

The effect of ethanol vapour exposure on atrial and ventricular walls of chick embryos

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Abstract

Objective: To study the effects of ethanol vapour exposure on atrial and ventricular walls of heart in chick embryo.

Methods: The study design was experimental, conducted at Islamabad Centre of College of Physicians and Surgeons, Pakistan. One hundred and eighty chicken eggs were divided into two groups, experimental and control, of 90 eggs each. Each group was subdivided into three subgroups of 30 eggs each based on the day of sacrifice. Experimental group was exposed to ethanol vapours and then compared with age matched controls.

Results: The thickness of atrial and ventricular walls along with lengths of valvular cusps increased in hearts of day 7 and day 10 chick embryos in experimental group. There was thinning of walls and decreased length of valvular cusps in hearts of experimental chicks on hatching as compared to age matched controls.

Conclusion: Ethanol vapour exposure during development causes cardiac and septal wall thickening during initial days of development followed by cardiac and septal wall thinning which is a classical picture of alcohol induced cardiomyopathies.

Keywords: Ethanol vapour, chick embryo, atrial and ventricular walls. (JPMA 66: 1227; 2016)

Introduction

Alcohol drinking has ill-effects on health of humans. Alcohol misuse results in damage to almost every organ system of the body potentially resulting in serious illnesses like liver disease¹ and alcoholic brain disease.² Prenatal alcohol exposure can lead to foetal abnormalities. Alcohol intake exerts teratogenic effects on the developing foetus known as foetal alcohol syndrome.³ Alcohol drinking also impedes the functions of the heart, a condition referred to as alcoholic cardiomyopathy.⁴

Currently another method of alcohol abuse has been utilized through a machine known as "Alcohol without Liquid Vaporizer" which allows users to inhale alcohol vapours instead of drinking alcohol.⁵ Another device is used in Canada for the same purpose named as "Vaportini". The two apparatuses have different methodologies but serve the same purpose that is inhaling alcohol vapours rather than drinking. Product manufacturers emphasize that vapour inhalation does not have any ill effects on health like drinking alcohol but researchers are of the opinion that inhaling alcohol vapours bypasses its passage through stomach and delivers alcohol directly to the brain.⁶ Various studies are now in progress, in which animal models are exposed to ethanol vapours resulting in serious consequences.⁷ The

effects of ethanol vapour exposure during gestation have also been under research lately to see whether ethanol vapour exposure has same effects on the foetal heart as drinking ethanol.⁸ This research was designed to study the effects of ethanol vapour exposure on atrial and ventricular walls of chick embryo.

Materials and Methods

This research was conducted at Department of Anatomy, College of Physicians and Surgeons, Islamabad from 2006 to 2007. The design of this study was experimental. One hundred and eighty Desi Chicken eggs were divided into an experimental group B and control group A comprising 90 eggs each. Each group was further subdivided into three subgroups 1, 2 and 3 containing 30 eggs each. Subgroup 1 was sacrificed on day 7, subgroup 2 on day 10 of incubation and subgroup 3 on hatching or day 22 whichever was earlier. The day when eggs were placed in the incubator was taken as day 1. The temperature was maintained at 102°F and the relative humidity was kept between 70% and 80%.⁹

Group B was exposed to ethanol vapours produced in a tightly sealed glass chamber. The level of ethanol vapours was monitored and maintained with the help of a breathalyzer.⁷ Range of ethanol vapours was kept between 0.75mg/l to 1.5mg/l. Subgroup B1 was exposed to ethanol vapours from day 1 to day 6. Subgroup B2 and B3 were exposed to ethanol vapours from day 1 to day 9.

Chick embryos were sacrificed on their respective days and hearts were processed for paraffin embedding. Serial

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sections of 15 to 20µm thickness were taken and stained with haematoxylin and eosin to measure the atrial, ventricular and septal wall thickness. The lengths of right and left atrioventricular valvular cusps were also measured.

The thickness of interatrial and interventricular septa was measured by taking the maximum transverse distance between two points on the septal walls. The thickness of left and right atrial and ventricular walls was measured by taking the maximum transverse distance between two points on the respective walls. Right atrioventricular valve has only one cusp. Length of right atrioventricular valve cusp was measured by taking the distance between the free and attached end of the cusp. Left atrioventricular valve has two cusps. Length of left atrioventricular valve cusps was measured by taking the distance between attached and free end of the respective cusp. (Figure-1, 2)

The data was analyzed statistically using SPSS version 10. Student t test was applied to the quantitative data.

Results

The thickness of interatrial and interventricular septa of experimental group B1 was more than that of control

group A1. This difference was not statistically significant for interatrial septa but was significant in case of interventricular septa (Table-1).

The thickness of right ventricular and left atrial wall of experimental group B1 was more than that of control group A1. This difference was not statistically significant for left atrial wall but was significant in case of right ventricular wall. The thickness of left ventricular wall and right atrial wall of control group A1 was more than that of experimental group B1 but this difference was statistically not significant (Table-1). The length of right atrioventricular valve was more in control group A1 than that of experimental group B1; however this difference was not statistically significant. The length of right cusp of left atrioventricular valve was significantly greater in experimental group B1 than that of control group A1. The length of left cusp of left atrioventricular valve was more in control group A1 than in experimental group B1; however this difference was not statistically significant (Table-1).

The thickness of interatrial and interventricular septa of experimental group B2 was more than that of control

Table-1: Different parameters of day 7 control heart (A1) and day 7 alcohol exposed heart (B1).

Parameter	Number of chick embryos		Mean±SD		P-value of difference
	A1	B1	A1	B1	
Thickness of left atrium(µm)	30	29	87.25±44.08236	111.034±52.12244	P=0.802
Thickness of right atrium(µm)	30	29	121.083±51.91041	98.534±35.84140	P=0.983
Thickness of left ventricle(µm)	30	29	331.916±55.459222	329.137±53.281191	P=0.063
Thickness of right ventricle(µm)	30	29	287±47.20827	315.655±60.69495	P=0.05*
Thickness of Interatrial septum (µm)	27	29	75.74±31.87879	77.931±33.18017	P=0.845
Thickness of Interventricular septum(µm)	30	29	366.083±100.90044	366.551±61.67460	P=0.047*
Length of right. AV valve cusp(µm)	29	29	506.206±81.54102	482.672±104.33876	P=0.343
Length of rt.cusp of lt.AV valve(µm)	30	29	371.416±101.56891	471.81±171.53183	P=0.008*
Length of lt.cusp of lt.AV valve(µm)	30	29	308.333±96.00946	267.844±57.41735	P=0.055*

SE= Standard Error of the Mean. *= Significant.

Table-2: Different parameter of day 10 control heart (A2) and day 10 alcohol exposed heart (B2).

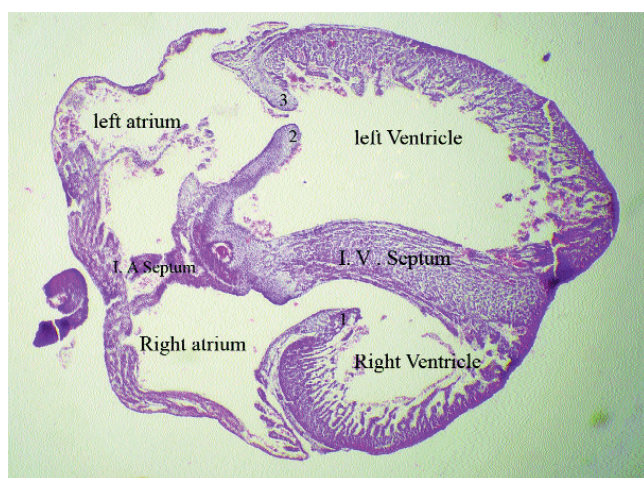
Parameter	Number of chick embryos		Mean±SD		P-value of difference
	A2	B2	A2	B2	
Interatrial septum (µm)	28	21	177.928±95.768	195.095±46.121	P=0.453
Interventricular septum(µm)	29	23	501.62±79.037	681.818±125.419	P<0.0001*
Thickness of left atrium(µm)	29	23	196.27±74.001	296.000±131.199	P=0.001*
Thickness of right atrium(µm)	29	23	162.06±52.039	187.045±101.520	P=0.258
Thickness of left ventricle(µm)	29	23	514.82±673.519	495.227±90.468	P=0.893
Thickness of right ventricle(µm)	29	23	289.72±63.688	382.500±74.125	P<0.0001*
Length of rt. AV valve cusp(µm)	29	23	589.82±110.877	873.863±152.689	P<0.0001*
Length of rt.cusp of lt.AV valve(µm)	29	23	427.24±85.477	480.454±125.251	P=0.078
Length of lt.cusp of lt.AV valve(µm)	29	23	285.24±71.311	341.363±86.701	P=0.014*

SE= Standard Error of the Mean. *= Significant.

Table-3: Different parameters of newly hatched chick hearts of control (A3) and newly hatched alcohol exposed chicks hearts (B3).

Thickness	Number of chicks		Mean± SE		P-value of difference
	A3	B3	A3	B3	
Interatrial septum (µm)	29	16	437.777±139.451	337.596±173.184	P=0.04*
Interventricular septum(µm)	30	21	1677.811±199.447	1655.945±223.833	P=0.716
Thickness of left atrium(µm)	30	21	877.066±188.432	810.804±276.075	P=0.313
Thickness of right atrium(µm)	30	20	587.381±174.99	545.215±127.35	P=0.359
Thickness of left ventricle(µm)	30	21	1514.205±260.546	1404.029±432.083	P=0.262
Thickness of right ventricle(µm)	30	21	834.984±203.170	791.528±45.207.459	P=0.460
Length of rt. AV valve cusp(µm)	30	20	2152.692±55.334	2135.699±108.245	P=0.879
Length of rt.cusp of lt.AV valve(µm)	30	19	1040.167.266188	875.799±294.297	P=0.049*
Length of lt.cusp of lt.AV valve(µm)	30	19	728.64±26.483	654.471±51.351	P=0.165

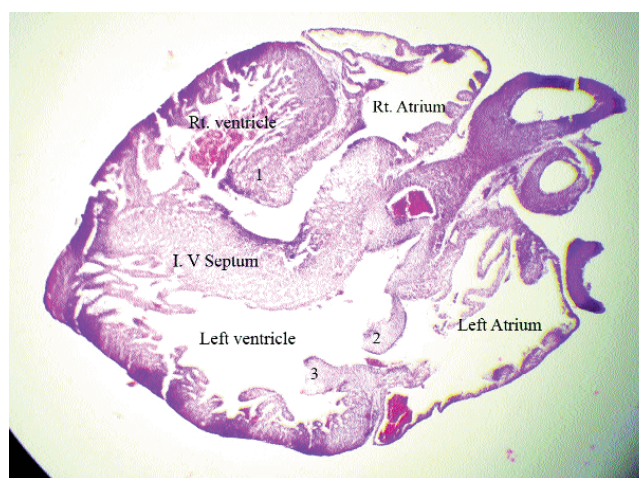
SE= Standard Error of the Mean. *= Significant.



1. Right atrio ventricular valve.
2. Left atrio ventricular valve-right cusp
3. Left atrio ventricular valve-left cusp

Figure-1: Normal chick heart at day 10 of development.

group A2; however the difference was statistically not significant (Table-2). The thickness of left atrial wall and right ventricular wall was significantly more in experimental group B2 than that of control group A2. The thickness of right atrial wall was more in experimental group B2 than that of control group A2 but the difference was not statistically significant. The thickness of left ventricular wall was more in control group A2 than that of experimental group B2 but this difference was not statistically significant (Table-2). The length of right atrioventricular valve and left cusp of left atrioventricular valve was significantly more in experimental group B2 than that of control group A2. The length of right cusp of left atrioventricular valve was more in experimental group B2 than that of control group A2 but this difference was not statistically significant (Table-2).



1. Right atrio ventricular valve.
2. Left atrio ventricular valve-right cusp
3. Left atrio ventricular valve-left cusp.

Figure-2: Experimental chick heart at day 10 of development.

The thickness of interatrial septum of newly hatched chicks of control group A3 was significantly more than that of experimental group B3. The thickness of interventricular septum of newly hatched chicks of control group A3 was more than that of experimental group B3; however, this difference was not statistically significant. The thickness of left and right atrial wall was more in control group A3 than experimental group B3 but these differences were not statistically significant (Table-3). The thickness of left ventricular wall and right ventricular wall was also more in control group A3 than that of experimental group B3 but again these differences were not statistically significant (Table-3). The length of right atrioventricular valve and left cusp of left atrioventricular valve was more in control group A3 than that of experimental group B3; however, this difference was not statistically significant. The length of right cusp of

left atrioventricular valve was significantly more in control group A3 than that of experimental group B3 (Table-3). No identifiable anomaly was seen.

Discussion

Interventricular, interatrial and septal thickness increased in alcoholic chicks at day 7 showing enhanced growth. The length of right atrioventricular (AV) valve and left cusp of left atrioventricular valve (AV) were longer in chick embryos belonging to control group as compared to those in experimental group but this was not statistically significant.

The hearts of ethanol-exposed chick embryos at day 10 had increased thickness of walls and septa. The valves were also longer in length in experimental group as compared to the control group which depicted enhanced growth.

The thickness of interatrial septum of newly hatched chicks of control group A3 was significantly more than that of experimental group B3. The thickness of interventricular septum of newly hatched chicks of control group A3 was more than that of experimental group B3; however, this difference was not statistically significant. In the present study, the length of right atrioventricular valve and both the cusps of left atrioventricular valve was less in the experimental group of B3 as compared to control group A3 again showing depressed growth.

The thickness of atrioventricular walls and septa increased in experimental groups during initial ten days of incubation. The thickness of atrioventricular walls and septa was less in experimental group B3 as compared to the control A3 group showing that the experimental hearts had thinning of their walls. This is in accordance with the previous studies which show that initial response to drinking was an increased thickness of the chamber walls followed later on by thinning of walls creating a classical picture of alcoholic cardiomyopathy.¹⁰

It is seen in previous researches that Alcoholic Heart Muscle Disease (AHMD) occurs in phases, starting with an asymptomatic phase, succeeding to a symptomatic phase and ultimately heart failure. In general, initial signs of Alcoholic cardiomyopathy (ACM) appear to be increased left ventricular mass along with increased posterior and septal wall thickening. This was in accordance with the findings of this study in which on day 7 and 10 the alcoholic heart had increased in thickness of atrial, ventricular and septal walls but this was statistically not significant. After day 10 alcohol was withdrawn, and on hatching it was seen that the alcoholic hearts had wall

thinning as compared to controls which is the classical picture of alcoholic cardiomyopathy. These findings were consistent with earlier work on alcoholic cardiomyopathies.¹¹ Alcohol causes widening of heart chambers, cardiac muscle cell hypertrophy, reduced ventricular contractility, disorganized myofibrillary architecture and diminished ejection volumes.¹² So even after ethanol withdrawal the alcoholic heart is in a compromised state with decreased myocardial contractility due to disorganized myocardial architecture. Reduced ejection fraction enhances end systolic volume,¹³ increasing volume overload resulting in dilatation of heart chambers as well as cardiac wall thinning.

Another possible cause of cardiac wall thinning on hatching could be ethanol withdrawal after day 10. Withdrawal has damaging effects which might have resulted in depression of growth of the experimental hearts leading to cardiac wall thinning. It was seen in certain studies that chronic exposure to ethanol and sudden ethanol withdrawal produced toxic effects and oxidative stress leading to neuronal death in rat brains.¹⁴ Substantial losses of hippocampal neurons have also been noted in rat brains at various times following ethanol withdrawal.¹⁵ Chronic exposure to ethanol and its withdrawal produces an increase in reactive oxygen species triggering activation of protein kinase C. This later on aggravates apoptotic events producing DNA breakup¹⁴ ultimately causing tissue and organ damage. It has been shown that ethanol-induced apoptosis is probably the basic mechanism underlying ethanol-induced disorders such as foetal alcohol syndrome. Both humans and animal models of alcohol cardiomyopathy show that myocyte loss is the major etiological factor underlying alcohol induced cardiac dysfunction.¹⁶

There are many studies verifying the adverse effects of chronic alcohol consumption on the function of mitochondria and sarcoplasmic reticulum function which could be another cause of alcoholic cardiomyopathy.^{17,18} Alcohol decreases the amount of protein synthesis in cardiac muscle which lessens the rate of contraction¹⁹ thus enhancing end systolic volume ultimately leading to cardiac wall thinning. Alcohol also decreases the rate of contraction by interfering with excitation contraction coupling at the muscle cell membrane by disturbing the calcium transients.²⁰

Persistent raised levels of norepinephrine produce adverse effects on the myocardium, some of which include myocyte hypertrophy, toxicity, and apoptosis. Adams and Hirst demonstrated in male rats that alcohol intoxication for 2 to 4 days (via gastric intubation) was

accompanied by marked increase in urinary norepinephrine and epinephrine. These researchers proposed a direct role of catecholamines in alcohol-induced myocyte hypertrophy.²¹ With marked ethanol consumption, there is higher occurrence for hypertension, cardiomyopathy, and arrhythmias.²²

This study shows that ethanol vapour exposure is as hazardous to developing heart as drinking alcohol.

Conclusion

Ethanol vapour exposure during development causes cardiac and septal wall thickening during initial days of development followed by cardiac and septal wall thinning which is a classical picture of alcohol induced cardiomyopathies.

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Conflict of Interest: No.

Funding Sources: No.

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