

EFFECT OF MOSQUITO COIL SMOKE ON THE DEVELOPING CHICK EMBRYO (GALLUS GALLUS)

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ABSTRACT

The attempt was made to evaluate the effect of mosquito coil smoke on the development of chick embryo under laboratory conditions. It was reported earlier that the burning of coil causes problems in rats like inflammation of the tracheal epithelium, atelectasis of the lung parenchyma, emphysema and increase of alveolar macrophages in the alveolar space and perivascular infiltration of polymorph nuclear cells, which was studied by the “Department of Anatomy” and “ Department of Biology” (BAPTIST College)-Hongkong. In the present endeavor we try to evaluate the effect of D-trans allethrin which is a

component of in the mosquito coil. The compound has adverse effect on various stages of chick development. Microsomia and Tachycardia are the major deformities observed due to d-trans allethrin. The compound was found to induce certain structural anomalies in the developing embryo. The characteristic deformities during this study have been co-related with the results which we obtained from current investigation. In conclusion the mosquito coil smoke is embryo toxic. The continuous use of mosquito coil may lead to undesirable consequences in vertebrates. According to us the best option to get rid of mosquito bite is to use the mosquito net.

KEY WORDS: Mosquito coil, d-trans Allethrin, Embryo toxic, Microsomia, Tachycardia.

INTRODUCTION

There are various types of insects around us in our houses and living area. Insects such as termites, cockroaches, bed bugs, house flies and mosquitoes are commonly found around us. But among these the mosquito is the most harmful to human beings as it causes many severe diseases like malaria, chickenguniya etc . In order to prevent them from invading our houses and causing above mentioned diseases there are various mosquito repellent available in the

market. On such type is the mosquito coil. Mosquito coil is the pesticide which is used indiscriminately to get off mosquitoes. There is always impetus to find out the safer pesticides. In India, insecticide account for 80% of the total pesticide used while the herbicides usage is insignificant.(Abilash P.C;Singh N. 2009). An attempt was made to evaluate the effect of mosquito coil smoke on the development of chick embryo under laboratory conditions. It was reported earlier that the burning of coil causes problems in rats like inflammation of the tracheal epithelium, atelectasis of the lung parenchyma, emphysema and increase of alveolar macrophages in the alveolar space and per vascular infiltration polymorph nuclear cells which was studied by the “Department of Anatomy” and “Department of Biology” (Baptist College) –Hongkong. In the present endeavor we try to evaluate the effect of D-trans allethrin which is used in the mosquito coil.

Mosquito coil is widely used as an efficient mosquito repellent. The major active ingredients of the mosquito coil are Pyrethrins, accounting for about 0.3 to 0.4 % of coil mass (**Lukwa and Chandiwana 1998**).When a mosquito coil is burnt, and the insecticides evaporate with the smoke, which prevents the mosquito from entering the room. Pyrethrins are of low chronic toxicity to humans and low reproductive toxicity in animals; although headache, nausea and dizziness were observed in male sprayers exposed to 0.01 to 1.98 microgram per meter cube Pyrethrins for 1 to 5 hours (**Zhang et al.1991**). No carcinogenic and mutagenic effects have been found for these insecticides (**Ecobichon 1995**).The lowest lethal oral dose of Pyrethrum is 750 mg/kg for children and 1,000 mg/kg (Occupational Health Services (OHS)1987. The combustion of the remaining materials generates large amounts of sub micrometer particles and gaseous pollutants. These sub micrometer particles can reach the lower respiratory tract and may be coated with a wide range of organic compounds, some of which are carcinogens or suspected carcinogens, such as Polycyclic Aromatic Hydrocarbons(PAHs) generated through incomplete combustion of biomass(mosquito coil base materials).Researchers have found that the gas phase of mosquito coil smoke contains some carbonyl compounds with properties that can produce strong irritating effects on the upper respiratory tract—for example, Formaldehyde and Acetaldehyde (**Chang and Lin 1998**). Epidemiologic studies have shown that long-term exposure to mosquito coil smoke can induce asthma and persistent wheeze in children (**Koo and Ho 1994**). Toxicological effects of mosquito coil smoke on rates include local declination of the tracheal epithelium, metaplasia of epithelial cells, and morphologic alteration of the alveolar macrophages (**Liu and Sun 1988; Liu and Wong 1987**). In addition, the levels of total protein and lecithin and

the activities of lactate dehydrogenase, acid phosphatase and beta-glucuronidase in the lung fluid of rats were significantly higher than those in a control group that was exposed to air for the same exposure duration (Liu et al. 1989).complete

The common active ingredients found in mosquito coils are as

1. Pyrethrum
2. Pyrethrins
3. Allethrin
4. Esbiothrin(a form of allethrin)
5. Dbutyl hydroxyl toluene (BHT) (An optional additive to prevent parathyroid from oxidizing during burning)
6. Piperonylbutoxide(PBO) (An optional additive to improve the effectiveness of pyrethroid)
7. N-(2-ethylhexyl)-bicyclo-(2, 2, 1) hept-5-ene-2,3-di carboxyimide.
8. D-trans allethrin

Structure Of D-Trans Allethrin

MATERIALS AND METHODS

Materials

Fertilized eggs .Usual laboratory Glassware& Instruments , Incubator Autoclave Sterile wooden chamber, Preservation jar, Dissecting microscope , Water tray, Cotton swabs, Sterilized gloves Mosquito coil (MORTEIN POWER GUARD) Dissection box

Chemicals:- Avian saline (0.9% NaCl solution) Formalin Absolute alcohol

Methodology

Before the experimental set up, the glassware to be used was properly cleaned, dried and then autoclaved as per the sterilization procedures. The working area was neatly sterilized by wiping with 70% alcohol. This provides a contaminant free environment for culturing chick embryos. These precautions are taken to minimize the possibility of contaminant Induced mortality and abnormalities in the developing embryos which will interfere with the experimental results.

Preparation of Experiment

Fertilized eggs were obtained from villager women in Bhopal city. Then they were cleaned and incubated at 37.5 Celsius with a relative humidity of 70-80%. Fertilized eggs that are to

be used for experimental process must be placed in the horizontal position 15 to 20 minutes before carrying out the culturing process, so as to ensure the position of the embryo on the top. The eggs must be surface sterilized by wiping their shell with the help of cotton, dipped in 70% alcohol. These eggs were then transferred to the incubator. While handling the eggs, hands must be thoroughly wiped with absolute alcohol then I have divided them into three batches of 15 eggs each.

Batch A

The fertilized eggs were used and a window was made of approximately 0.5cm so that the developing embryo should come in contact with the burning coil smoke. The coil was burnt in the incubator for 6 hrs for 6 days in a week (these were experimental eggs with window).

Batch B

These fertilized eggs were kept as it is, (this was experimental eggs without window).

Batch C The fertilized eggs were kept as normal in another incubator to study the normal development of chick embryo and comparing it with the experimental batches mentioned above. Fertilized eggs were incubated at 37.5 degree Celsius and relative humidity of 70-80% then burn the mosquito coil and keep it in the incubator. The mosquito coil was kept in the plate so that no ashes would fall in the incubator. The mosquito coil was the same which we use in the houses [MORTEIN POWER GUARD mosquito coil]. Finally the incubator was closed and the mosquito coil was allowed to burn till it ends. This procedure was repeated for 20 days till the eggs get hatched. The eggs were dissected during various stages of development to observe the abnormalities. The embryos were studied at the different stages, i.e. 10hrs, 14 hrs.

RESULTS AND DISCUSSION

The pilot experiment was performed so as to observe the mortality rate of the chick embryo at the laboratory conditions. This has been helpful for us to study the development of the chick embryo at 37.5°C

Table 1.1 shows pilot reading for percent survival from 1-5 days of incubation.

Type	No. of embryos Cultured	Duration of Incubation	No. of embryos survived	% of Survival
With window	15	1-5 days	9	60%
Without window	15	1-5 days	10	66%
Normal	15	1-5 days	13	86%

Table 1.2 shows pilot reading for percent survival from 5-10 days of incubation.

Type	No. of embryos cultured	Duration of Incubation	No. of embryos survived	% of Survival
With window	15	5-10 days	7	46%
Without window	15	5-10 days	9	60%
Normal	15	5-10 days	11	73%

Table 1.3 shows pilot reading for percent survival from 10-15 days of incubation.

Type	No. of embryos cultured	Duration of Incubation	No. of embryos survived	% of Survival
With window	15	10-15 days	4	26%
Without window	15	10-15 days	5	33%
Normal	15	10-15 days	8	53%

Table 1.4 shows pilot reading for percent survival from 15-21 days of incubation.

Type	No. of embryos cultured	Duration of Incubation	No. of embryos survived	% of Survival
With window	15	15-21 days	1	6%
Without window	15	15-21 days	2	13%
Normal	15	15-21 days	5	33%

Table 1.5 Effect of D-trans allethrin on developing chick embryo, approximately on 10th day

Experimental Embryos	Days of incubation Approx at 10 th day.	Total length in cm	Hind limb in Cm	Fore limb in cm	Beak in Cm	Diameter of eye in cm
With window	Approx at 10 th day	3.8	1.3	1.0	0.4	0.4
Without window	Approx at 10 th day	4.2	1.5	1.2	0.6	0.5
Normal	Approx at 10 th day	4.5	1.8	1.4	0.8	0.7

Table 1.6-Effect of D-trans allethrin on developing chick embryo, approximately on 14th day

Experimental Embryos	Days of incubation Approx at 14 th day.	Total length in cm	Hind limb in cm	Fore limb in cm	Beak in Cm	Diameter of eye in cm
With window	Approx at 14 th day	4.5	1.3	1.0	0.5	0.8
Without window	Approx at 14 th day	6.8	3.0	1.9	0.9	1.0
Normal	Approx at 14 th day	7.2	3.4	2.5	1.2	1.4

Table 1.7-Effect of D-trans allethrin on developing chick embryo, approximately on 18th day

Experimental Embryos	Days of incubation Approx at 18 th day.	Total length in cm	Hind limb in Cm	Fore limb in cm	Beak in Cm	Diameter of eye in cm
With window	Approx at 18 th day	7.5	2.9	2.3	0.7	1.2
Without window	Approx at 18 th day	7.5	4.5	2.8	1.1	1.4
Normal	Approx at 18 th day	10.1	4.3	3.1	1.6	1.7

A: Treatment With D-Trans Allethrine (96hrs)

The experimental embryo (approx.96hrs) was treated with a mosquito coil and the following result was observed;

1: Experimental With Window-

1. Increase heart beat 80/min.
2. Reduction in total length was the most prominent abnormality.
3. Embryos also showed decreased total body wet weight as compared to without window & the control embryos.
4. Total length =1.5cm.
5. Wet weight of embryo =0.225gm.
6. No prominent blood vessel.
7. 2: EXPERIMENTAL WITHOUT WINDOW:
8. Heart beats as compared to experimental(with window)was
9. 72 beats/min.
10. Wet weight of embryo=0.258gm.
11. Total length=2cm.
12. Prominent blood vessels.
13. 3: CONTROL
14. Heart beat=60-65beats/min.
15. Total length =2.4cm
16. Wet weight of embryo=0.350gm.

Reduction in the body length as well as in the weight of the embryo indicates that d-trans allethrin affects the growth rate of an early embryo. During this stage were the embryos are at its differentiating best, the d-trans allethrin prove to be a hindering agent for this process. Improper development Of Limbs poor eye development **9 days chick embryo**

B: Treatment Of Embryo With D-Trans Allethrine(5-10 Days)

When chick embryo was treated with d-trans allethrin (5-10days)

The following abnormalities' were observed. Along with a reduced body length as well as the deformities observed during heart beat/min. With respect to all this the following results were also observed.

1: Experimental With Window

1. Similarly during 96 hrs chick embryo reduced body weight was again observed in this condition.
2. The embryo which was observed at this stage showed one additional abnormality i.e.; the development of body feathers was not seen.
3. Compared to that of the window, without window and control; the abnormal feather development was clearly noticed.
4. With respect to the limbs poor development of hind limb and fore limb was also clearly distinct in embryo`s with window.
5. Poor development of the beak was also noticed when compared with without- window as well as with the normal embryo.
6. The reduced diameter of eye was also noticed with embryo which was having a window

2: Experimental Without Window

1. Less development of feathers were observed in the embryo with, without window.
2. Similarly, the poor development of fore limb and hind limb was observed.
3. The beak deformities were also observed
4. Well development of feathers
5. Small beak
6. Eyes not well developed
7. Limbs not well developed
8. Reduced body length

14 Days Chick Embryo Effect Of D-Trans Allethrin On Developing Chick Embryo (10-15 Days)

When developing chick embryo were treated with d-trans allethrin the embryo of 10-15 days were having the following deformities.

1: Experimental With Window

1. During further development of chick embryo deformities were
2. Symptoms indicating retardation in the growth was one of the important aspect which was noticeable
3. With respect to this decreased means body weight was observed.
4. Reduction in total body length was also seen when compared with, without window and normal.

2: Experimental Without Window

1. As compared to that the embryo with window, the embryo without window was well developed.
2. Similarly when compared with the normal embryo the embryo with, without window showed the abnormalities in feathers development.
3. □ 3 at the same time poor development of fore limb and hind limb was also noticed.

Treatment of Chick Embryo With D-Trans Allethrin (15-21 Days)

This was the final stage so as to expose the harmful effect of d-trans allethrin on the chick embryo which was almost in the hatching condition.

Experimental With Window

1. The embryo which was hatched showed us very complicated body structure.
2. One of the aspects that were studied during this period was that the mortality of the developing chick embryo was much higher.
3. Even if the embryo from these 15-21 days were able to hatch they haven't shown proper development of whole body.
4. Similarly effect regarding poor development of feathers has been a major effect due to the d- Trans allethrin
5. Same parallel effect was noticed with respect to the abnormal beak development.
6. With concern to this effect the poor development was seen in forelimb and hind limb.

Experimental Without Window

1. The embryo which was hatched showed little development of the feathers as compared with the embryos of without window and normal.
2. But when compared with the normal embryo parallel condition of poor development of the feathers was observed.

3. Were the development of the beak was concerned, the embryos with, without window showed some abnormalities with regard to the length.
4. Length of the fire limb was also stunted which showed the retarded ossification of the long bones.

Normal Without Window With Window

1. Beak small in size
2. Reduced body size
3. Weak limb development
4. 18 days of chick embryo

D- trans allethrin has been reported as one of the harmful substances which are present in the mosquito coil (19,20 and 21). D -Trans allethrin has been demonstrated to be a teratogen inducing a range of abnormalities in the chick embryo bring exposed to it during the early stages of development. The concentration of d-Trans allethrin which exposed to the approximately 96 hour's old embryo several abnormalities was observed which indicates the fact that the d-trans allethrin is definitely affecting the growth in some way or the other. The decreased size of the chick embryo as well as the reduced body length can be co-related with the decreased fetal weight which has also been accounted in the case of human embryos i.e.; prenatal growth retardation.

The embryos which are observed on 10th day a more drastic intensity of deformities were also noticed. Feathers abnormalities as well as the total body length was one of the major abnormalities noted in the o the embryos exposed to the said- Trans allethrin. The above mentioned abnormalities were indicative of the possibility that it can lead to a variety of different deformities ranging for the poor feathers development in embryo which were with window. Similarly less effect was seen in chicks in without window compared with the normal embryo.

Microsomia is another abnormality observed in the embryos during the present study. The overall abnormal smallness of the whole body as in dwarfism as compared to that of the control embryo is confirmative of this condition. This can be due to a variety o factors such as uneven accelerating or accelerated cell differentiation. The observed embryos with window showed us the Microsomia affecting the abnormal development of the whole body.

Tachycardia was also one of the condition observed during the experimental study as a result and effect of d trans allethrine. The heart beat rate which was observed in the embryo with window was slightly higher than that of the without window and finally compared with normal embryo heart beat/min. During 5-10 days o chick embryo development various changes in the hind limb and as well as in the fore limb was noticed, resulting poor development of both hind limb and fore limb. Similarly when the embryos from 10-15 days and 15-21 days were observed one main criterion taken into consideration was development of beak. During this experimental study developing embryo with window showed reduced beak length. The reduced beak length was also observed in the developing chick embryo without window which was further compared with that of the normal embryo.

Considering other abnormalities the embryo displayed the same effect all over again. This indicates that d-Trans allethrin is highly toxic and causes various deformities in developing stages of chick embryos. These toxic results also affect humans via; d-Trans allethrin is a central nervous system stimulant. Heavy respiratory exposure caused in coordination and the urinary incontinence in mice or rats (Liu & Sun (1988), Liu & Wong (1987)). Thus for the experimental study we can conclude that d -Trans allethrin induced malformation observed in chick embryo, which is comparable with those reported in human embryos. In the present investigation, it has been shown for the first time that d- Trans allethrin induced malformation which can be demonstrated and examined in the developing chick embryo.

Nevertheless it is difficult to compare the result obtained in the experimental study here using the chick embryo system with that of the human physiology. While considering whole body development, metabolism and biochemical mechanism during human embryonic life. In summary the present results demonstrate that d- Trans allethrin is one of the harmful compounds used to repel mosquito in mosquito coil. Last but not the least keeping in mind the harmful effects of d- Trans allethrin it is rather useful to recommend mosquito net as a best and cheap method to repel mosquito.

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