OPEN ACCESS

Saudi Journal of Medical and Pharmaceutical Sciences
Abbreviated Key Title: Saudi J Med Pharm Sci
ISSN 2413-4929 (Print) | ISSN 2413-4910 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article **Zoology**

Effect of *Morinda citrifolia* (Noni) Fruit Extract on the Teratogenecity in Female Wistar Rats

Shailesh A. Tawade[1*]

[1]Department of Zoology, S. S. & L.S. Patkar College of Arts & Science & V. P. Varde College of Commerce & Economics S. V. Road, Goregaon (west), Mumbai - 400104, Maharashtra, India

DOI: 10.36348/sjmps.2024.v10i03.011 | **Received:** 18.02.2024 | **Accepted:** 26.03.2024 | **Published:** 29.03.2024

***Corresponding author:** Shailesh A. Tawade
Department of Zoology, S. S. & L.S. Patkar College of Arts & Science & V. P. Varde College of Commerce & Economics S. V. Road, Goregaon (west), Mumbai - 400104, Maharashtra, India

Abstract

The raw fruit extract of *Morinda citrifolia* plant and its commercially available formulation in the market as Noni max (Best-selling brand of Brihans pharmaceutical in India and abroad) were obtained from Brihans Pharmaceuticals Pvt. Ltd. Mulund, Mumbai, India. 80 Wistar rats of either sex weighing between 200 to 250 gms were used for experimentation. In the present study, it was found that the *Morinda citrifolia* fruit extract and its formulation found safe for the developmental stages of foetus. It was also found that there was no significant difference was noted in body weight and the relative weight of the ovary, uterus and placenta of female rat. There was no structural and functional manifestation was noted in the developing foetusin the pregnant female rats. From the present study it is confirm that, both *Morinda citrifolia* fruit extract and its formulation (Noni max) did not produce any teratogenic effect in rats up to 1000 mg/kg and 40 ml/kg respectively.

Keywords: Fruit extract, formulation, foetus, placenta, teratogenic effect.

INTRODUCTION

Teratology, the study of abnormal prenatal development and congenital malformations induced by exogenous chemical or physical agents, continues to be a growing area of medical research in the quest for the eradication of preventable birth defects. Identification of agents with teratogenic potential from the excess of drugs and chemicals that human beings come into contact with in their everyday environment is crucial; although only some 10% of congenital anomalies are thought to be caused by teratogens [1]. Representing roughly one in every thousand live births, they compromise the quality of life for millions of individuals worldwide and cost billions of dollars in health care every year. Knowledge of the most hazardous substances would enable medical professionals and would-be mothers to minimize foetal exposure to them, helping to achieve the laudable goal of abolishing teratogen-induced malformations. The burden of this goal currently rests heavily upon animal-based testing [2]. Birth defects are known to occur in 3-5% of all newborns. They are the leading cause of infant mortality in the United States, accounting for more than 20% of all infant deaths. Seven to ten percent of all children will require extensive medical care to diagnose or treat a birth defect [3]. And although significant progress has been made in identifying the etiology of some birth defects, approximately 65% have no known or identifiable cause [4]. It was previously believed that the mammalian embryo developed in the impervious uterus of the mother, protected from all extrinsic factors. However, after the thalidomide disaster of the 1960s, it became apparent and more accepted that the developing embryo could be highly vulnerable to certain environmental agents that have negligible or non-toxic effects to adult individuals. A review published in 2010 identified 6 main teratogenic mechanisms associated with medication use: folate antagonism, neural crest cell disruption, endocrine disruption, oxidative stress, vascular disruption and specific receptor- or enzyme-mediated teratogenesis [5].

Teratology as a science was born in the 1920s and 30s, when the birth of malformed piglets from mothers fed an experimental diet high in fat or deficient in vitamin A elicited the shocking realization that the conceptus was not, as had been believed, in a privileged and highly protected position when within the mother's 'impervious womb,' but was susceptible to environmental conditions with potentially serious effects [6, 7]. All of these piglets suffered a variety of

malformations, predominantly a lack of eyes. Subsequent evidence came to light over the next two decades; correlation of particular birth defects of children with maternal Rubella infection in 1941 [8] and with environmental mercury contamination in 1956 [9]; malformed rats born following the inclusion of a growth-inhibiting amino-acid mimic in their mothers' diet [10] and malformed children born following failed aminopterin-induced abortions (a substance that shows no teratogenic effect in other primates, mice or cats) [11]. 'Then, infamously, the thalidomide catastrophe in 1961 [12, 13] represented the first case of a substance producing minimal toxicity in adults but considerable toxicity in the developing human embryo. The characteristic malformations of thalidomide embryopathy subsequently manifested themselves in 20 – 30% of children born to mothers who had taken the drug at any point during the four years it had been available; in some cases, even one single low dose was sufficient [14]. The confounding nature of many of the results from these animal-based studies has required these principles to be elaborated and revised [15, 16]. Many variables have been found to interfere with interspecies and animal-human comparisons, and these must be considered when designing developmental and reproductive toxicology studies [17, 18].

The least-developed area of clinical pharmacology and drug research is the use of medication during pregnancy and lactation. Almost every pregnant woman is exposed to some type of medication during pregnancy. Although the majority of pregnant and breast-feeding women consume clinically indicated or over-the-counter drug preparation regularly, only few medications have specifically been tested for safety and efficacy during pregnancy. There is scanty information on the effect of common pregnancy complications on drug clearance and efficacy. Often, the safety of a drug for mothers, their fetuses, and nursing infants cannot be determined until it has been widely used. Absence of this crucial information, many women are either refused medically important agents or experience potentially harmful delays in receiving drug treatment. Conversely, many drugs deemed safe are prescribed despite evidence of possible teratogenicity. Recreational, non-prescription, and prescription drug use in pregnancy is common. A WHO survey found that 86% of women took medications during their pregnancy; with an average of 2.9 (range 1–15) prescriptions [19]. Andrade *et al*., [20] found that 64% of pregnant women received a drug prescription, not including vitamins or minerals, within 270 days of delivery. Approximately 50% of these prescriptions were in the risk categories C, D, or X. (A: 2.4%, B: 50%, C: 37.8%, D: 4.8% X: 4.6%) Over-the-counter medications commonly used in pregnancy include acetaminophen (65%), ibuprofen (10%), and pseudoephedrine (15%). [21] Lo *et al*., found that teratogenic risks were undetermined for 91.2% of drug treatments approved in the United States from 1980 to 2000. There was inadequate information to determine whether the benefits exceed the teratogenic risks for most drug treatments introduced in the past 20 years. Marcus and Snodgrass [22] recommend that obstetricians advise women not to expose their fetuses to the risks of herbal medications. The quality control is variable and there is inadequate information regarding toxicity. Friedman [23] emphasizes that dietary supplements cannot be assumed to be safe for the embryo or fetus. Dietary supplements should not be labeled for use in pregnancy unless they have been shown to be safe by standard scientific methods.

Morinda citrifolia L (Noni) is one of the most important traditional Polynesian medicinal plants. Remedies from isolated Polynesian cultures, such as that of Rotuma, illustrate traditional indications that focus upon leaves, roots, bark, and green fruit, primarily for topical ailments. Ralph M. Heinicke promoted a wide range of claims about Noni, and these seem to have fueled much of the current commercial interest in the plant. Recent studies of the proliferation of commercial products have shown that Noni product manufacturers are promoting a range of therapeutic claims. These claims are based upon traditional Polynesian uses. A previous study of plant extracts in mice revealed that sub-cutaneous injection of an aqueous extract from the Noni plant did not have any adverse effect on litter sizes and produced no gross abnormalities in pups [24]. Another study on Sprague Dawley rats shows no teratogenic effect of freeze-dried Noni fruit puree of *Morinda citrifolia* from French Polynesia [25]. Hence the objective of present work is to evaluate the teratogenic effect of *Morinda citrifolia* fruit extract and its formulation (Noni max) in pregnant female rats which are exposed to test material by oral route during gestation period of their pregnancy i.e. from day 5 to day 21 of pregnancy.

MATERIALS AND METHODS

a) Collection of Samples and Animal Procurement:

The raw fruit extract of Morinda citrifolia plant and its commercially available formulation in the market as Noni max (Best-selling brand of Brihans pharmaceutical in India and abroad) were obtained from Brihans Pharmaceuticals Pvt. Ltd. Mulund, Mumbai, India. 80 Albino Wistar rats, 40 male rats and 40 female rats for each experimental set up i.e. for Morinda citrifolia extract and its formulation, weighing between 200 to 250 gm, were procured from Haffkine Biopharmaceuticals limited, (Haffkine BPCL), CPCSEA Registered breeder & Supplier No: 200/2000/CPCSEA Parel, Mumbai.

b) Experimental Design:

The animals were acclimatized, maintained and housed in the laboratory for a week. The controlled humidity and temperature at 22+3^{0}C, humidity 50-60 %, and illumination cycle set to 12-hlight/12 hrs dark cycle was also maintained. Five rats per cage were housed in polypropylene cages with stainless steel grill top,

facilities for commercial Pallet food 'Amrut' brand Pellet manufactured by M/s. Pranav Agro Industries Ltd., Sangli and water bottle with ad-libitum, and bedding of clean paddy husk.

c) Administration of Test Sample:

The Groups of animals at each dose level were identified by cage number and were individually colour coded, stained with diluted picric acid. After grouping, all the animals were kept in quarantine for an additional period of two weeks for acclimatization to the experimental environment, prior to mating and allocating to different treatment groups. The oral route was selected for dosing, The animals from each group were administered with doses of 250 mg, 500 mg, and 1000 mg of *Morinda citrifolia* fruit extract per kg body weight and 10 ml, 20 ml and 40 ml of *Morinda citrifolia* formulation per kg body weight, respectivelyas it is the most recommended route of exposure in humans. Animals were administered a dose of test substance daily from day six to fifteenth day of pregnancy. The doses were administered at similar times each day and were calculated on the basis of bodyweights determined on the day of dosing. The control group was also maintained separately for each set along with treated groups by feeding commercial pallets food and water bottle with ad-libitum, and bedding of clean paddy husk.

d) Mating and treatment procedure:

After quarantine period all the females were introduced in to the male cages. The animals were paired on one-to-one ratio basis. The morning vagina of each female rat was examined to retained intra-vaginal copulatory plug using a drop saline solution to check the pregnancy. The presence of sperms in the vaginal smear is considered as successful mating and leads to pregnancy by examining under the light microscope. At a particular day when the sperm/vaginal plugs were detected could be considered day-0 of pregnancy. All the pregnant female rats were allocated individually (i.e. one female rat per cage) to their respective treatment groups. A body weight of each female was also recorded.

RESULTS AND DISCUSSIONS

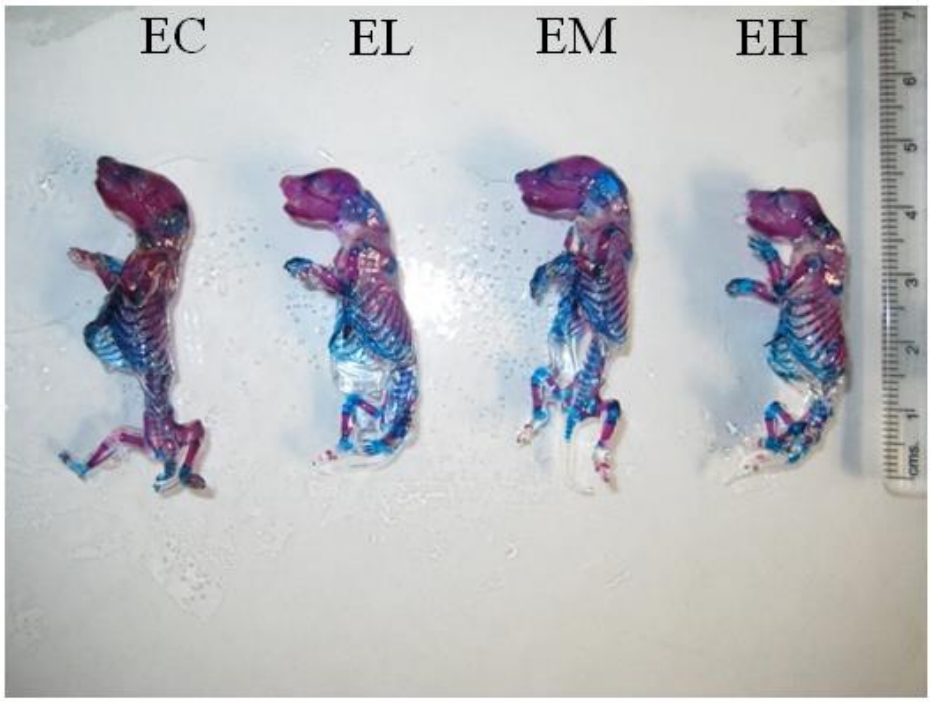
Table 1: Showing the effect of *Morinda citrifolia* (noni) aqueous extract on the teratogenecity in male and female *Wistar rats*

Sr. No	Developmental Parameters		Treatment Groups (Dose) for Extract			
			Control (Vehicle Alone)	250 mg/kg	500 mg/kg	1000 mg/kg
1.	Number of dams mated		20	20	20	20
2.	Number of pregnant		20	20	20	20
3.	Number of animals surviving		20	20	20	20
4.	Number of animals aborting		00	00	00	00
5.	Number of animals delivering early		00	00	00	00
6.	Number of live offspring (Mean)		13	12	14	12
7.	Sex ratio	Male	7.2	7.0	8.5	5.9
		Female	6	7	6.4	7.1
8.	Mean Foetal body weight by sex M/F (g)	Male	4.4	4.3	3.9	4.0
		Female	4.0	4.2	4.1	4.2
9.	Mean No. of corpora lutea Right/left (Mean± SD)		9.46± 2.54 8.92± 2.50	8.76 ± 2.79 9.07 ± 2.73	9.16 ± 2.67 8.78 ± 2.70	8.82 ± 2.45 8.02 ± 2.50
10.	No. of implantations (Mean± SD)		13.2± 3.0	14.0± 1.7	12.9± 1.9	13.0± 3.3
11.	Mean No. of live foetuses		13.2 ± 2.8	14 ± 1.5	12.9 ± 1.7	13 ± 2.5
12.	No. dead foetuses (Mean± SD)		00	00	00	00
13.	No. of Resorptions		00	00	00	00
14.	No. & % of pre- & post-implantation losses.		00	00	00	00
15.	Mean Body weight(g) ($\overline{X}$ ± SD)		272.85 ±35.22	273.21 ± 36.34	269.99 ±34.03	277.05 ± 33.74
16.	Mean Food consumption (g/100g/Day) ($\overline{X}$)		9.72 + 0.71	10.2 ± 0.70	10.25 + 0.90	9.88 + 1.06
17.	Comparison of change in absolute values mean organ weights between the groups (dams) Mean Organ weight(g) ($\overline{X}$ ± SD)	Body wt.(g)	327.30±7.85	326.08 ±17.15	322.17 ±22.99	328.42 ±16.77
		Uterus (g)	53.85±6.60	55.80 ±6.78	59.85 ±7.58	64.60 ±6.51
		Ovaries mg)	73.50±10.73	72.52 ±8.59	76.85 ±7.66	76.14 ±4.66
18.	Comparison of change in relative values mean organ	Body wt.(g)	327.30±7.85	326.08 ±17.15	322.17 ±22.99	328.42 ±16.77
		Uterus (g)	16.44	17.16	18.65	19.73

Sr. No	Developmental Parameters		Treatment Groups (Dose) for Extract			
			Control (Vehicle Alone)	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
	weights between the groups (dams)		± 1.87	± 2.31	± 2.53	± 2.33
	Mean Organ weight(g) ($\bar{X}$ ± SD)	Ovaries(mg)	22.49 ± 3.50	22.35 ± 3.25	23.95 ± 2.80	23.26 ± 2.20

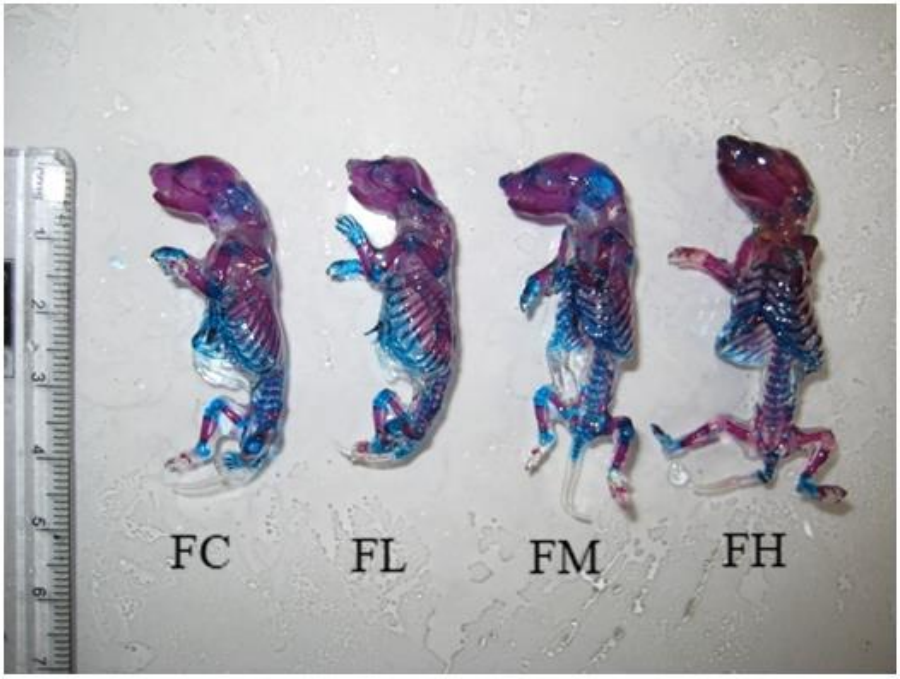
Table 2: Showing the effect of *Morinda citrifolia* (noni) formulation on the teratogenecity in male and female *Wistar rats*

Sr. No	Developmental Parameters		Treatment Groups (Dose) for Formulation			
			Control (Vehicle Alone)	**10 ml/kg**	**20 ml/kg**	**40 ml/kg**
1.	Number of dams mated		20	20	20	20
2.	Number of pregnant		20	20	20	20
3.	Number of animals surviving		20	20	20	20
4.	Number of animals aborting		00	00	00	00
5.	Number of animals delivering early		00	00	00	00
6.	Number of live offspring (Mean)		15.2	14.0	13.0	13.5
7.	Sex ratio	Male	7.2	7.0	6.5	6.9
		Female	8.0	7.0	6.5	7.6
8.	Mean Foetal body weight by sex M/F (g)	Male	4.5	4.0	4.6	4.2
		Female	4.4	4.3	4.5	4.6
9.	Mean No. of corpora lutea Right/left (Mean± SD)		9.50 ± 2.30 8.92 ± 2.40	8.76 ± 2.69 9.07 ± 2.73	9.16 ± 2.91 8.73 ± 2.85	8.80 ± 2.88 8.00 ± 2.73
10.	No. of implantations (Mean± SD)		15.2 ± 3	14 ± 1.7	13 ± 1.9	13.5 ± 3.3
11.	Mean No. of live foetuses		15.2 ± 2.5	14 ± 1.32	13 ± 1.16	13.5 ± 2.64
12.	No. dead foetuses (Mean± SD)		00	00	00	00
13.	No. of Resorptions		00	00	00	00
14.	No. & % of pre- & post-implantation losses.		00	00	00	00
15.	Mean Body weight(g) ($\bar{X}$ ± SD)		273.95 ± 35.22	274.30 ± 36.35	271.09 + 34.03	277.56 + 32.46
16.	Mean Food consumption (g/100g/Day) ($\bar{X}$)		9.95 ± 0.71	10.42 ± 0.71	10.55 ± 1.00	10.12 ± 1.06
17.	Comparison of change in absolute values mean organ weights between the groups (dams)	Body wt.(g)	328.40 ±7.85	327.18 ±17.15	323.27 ±22.99	324.47 ± 25.24
		Uterus (g)	55.85 ±6.86	57.30 ±7.15	61.05 ±7.58	68.20 ± 6.51
	Mean Organ weight(g) ($\bar{X}$ ± SD)	Ovaries(mg)	74.60 ±9.95	75.43 ±8.15	78.05 ±7.66	79.74 ± 4.66
18.	Comparison of change in relative values mean organ weights between the groups (dams)	Body wt.(g)	328.40 ± 7.85	327.18 ± 17.15	323.27 ± 22.99	324.47 ± 25.24
		Uterus (g)	16.99 ± 1.95	17.55 ± 2.34	18.96 ± 2.53	21.13 ± 2.46
	Mean Organ weight(g) ($\bar{X}$ ± SD)	Ovaries (mg)	22.75 ± 3.25	23.18 ± 3.25	24.24 ± 2.80	24.76 ± 2.84



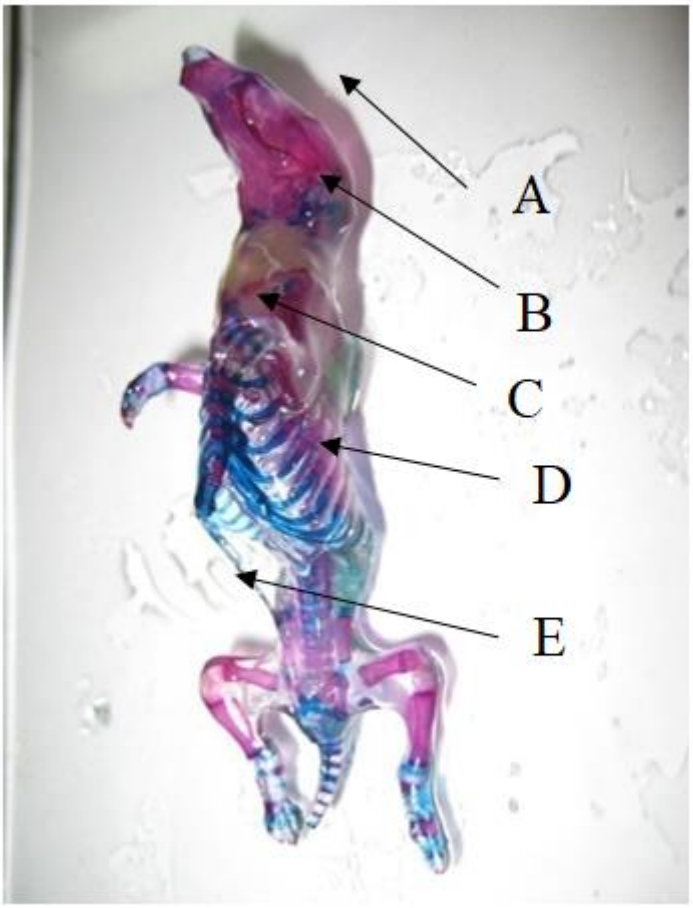
Photograph No. 1: Showing the effect of *Morinda citrifolia* (noni) aqueous fruit extract on the fetal skeletal developmentof female *Wistar* rats

Photograph of fetal skeleton from extract, treatment group (ie. EL, EM, and EH) showing normal length as compare to control (E – Extract, C – Control, L – 250 mg/kg, M – 500 mg/kg, H – 1000 mg/kg)



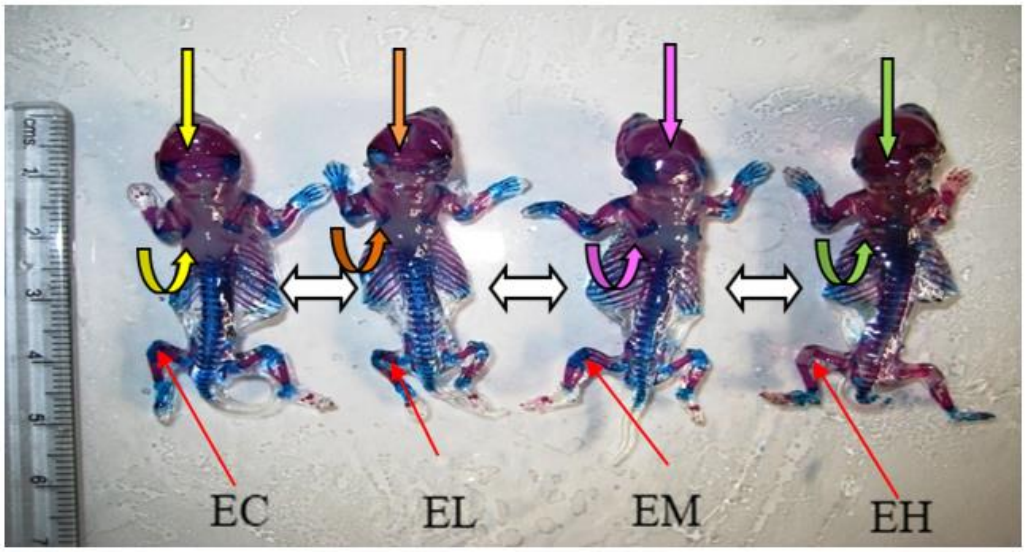
Photograph No. 2: Showing the effect of *Morinda citrifolia* (noni) formulation on the fetal skeletal development of female *Wistar* rats

Photograph of fetal skeleton from formulation (Noni Max) treatment group (ie. FL, FM and FH) showing normal length as compared to control group (F – Formulation, C –Control, L – 10 ml/kg, M – 20 ml/kg, H – 40 ml/kg)



Photograph No. 3: Showing the effect of *Morinda citrifolia* (noni) aqueous fruit extract on the fetal skeletal development of female *Wistar* rats

Photograph of fetal skeleton from Control group (formulation) stained with Alcian blue and Alizarin red, showing normal skull structure (A), Ossified parts of scapula (B), ribs (C), vertebrae (D), tibia and fibula of the hind limb(E)



Photograph of fetal skeleton from extract Control group, treatment group showing normal skull structure (down arrow), Ossified parts of scapula (curved up arrow), ribs (left-right arrow), tibia and fibula of the hind limb (red arrow) (E – Extract, C – Control, L – 250 mg/kg, M – 500 mg/kg, H – 1000 mg/kg)

Since time immemorial, man has made use of plants in the treatment of disease. The history of medicinal plants dates back to the Rigveda and Ayurveda era (about 2500 BC), which gives a detailed account of many drugs. Herbal medicines are defined as plant derived material or preparations with therapeutic benefits, and contain raw or processed ingredients from one or more plants [26]. The use of herbal medicines during pregnancy is common, ranging from 7.0 to 55.0% [27]. Teratogenicity is a study of the effects of intrinsic and extrinsic factors, which cause permanent structural and functional deviations during embryogenesis. A teratogenic agent can either induce or increase the incidence of congenital abnormalities. There is a dearth of data on teratogenicity or toxicity of herbal preparations, as they are believed to be completely safe for human use. Several studies have found herbal medicine use in pregnancy is associated with congenital malformations [28-30], intrauterine growth retardation, decreased fetal survival rates [31], low birth weight [32], fetal distress [33], fetal hypoxia and premature delivery as a result of uterine hyperstimulation [34], which may lead to perinatal mortality. The present study deals with one of the herbal supplements *Morinda citrifolia* commonly known as Noni. It has been used by Polynesians throughout recorded history in a variety of medicinal preparations and as a famine food. In recent years, interest in Noni has grown to such an extent that a large and lucrative market now exists. Noni has been promoted as a treatment for a vast array of medical conditions ranging from cancer to sexual dysfunction. Relatively few studies have ever been done concerning the efficacy of Noni in these conditions. Hence the present work evaluates the teratogenic effect of *Morinda citrifolia* fruit extract and its formulation (Noni max) in pregnant female rats which are exposed to test material by oral route during gestation period of their pregnancy i.e. from day 5 to day 20 of pregnancy.

In the present study no treatment related toxic signs were observed in any females administered with 250mg, 500mg and 1000mg of *Morinda citrifolia* fruit extract and 10 ml, 20 ml and 40 ml of *Morinda citrifolia* formulation. No toxic symptoms were observed in the females from the control groups of both the experiment sets with any clinical signs of toxicity as compared that of control group. There was no mortality in any of the groups studied.

There was no significant difference in the body weight gain in low dose group of *Morinda citrifolia* fruit extract and all treated groups of formulation (Noni max) with comparison to their respective control whereas there is decreased in the body weight of mid (500 mg/kg) and high (1000 mg/kg) dose groups as compare to control but this decreased is statistically non-significant. There was no significant difference in food consumption of all treated groups as compared with those in the control group.

In female, there was increased in uterus weight in all the treated group but the values are statistically non-significant as compare to control group. There was also statistically non-significant increase in the ovarian weight in all treated groups as compare to control.

There were no gross morphological changes seen on any of the reproductive organs of both male and female rats. There was no abnormality and no clinical signs of toxicity evident in any of the randomly selected pup/sex/litter, examined macroscopically.

Post-Mortem Examination

Females were killed one day prior to the expected day of delivery. None of the females in any of the treatment groups showed any signs of abortion or premature delivery prior to scheduled kill. At the time of termination or death during the study, none of animals in any treatment groups showed any macroscopical or structural abnormalities or pathological changes when compared to that of control group animals.

Developmental Endpoints (Implants):

On exposing the gravid uterus at the terminal sacrificed of all surviving females from all dose groups on day 20st 0f gestation by caesarean sectioning. No significant changes in the number of corpora lutea on either uterine horn were observed in any of the dose groups. Subsequently, there were no significant changes in the number of resorption sites observed in any of the uterus of the female from all treatment groups up to 1000 mg/kg for M. citrifolia fruit extract and up to 40 ml/kg for formulation (Noni max). None of the animals from any of the treatment groups showed any significant changes in, numbers of embryonic or foetal deaths and viable fetuses when compared to that of control group animals.

Developmental Endpoints (live fetuses):

All animals from different treatment groups showed no significant differences in number and percent of live offspring, sex ratio, foetal body weight and total number and percent of foetuses as compared to control. On examining the viscera of the pups which include the abdominal and thoracic viscera, all the organs viz., liver, kidney, intestine, stomach gonads, pelvis, thoracic cavity, trachea, oesophagus, heart and lungs were found to be of normal size and at normal position from all the dose groups up to 1000 mg/kg for M. citrifolia fruit extract and up to 40 ml/kg for formulation (Noni max). No abnormality in any of the organs was detected in control group.

Skeletal and Histological Findings

The processed and stained skeleton of the pups from the female of the treatment group did not demonstrate any detrimental effects on the bone structure due to treatment of *Morinda citrifolia* fruit extract up to dose of 1000 mg/kg and of formulation (Noni max) up to dose of 40 ml/kg. Normal head

structure and proper symmetry of nasal, frontal parietal bone, frontal suture, anterior fontanella and posterior fontanella plates was observed in the animals treated up to high dosed group. On examining the ribs and vertebral column of the pups it was observed that the ribs and vertebral column of the pups up to high dose group demonstrating normal vertebral column, normal ribs, normal tail structure and normal hind limb bones showing normal tibia and fibula. There were no pathological changes seen in any of the reproductive organs in female rats attributable to the treatment.

CONCLUSION

To conclude in the present study, *Morinda citrifolia* fruit extract and its formulation up to a dose level of 1000 mg/kg body weight, did not manifest any structural and functional alteration in development stage (organogenesis) during transition from embryo to foetus in the pregnant female rats. Hence both *Morinda citrifolia* fruit extract and its formulation (Noni max) did not produce any teratogenic effect in rats up to 1000 mg/kg and 40 ml/kg respectively. Increased uterine and ovarian weight did not cause any disturbances in the embryonic development in this study thus further study is to be carried out at the same dose level or at high dose level to find out any hormonal imbalance during pregnancy which may show impairment during development.

ACKNOWLEDGEMENT

Author is thankful Director, the Haffkine, Biopharmaceuticals limited, Mumbai, for the approval of experimental protocol and providing the Wister rats as per the CPCSEA guidelines No: 200/2000/CPCSEA Parel, Mumbai. Thanks are due to Brihans Pharmaceuticals Pvt. Ltd. Mulund, Mumbai, India for providing the commercially formulation of Noni max.

Conflicts of Interest: There are no conflicts of interest

REFERENCES

1. Brent, R. L. (1995). The application and principles of toxicology and teratology in evaluating the risks of new drugs for treatment of drug addiction in women of reproductive age. *NIDA Res Monogr*, 149, 130-184.
2. Wilson, J. G. (1975). Reproduction and teratogenesis: current methods and suggested improvements. *J Assoc of Anal Chem*, *58*(4), 657-67.
3. Dicke, J. M. (1989). "Teratology: principles and practice". *Med Clin North Am*, *73*(3), 567–82. PMID 2468064.
4. O'Rahilly, R., & Fabiola, M. (2001). Human embryology & teratology. New York: Wiley Liss. ISBN 0-471-38225-6.
5. Van Gelder, M. M., Van Rooij, I. A., Miller, R. K., Zielhuis, G. A., de Jong-van den Berg, L. T., & Roeleveld, N. (2010). Teratogenic mechanisms of medical drugs. *Human reproduction update*, *16*(4), 378-394. doi:10.1093/humupd/dmp052. PMID 20061329.
6. Hale, F. (1933). Pigs born without eyeballs. *J Hered*, 24, 10.
7. Schardein, J. L. (1993). Chemically induced birth defects. 2nd ed. Rev. New York: Marcel Dekker.
8. Gregg, N. M. (1941). Congenital cataract following German measles in the mother. *Trans Ophthalmol Soc Aust*, 3, 35-46.
9. Igata A. (1993). Epidemiological and clinical features of Minamata disease. *Environ Res*, *63*(1), 157-169.
10. Murphy, M. L., & Karnofsky, D. A. (1956). Effect of azaserine and other growth-inhibiting agents on foetal development of the rat. *Cancer*, 9, 955-962.
11. Thiersch, J. B. (1956). Therapeutic abortions with folic acid antagonist 4-amino pteroylglutamic acid (4-amino P.G.A.) administered by oral route. *Am J Obstet Gynecol*, 63, 1298-1304.
12. McBride, W. G. (1961). Thalidomide and congenital abnormalities. *Lancet*, 2, 1358.
13. Lenz, W. (1966). Malformations caused by drugs in pregnancy. *Am J Dis Child*, 112, 99-106.
14. Toms, D. A. (1962). Thalidomide and congenital abnormalities. *Lancet*, 2, 400.
15. Wilson, J. G. (1977). Current status of teratology. General principles and mechanisms derived from animal studies. In: Handbook of teratology. New York, NY: Plenum Press; 1-47.
16. Finnell, R. H. (1999). Teratology: general considerations and principles. *Journal of allergy and clinical immunology*, *103*(2), S337-S342.
17. Nielsen, E., Thorup, I., Schnipper, A., Hass, U., Meyer, O., Ladefoged, O., ... & Østergaard, G. (2001). Environmental project number 589: Children and the unborn child. *Exposure and susceptibility to chemical substances—an evaluation, Danish Environmental Protection Agency Website*.
18. Palmer, A. K. (1986). A simpler multigeneration study. In *International Congress of Pesticide Chemistry* (pp. 1-20).
19. Igata, A. (1993). Epidemiological and clinical features of Minamata disease. *Environ Res*, *63*(1), 157-169.
20. Murphy, M. L., & Karnofsky, D. A. (1956). Effect of azaserine and other growth-inhibiting agents on foetal development of the rat. *Cancer*, 9, 955-962.
21. Thiersch, J. B. (1956). Therapeutic abortions with folic acid antagonist 4-amino pteroylglutamic acid (4-amino P.G.A.) administered by oral route. *Am J Obstet Gynecol*, 63, 1298-304.
22. McBride, W. G. (1961). Thalidomide and congenital abnormalities. *Lancet*, 2, 1358.
23. Toms, D. A. (1962). Thalidomide and congenital abnormalities. *Lancet*, 2, 400.
24. Polifka, J. E., & Friedman, J. M. (1999). Clinical teratology: identifying teratogenic risks in humans. *Clin Genet*, 56, 409-420.

25. Finnell, R. H., Waes, J. G., Eudy, J. D., & Rosenquist, T. H. (2002). Molecular basis of environmentally induced birth defects. *Annu Rev Pharmacol Toxicol*, 42, 181-208.
26. Finnell, R. H., Waes, J. G. V., Eudy, J. D., & Rosenquist, T. H. (2002). Molecular basis of environmentally induced birth defects. *Annual review of pharmacology and toxicology*, *42*(1), 181-208.
27. Bracken, M. B., & Holford, T. R. (1981). Exposure to prescribed drugs in pregnancy and association with congenital malformations. *Obstetrics & Gynecology*, *58*(3), 336-344.
28. King, C. R. (1986). Genetic counseling for teratogen exposure. *Obstetrics & Gynecology*, *67*(6), 843-846.
29. Wilson, J. G. (1975) Reproduction and teratogenesis: current methods and suggested improvements. *J Assoc of Anal Chem*, *58*(4), 657-667.
30. Matsui, A. S., Rogers, J., Woo, Y. K., & Cutting, W. C. (1967). Effects of some natural products on fertility in mice. *Medicina et Pharmacologia Experimentalis*, *16*(5), 414-424.
31. West, B. J., Su, C. X., & Jensen, C. J. (2008). Prenatal toxicity test of Morinda citrifolia (noni) fruit. *The Journal of Toxicological Sciences*, *33*(5), 647-649.
32. Christian, M. S. (2001). Test methods for assessing female reproductive and developmental toxicology. In: Hayes, A. W. (Ed.), Principles and Methods of Toxicology (4th Edition). Taylor & Francis, Philadelphia, pp. 1301-1381.
33. World Health Organization. (2000). General guidelines for methodologies on research and evaluation of traditional medicine document. Geneva: World Health Organization.
34. Tiran, D. (2003). The use of herbs by pregnant and childbearing women: a risk–benefit assessment. *Complementary Therapies in Nursing and Midwifery*, *9*(4), 176-181.