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Effect of Ethyl Alcohol on Chick Embryo: A study on Fetal Alcohol Syndrome

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Abstract

Prenatal alcohol exposure exerts teratogenic effects on the developing foetus, mainly manifest by growth retardation, abnormal facial features and CNS damage collectively known as the Fetal Alcoholic Syndrome. The present experiment investigates the phenotypic responses of FAS at different treatment doses in the chick model. Fertilized eggs were collected from a poultry farm and was then kept in the incubator at 38.5° for 27 hours. After 27 hours; a 250 µl injection of 0% (control), 5%, 10% and 15% ethanol (in Howard Ringers solution) was introduced directly into the yolk using a 1ml syringe. No significant differences were seen among the control group of chick embryos and the group administered with 5% solution of ethanol . Both 10% and 15% groups showed quite significant effects overall in their total body mass, body length, head diameter and beak length. The results of the present study proves that a single exposure of ethanol to developing embryos can cause serious defects leading to the condition known as fetal alcohol syndrome. It was also proved that on increasing the concentrations of ethanol administered, the associated morphological defects also increased significantly. Thus alcohol consumption during pregnancy can lead to this serious anomaly.

1. Introduction

Fetal Alcohol Syndrome (FAS) refers to a pattern of birth defects that may develop in children whose mothers drank alcohol during pregnancy. FAS is a disability characterised by facial anomalies, low birth weight, mental handicaps or learning disabilities, central nervous system dysfunction (poor condition, hyperactivity, attention problems etc)and varying degrees of damage o malfunction of internal organs (Checky *et al.,* 2000).

Fetal Alcohol Syndrome (FAS) is the leading cause of mental retardation in many countries (Check *et al.* 2000). Alcohol damage to the foetus will vary greatly due to the volume of alcohol ingested, the time alcohol is ingested during the pregnancy, peak blood alcohol level, genetics and environmental factors. Studies using human models, such as the chick, can be used to promote the understanding and future treatment of this preventable condition (Zagory, 2004).The chick is a suitable mode for studies on FAS, since it allows for the determination of direct effects of ethanol in early pregnancy, without the influence of maternal under nutrition, concurrent drug use, acetaldehyde formation or impaired placental formation. FAS in chick models are comparable to those in humans (Cartwright and Smith, 1995).

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The neural crest cells (NCC) are mainly affected due to the exposure of alcohol (Zagory, 2004). The exposure of alcohol targets the proliferating cells in the developing central nervous system that interrupts the proliferate activities of glial and neural precursors. The inhibitory effects of alcohol on cell proliferation directly interfere with the growth factor to the developing embryo (Tavares, 2015).

Alcohol exposure also interferes with signal transduction. Thus alcohol exposure during development directly affects sonic hedgehog signalling (Tavares, 2015). Sonic hedgehog is a protein that in humans is encoded by SHH (sonic hedgehog) gene. Both the gene and the protein may also be found notated alternatively as "Shh". Sonic hedgehog is one of the three proteins in the mammalian signalling pathway family called hedgehog. It is important to neural development because it is a secreted protein necessary for embryogenesis (Sulik, 2005). Shh also expresses the pattern in the developing head and inducing the development of neural crest cell migration (Ahlgren *et al.,* 2002). Several studies have revealed that fetal alcohol exposure has a negative effect on the expression of genes required to facilitate proper embryonic development. Based on the information from various studies, the present experiment investigates the phenotypic responses of FAS at different treatment doses in the chick model.

2. Materials & Methods

Fertilized eggs were collected from a poultry farm. Total 20 eggs were collected and was then kept in the incubator at 38.5° for 27 hours. After 27 hours; a 250 µl injection of 0% (control), 5%, 10% and 15% ethanol (in Howard Ringers solution) was introduced directly into the yolk using a 1ml syringe. Ringers solution is a solution of several salts dissolved in water for the purpose of creating an isotonic solution relative to the body fluids of an animal. Howard ringer solution is prepared by mixing 7.2 NaCl, 0.17 CaCl2 (anhydrous) or 0.23gCaCl2.2H2O and 0.37g KCl to 1 litre of distilled water. A small puncture is made at the blunt end of the egg carefully using a needle. Then each concentration of ethanol was injected into a set of 5eggs using 1ml syringe. The hole was then sealed using the cello tape tightly. Care should be taken while taking the eggs out of the incubator for injection. The eggs must be kept above a piece of cotton kept in an embryo cup. This is done in order to prevent any physical disturbance and thus to prevent distortion of blastodisc and the developing embryo. After injection each of the eggs are placed back into the incubator. The eggs were then incubated at 38.5°c for 16 days. At day 16 post-injection the development of embryos were terminated and the effect of ethanol were noted. Eggs were opened carefully and chicks were qualitatively compared to one another. Additionally, quantitative data was also gathered. The total body mass of each embryo was measured using an electronic weighing balance. Total body length, beak length and head diameter were measured for each set of embryos. The obtained data was compared with other previous works to draw various conclusions.

3. Results & Discussion

The present study was aimed to investigate the effect of early dose of alcohol on developing embryo via embryological studies using chicken egg. The study investigates the phenotypic responses of FAS at different treatment doses in the chick model. Initially all the embryos under the four different experimental groups were qualitatively analyzed and significant morphological differences were noted in all the four groups. Data were collected and analyzed . The total body mass, body length, head diameter and beak length was measured for each group of embryos. Morphologically the defects observed were those associated with development of limbs, beaks and other externally visible features.

No significant differences were seen among the control group of chick embryos and the group administered with 5% solution of ethanol . Both 10% and 15% groups showed quite significant effects overall in their total body mass, body length, head diameter and beak length. The chick embryo administered with 5% ethanol showed negligible adverse effects. The group of embryos which were given 15% ethanol solution displayed the most notable superficial difference.

The total body mass of the various groups displayed a decline on increasing the concentration of ethanol. No significant decline was observed between the control group and the group administered with 5% ethanol solution. While the experimental group administered with 15% ethanolic solution displayed quite significant reduction in body mass as well as reduction in body length when compared to the control group. Thus the present study proves that increasing concentration of ethanol when administered to the developing embryo causes increased physical and morphological anomalies. As the concentration of ethanol increased; the extent of morphological defects also increased. The results obtained were further compared with other similar research works.

Table: 1 illustrates the total body mass , body length, head diameter and beak length of the various experimental groups.

The morphological effects induced by various doses of ethanol was interpreted and was then analysed statistically by measuring the total body length, head diameter, beak length and total body mass. As depicted in Fig:1 the overall growth of embryos retarded with increased concentrations of ethanol. The group administered with the highest dose of ethanol (15%) showed the most significant effects like abnormal decrease in body length, beak length and overall development.

Table: 1 Table showing the total body mass , body length, head diameter and beak length of the various experimental groups

	Eggs	Total body mass(gm)	Body length (cm)	Head diameter (cm)	Beak length (cm)
CONTROL	1	21.277	11.50	1.90	1.21
	2	20.371	10.80	1.85	1.10
	3	20.732	10.35	1.83	1.00
5%	1	19.363	8.01	1.72	0.85
	2	19.467	8.30	1.75	0.89
	3	19.966	8.70	1.75	0.90
10%	1	18.641	7.90	1.68	0.70
	2	17.360	7.75	1.65	0.70
	3	16.050	7.65	1.62	0.65
	4	16.227	7.65	1.61	0.70
15%	1	13.732	7.0	1.62	0.67
	2	7.978	5.25	1.337	0.60
	3	6.670	4.91	1.369	0.60
	4	7.309	5.50	1.242	0.60

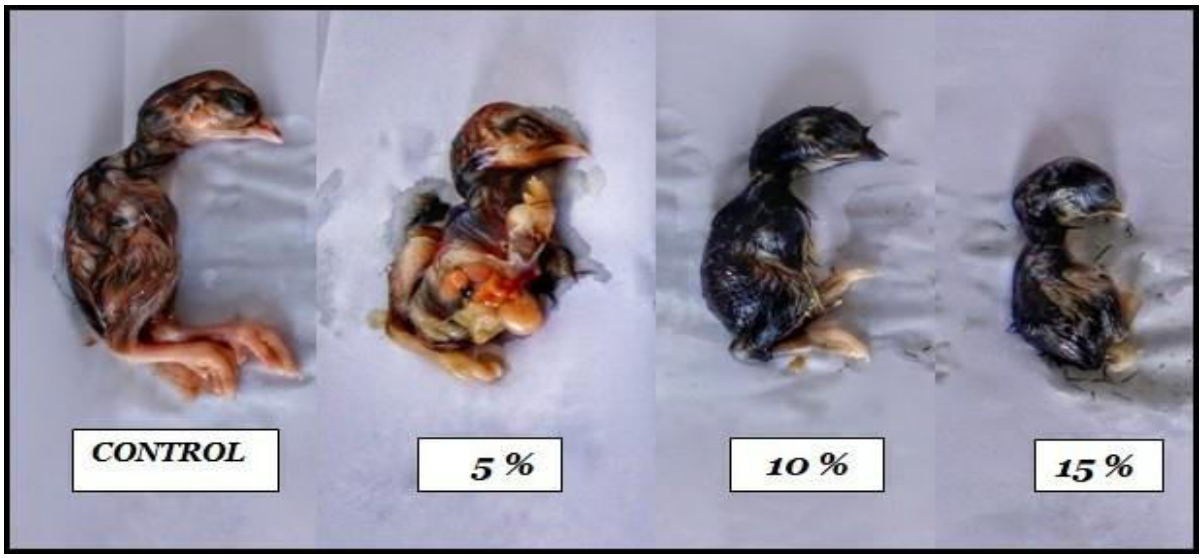
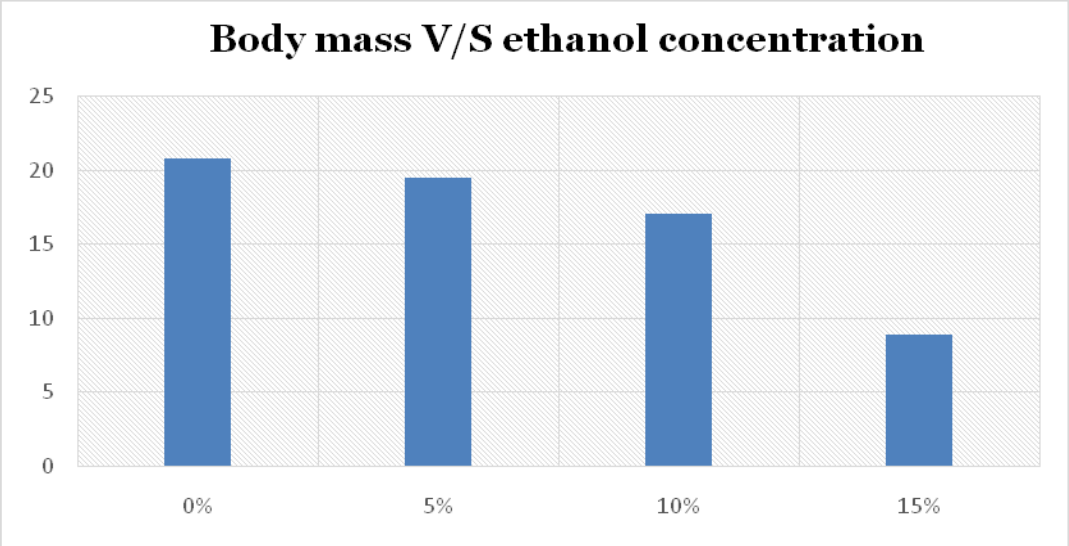


Fig:1 Morphological difference between experimental groups

Table: 2 Average body mass of experimental groups with respect to ethanol concentrations

EXPERIMENTAL GROUP	AVERAGE BODY MASS (g)
Control	20.79
5%	19.5
10%	17.06
15%	8.9

The following graph depicts the decrease in average body mass with respect to increased ethanol concentrations.



Graph:1 Change in body mass with respect to ethanol concentrations

The most notable difference was observed between the control group and 15% ethanol administered chick embryos. So the control and 15% treated embryos were further compared and observed to arrive at various conclusions regarding their decrease in head diameter , beak length, body length and total mass. Many more morphological effects like defective limb development , beak development also were noted in 15% treated embryo when compared with the control.The Fig:2 illustrates the difference in head diameter as well as the difference in beak length between control and 15 % chick embryo.

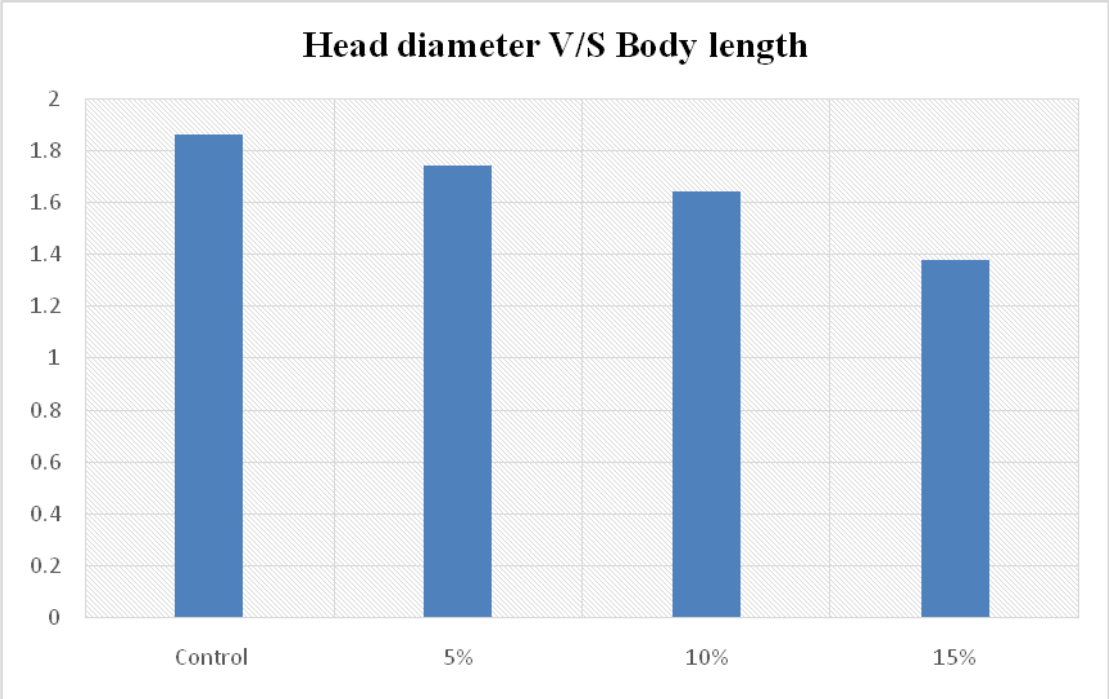
Fig: 2 Difference in head diameter and beak length between control and 15% group.

The average head diameter of various experimental groups was calculated and was then plotted against the various doses of ethanol administered. The following table shows the average head diameter of chick embryos belonging to various experimental groups with respect to the ethanol concentrations.

Table 3: Average head diameter of experimental groups with respect to ethanol concentration.

EXPERIMENTAL GROUP	AVERAGE HEAD DIAMETER(cm)
Control	1.86
5%	1.74
10%	1.64
15%	1.38

The following graph depicts the variations in average head diameter of various experimental groups with respect to increased concentrations of ethanol.



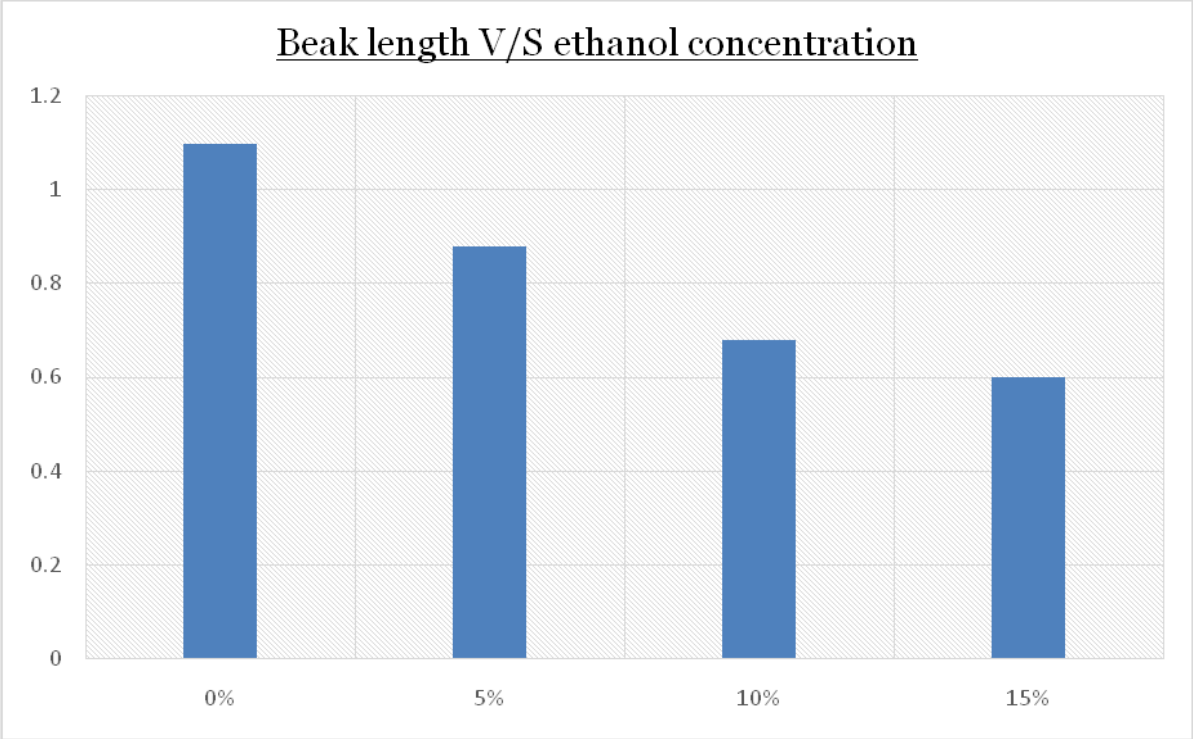
Graph:2 Difference in head diameter with respect to various ethanol concentrations.

The average beak length of various experimental groups was calculated and was then plotted against the various doses of ethanol administered. The following table shows the average beak length of chick embryos belonging to various experimental groups with respect to the ethanol concentrations.

Table: 4 Average beak length of experimental groups with respect to ethanol concentrations.

EXPERIMENTAL GROUP	AVERAGE BEAK LENGTH(cm)
Control	1.1
5%	0.88
10%	0.68
15%	0.60

The following graph displays the decreasing trend of beak length with increased concentrations of ethanol.



Graph: 3 Difference in average beak length with respect to ethanol concentrations

Among all the experimental groups considered the group administered with 15% ethanol showed the most significant effects. So it is further compared both morphologically and statistically.The following figure shows the difference between control (0% ethanol) and 15 % ethanol administered chick embryo:

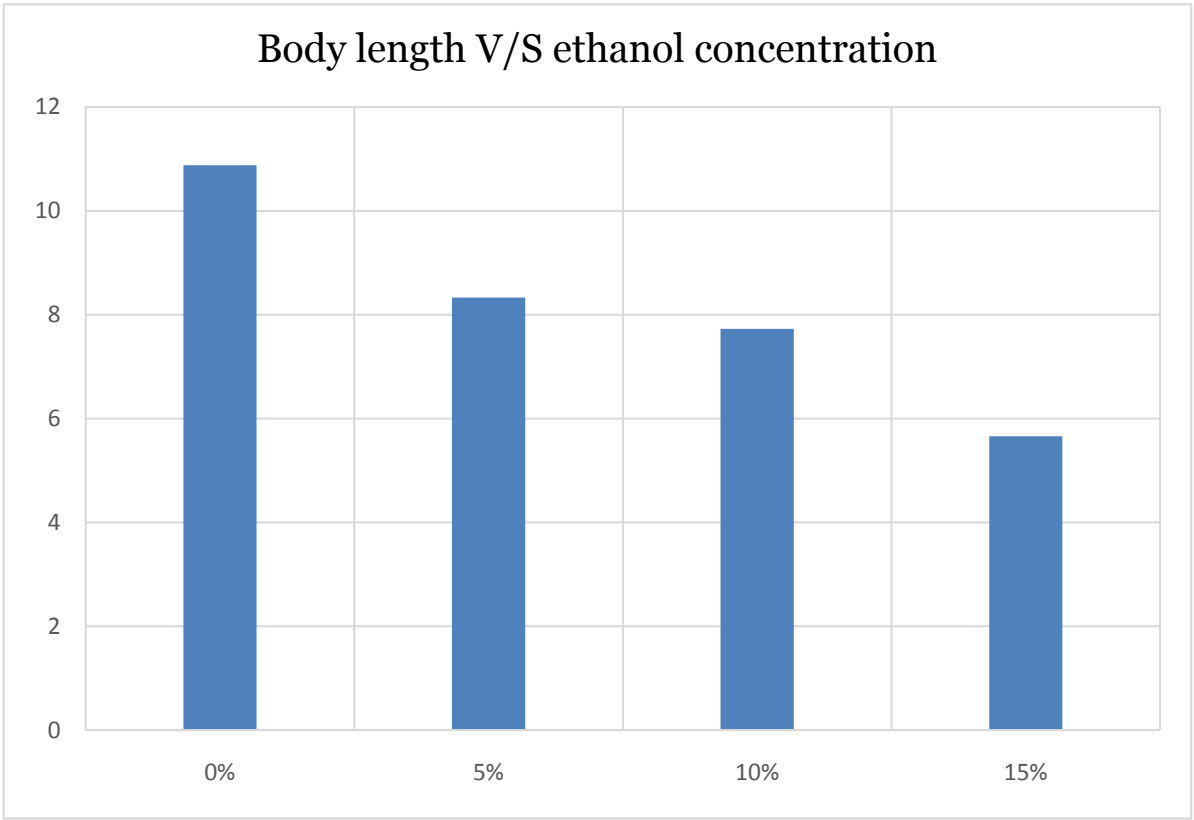
Fig: 3 Difference between control and 15% group.

The average body length of various experimental groups was calculated and was then plotted against the various doses of ethanol administered. The following table shows the average body length of chick embryos belonging to various experimental groups with respect to the ethanol concentrations.

Table: 5 Average body length of chick embryos with respect to body length.

EXPERIMENTAL GROUP	AVERAGE BODY LENGTH(cm)
Control	10.88
5%	8.33
10%	7.73
15%	5.66

The following graph depicts the variations in average body length in accordance with the increasing concentrations of ethanol administered to the various groups.



Graph: 4 Difference in average body length with respect to ethanol concentrations.

The effect of ethanol on body mass

The graph 1 illustrates a decrease in mass as ethanol dose increases. There is no significant difference observed between the control group and 5% ethanol administered chick embryo. A very significant decrease was observed between the control group and 15% administered chick embryo (Figure: 3). An unsubstantiated argument for the observed decline in mass is that perhaps all body cells are affected by ethanol treatment to varying degrees. The study conducted by Zagory *et al.,* (2004) also reveals the same fact that there is a decline in body mass as the ethanol dose increases. Continued researches using a larger sample size could determine if the observed amount of variation is acceptable or not. The study conducted by Fernandes (2013), also reveals the fact that exposure to increasing doses of ethanol has a significant impact upon the severity of phenotypic malformations produced in the developing embryo. The present study proves that single dose of increased ethanol concentrations induces decreased body mass of the embryo.

The effect of ethanol on head diameter

A significant difference can be observed in the head diameter between the different ethanol exposed groups to the control. So it can be concluded that the cranial NCC are affected adversely by the single dose of ethanol treatment. This supports previous research in this area (Zagory *et al.,* 2004 and Flynn, 2015). This result is in accordance with the fact that the cranial NCC are determined at emigration and there seems to be a critical window of 18-36 hours of incubation where these cells are most sensitive to alcohol. The embryos of the present study treated at 27 hours fit into this critical window and so there is much neural crest apoptosis. Due to the neural crest cells apoptosis , both brain and central nervous system gets adversely affected. As a result a significant reduction in head diameter is observed. The extent of reduction was high in case of the eggs treated with 15% ethanol. Here also, continued research using a larger sample size could determine if the observed amount of variations are acceptable.

The effect of ethanol on beak length

Beak tissues are derived from NCC (Gilbert, 2003). As a result the beak lengths of various experimental groups displayed a significant reduction. The beak length decreased with increasing ethanol concentrations. These results can be correlated with the results observed by Zagory *et al.,* (2004). The reason for this reduction in beak length is that the beak tissues are derived from NCCs and the NCCs undergo apoptosis when ethanol is administered during the critical window of development that is 18-36 hrs .The study conducted by Yang *et al.,* 2012 and Tavares, 2015 also support the fact that increased ethanol concentrations when administered to developing embryo produced increased level of adverse effects.

The effect of ethanol on body length

The results show that alcohol affects the body length of chick embryos. The decline in body length can be correlated with the decrease in body mass. The overall development is

affected by alcohol exposure. The reason for the decrease in body length with increased concentration of ethanol is that alcohol exposure during development effects some cell signals that are important for neural development (Ahlgren *et al.,* 2002).

4. Conclusion

Numerous birth defects can occur when alcohol is entered into the foetus's blood.The phenotypic responses of FAS at different treatment doses were investigated in the chick model. The various experimental groups showed varying degree of morphological changes as well as other defects with respect to increasing concentrations of ethanol. No significant differences were seen among the control group of chick embryos and the group administered with 5% solution of ethanol. Both 10% and 15% groups showed quite significant effects overall in their total body mass, body length, head diameter and beak length. The group of embryos which were given 15% ethanol solution displayed the most notable superficial difference. Thus it can be concluded from the present study that a single exposure of ethanol to developing embryos can cause serious defects leading to the condition known as fetal alcohol syndrome. This is because alcohol exposure also interferes with signal transduction. Thus, alcohol exposure during development directly affects sonic hedgehog(shh) signalling which is important in cell signalling.With increased concentrations of ethanol ; the phenotypic anomalies also increased. Thus alcohol consumption Thus when the cell signalling gets disrupted, the overall development also gets affected. Thus the result of the present investigation is also in accordance with the studies conducted by other researchers (Zagory *et al*., 2004 and Tavares, 2015). during pregnancy can lead to this serious anomaly.

5. Acknowledgements

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