanol on nitrites level

The effects of ethanol consumption on nitrites levels in various tissues investigated in the present study are summarized in Figure 5. The results indicate that the level of nitrites in ethanol hypertensive rats was significantly reduced as compared with their normal control respectively, apart from aorta and heart of rats which received the 6 g/kg dose. The lower

dose of ethanol daily administered for 12 weeks in the present study induced the decrease ($P<0.01$) of nitrites content by 50.47% in aorta, 41.41% in heart, 66.90% in liver and 18.67% in kidney when compared to their normal control group, respectively. The higher dose of ethanol administered for 2 weeks caused the decline of the nitrites levels in homogenates tissues by 11.43% ($P>0.05$) in aorta, 7.58% ($P>0.05$) in heart, 43.98% ($P<0.01$) in liver and 65.31% ($P<0.01$) in kidney as compared to rats receiving distilled water during the experimental period.

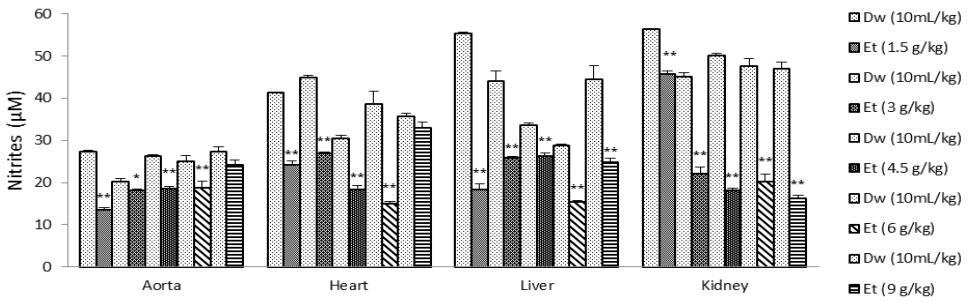

Fig. 5: Effects of various doses of ethanol on nitrites levels.

Each bar represents means ± S.E.M. of 5 rats; $^{**}P<0.01$, significantly different compared to rats treated with distilled water. Dw: distilled water, Et: Ethanol.

DISCUSSION

Hypertension is a chronic non communicable disease and often asymptomatic medical condition in which the pressure exerted by the blood on the wall of the artery is elevated.[16] Many kind of animal models of hypertension have been developed in order to understand the pathogenesis of this disease and to test novel therapeutic agents.[7] Therefore, the present study was designed to characterize the animal model of hypertension induced by ethanol. Currents findings indicate that ethanol has biphasic effect on body weight dependently of the daily dose administered. Higher doses induced a decrease in body weight while lower doses caused the increase of this anthropometric parameter during the experimental period. Several mechanisms could have contributed to lower body weight in high dose ethanol hypertensive rats. Moreover, alcohol impairs nutrient absorption by damaging cells lining the stomach and intestines, and disabling transport of some nutrients into the blood.[17] Alcohol also inhibits the breakdown of nutrients into usable substances, by decreasing the secretion of digestive enzymes from the pancreas.[18] Chronic ethanol intake enhanced triglyceride degradation in adipose tissue through a reduction of the antilipolytic action of insulin.[19] Our results indicate that the administration of ethanol is able to induce hypertension and that, the daily dose

ingested affects the time of induction. The higher doses of ethanol used in the present study caused increase in systolic arterial blood pressure early than lower doses. The observed elevations in blood pressure in animals receiving heavy amount of ethanol could be due to alterations in contractile/relaxant properties of the vascular smooth muscle, impairment of baroreflex activity, excessive central-nervous-system excitability, sympathetic activation and activation of the renin-angiotensin-aldosterone system.[20,21,22] Furthermore, in chronic alcoholics, insulin resistance, leading to sodium retention, vascular smooth-muscle hypertrophy, and increased cytosolic Ca^{++} levels, all of which trigger hypertension, has been reported as well as hyperdynamic circulation with high cardiac output.[23]

Current study also indicate significant increase of the duration of QT interval and ST segment in some ethanol hypertensive groups. The prolonged QT interval may be a manifestation of the intraventricular or interventricular conduction delay or block, or it may be due to the down regulation of several potassium currents responsible of repolarization.[24] ST segment elevation has been described in many conditions, including acute myocardial infarction, Brugada syndrome, early repolarization, and acute pericarditis.[25] Abnormalities in plasma lipids and lipoprotein metabolism play a central role in the pathogenesis of hypertension. The presence of hyperlipidemia is so common in patients with hypertension that many have argued that the high blood pressure itself may play a role in altering lipid metabolism, resulting in abnormalities.[26] The most common lipid abnormalities during chronic alcohol consumption are hypercholesterolemia and hypertriglyceridemia, which were confirmed in the present study.[27] The increased cholesterol level during alcohol ingestion is attributed to the increased alpha-hydroxyl methyl glutaryl CoA reductase activity, which is the rate limiting step in cholesterol biosynthesis.[28] Increased triglycerides (TG) levels after ethanol ingestion may be due to the increased availability of free fatty acid, glycerophosphates, decreased TG lipase activity and decreased fatty oxidation. Also, in ethanol-treated rats, LDL was increased in the serum, while HDL was found to be reduced in high doses. It has been reported that high serum abnormally levels of total cholesterol and LDL are associated with an increased risk for atherosclerosis.[29]The present study also shows that administration of ethanol induces hepatic damage that elevates intracellular enzymes, such as transaminases, alkaline phosphatase and gamma glutamyl transferase. The increase of theses enzymes was very important in the high doses groups of ethanol-treated rats. Indeed, the transaminases (AST and ALT) are well-known enzymes used as good indicators of liver function and as biomarkers predicting possible toxicity.[30,31] In addition, AST found in the serum is of both

mitochondrial and cytoplasmic origin and any rise can be taken as a first sign of cell damage that leads to the outflow of the enzymes into the serum.[32] Thus, the significant increases observed in ALT and AST activities strongly suggest the common accepted view that the chronic administration of ethanol did alter the hepatocytes and consequently the metabolism of the rats. The increase of serum-glutamyl transferase (GGT) in ethanol hypertensive rats in this study may also indicate liver dysfunction.[33] Equally, there was also a significant rise in creatinine in the high dose of ethanol when compared to the control. Indeed, creatinine is known as a good indicator of renal function.[30] Any rise in creatinine levels is only observed if there is marked damage to functional nephrons.[34]

Oxidative stress has been shown to be one of the main components in ethanol-induced cell injury and apoptosis, resulted from the excessive exposure to reactive oxygen species (ROS).[35]The results of the present study indicate a significant decrease in the levels of catalase, reduce glutathione and SOD in the most of homogenates organs of ethanol hypertensive rats. The decrease in enzymatic and non-enzymatic antioxidants activities in hypertensive humans and in animal models of hypertension has been widely reported.[36] Similarly, current finding also indicate significant increase in the levels of MDA, the major product of lipid peroxidation in investigated tissues of ethanol treated rats. Oxidative stress induced by ethanol feeding in the present study may directly cause hypertension by mechanisms such as quenching of the vasodilator nitric oxide, generation of vasoconstrictor lipid peroxidation products. We can also suggest a depletion of tetrahydrobiopterin (BH4), damage of endothelial cells and vascular smooth muscles cells, increase in the intracellular free calcium, the endothelial permeability and the stimulation of growth signaling events.[37] The effects of ethanol feeding on endothelial function in the present study were evaluated by the determination on the levels of nitrites in investigated tissues. The present investigation showed that ethanol reduced the level of nitrites mainly in aorta, indicating endothelium dysfunction. This reduction of nitrites contain may in part be due to its conversion in peroxynitrites by reacting with superoxide. Actually, most of the cytotoxicity attributed to nitric oxide is due to peroxynitrite which is implicated in the pathogenesis of cardiovascular disease and complication of diabetes.[38,39,40] Globally, current findings are consistent with findings in humans, where alcohol consumption abuse is associated with the development hypertension and other metabolic disorders such as endothelial dysfunction, liver and kidney damages, dyslipidemia and oxidative stress.

CONCLUSION

In conclusion, the present study demonstrates that ethanol was effective to induce hypertension in Wistar rats from the daily dose of 1.5 mg/kg and the time of induction is closely related to the dose. Taken together, the data from the present study also indicates a possible implication of oxidative stress, dyslipidemia, liver and kidney damages as well as endothelial impairment in the development of ethanol induced hypertension. The present model of hypertension has characteristics similar to human and can then be used to test antihypertensive drugs.

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Conflict of Interest

The authors declare no conflicts of interest relevant to this manuscript.

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