EFFECT OF TETRACYCLINE ON EARLY DEVELOPMENT OF CHICK EMBRYO

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ABSTRACT: **this study aimed on evaluating the possible effects of tetracycline administered to chick embryo early development of their offspring. At intervals from 2 to 13 days of incubation, 0.1 to 0.2 mg of tetracycline hydrochloride was injected into the yolk sac of chick embryos. The femurs and mandibles were examined histologically at intervals between 8-10 days of embryonic age.**

Chick embryo was chosen as primary objective because of several advantages. Fertilized eggs are easily available from the local hatchery at all seasons of the year. Chick embryos are easier to dissect, as they are larger than mouse embryo at the equivalent stage of development. Because of its larger size chick embryo is easier to dissect out individual organs to test specific tests for different organs such as liver, heart and brain.

Chick embryos are easier to incubate at laboratory level to get specific stage of development to which we have incubated with 85% relatively humidity and 37±1^{o}C for 8- 10 days to get chick embryo. Tetracycline's are commonly used antibiotics in hatchery with chicken feed to minimize the risk of infection in chicks as well as eggs. These findings promoted a study of effect of Tetracycline on development of chick embryo. While tetracycline does cause some retardation in the rate of osteoid deposition, the drug appears to affect intramembranous bone formation in the mandible and femur primarily by retarding or temporarily inhibiting the rate of mineralization of the osteoid matrix.

The results of this study indicate that the effects produced by tetracycline on developing bones are dependent upon the concentration of the drug and not upon the time of administration.

Keywords: Tetracycline and chick embryo development

INTRODUCTION

Antibiotics are drugs of natural or synthetic origin that have the capacity to kill or to inhibit the growth of micro-organism. The term antibiotic was coined by Selman Waksman in 1942 to describe any substance produced by a microorganism that is antagonistic to the growth of other microorganisms in high dilution (Waksman et al., 1947).

Tetracycline's are generally absorbed primarily from the stomach and duodenum and to a lesser degree from the small intestine. Maximum plasma levels are attained after two to four hours and begin to decline after approximately six hours, although traces can be detected after 24 to 30 hours. (Hirsch and Finland, 1959) A variable proportion of absorbed Tetracycline is bound to plasma proteins and later deposited in unbound form in the tissues. (Kunin and Finland, 1961) These compounds are concentrated in the liver, and excreted by the biliary tract into the intestine from which they are partially reabsorbed. Administered Tetracycline's may also be detected in the brain, saliva, cerebrospinal fluid and maternal milk, in about half the concentration achieved in plasma (Dowling, 1955).

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The objective of the present study was to check the effect of tetracycline on developing chick embryo and the estimation of total protein content, catalase enzyme activity, estimation of liver marker enzymes activity such as AST and ALT in organs of tetracycline treated chick tissues. (Liver, Brain and Heart)

III. MATERIAL AND METHODS

Fertilized eggs of 8th day were made available by a local hatchery; eggs were divided in to three groups. The 1st group received Distilled water (100µl) and served as control. Second group of eggs received 0.1 mg of tetracycline (Pfizer) and the third experimental group received 0.2 mg of tetracycline. Injection site is marked and perforated after injection site is sealed with cello tape. The working solution of Tetracycline was made in distilled water, so the control eggs received same amount of distilled water. After treatment eggs were kept in humid incubator (Jyoti Scientific) with 85 % relatively humidity and 37±1 ^{0}C temperature in tilting position. The positions of eggs were changed in every 2-3 hours. After 3 days of incubation the eggs were sacrificed and processed dissection of eggs, Catalase taste (Luck et.al. 1974), estimation of AST enzyme concentration, estimation of ALT enzyme concentration and quantitative estimation of protein by Folen-Lowry Method (Lowry et. al. 1951).

IV. RESULTS

Chick embryo treated with 0.2 mg of tetracycline and the control embryos did not show any fluorescent under UV light**.**

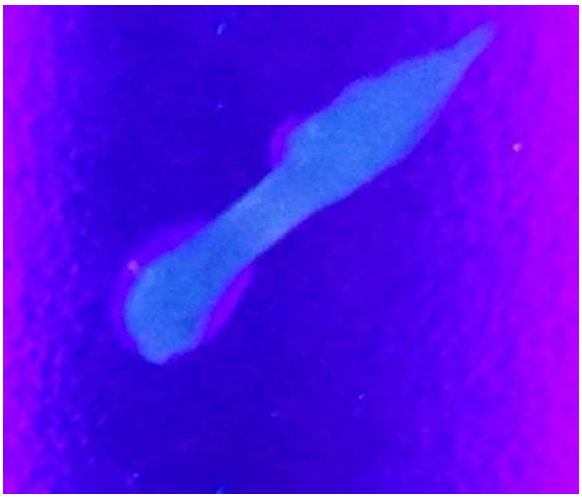


Figure No. 1. Observation of chick embryo bone under UV-light, after treated with 0.2 mg of tetracycline.

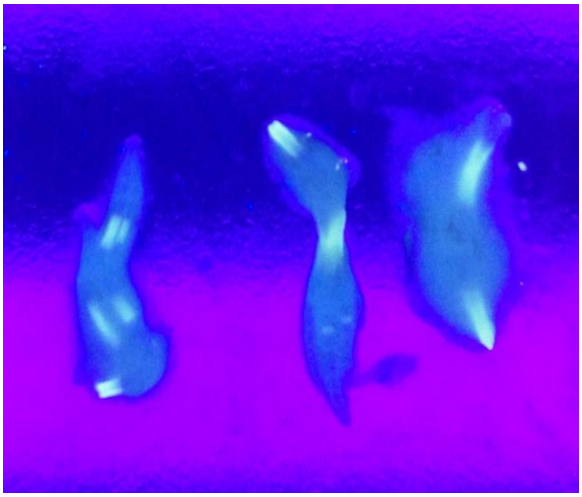


Figure 2: - observation of chick embryo bone under UV- light, after treated with 0.1 mg of tetracycline.

The bones of chick embryos, treated with 0.1 mg of tetracycline showing fluorescent when visualized under UV- light. This effect showed the absorption of tetracycline into bones of chick embryos.

Total protein estimation:-

Total protein content is estimated by Lowry method in all the tissues (liver, heart and brain) of chick embryos treated with tetracyclines and results were compared with values found in control embryos.

Figure 3-5 represents the toxic effect of tetracycline in total protein content in liver, heart and brain of chick embryos. Total protein contents were reduced in all the organs after treatment of tetracycline at the dosage of 0.1 mg/kg and 0.2 mg/kg. Maximum inhibition in the protein content was seen in brain tissue in comparison to liver and heart.

Figure (3 -5):- Effect of tetracycline on total protein content in liver, brain and heart.

Figure -3

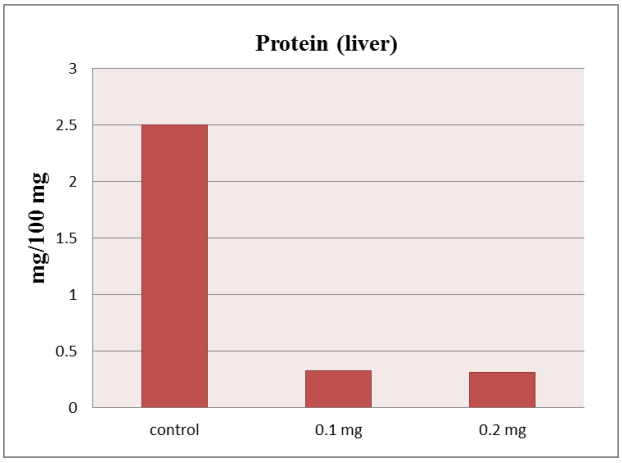


Figure-4

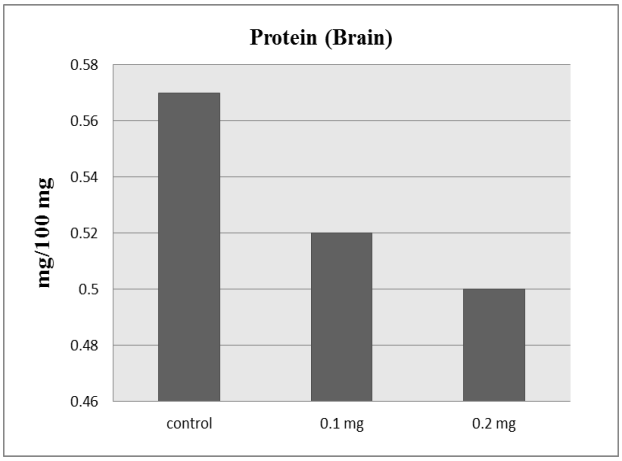
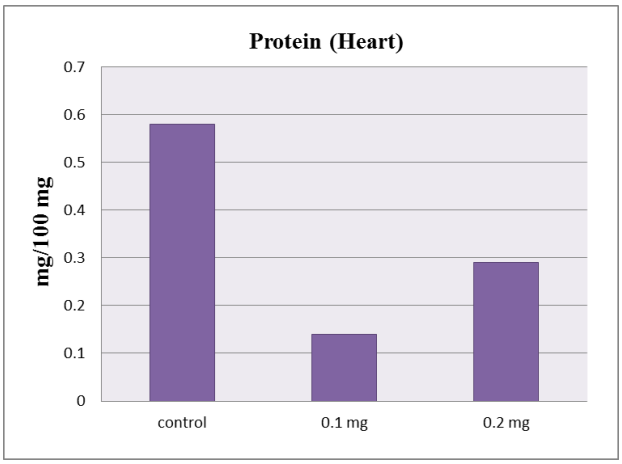


Figure-5



Catalase enzyme activity:-

Catalase enzyme activity is observed in liver and brain tissues of chick embryos which are treated with 0.1 mg/kg and 0.2 mg/kg of tetracycline. Figure 6 & 7 represents the toxic effect of tetracyclines on catalase enzyme activity in liver and brain of chick embryos. Catalase enzyme activity is reduced in both the tissues of chick embryo after treatment. Maximum fall in catalase activity was seen in brain tissue in comparison to liver.

Figure 6-7:- Effect of tetracycline on Catalase enzyme activity in liver and brain.

Figure-6

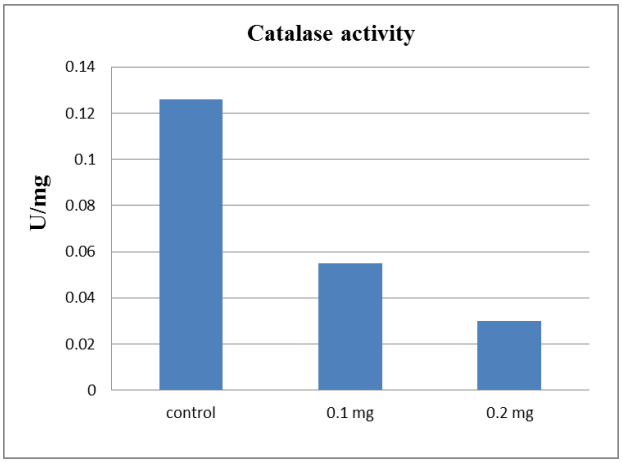
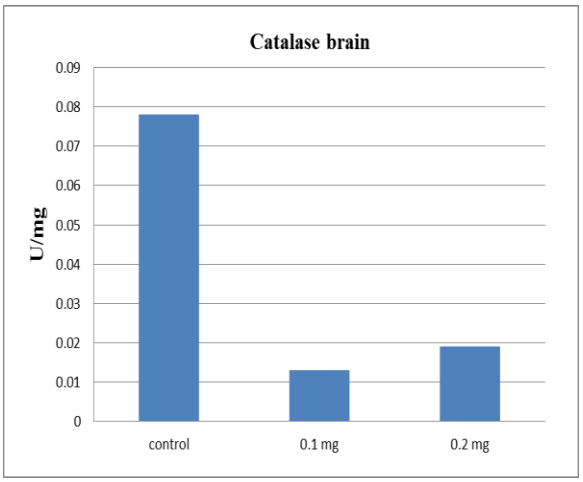


Figure-7



Liver markers enzymes activities: -

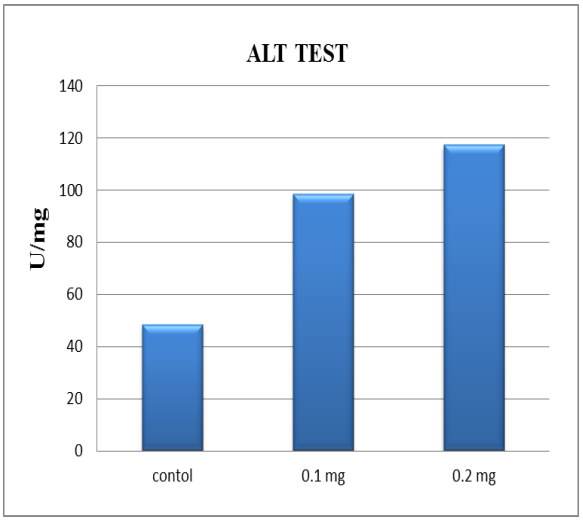
(A) ALT

(B) AST

ALT enzyme activity was observed in liver tissues of chick embryos treated with 0.1 mg/kg and 0.2 mg/kg of tetracycline. Figure 10 represents the toxic effect of tetracycline in ALT enzyme activity. Enzyme activity is increased in both the dosages in comparison to control embryos.

Figure 8 & 9:- Effect of tetracycline on liver marker enzymes activities

Figure-8



FigureNo.9

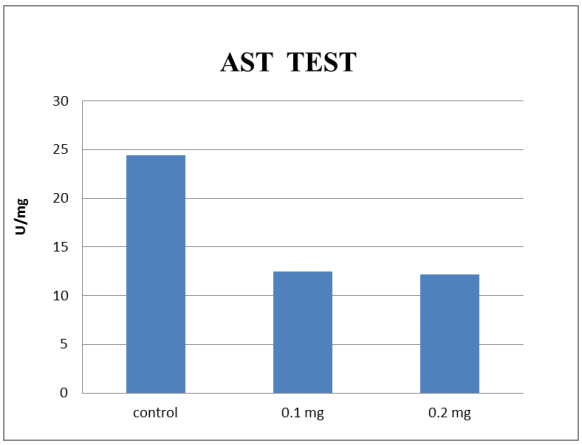


Figure 9:-represents the toxic effect of tetracycline on AST enzyme activity. The enzyme activity is observed in liver tissues of chicks treated with 0.1 mg/kg and 0.2 kg/ kg of tetracycline. The enzyme activity is highly reduced in both the dosage in comparison to control tissues.

Effect of tetracycline on average weight: - (values are in gm)

Treatments	Total body weight	Organ weight		
		Brain	Liver	Heart
Control	3.853	0.253	0.104	0.060
Tetracycline (0.1 mg/ kg)	2.740	0.193	0.069	0.052
Tetracycline (0.2 mg/ kg)	2.933	0.217	0.089	0.056

The data showed that approx 1 gm of weight was reduced in total body weight of chick embryos treated with two different dosage of tetracycline. The weight of brain and liver was also reduced in both the dosage but the weight of both tissues was more reduced at 0.1 mg/kg and lower reduction was found at 0.2 mg/kg. Reduction in heart weight was slightly decreased in both the dosages.

DISCUSSION

In present study two different dosages (0.1 mg and 0.2 mg) of tetracycline were injected into yolk sac of fertilized eggs and after 3 day of incubation chick

embryos were used for enzyme assay and protein estimation.

All the embryos treated with 0.1 mg of tetracycline showed binding of tetracycline in their bones under ultra violet light whereas the embryos treated with 0.2 mg of tetracycline did not showed any binding of tetracycline.

The similar result was demonstrated in the experiment of Milch *et al*., (1957) who showed the fluorescence of tetracycline in bones of treated chicks and discovered a particular fluorescence in the skeleton of animals that received tetracycline. This fluorescence could be observed for weeks or months after cessation of drug administration.

Similar result was showed by Bevelander (1964). They injected 0.1 mg of tetracycline into vitelline membrane of chick embryo between 4th – 8th days of embryonic life and detected the antibiotic by its fluorescence. Increasing the dose of tetracycline from 2.5 – 5.0 mg on the 6th – 8th day showed result in marked inhibition of bone growth.

In 1960 Frost and Villanue found that radioactive tetracycline were deposited in mineralized bones. There is no specific mechanism by which tetracycline fixed in bones, this theory is remain controversial. Scientist hypothesized that this is due to chelation of salts. All tetracycline produce a yellowish fluorescence in bones, these effects may be observed in foetus whose mother have treated with tetracycline during pregnancy.

The average body weight of chick embryos treated with 0.1 mg and 0.2 mg of tetracycline was 2.74 gm and 2.99 gm respectively. Whereas the average body weight of control embryos was 3.68 gm that showed the reduction of total body weight.

E. Tubaro (1964) found that the average body weight of chick embryo treated with chloro-tetracycline was 9.7 gm whereas 10.4 gm in the embryo which is treated with tetracycline.

No mortality was found at the concentration of 0.1 mg and 0.2 mg of drug during our experiment. In the experiment of Hughes *et al* (1964) the death rate at 2.5 µg was 21 out of 103 or about 20% and at 25 µg it was 34 out of 84 or about 40%.

Enzymes AST, ALT, Catalase activity and total protein content were tested during our experiment and we found reduced enzymatic activity of all these enzymes, except ALT enzyme level is increased. Total protein content was also reduced in all tissues (liver, brain and heart) but the protein level of brain is highly reduced in comparison to liver and heart.

Similar result was found in the experiment of Maity *et al* (2007). They used paracetamol in rats and found reduced activity of AST, ALT, and Catalase activity and reduced total protein content.

SUMMARY

The aim of present experiment was to study the effect of tetracycline on chick embryo development. Chick embryos were selected because they are easier to incubate at laboratory level and easier to dissect. Chick embryos are larger than mouse embryo at equivalent stage of development.

The objective of this experiment was to evaluate the total protein content and activity of AST, ALT and catalase after treatment of tetracycline. A specific dose of antibiotic is a defense line between effectiveness and non-effectiveness, therefore a specific dose of tetracycline was administered at the 8th day of embryonic life and after 3 day of incubation embryos were examined.

All the embryos showed reduced concentration of protein in internal organs such as liver, heart and brain. As well as enzyme activity of AST and catalase were also reduced, however ALT enzyme activity was increased.

The present experiment showed that the exposure of tetracycline during development of chick embryo causes reduction of protein level and enzymatic activities.

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