

Effect of Mosquito Repellent (Odomos) on the Developing Chick Embryo (Gallus Gallus)

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Abstract: *The attempt was made to evaluate the effect of mosquito repellent i.e. odomos on the development of chick embryo under laboratory conditions. There are several species of insect which cause problem of human health directly and indirectly. It can be controlled by chemical such as DDT, B.H.C etc. In the present endeavour we try to evaluate the effect of N, N Dimethyl benzamide which is component of mosquito repellent. The compound has adverse effect on various stage of chick development. Tachycardia and Micosomia are major deformities observed due to N,N Dimethyl benzamide. The compound was found to certain abnormalities seen in the development of embryo. The characteristics deformities during this study have been co-related with the result which we obtain from current investigation. The continuous use of odomos may lead to undesirable consequence in vertebrates. According to us the best option to get rid of mosquito bite is to use the mosquito net.*

Keywords: Odomos, Tachycardia, N, N Dimethyl benzamide, Chick embryo, Microsomia

1. Introduction

There are several species of insect which cause problem of human health directly and indirectly. Some of the insects which cause damage to human health are bed bug, house fly, Drosophila, head louse, mite, ticks, mosquitos etc. It can be controlled by chemicals such as chlorinated hydrocarbon like DDT, B.H.C etc. But these chemicals also not safe for human health. These insects or majorly called mosquitoes can be repel by using mosquito repellent cream, here we use ODOMOS cream as a mosquito repellent whether it is safe or not to observe, its effect on development of chick embryo (*Gallus gallus*). One of the greatest miracle of nature is transformation of the egg into the chick. A chick emerges after a brief three weeks of incubation. We study these three weeks of incubation at the different four stages after egg get hatched i.e after 96 hours, after 9 days, after 14 days and after 18 days.

After the hatching process, these eggs were divided into 2 categories i.e with window and without window. Before incubating we insure that all the instruments were sterilize with 70% alcohol to minimise surface contamination. We tested these eggs in presence of odomos cream which is are clinically proven mosquito repellent (P.K. Mittal, U. Sreehari, R.k. Razdan).

We studied chick embryo at different stages was use to observe the effect of N, N Dimethyl benzamide. A developing chick embryo, it is beneficial for experimentation with all stages of development. N, N Dimethyl benzamide has been reported to be safe at 12% concentration which is present in the odomos cream which is a mosquito repellent to observe several abnormalities in the chick embryo. In this work we analysed or demonstrate that the development of chick embryo can be used an alternative to the mammalian model for the research to check deformities in the various development stages under the laboratory conditions.

The active ingredients found in ODOMOS cream-

- 1) N, N Dimethyl benzamide.
- 2) DEET.

2. Materials and Method

Materials– Fertilized eggs, Usual laboratory glassware and instruments, Autoclave, Scalpel, Incubator, Sterile wooden chamber, Preservation jar, Dissecting microscope, Cardboard tray, Cotton swabs, Sterilized gloves, Water tray, weighing balance.

Chemicals- Avian saline (0.9%NaCl solution), Formalin, Absolute alcohol, N, N Dimethyl benzamide.

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

Freshly fertilized eggs obtained from a local poultry were cleaned and incubated for 24 hours at 37 degree Celsius with a related humidity of 70.80%. Eggs were placed in horizontal position 15 to 20 minutes before the incubation. So as the insure the position of the embryo on the top. Before incubating the eggs were divided into two categories i.e with window and without window. In 1st category of eggs the window was made and in 2nd category they were incubated without window. The surface sterilization of these eggs must be wiped then with 70% alcohol to minimise surface of contamination of the cultures. These eggs were then transferred to the incubator. While handling the eggs, gloves are used.

Eggs were incubated at 37.5 degree Celsius and relative humidity of 70-80% and put them in incubator. Then odomos cream was taken and weighed 1 gram cream on weighing balance. The cream measured was put on a petri

plate. Then, Aluminium foil was taken and wrapped it over the paper. The length and width of the paper was measured to be 25cm and width 20cm respectively. Then, the cream was taken and was applied uniformly on the aluminium foil. Then, this foil was paced on the side of the eggs in the incubator. Then this method was repeated for 20days till the eggs get hatched. Then, the eggs were also dissected out on the stages of development to observe the complications. Embryos were studied at the different stages.i.e 96 hours, 9 days, 14 days, 18 days.

3. Result and Discussion

The pilot experiment was performed so as to observe the mortality of rate of 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 present survival at 96hrs in incubation

Type	Number of embryos cultured	Duration of Incubation	No. of embryos survived	% of survival
With window	30	96 hrs	20	66.66%
Without window	30	96 hrs	24	80%
Normal	30	96 hrs	28	93.33%

Table 1.2: Shows pilot reading for present survival at 9 days incubation

Type	Number of embryos cultured	Duration of Incubation	No. of embryos survived	% of survival
With window	30	9 Days	16	53.33%
Without window	30	9 Days	21	70%
Normal	30	9 Days	23	76.66%

Table 1.3: Shows pilot reading for present survival at 14 days incubation

Type	Number of embryos cultured	Duration of Incubation	No. of embryos survived	% of survival
With window	30	14 Days	9	30%
Without window	30	14 Days	11	36.66%
Normal	30	14 Days	18	60%

Table 1.4: Shows pilot reading for present survival at 18 days incubation

Type	Number of embryos cultured	Duration of Incubation	No. of embryos survived	% of survival
With window	30	18 Days	2	6.66%
Without window	30	18 Days	4	13.33%
Normal	30	18 Days	15	50%

Table 1.5 Effect of N, N Dimethyl benzamide on developing chick embryo, approximately on 7th day

Embryos with	Day of Incubation	Hind Limb (cm)	Fore Limb (cm)	Beak (cm)	Diameter of Eye (cm)	Total length (cm)
With window	Approx at 7 th days	1.2	0.9	0.3	0.3	3.9
Without window	Approx at 7 th days	1.6	0.9	0.4	0.4	4.1
Normal	Approx at 7 th days	1.9	1.1	0.7	0.7	4.5

Table 1.6: Effect of N, N Dimethyl benzamide on developing chick embryo, approximately on 14th day

Embryos with	Day of Incubation	Hind Limb (cm)	Fore Limb (cm)	Beak (cm)	Diameter of Eye (cm)	Total length (cm)
With window	Approx at 14 th days	2.3	1.2	0.5	0.7	5.2
Without window	Approx at 14 th days	2.7	1.8	0.9	0.8	6.1
Normal	Approx at 14 th days	3.5	2.4	1.2	1.3	7.5

Table 1.7: Effect of N, N Dimethyl benzamide on developing chick embryo, approximately on 18th day

Embryos with	Day of Incubation	Hind Limb (cm)	Fore Limb (cm)	Beak (cm)	Diameter of Eye (cm)	Total length (cm)
With window	Approx at 18 th days	3.1	2.2	0.6	1.0	7.0
Without window	Approx at 18 th days	4.6	2.6	2.6	1.3	9.0
Normal	Approx at 18 th days	5.0	3.0	3.0	1.6	10.0

A) 96 hours chick embryo treated with 1gm N, N Dimethyl benzamide

1) With window-

- The beats of the embryo was recorded to be 85 beats per minute.
- The length of the embryo was measured as 1.2 cm.
- The weight of the embryo was 0.202 gms.
- The mortality rate was observed to be 55%
- Tachycardia.
- Microcephaly.
- Blood clot formation.
- Eye were less prominent.

2) Without window-

- The beats of the embryo was recorded to be 75 beats per minute.
- The length of the embryo was measured as 1.4 cm.
- The weight of the embryo was 0.923 gms.
- The blood clot formation in the tail bud.

3) Normal embryo -

- The beats of the embryo was recorded to be 60-62 beats per minute.
- The length of the embryo was measured as 2.3 cm.
- The weight of the embryo was 0.335 gms.

So the deformities like clot formation, hydrocephaly i.e the presence of the water cephalon. Tachycardia, microcephaly was observed at the stage of 96 hrs chick embryo. At this stage although the embryo is differentiating best but the odomos proves to be hindering object for this process.

B) 9 days chick embryo treated with 1gm N, N Dimethyl benzamide

1)With window

- The heart beat of the embryo was recorded to be 72 beats per minute.
- The length of the embryo was measure as 2.5 cm.
- Blood clot formation was seen near the brain region.
- The eye were less prominent.
- The mortality rate was observed to be 56%.

- The feather development was weak.
- The body of the embryo was not properly oriented as it shows weak development of the hind limb.

2) Without window-

- The heart beat of the embryo was recorded to be 72 beats per minute.
- The length of the embryo was measure as 2.8 cm.
- The weight of the embryo was 0.999 gms.
- The blood clot were observed throughout the body of the embryo.
- The feather development was weak.

The deformities seen in the 9 days chick embryo was-

Blood clots observed near the brain. Rupturing of blood vessels throughout the body. Reduced body size. Weak feather development, Weak development of hind limbs.

C) 14 days chick embryo treated with 1gm N, N Dimethyl benzamide-

1) With window-

- The length of the embryo was measured as 4cm.
- Blood clot formation was seen near the ear and brain region.
- The eyes were less prominent.
- The mortality rate was observed to be 67%.
- The feather development was weak.
- The beak development was less prominent.
- The hind limb and fore limb were also reduced in size when compared with normal embryo,

2) Without window-

- The length of the embryo was measured as 4.3cm.
- Blood clot formation was seen near the ear region.
- The eyes were less prominent.
- The feather development was weak.
- The beak development was less prominent.

The chick embryo with window and without window showed reduced body size, less growth of the feathers, less prominence of the eyes, as well as fore limb and hind limb.

D) 18 days chick embryo treated with 1gm N, N Dimethyl benzamide-

1) With window-

During further development of chick embryo the deformities which were –

- Symptoms indicating retardation in growth was one of the important aspect which was noticeable.
- With respect to this decreased mean body weight was observed.
- Reduction of the total body length was also see when compare with, without and normal.

2) Without window –

- Less development of the feathers were observed in the embryo with, without window.
- Similarly, poorly development of fore limbs and hind limbs as observes.
- Finally the beak deformities were also observed.

In the present study, the chick embryos of different stage was used to observed the effect of N,N Dimethyl benzamide on the developing chick embryo (6). It is beneficial for experimentations with all the stages of the development.

Its useful lies in the fact that it allow studies to be carried out at an early stage of the chick embryos where organogenesis take place as well as the later stage when morphological changes occurs in the chick embryo. It also gives us the tremendous advantage of providing easy access to embryos(10). As well as extra embryonic membrane for the observation of morphogenesis and growth. The applications of the different agents under study and inoculations of viruses in specific extra embryonic membrane(7), such as the yolk sac, omnion and chiroallontonic membrane(15). N,N Dimethyl benzamide has been reported to be a safe (12,14,16) at the 12% concentration which is present in the odomos cream which is mosquito repellent. The concentration of 1gm N,N Dimethyl benzamide when exposed to the approximately 96 hrs old embryo several abnormalities were observed that gives us some indication that it if repels away the mosquito but may show certain effects of the human also.

When the embryos were discarded at an approximate stage 96hrs its showed tachycardia that is abnormal cardiac rate. The parallel condition is known as ‘Cardiac murmur’ in the human embryo(18). Hydrocephaly i.e the accumulation of fluid in the head region leading to pressure being created on the developing brain was also not prominent. It was observed that the blood clot were present in the blood vessels thus blocking the whole functioning of circulatory system.

The embryo which were discarded at the 8 days stage more deformities were observed. The feather development as well as other deformities like limb i.e fore limb and hind limb were weakly developed. The feather development was major deformity observed it may have be because of the repellent were applied externally on the skin of humans and by the observation we may conclude that it may show the hair removing properties both window as well as without window. The less feather development may be because of the repellent. That is may interfere with the protein metabolism of feather protein leading to be the abnormality in the feather development process(20).

Similarly for the 14 and 18 days chick embryo which when compared with the normal both window and without window showed the weak feather and limb development (21). Hence opening eggs from early to later stage it has been observed that the mortality rate has shown a prolific increase i.e in the early stages of 96hrs and 8 days less mortality rate but as the later stage were observed the mortality rate ware seen to increase i.e in the four embryos discarded at a particular stage showed the 50% mortality. It was also seen that in 18 days embryo the mortality rate higher than the previous stage.

Microsomia is another abnormalities observed in the embryo during the present study. The overall abnormal smallness of the whole body as in dwarfism as compare to that of control embryo is confirmative of this condition. This can be due a

variety of factor such as uneven accelerating or a accelerated cell differentiation(22). The observed embryo with window showed we the micosomia affecting the abnormal development of the whole body.

Similarly when the embryos of 14 and 18 days were observed one main criterion was taken into consideration that is the development of the beak. During this studies developing embryo with window showed reduced beak length. In the present investigation, it has been shown that the N,N Dimethyl benzamide induced malformations which can be demonstrated and examined the developing chick embryo. Nevertheless it is difficult to compare the result obtained in the study here using the chick embryo system of that the human physiology. Considering whole body development, the metabolism and biochemical mechanism during human embryonic life (23). In this regards, a 21 days incubation period of the chick embryo is comparable to the 21 days gestation periods of mice. The 4-5 days incubation in chick do then correspond to 2-5 weeks of post conception ages in human embryo(17). Some way such as relation in total body length, abnormalities of feather development , tachycardia etc.. association with human being. Further studies regarding various hormonal changes as well as the genetic changes caused by the N,N Dimethyl benzamide is required to support these findings. In summary the present results demonstrate that the development of chick embryo can be used an alternative to the mammalian models for the research to check deformities in the various development stages.

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