International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Evaluation of Teratogenic Effect of Methanolic Extract of Fruits of Momordica dioica in Rat Model

Blessy Elizabeth Babukutty[1], Deepak Kumar Jha[2], Ali Salman Al-Shami[3], Jeevan Khadka[4]

[1,3,4]Student, Karnataka college of pharmacy
[2]Assistant Professor, Karnataka college of pharmacy

ABSTRACT

Background: Teras- 'Monster' and Genesis- 'Producing.' Teratogenecity is the ability to cause fetal abnormalities when administered to the pregnant mother at any stage of pregnancy. *Momordica dioica*, commonly known as spiny gourd, is a species of flowering plant in the Cucurbitaceae family. It has commercial importance and is exported as well as used locally as a vegetable in all regions of India. Objective: The main objective of this research was to evaluate the teratogenic activity of methanolic extract of fruits of *Momordica dioica* in rat model. Methodology: The dose selection was made based on acute toxicity studies. For evaluating teratogenicity, the pregnant animals were divided into 5 groups and drug administration was done from day 6-17 of gestation. All groups were subjected to laparotomy and then were sacrificed for teratogenic examination which involves physical parameters, morphological analysis and histopathological studies of the foetuses. Results: The rats treated with methanolic extract of fruits of *Momordica dioica* did not show any teratogenic activity which was evident by observing the physical parameters, morphological analysis and histopathological study. Conclusion: From the results we can conclude that methanolic extract of fruits of Momordica dioica administered at a dose of 250, 500, and 1000mg/kg bodyweight was not teratogenic.

Keywords: *Momordica dioica fruits*, teratogenicity, pregnancy.

INTRODUCTION

Teratogenicity refers to the potential of certain substances to induce abnormalities or malformations in the developing fetus during pregnancy.[1] Every chemical substance may be teratogenic, this effect depends on quantity. A human teratogen is an agent that alters the growth or structure of the developing embryo or fetus, thereby causing birth defects. [2] It can induce chromosomal abnormalities, prevent implantation, and cause abortion of the early embryo, late fetal death, congenital malformations, or intrauterine growth retardation. [3] Teratology is the science that studies the causes, mechanisms, and patterns of abnormal development. [4] Approximately 3-5% of live births are complicated by a birth defect each year totaling around 120,000 babies. Since the 1960s, drugs have been known to cause teratogenic effects in humans. Such teratogenicity has been postulated to be influenced by genetics. [5] Study of human teratogenic exposures is a relatively new one, emerging in the past 70 years. Before,

maternal rubella infection during pregnancy was identified as a cause of birth defects and developmental disabilities in 1941 by an ophthalmologist, birth defects were generally believed to be inherited. The uterus was thought to serve as a barrier, protecting the infant from the effects of external factors. But recognition of maternal rubella syndrome and subsequently, of thalidomide as a cause of birth defects in 1961 by McBride and Lenz, increased awareness of the effects that non-genetic factors could have on the development of embryo or fetus. [6] Teratogenecity testing came into being since thalidomide tragedy, 1961. During the early 1960s, thalidomide was prescribed as a medication for morning sickness.[7] The use of thalidomide as a medication for morning sickness resulted in over 10,000 children worldwide being born with severe birth defects between 1957 and 1962, and determining the mechanisms by which it caused these abnormalities has been a longstanding challenge. [8]Teratogenicity can result in various manifestations, such as structural malformations, growth restriction, functional deficits, and even death, which can be classified as abortion, fetal death, or stillbirth in humans, and fetal resorption in animals, depending on the timing of the event.[8] Teratogenic substances can often hinder cell division and result in embryonic death during blastocyst formation, although the embryo typically survives, and its development may not be significantly affected. However, exposure to teratogens during organogenesis (Day 17-60) can cause noticeable structural abnormalities, and the specific type of malformation depends on the timing of exposure and the stage of embryonic development.[9]

Valproic acid, primarily used as an anticonvulsant and mood stabilizer, has also found applications in treating migraines and schizophrenia, but studies indicate that it poses a significant risk to the developing fetus, with dose-dependent increases in both anatomical and behavioral teratogenic effects observed in multiple pregnancy registries and studies. [10] While the mechanism of action for VPA is not yet fully understood, some research suggests that it may increase levels of γ-aminobutyric acid in the brain by inhibiting its breakdown, inhibit voltage-gated sodium channels, or function as a histone deacetylase inhibitor by binding to the catalytic domain of these proteins.[11]

Momordica dioica or spiny gourd, also known as kakrol or teasle gourd, is a climbing vine that belongs to the cucurbit family, is dioecious (separate male and female plants), and is native to Asia, particularly India and Bangladesh, where it is widely distributed. [12] The plant species can be found extensively across South Asia, including India, Pakistan, Bangladesh, and the Himalayan region down to Ceylon, and has been reported at altitudes of up to 1500m in certain areas such as Assam and the Garo hills of Meghalaya, as well as in West Bengal, Uttar Pradesh, Bihar, Maharashtra, Madhya Pradesh, Gujarat, and many parts of southern India, including the Andaman Islands, while also being found in wild forms in Japan and Malaysia. [13,14] For thousands of years, this plant has been utilized not only as a preventative and curative measure for various ailments but also as a vegetable with high nutritional value, with the fruit being yellow in color, shortly beaked and obtuse with a red inner kernel, densely covered in soft spines that are green and yellow in color when mature.[13,15] Ayurveda recognizes the multifaceted medicinal properties of the fruit and leaves of this plant, including its ability to act as a diuretic, laxative, hepatoprotective, antivenomous, anti-inflammatory, antiasthmatic, antipyretic, antileprosy, antidiabetic, and antidepressant, as well as its antioxidant, nephroprotective, neuroprotective, antiallergic, antiulcer, anticancer, antimicrobial, antimalarial, antifertility, and antiedemic effects. [16]

MATERIALS AND METHODS

Plant material collection

Fresh fruits of *Momordica dioica (MD)* were collected from the local vendor, Bangalore in the month of June. All damaged or fungus infected fruits were removed.

Extraction of phytochemicals

The fruits were washed thoroughly with water to remove the adhering soil, mud, and debris. Then chopped, shade dried, powdered, and sieved. The powder was stored in an airtight container and was protected from light. Each 100 g of coarse powder was transferred in to the extraction glass and the plant material was loaded into the main chamber of the soxhlet extractor. The grinded course powder was packed tightly in the soxhlet extractor and was subjected to extraction with 1 litre methanol in a reflux condenser for 3 cycles, 7 hr. each, till the volume was reduced to half. The extract was filtered and evaporated to get constant weight. [18]

Dose selection

Acute toxicity studies were already conducted according to OECD guidelines (425),[19] up and down procedure, single dose administration of 5000mg/kg body weight showed no mortality and so upon calculation of ED50 the dose of methanolic extract of fruits of *Momordica dioica* was fixed as 1/5th, 1/10th and 1/20th of 5000mg/kg as high dose, mid dose and low dose respectively.[20]

Experimental Animals

Wistar albino rats weighing (150-200 gm) of both sex (male and female) were used in this study. The animals were acclimatized for two weeks, under standard conditions of temperature and illumination (12 hr light and 12 hr dark) cycle in standard polypropylene cages. Animals had free access to food (standard rat diet) and water. Care of animals were according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on animals. The study was conducted after obtaining the ethical committee clearance from the Institutional Animal Ethics Committee.

Experimental Design

The female animals were checked for their estrous cycle. Then were allowed for mating 3:1 (3 females and 1 male) and successful mating was determined by microscopic observation.

Animals were divided into 5 groups each group consist of 10 animals. Group I: Normal control- Animals received Normal saline (10ml/kg/day/oral) from day 6-17. Group II: Disease control- Animals received Valproic acid [21] (1000 mg/kg./I.V) from day 6-17. Group III; Test group I- Animals received MEFMD (250mg/kg/p.o) from day 6-17. Group IV; Test group II -Animals received MEFMD (500mg/kg/p.o) from day 6-17. Group V; Test group III– Animals received MEFMD (1000mg/kg/p.o) from day 6-17.

Method: The selected doses of *Momordica dioica,* was administered via oral gavage and Valpro*ic acid* via intravenous route respectively to experimental animals from day 6 – 17 once daily. The pregnancies were interrupted just prior to calculated date of delivery at day 20 of gestation and all groups were subjected to laparotomy under euthanization using pentobarbital and then were sacrificed for teratogenic examination. [22]

A. Physical parameters:

No. of pups [23]

The number of pups of each group are counted at the end of the experiment.

No. of alive foetus and weight [24]

The number of alive foetus and their weights are measured at the end of the experiment.

No. of dead foetus [24]

The number of dead foetus are counted at the end of the experiment.

Mean organ weight

Mean organ weight of the heart, liver, lungs, kidney and spleen were observed.

B. Morphological analysis

The fetuses were dissected and morphology was analaysed, along with mean organ weight.

C. Histopathological study

The heart, liver, lung, spleen and kidney was isolated and preserved in 10% formalin solution and the sections at 5 μm thickness were observed under light microscope to assess the architectural changes.

RESULTS

A. Assessment of physical parameters

- Total number of pups, total number of alive pups, total number of dead pups were observed. The findings are listed out in table1.1

Table 1.1: Assessment of physical parameters of pups

Group	Treatment	Total No. of pups	Total No. of alive pups	Total No. of dead pups
I	Normal control	10	10	0
II	Disease control	6	4	2
III	Test group I- MEFMD	11	11	0
IV	Test group II- MEFMD	9	9	0
V	Test group III- MEFMD	9	9	0

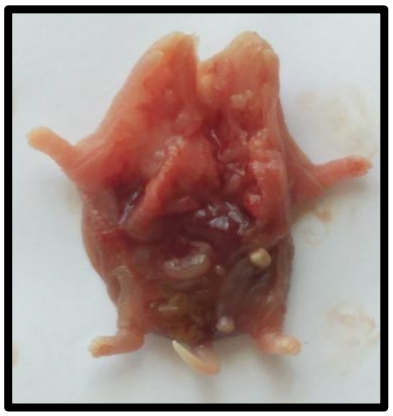
- Mean organ weight of the heart, liver, lungs, kidney and spleen were observed. The findings are listed out in table 1.2

Table 1.2: Assessment of mean organ weight

Group	Treatment	Mean Organ Weight (g)				
		Heart	Liver	Lungs	Kidney	Spleen
I	Normal control	0.76	12.1	1.32	1.95	0.85
II	Disease control	0.50	9.98	0.95	0.87	0.67
III	Test group I- MEFMD	0.85	12.3	1.40	1.99	0.84
IV	Test group II- MEFMD	0.79	12.1	1.38	1.96	0.83
V	Test group III- MEFMD	0.70	11.8	1.30	1.92	0.80

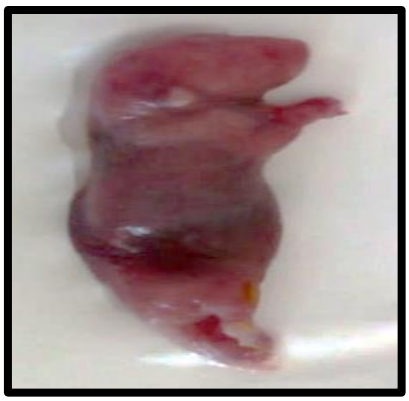
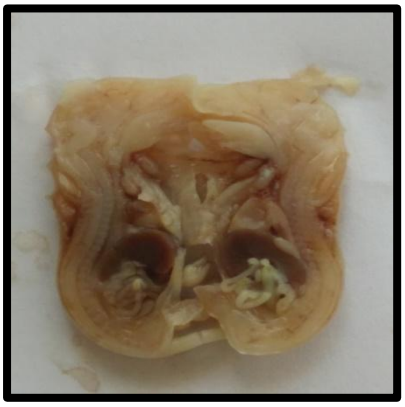
Morphological Examination

Group I : Normal control



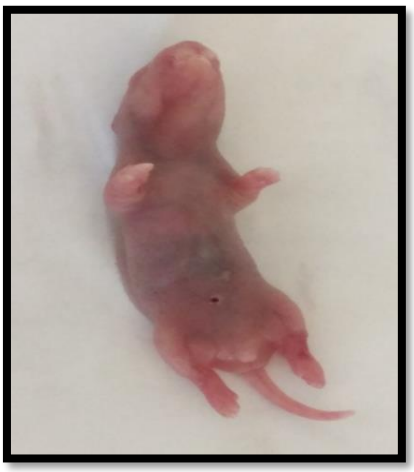
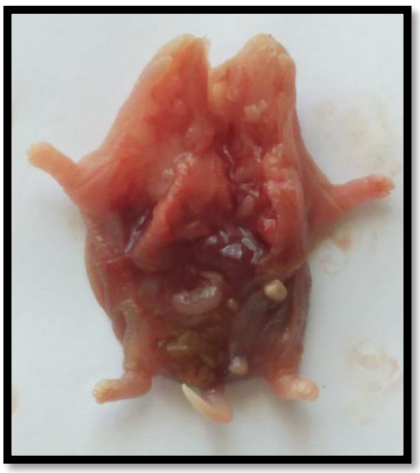
Showed no malformations

Group II : Disease control

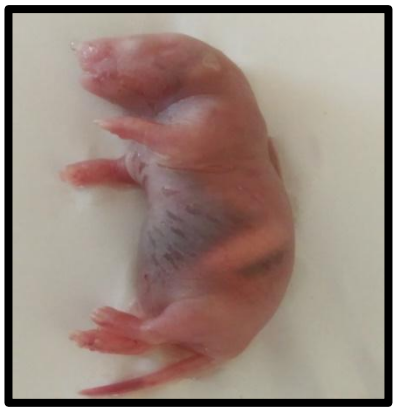
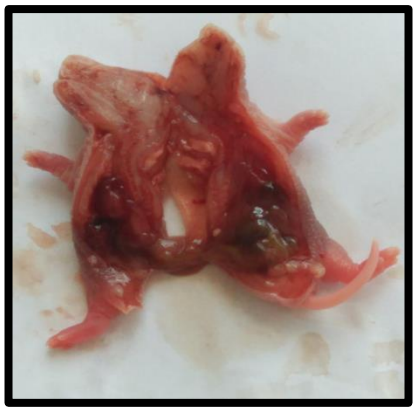
Shortness of the upper limbs, lower limbs meant irregular shape, low birth weight, growth retardation and Internal to the viscera, and internal organ defects, focal necrosis was detected, vacuolar degeneration

GROUP III : Test group I

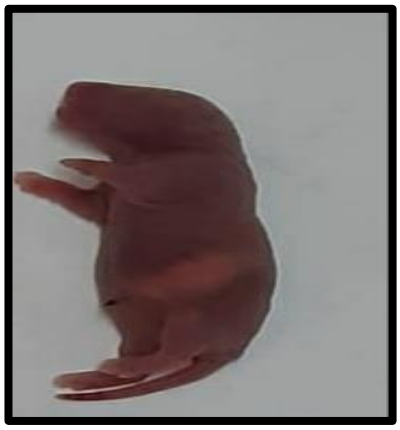
Showed no malformations.

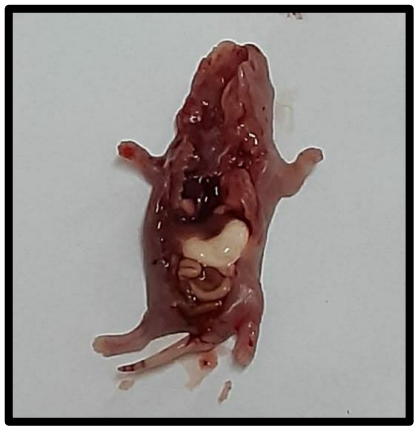
GROUP IV: Test group II

Showed no structural malformation. And also found no effect on the duration of gestation or offspring body weight alteration. No evidence of Teratogenic effect.

GROUP V : Test group III

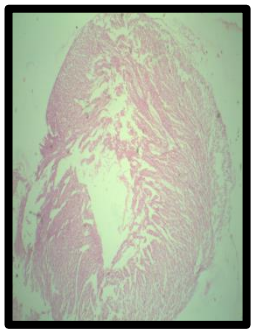
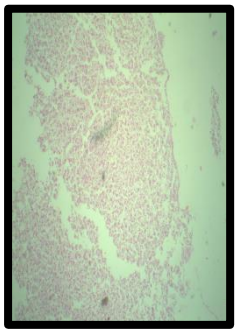
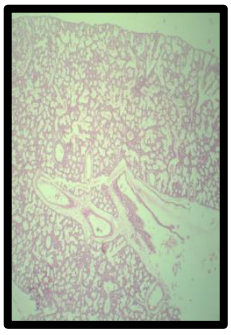
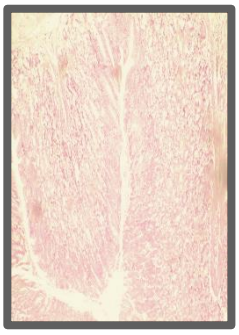
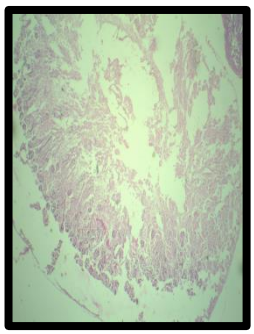




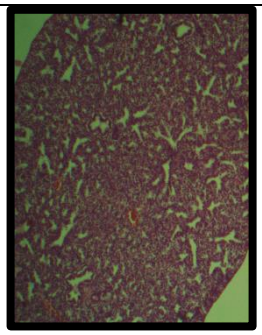
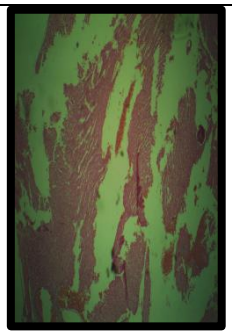
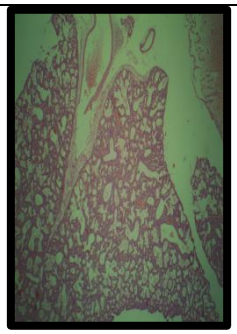
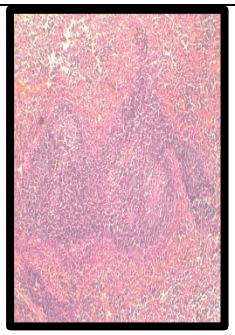
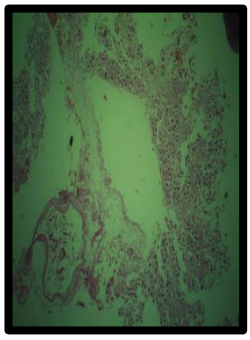
Showed no malformations.

HISTOPATHOLOGICAL ANALYSIS

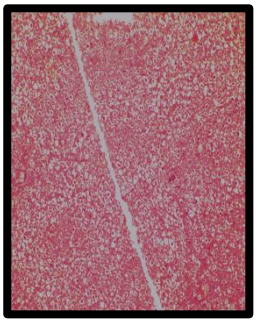
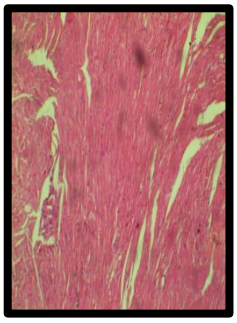
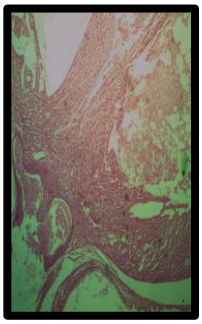
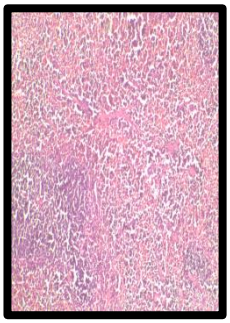
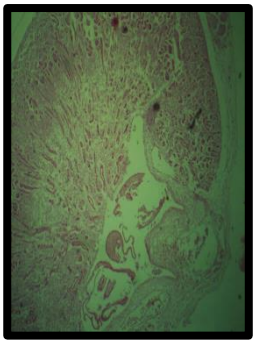
Normal control

A. 	**B.** 	**C.** 	**D.** 
E. 	**A. Heart:** Normal auricles and ventricles, no observable histological changes **B. Liver:** Normal architecture seen with extramedullary haematopoiesis. Normal hepatocytes with pink staining cytoplasm and hepatocytes. **C. Lungs:** Normal alveoli, bronchioles and vasculature **D. Spleen:** Spleen showed no observable histological changes **E. Kidney :** Cortex and medulla are normal with normal glomeruli and tubules.		

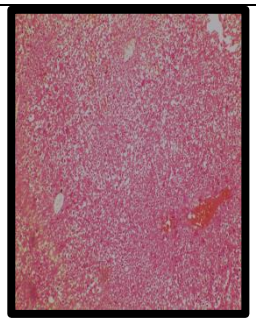
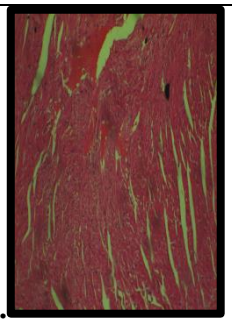
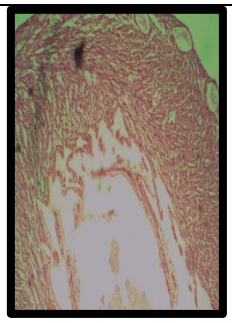
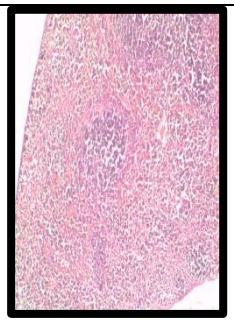
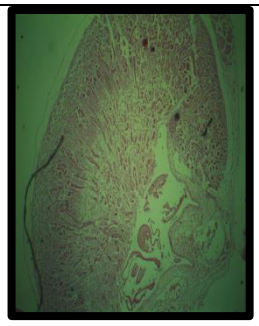
Disease control

<table>
<tr><td>A.  </td><td>B.  </td><td>C.  </td><td>D.  </td></tr>
<tr><td>E.
  </td><td colspan="3">A. Liver : Degenerative changes seen in hepatocytes with condensed nucleus and distortion in the architecture. B. Heart : Normal auricles and ventricles, no observable histological changes. C. Lungs : Normal alveoli, bronchioles and vasculature. No apparent histological changes seen D. Spleen: Spleen - usual pulp, but congested red pulp, verified normal architecture. E. Kidney : Cortex and medulla are necrotic, degeneration with loss of capillaries surrounded by Bowman’s capsule.</td></tr>
</table>

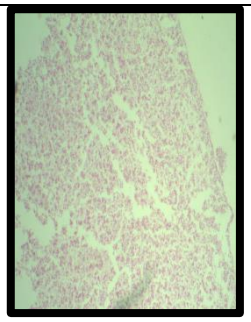
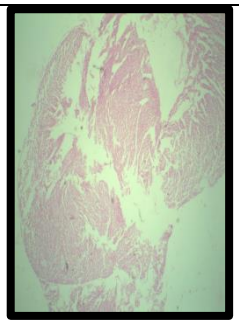
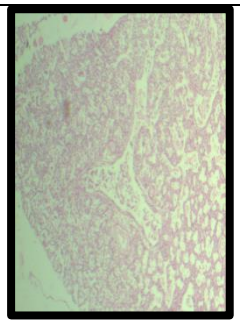
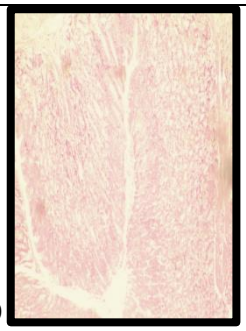
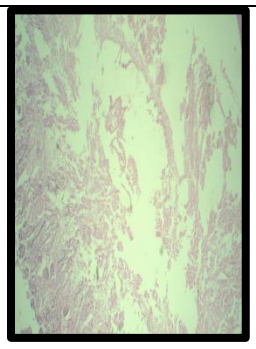
Test group I MEFMD

<table>
<tr><td>A.
  </td><td>B.
  </td><td>C.
  </td><td>D.
  </td></tr>
<tr><td>E.
  </td><td colspan="3">A. Liver : Normal hepatocytes with pink staining cytoplasm and hepatocytes arranged in the cord like fashion surrounding the central vein. B. Heart : Normal auricles and ventricles, no observable histological changes. C. Lungs : Normal alveoli, bronchioles and vasculature D. Spleen: Spleen showed white pulp in lymphocytes and red pulp including regular RBCs. E. Kidney : Cortex and medulla are normal with normal glomeruli and tubules.</td></tr>
</table>

Test group II MEFMD

<table>
<tr><td>A.  </td><td>B.  </td><td>C.  </td><td>D.  </td></tr>
<tr><td>E.  </td><td colspan="3">A. Liver : Normal hepatocytes with pink staining cytoplasm and hepatocytes arranged in the cord like fashion surrounding the central vein. B. Heart : Normal auricles and ventricles, no observable histological changes. C. Lungs : Normal alveoli, bronchioles and vasculature D. Spleen: Spleen showed white pulp in lymphocytes and red pulp including regular RBCs. E. Kidney : Cortex and medulla are normal with normal glomeruli and tubules.</td></tr>
</table>

Test group III MEFMD

<table>
<tr><td>A.  </td><td>B.  </td><td>C.  </td><td>D  </td></tr>
<tr><td>E.  </td><td colspan="3">A. Liver : Liver showing normal hepatocytes, mild cell necrosis and degenerative changes was seen in parenchyma. B. Heart : Normal auricles and ventricles, no observable histological changes. C. Lungs : Normal alveoli, bronchioles and vasculature D. Spleen: Spleen showed white pulp in lymphocytes and red pulp including regular RBCs. E. Kidney : Glomeruli normal, tubules are necrotic and loss of tubular</td></tr>
</table>

DISCUSSION

Plants have long been utilized for their medicinal properties, as they offer a wide range of potential health benefits, including treating illnesses and promoting overall wellness. However, like all drugs, natural compounds derived from plants can have harmful effects if not used properly. Therefore, it is

crucial to identify natural products that have a favorable balance between their therapeutic effects and their potential toxicity. To achieve this, a variety of tests must be conducted to evaluate the safety and efficacy of natural products.[25]

The purpose of this study was to evaluate the teratogenic effect of methanolic extract of fruits of *Momordica dioica* on wistar albino rats.

It was investigated that the fruits have reported diuretic, laxative, hepatoprotective, antivenomous, antihypertensive, anti-inflammatory, antiasthmatic, antipyretic, antileprosy, antidiabetic, antidepressant properties and so on. However no information was available regarding teratogenic activity. Therefore the current attempt has been made to investigate the teratogenic activity of methanloic extract of fruits of *Momordica dioica.*

The methanolic extract of fruits of *Momordica dioica* potentially did not induce teratogenicity in the foetus which was clearly evident from the physical parameters, morphological analysis, and histopathological studies when administered at a dose of 250mg/Kg, 500mg/Kg and 1000mg/kg for a period of 12 days starting from day 6-17 of gestation.

Therefore it can be postulated that methanolic extract of fruits of *Momordica dioica* are not teratogenic.

CONCLUSION

A clear negative effect on the induction of teratogenecity by *Momordica dioica* was found. The results of the present study clearly showed that the drugs had no teratogenic effect in rats. However, further studies are needed in other test systems to conclude they are completely safe. The study was conducted to find out whether the phytoconstituents are having any teratogenic effect or not and also whether they improve the quality of life by finding these phytoconstituents are harmful or not to our mankind. Therefore, the methanolic extract of fruits of *Momordica dioica* was evaluated for teratogenic activity. The acute toxicity study revealed that the extract was devoid of major toxic effect at a dose of 5000mg/kg. The methanolic extract of fruits of *Momordica dioica* did not show any teratogenic effect.

ACKNOWLEDGEMENTS

We are grateful to all the staffs of Department of Pharmacology, Karnataka College of Pharmacy, Bangalore.

REFERENCES

1. Aral H, Vecchio-Sadus, Nriagu J.O. A. Lithium: environmental pollution and health effects. Encyclopedia of Environmental Health, *Elsevier.* 2011: 499-508.
2. Alwan S, Chambers CD. Identifying Human Teratogens: An Update. *J Pediatr Genet.* 2015; 4(2): 39-41.
3. McElhatton P.R, Principles of teratogenicity. *Current Obstetrics & Gynaecology.* 1999; 9(3): 163-169.
4. Ujházy E, Mach M, Navarová J, Brucknerová I, Dubovický M. Teratology past, present and future. *Interdiscip Toxicol.* 2012;5(4):163-8.
5. Amaral G.J.D, Willoch O.E, Woycinck K.T, Kristina G, Vianna Fernanda Luiz V F et al. Genetic Susceptibility to Drug Teratogenicity: A Systematic Literature Review. *Frontiers in Genetics.* 2021;12
6. Rasmussen SA, Friedman JM. Emerging issues in teratology: an introduction. *Am J Med Genet C*

Semin Med Genet. 2011;15:157C(3):147-9.
7. Alliance G: Teratogens/prenatal substance abuse. A district of columbia guide for patients and health professionals. *Genetic Alliance.* 2010.
8. Vargesson Neil, Ramesh C. Thalidomide, Reproductive and Developmental Toxicology. *Academic Press.* 2022; 3: 423-437.
9. Jeanette R. Bauchat, Marc V.D.V. Nonobstetric Surgery during Pregnancy. *EDRA Chestnut's Obstetric Anesthesia.* 17; 368-391
10. Donnai D, Sydney B, Jefferey H. Congenital Disorders. Encyclopedia of Genetics. *Academic press.* 2001; 448-449.
11. Hill DS, Wlodarczyk BJ, Palacios AM, Finnell RH. Teratogenic effects of antiepileptic drugs. Expert Rev Neurother. 2010 Jun;10(6):943-59. doi: 10.1586/ern.10.57. PMID: 20518610; PMCID: PMC2970517.
12. Fathe K, Palacios A, Finnell RH. Brief report novel mechanism for valproate-induced teratogenicity. Birth Defects Res A Clin Mol Teratol. 2014 Aug;100(8):592-7. doi: 10.1002/bdra.23277. Epub 2014 Jul 26. PMID: 25066307; PMCID: PMC4396868.
13. Talukdar SN, Hossain MN. Phytochemical, phytotherapeutical and pharmacological study of Momordica dioica. *Evidence-Based Complementary and Alternative Medicine.* 2014 (11).
14. Bawara B, Dixit M, Chauhan NS, Dixit VK, Saraf DK. Phyto-pharmacology of Momordica dioica Roxb. ex. Willd: a review. *International Journal of Phytomedicine.* 2011;2(1).
15. D, Kalloo G, Banerjee MK. Popularizing kakrol and kartoli: the indigenous nutritious vegetables. *Indian Horticulture*. 2002; 6:9-11.
16. Anjana M, Swathi V, Ramya Sai A, Divya N, Sunisha Y. A Review on Momordica dioica fruits. *J Adv Plant Sci*. 2019; 2:201.
17. Jha DK, Koneri R, Samaddar S. Potential Bio-Resources of Momordica dioica Roxb: A Review. *Int J Pharm Sci Rev Res*. 2019; 45(2): 203-209.
18. Redfern J, Kinninmonth M, Burdass D, Verran J. Using soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. *J Microbiol Biol Educ.* 2014; 15(1): 45-6.
19. OECD test No 425: Acute oral toxicity: Up-and-down procedure. OECD guidelines for the testing of chemicals 2008.
20. Jha DK, Koneri R, Samaddar S.. Toxicity studies of a saponin isolated from the fruits of Momordica dioica in rats. *IJPSR.* 2019.
21. Khera KS. Valproic acid induced placental and teratogenic effects in rats. *Teratology*. 1992; 45(6): 603-10.
22. Sukandar EY, Safitri D. Evaluation of teratogenic effect of tempuyung
23. Uche-Nwachi, Edward O, Carol McEwen. Teratogenic effect of the water extract of bitter gourd Momordica charantia on the sprague dawley rats. *African journal of traditional, complementary, and alternative medicines*. 2010;7(1): 24-33.
24. Essiet GA, Essien AD, Akuodor GC. Embryotoxic and teratogenic potentials of Salacia lehmbachii in rats. *IOSR-JPBS*. 2017; 12(2): 43-8.
25. Wongpa S, Himakoun L, Soontornchai S, Temcharoen P. Antimutagenic effects of piperine on cyclophosphamide-induced chromosome aberrations in rat bone marrow cells. *Asian Pacific Journal of Cancer Prevention.* 2007; 8(4): 623-7.