Evaluating Developmental And Reproductive Teratogenicity Of Some Drugs And Herbal Suspension In Chick Embryo Model.

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Abstract

Teratogenicity refers to the study of birth blights and their causes. The teratogen information system (TERTS) and Shepard's roster of teratogenic agents are precious coffers for experimenters in this field. It is important to completely test all medicines for their implicit to beget birth blights, as utmost medicines can cross the placenta and affect the developing foetus. The major birth blights include natural heart blights, neural tube blights, Down pattern, hemoglobinopathies, and glucose-6-phosphate dehydrogenase insufficiency. still, medicines can also laterally increase the threat of birth blights by depleting essential nutrients. juvenile development is divided into three ages fertilization to blastoderm conformation, embryogenesis, and embryonic growth. The juvenile embryo model is generally used for toxin studies, including genotoxicity studies to determine the eventuality of a substance to beget inheritable damage. juvenile embryos also give perceptivity into cytotoxicity and embryo toxin. The parallels between juvenile embryos and other beast models, including humans, make them a precious tool for studying medicine goods. Factors similar as egg storehouse temperature, time of incubation, and route of medicine administration can impact toxin results in juvenile embryos. Herbal drugs have been used since ancient times, with India being a mecca for traditional factory- grounded drug systems. Tecoma undulata is a medicinal condiment used in Ayurvedic drug for colourful purposes similar as treating urinary diseases, liver diseases, excrescences, and promoting crack mending. It has also been traditionally used for syphilis and painful bumps. The dinghy of Tecoma undulata has shown pharmacological conditioning similar as hepatoprotective exertion, analgesic exertion, antibacterial exertion, antifungal and anti-termite exertion and anti-helminthic exertion. In conclusion, teratogenicity exploration plays a pivotal part in understanding birth blights caused by medicines. The juvenile embryo model provides precious perceptivity into medicine toxin studies. Herbal drugs like Tecoma undulata have been used for centuries with colourful remedial parcels. Understanding the pharmacological conditioning of herbal drugs can help uncover their implicit benefits in ultramodern drug.

Keywords: **Teratogenicity, chick development, toxicity studies, herbal medicine, *Tecoma undulata*, extract, embryo development.**

1.Introduction

Teratogenicity: Teratology is the science of studying and investigating birth defects and their etiologies [1]. The teratogen information system (TERTS) and the online version of Shepard's catalogue of teratogenic agents are available at Web resources 8-15, TERTS is an online database containing series of agent summarizes each of which is based on a thorough review of published clinical and experimental literature summarizes which may be accessed using either generic or brand names [2]. In 1937, the US company marketed an elixir of sulphonamide in which the main ingredient is diethylene glycol, and due to this product more than 100 people and children were died [3]. All drugs which are on market are hardly tested for their teratology data [4]. The great majority of drugs cross the placenta through simple diffusion and the most significant factors influencing the placental diffusion are lipid solubility and molecular size [5]. Each drug needs to be considered both for its teratogenic potential and for its ability to create serious consequences during the pregnancy. The major birth defect contains congenital heart defects, neural tube defects (NTD) and Down syndrome, Hemoglobinopathies, and glucose-6phosphate dehydrogenase deficiency [6]. The situation becomes even more complex when one considers that the drug may not have either of these effects but may indirectly increase the risk of birth defects or pregnancy complications by depleting an essential vitamin, nutrient or co-factor that is essential for foetal development or a normal pregnancy outcome [7].

Chick development: The cleavage pattern of chicken is meroblastic, in which the only one part of egg is destine to become and other part i.e. yolk portion serves as nutrition [9].

Chick development has been divided into three periods.

1. Fertilization to blastoderm formation- lasts for one day.
2. CC Embryogenesis- lasts for three days.
3. Embryonic growth- for eighteen days [10].

The CNS in the chick embryo begins to develop on the second day of incubation and matures before hatching on day 21 [11].

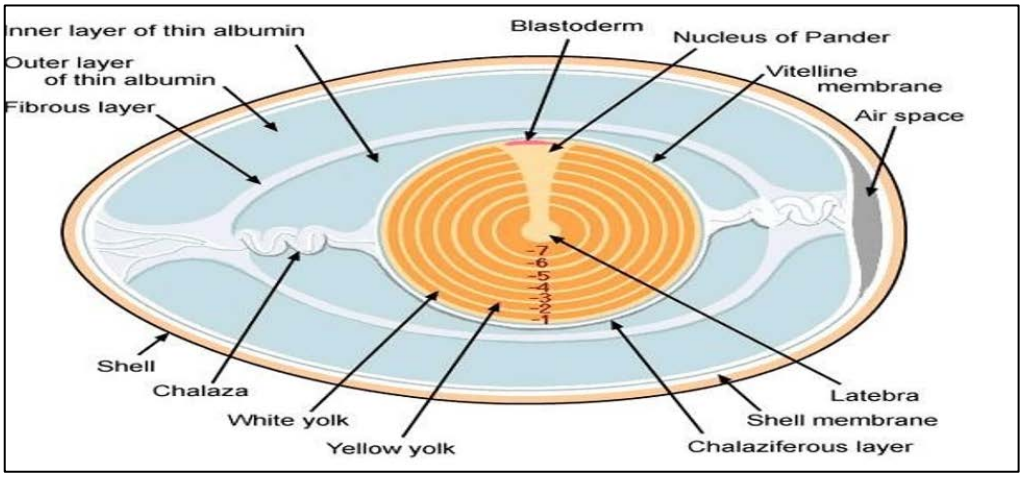

Fig 1. Chick development is described by Hamburger Hamilton in different stages.

Mechanism of hatching

Day 18:- the embryo rotates its body to the right position i.e. neck and head are below the air space membrane in folded condition.

Day 19-Day 20:- hatching behaviour is more active and due its head movements the beak penetrates the membrane (called as internal piping). At the beginning the allantois membrane acts as mode of gas exchange, now it dries up as the chick starts pulmonary respiration [11].

Essence of toxicity studies in chick embryo

1) Genotoxicity studies- Chick embryo model is used for Genotoxicity study of karamecyne (KACY). Genotoxic potential of KACY is explored using an *in-vivo* assay which determines the MN and SCE.
2) Cytotoxicity studies-chick embryo model is also used for bone marrow cytotoxicity study [12].
3) Embryo toxicity studies [13].

2.Similarity In Results Of Chick Embryo And Other Animal Study:

At 20 hour of incubation the chick embryo resembles to a human embryo of two weeks old [8]. Cross link between avian host and mammalian graft was elegantly illustrated in the study of tooth bud formation, which involves a sequence of reciprocal interactions between the oral epithelium and neural crest derived mesenchyme [14] Deformities produced by a given drug in the chick are identical to those mentioned in other species including man [8]. The gene sequencing in the chickens have more similarity with that of humans, with a great level of sequence conservation [15].

3. Factors Influencing Toxicity Of Drugs In Chick Embryo Model

The number of factors is known to influence the toxicity of substances administered by infection into the hen eggs. For e.g., the age, diet and strain of hen can affect the results of tests on the egg. The temperature at which the eggs are stored and the time when the egg is laid and the start of incubation affects the fertility. The route of injection of the test

compound that is administration of drug into the yolk sac, albumen, air-sac, chorioallantoic space, or on the surface of the chorioallantoic membrane, has a specified effect on the toxicity of the compound [16].

4.Herbal Medicines:

Tecoma undulata

Since ancient times, most of the people have been exploring the nature particularly plants in search of new drugs. This has resulted in the use of large number of medicinal plants with curative properties to treat various diseases. According to the present scenario it is estimated that 40% of the world populations depends directly on plant-based medicine for their health care. India is the birth place of renewed system of indigenous medicine such as Unani, Ayurveda, Homeopathy and Siddha. Traditional systems of medicine are prepared from a single plant or combinations of number of plants, the efficacy depends on the use of proper plant part and its biological potency which in turn depends upon the presence of required quantity and nature of secondary metabolites in raw drug. According to the world Health Organization, over 80% of the world's population or 4.3 billion people rely upon traditional plant-based systems of medicine for primary health care. In India, almost 95% of the prescriptions were plant based in the traditional systems.

Tecoma undulata belong to family Bignoniaceae commonly known as "Rohira," "Rohitaka" and "Rakta – Rohida is an important medicinally herb in ayurvedic medicine, the bark of *Tecoma undulata*, is claimed to be useful in the treatment of urinary discharges, enlargement of spleen, leucorrhoea, leukoderma, tumours, liver disorders, gonorrhoea, gout and promotes wound healing in Indian traditional system of medicine. Also, the bark is declared to act as relaxant, cardiotonic and choleretic in the Dictionary of Indian Medicinal Plants. It has also been traditionally used in the treatment of syphilis, painful swellings and cancer. The methanolic extract of the whole plant of *Tecoma undulata* has been reported to possess analgesic activity [17].

Pharmacological Activity:

Till the date, the activities such as Hepatoprotective Activity, Analgesic Activity, Antibacterial Activity, Antifungal and Anti Termite Activity, Anti helminthic activity are reported [19].

Fig 2. Tecomella undulata growing: A naturally and B under agroforestry system; trees bearing (C, D) red, E, F orange, and G, H yellow-coloured flowers, I almost leafless tree in full bloom closeup view J flower, K immature pods, L tree bearing mature pods, and M wing pods. (by own source)

5.Material And Methods

5.1 Plant collection and authentication

Tecoma undulata steam bark powder was purchased in the month of Feb 2023 from the Shree vishwambar Aaurvedic chikitsalaya , A/P Nasrapur , Tal –Bhor, Dist –Pune,412 213. Sample were authenticated in the Dr. Swapnil S. Jagatap. In an Ayurvedacharya and Ayurvedic padvika, Nasrapur, Pune.

Figure 3. Bark of *Tecoma undulata*

Figure 4. Powder of *Tecoma undulata*

5.2 Preparation Of Plant Powder

The plant material was powdered using mechanical grinder. To get uniform sized powder, the powder was passed through the sieve No. 40. Then the powdered is stored in airtight container at dry place which is to be used for further procedure.

5.3 Preparation Of Extract

The extraction of Tecoma undulata was done by using two methods, namely Soxhlet and maceration.

5.3.1 Soxhlet Method

The Soxhlet extraction method is used for the extraction of secondary metabolites, the measured 50 gm of *Tecoma undulata* powder was successively extracted in a Soxhlet extractor using 500ml of hydroalcoholic solvent mixture of ethanol and water (50:50). The extraction was done for 18-hour time, where the extraction followed the 21 cycles. To minimize the solvent loss, the solvent is recovered using simple distillation method. Then the extracts were dried in rotary evaporator. The % extractive value was calculated after drying, and the extracts were stored in stock vials and maintained in the refrigerator for usage during the experiment.
Percent Yield was calculated according to the following formula.

Percent Yield = W1/W2 × 100
Where,
W1= Net weight of powder in grams after extraction
W2= Total weight of powder in grams taken for extraction.

Fig 5. Soxhlet extraction

5.3.2 Maceration Method-

100 gm of powder of *Tecoma undulata* was blended with 1000 ml of solvent, in a ratio of [1:10]. 1000 ml of each solvent were used-chloroform, ethanol, and water for different periods (14, 24, 48 hours respectively) with frequent agitation at room temperature. After the complete maceration, extracts were taken and filtered by using a muslin cloth. Then, these extracts were kept to gets evaporated at room temperature to obtain concentrated final product. Finally, the concentrated extracts were weighted and stored at 40℃ till their usage in the different test.

Figure 6. Maceration

5.4 Phytochemical Screening

The various extract obtained after extraction were subjected for phytochemical screening to determine the presence of following various phytochemical present in the extract.

5.5 Development Of Suspension With Herbal Extract

Suspension formulation

Techoma undulata, suspending agent, distilled water.

The dried extract of herbal drug was taken to be 1.5mg, which was then triturated to make a fine paste and reduce the particle size.1gm of suspending agent(acacia) was added. Later 12.5ml of water was added to it, with continuous stirring. And then shaking for 2 mins to get homogenous distribution of particles. Later the volume was made up to 25ml. this was 1% w/v of herbal suspension.

Evaluation parameter

5.5.1 Viscosity: Using Ostwald viscometer.

5.5.2 Sedimentation volume [19].

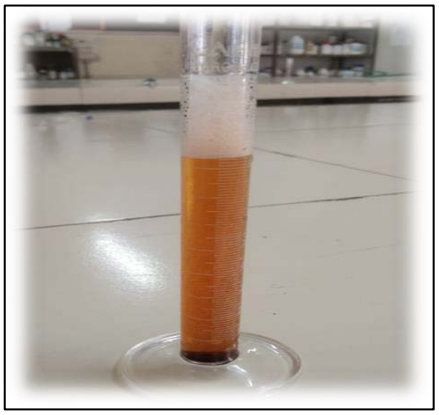

Fig 7. herbal suspension

• Anthelmintic activity of *techoma undulata*:

The activity was checked on the earthworms, by taking them in a petri dish. Each petri dish contained 10 earthworms. One was the control group treated with no drug. Next was albendazole doses, and the third was the herbal suspension treated.

Fig 8. Herbal suspension treated

Fig 9. Group A: Albendazole400mg,

Group B: Albendazole 200mg

Group C: control, Group D: Herbal suspension

5.6 Procedure For Teratogenicity Study

Gallus Domesticus strain was used for this experiment. Eggs weighing greater than equal to 50gm were selected and the eggs were obtained from venkys hatcheries girinagar Pune. The eggs were placed in plastic tray in incubator at 37.5-37.8°C and 50-60% RH (Relative humidity). After stabilization of eggs, eggs were candled and weighed. The rotten eggs were removed. The doses for the eggs were calculated based on brine shrimp toxicity model and the protocol for drug formulation administration in chick embryo. As some drugs were slightly soluble in water so that acacia was used as suspending agent. On third day on incubation drug was injected through yolk inoculation route. The drug was administered by windowing to egg shell using scissor tip or concentrated HCL. After drug injection the window was sealed using surgical cello tape after that eggs were re-incubated. On every 5^{th}, 10^{th}, 15^{th}, 20^{th} days of incubation candling was done to remove rotten eggs and also to observe development of chick embryo. When 21 days of incubation completed, eggs were removed and manually hatched and chicks were observed whether they alive or dead. Various evaluation parameters were performed and noted. The all data were extrapolated on control; teratogenic and safe group data and observed the toxicity of drug on morphology of chick and on every organ of chicken. The organs were dissected weighed and sent to pathology laboratory to prepare slides and blocks.

Experimental Steps

5.6.1 Procurement of eggs

Gallus Domesticus strain was selected for the research and the eggs were obtained from venkys hatcheries girinagar Pune.

5.6.2 Incubation conditions

The eggs were stabilized for two days at 37.5-37.8°C and 50-60% RH. The incubator used was Meta-Lab Company. After stabilization drug is administered and re-incubated at 37.5-37.8°C and 50-60% RH for 21 days with horizontal turning to 45° after period of 4 days.

Fig 10. Incubation of egg

5.6.3 Candling of eggs

It is the method to remove rotten eggs. After stabilization period rotten eggs were removed. Candling was done on 5th, 10th, 15th, 20th day of incubation and development of chick embryo was observed nakedly.

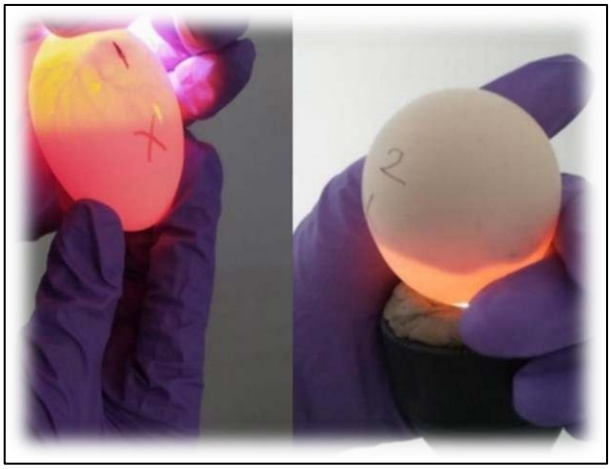

Fig 11. Candling of the eggs

5.6.4 Windowing to eggs

Window to egg was made using scissor and concentrated HCL.

Fig 12. Windowing of the eggs

Fig 13. Drug administration

5.6.5 Drug administration to embryo

Drug was administered to embryo through yolk inoculation route. Based on the brine shrimp toxicity lethal dose was calculated of drugs and herbal suspension, dose for chick embryo was calculated. The eggs were randomly assigned into nine groups (n=10). Hatch able eggs were placed at 37.5 °C temperature and 50-60% relative humidity in humidified incubator.

Different concentration of the suspensions was applied on the brine shrimp like 0.1ml,0.2ml,0.3ml,0.4ml,0.5ml, and it was found that at 0.3ml all the shrimp survived which led to the conclusion that 0.3ml is the therapeutic dose which can be administered to the chick embryo and these results also matched with protocol given for the dose administered to the chick embryo.

5.6.6 Sealing of window

Window of egg shell was closed using cello tape. For the perfect sealing of window two types of tape used, i.e. adhesive tape and plastic cello tape.

Fig 14. Sealing of window

5.6.7 Re-incubation

After drug injection and sealing of windows the eggs were re-incubated at 37.5-37.8°C and 50- 60% RH till 21 days of incubation.

5.6.8 Evaluations

After manual hatching of eggs following were the parameters performed.

5.6.8.1 Macroscopic evaluation:

Through eye observation

5.6.8.2 Body weight:

Embryo was weighed using electric balance. This weight denotes the development of embryo. Less weight than completely develop embryo i.e., control group.

5.6.8.3 Body size:

Body size was measured using 30cm of scale.[20]

6. Results

5.3 Phytochemical Screening

The various extract obtained after extraction were subjected for phytochemical screening to determine the presence of following various phytochemical present in the extract.

Table No. 1: phytochemical screening test

Phytochemical	**Phytochemical Test**	**Observation**
Saponins	**Foam test**- shake the drug extract or dry powder vigorously with water.	Stable foam observed.
Glycosides	**Legal's test**- alcoholic extract –add 1ml pyridine and 1ml sodium nitroprusside.	Pink to red colour appears.
Carbohydrate	Test for reducing sugar- **1]Fehling's test**- 1ml Fehling's A and Fehling's B solution –boil -1min heat to 5-10 min.	Brick red ppt.
	2] Molish's test- 2-3 ml extract add alpha naphthaol add H2so4.	Voilet ring present.
Alkaloids	**1] Wagner's test**- 1gm of extract dilute HCL, Filter and add 2-3 ml of Wagner's reagent	Reddish brown ppt
	2] Mayer's test- 2-3 ml filtrate with few drops Mayer's reagent	Orange ppt
Flavonoid	**Shinoda test**- dry extracted 5ml 95% ethanol – few drops conc. HCL and 0.5 g magnesium turning.	Orange –pink-red ppt

Tannins and phenolic compounds	1] Lead acetate test-	White ppt
	2] $Fecl_3$ test-	White ppt
	3] Acetic acid solution-	Red colour solution.

5.4 Development and Evaluation of suspension With Herbal Extract
Evaluation parameters:

1. Viscosity:

Using Ostwald viscometer was found to be 0.541kg/m-S.

2. Sedimentation volume:

F (%) = 0.76%.

Anthelmintic activity of herbal suspension:

The earthworms died within 5mins of exposure to the suspension which was comparable with marketed formulation.

Fig 15. Group A: Albendazole 400mg,
Group B: Albendazole 200mg
Group C: Control, Group D: Herbal suspension

5.5 Teratoginicity:

1. Macroscopic evaluation
2. Body weight: embryo was weighed using electric balance. This weight denotes the development of embryo. Less weight than completely develop embryo i.e., control group.
3. Body size: body size was measured using 30cm of scale.

1. Macroscopic Evaluation:

The all groups of embryos were evaluated for any lesions and abnormalities on external body surface.

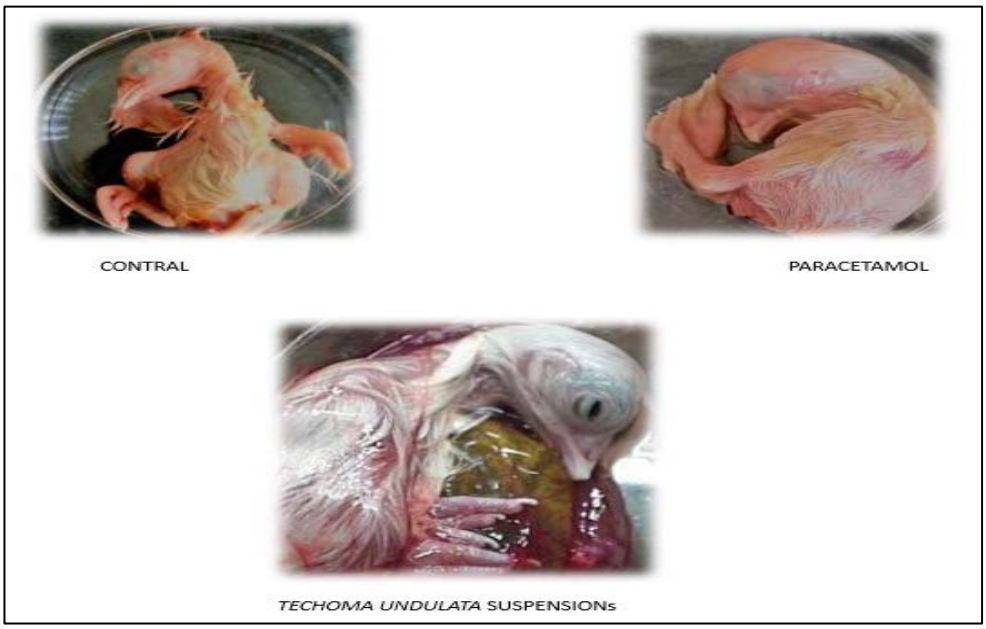

Fig 16. Chicken embryos treated with control, standard (Paracetamol), test (*techoma undulata*)

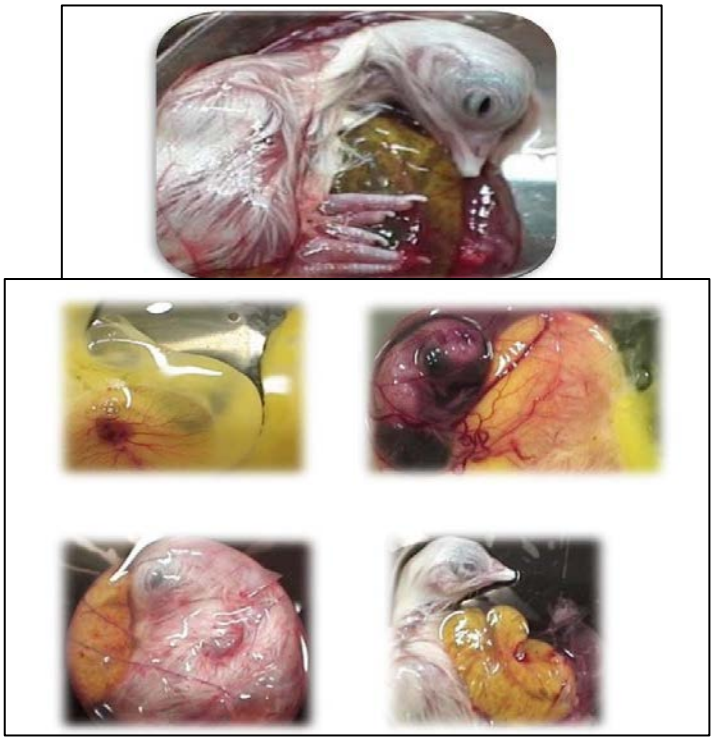

Fig 17. Herbal suspension treated chicken embryos

2) body weight and body size of chick embryo treated with control, standard, herbal extract.

Type	Body size in cm	Body weight in gm
1. Control	8.4 cm	39 gm
2. Standard (paracetamol)	7.46cm	35 gm
3. Test (herbal suspension)	8cm	38.9 gm

Table No.2: body size and body weight of the chicken embryos

Toxicological profile of number of potential drugs is not available and so that they are underutilized, hence an attempt was made to reveal the toxicological data of some drugs. When compared to other *in-vivo* animal models chick embryos possess many advantages as being cost- effective, produce faster results, easy availability of eggs, etc. and the data produced from this model will act as a predictive tool to perform the further study on other

models that will speed up the study. A broiler strain of chicken was selected for this study as this strain can grow faster than layer strains (Staphanie and Becker 1998).
Morphological parameters of the chick embryo are signifying whether test regimens affecting the development of embryo. Various morphological parameters were studied like body weight, body size, macroscopic characters. Significant changes in test group from the control and Paracetamol group indicated that the drug is affecting the developmental stages of the embryo. Herbal suspension showed no deformities and its result were comparable to the control. This study can be extended as biochemical study for chick embryo was not done as blood quantity is less, study on some vital organs and to study mechanism of action of toxicity.
No interaction of the suspending agent with the extract is reported and neither the quantity of suspending agent can cause any teratogenic effects.
Future scope: Safe dose calculation can be performed, a study of biochemical parameters using same drugs on chick embryo model and study of target organ toxicity.

8. Conclusion

- Drugs with unknown developmental toxicity were identified.
- The chick embryo model was developed to study the toxicity of selected drugs.
- Toxicological changes in test groups were compared with reference groups i.e., control, Paracetamol.

Broiler strain was selected for the study as broiler strain has faster development process than layer strains. The eggs were incubated at 50-60% RH and 37.5-37.8°C. paracetamol was taken as a standard drug for the study as it is widely used drug and easily available. The herbal suspension showed no deformities which was concluded by measuring various parameters and comparing it with the control group. And hence lastly at the end of the study it was concluded that *techoma undulata* is safe to be taken in pregnancy as per chick embryo model for teratogenicity study.

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