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EFFECT OF ETHANOL ON GLUCOSE, PROTEIN, CHOLESTEROL AND CALCIUM LEVEL IN THE AMNIOTIC FLUID OF CHICK EMBRYO

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ABSTRACT
Ethanol (CH_3CH_2OH) is the active ingredient in alcoholic drinks which is produced by fermentation. To observe the utero Ethanol exposure on the growth and development of the embryo, an embryonic chick model was used in the present study as an experimental animal model. In the present study the biochemical estimation of the amniotic fluid was recorded. The level of glucose and protein in the amniotic fluid of the embryos were observed to be increased significantly at 1% level in the amniotic fluid of experimental group II (Ethanol treated). The level of cholesterol and calcium in the amniotic fluid of the embryos were compared it was observed to be increased significantly at 1% level in the amniotic fluid of experimental group II (Ethanol treated). However the levels of cholesterol in the amniotic fluid of experimental group II (Ethanol treated) were decreased significantly at 1% level. The present study shows a change in the level of Glucose, protein, cholesterol and calcium in the amniotic fluid with single dose of 10% ethanol.

INTRODUCTION

Ethanol (CH_3CH_2OH) is the active ingredient in alcoholic drinks. It is produced by fermentation through the metabolism of carbohydrates by certain species of yeast in the absence of oxygen. The rate of Ethanol intake is increasing day by day irrespective of sex when taken during pregnancy it also passes from mother to the developing embryo. Suppression of foetal brain growth during pregnancy as the result of maternal intake of alcohol consumption leads to significant problems in the offsprings. Therefore to observe the utero Ethanol exposure on the growth and development of the embryo, an embryonic chick model was used in the present study as an experimental animal model.

Amniotic fluid or liquor amnii is the nourishing and protecting liquid contained by the amnion. The liquid contains proteins, carbohydrates, lipids and phospholipids, urea and electrolytes, all of which aid in the growth of the foetus. The level of these contents can be used as a diagnostic tool in the development of the foetus. Hence in the present study the biochemical estimation of the amniotic fluid was recorded.

Chaudhuri (2004) studied the effect of a single dose of ethanol on developing skeletal muscle of chick embryos. They observed, there was a significant retardation in crown rump length, head circumference, and body weight in ethanol-exposed chicks. This retardation was associated with significant and proportionate reductions in the weights of skeletal muscles. Microscopic examination of skeletal muscle showed areas of neutrophil infiltration and necrosis, suggestive of muscle damage in chicks exposed to 10% and 15% ethanol.

Pennington (1990) using a chick model observed that pharmacologically appropriate doses of ethanol (< 30 mm) inhibit brain growth and reduce CNS 3',5'-cyclic adenosine monophosphate (cyclic AMP) with an associated 50% decrease in the binding of cyclic AMP by the regulatory subunit (RII) of protein kinase A.

Illanes *et al.,* (1999) analyzed anomalies in avian embryos induced macroscopically and microscopically by ethanol (EtOH) during the first stages of development. Embryos treated with ethanol demonstrated a significant weight decrease. Microscopic analysis revealed that there was less type I collagen in trabecular bone in the embryos post-exposure to ethanol. Marco *et al.,* (2005) analyzed the effect of 60 hr ethanol ingestion on lipid composition of liver and brain membranes from 2-day-old chicks. Analysis of hepatic membrane cholesterol showed that ethanol induced a slight increase in microsomes exclusively due to free cholesterol. In brain, both fractions showed a clear increase in their cholesterol content.

Ramp *et al.,* 2007 observed hypocalcemia, hypermagnesemia and an elevated hematocrit in chicks exposed to high dose of ethanol (6g / kg body weight).

The objective of the present study is to study the level of Glucose, Protein, Cholesterol and Calcium level in the amniotic fluid of chick embryo when exposed to single dose of ethanol.

MATERIALS AND METHODS

Freshly laid Rhode Island Red strain fertilized eggs of zero days old were obtained from District animal husbandry office,

Table 1: Glucose, Protein, Cholesterol and Calcium level in the amniotic fluid of chick embryo on 8th day of incubation

	Group I (Control Howard Ringer's saline solution treated)	Group II (Experimental Ethanol treated)	Calculated t value	Tabulated t value at 5% level
Glucose level in the amniotic fluid On 8th day of incubation in mg%	54 ± 0.4656	63.2 ± 0.4360	5.33	2.23
Protein level in the amniotic fluid On 8th day of incubation in gm /100mL	16.0667± 0.2617	23.4667± 0.3757	6.46	2.23
Cholesterol level in the amniotic fluid On 8th day of incubation in mg / 100mL	37.2333 ± 0.6287	21 ± 0.4196	7.49	2.23
Calcium level in the amniotic fluid On 8th day of incubation in mg / 100mL	9.7 ± 0.1528	4.5667 ± 0.2028	5.35	2.23

(Values represent mean ± S.E.)

Amravati (M. S.). They were cleaned with wet cotton and placed in sterile egg incubator maintained at 37ºC with 65% relative humidity. Eggs were rotated manually once in a day and were examined through the candler every alternate day for proper growth and viability.

The eggs were coded into two groups. Each group is of six eggs.

Group I – Control – injected single dose of 100 μL of Howard Ringer's solution. (Saline treated) (Mathews and Schoenwolf, 1998)

Group II – experimental eggs were injected a single dose of 100 μL of 10% of Ethanol in Howard Ringer's solution per egg.

After windowing and injecting the control and experimental solutions, eggs were placed in an incubator at 37ºC for 8days.

On 8th day of incubation the amniotic fluid was taken out and the biochemical estimation of the supernatant was performed after centrifugation at 3000 rpm for 10 min.

Glucose was estimated by O-Toluidine method, the total Protein by Biuret method, the Cholesterol level was estimated by Liebermann Burchard method and Calcium was estimated by titration method using potassium permanganate (Samuel, 1999).

Statistical analysis was performed by using MS Excel. Data was analyzed using standard statistical methods (Zarr, 2005).

RESULTS

The mean value of Glucose in the amniotic fluid of embryos of group I was found to be 54 ± 0.4656. in group II it was found 63.2 ± 0.4360. The Protein in the amniotic fluid of embryos of group I was found to be 16.0667 ± 0.2617 in group II it was found 23.4667 ± 0.3757. The levels of Glucose and Protein in the amniotic fluid of the embryos were significantly higher at 1% level in the amniotic fluid of experimental group II (Ethanol treated).

The mean value of level of Cholesterol in the amniotic fluid of embryos of group I was found to be 37.2333 ± 0.6287 while in group II it was 21 ± 0.4196.

The Calcium content in group I was found to be 9.7 ± 0.1528 while in group II it was 4.5667 ± 0.2028.

The present study showed when the embryos were induced with single dose of 10% ethanol the level of Glucose and protein increased cholesterol and calcium in the amniotic fluid decreased significantly (Table 1).

DISCUSSION

The change in Glucose level is an indication of alterations on carbohydrate metabolism. It is also a major source of energy for the nervous system and erythrocytes. Protein being involved in the architecture and also in the physiology of the cell seems to occupy a key role in the cell metabolism (Yeragi *et al.,* 2003). The increased levels of glucose in amniotic fluid might be due to change in membrane permeability and diffusion of embryonic glucose into amniotic fluid. The elevated levels of proteins in amniotic fluid indicated the leakage of RBC into amniotic fluid. (Kishor *et al.,* 2009) The change in protein level is also due to tissue destruction by necrosis and disturbance of cellular fraction and consequent impairment of protein synthetic machinery (Brandbury *et al.,* 1987).

The decrease in Cholesterol and Calcium level in the amniotic fluid was also observed in Adriamycin treated and Cytarabine treated chick embryo and the level of cholesterol and calcium was retained back when treated with antioxidants (Mastan *et al.,* 2007; Kishor *et al.,* 2009). Similar results *i.e.* decrease in Cholesterol and Calcium level in the amniotic fluid were observed when the embryo was treated with ethanol.

These changes indicated towards abnormal development of the embryos due to ethanol induction.

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