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# Evaluate the Protective and Therapeutic Approach of Methanolic Extract of Fruits of *Momordica dioica* and Antioxidants like Vitamin C + Vitamin E Against Valproic acid-Induced Teratogenecity in Rats

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**ABSTRACT**

The main objective of this research was to evaluate the protective and therapeutic approach of methanolic extract of fruits of *Momordica dioica* and antioxidants like Vit. C and Vit. E against valproic acid-induced teratogenecity in rats. The dose selection was made based on acute toxicity studies. For evaluating protective and therapeutic approach of antioxidants, the animals were allowed for mating and successful mating was determined by microscopic observation. Pregnant sixty animals were divided into 6 groups as Group I - Normal control – Normal saline (1ml/kg/day/p.o), Group II– Positive control - Valproic acid (1000 mg/kg/day/I.V), Group III – Treatment control- Animals received Valproic acid (1000 mg/kg./I.V) + Vit.C (8.3mg/kg/p.o) + Vit E (100mg/kg/p.o). Group IV- Test I *MEFMD* (250mg/kg/day/p.o), Group V – Test II *MEFMD* (500mg/kg/day/p.o), Group VI – Test III *MEFMD* (1000mg/kg/day/p.o). The respective groups were administered with the respective drugs from day 6-17 of gestation. The pregnancies were interrupted just prior to the calculated date of delivery and all groups were subjected to laparotomy under euthanization using pentobarbital and then were sacrificed. The rats treated with methanolic extract of fruits of *Momordica dioica* did not show any teratogenic activity which was evident by an increase in glutathione peroxidase and superoxide dismutase level, and decrease in lipid peroxidation level when compared to the positive control and treatment control which was further calculated statistically to evaluate the antioxidant effect of methanolic extract of fruits of *Momordica dioica*. In conclusion, these results illustrate the protective and therapeutic effect of *Momordica dioica* and vitamin C + vitamin E through its antioxidant activity against valproic acid-induced tertaogenecity.

**Keywords:** *Momordica dioica* fruits, antioxidant activity, teratogenecity, valproic acid, oxidative stress, methanolic extract, vitamin C + vitamin E, pregnancy.

## INTRODUCTION

Teratogenecity refers to the potential of certain substances to induce abnormalities or malformations in the developing fetus during pregnancy. A human teratogen is an agent that alters the growth or structure of the developing embryo or fetus, thereby causing birth defects. Every chemical substance may be teratogenic depending on their quantity. [1] In the early 1960's, a drug known as thalidomide was used to treat morning sickness.[2] Thalidomide is responsible for the biggest medical disaster in history, causing severe birth defects in more than 10,000 children, globally, between 1957 and 1962. [3] Many teratogens have ability to inhibit cell division and kill embryo during cell division, which was involved in blastocyst formation. But most of time the embryo survives; its subsequent development does not generally seem to be compromised. Administration of teratogen during the period of organogenesis (Day 17-60) leads to gross malformations. [4] An antioxidant is defined as any substance that delays or inhibits oxidative damage to a target molecule. The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Herbal plants are considered as good antioxidants since ancient times. [5] Oxidative stress is the major driving factor responsible for the initiation and progression of cancer, diabetes mellitus, cardiovascular diseases, neurodegenerative diseases, and inflammatory diseases among other syndromes. The condition is brought by excessive generation of free oxygen and nitrogen species or their inefficient quenching in the cell. Free oxygen and nitrogen species are unstable molecules that are present in the environment (exogenous) and are also generated in the body (endogenous) during the normal aerobic metabolic processes in the body. [6]

Momordica dioica Roxb. Ex. Wild is a perennial, dioecious, cucurbitaceous climbing creeper (commonly known as kakrol, spiny gourd or teasle gourd). It contains many phytoconstituents like alkaloids, triterpenoids, flavanoids, glycosides, saponins, triterpenes of ursolic acid which is a dark brown semidrying oil and saturated fatty acids, ascorbic acids, vitamin A, thiamine, riboflavin, niacin, protein, carbohydrates, Momordica ursenol. The alkaloid present in seed is called momordicin and in root is called Momordica foetida.[7] Since ages it is used for prevention as well as for mitigation of various diseases and also as vegetable with a remarkable amount of nutritional content. [8] The fruits are yellow coloured shortly beaked, obtuse with inner red kernel and densely covered with soft spines.[9] The fruits have proven diuretic, laxative, hepatoprotective, antivenomous, antihypertensive, anti-inflammatory, antiasthmatic, antipyretic, antileprosy, antidiabetic, antiulcer, anticancer, antimicrobial, antimalarial, antifertility, and antiedemic activities.[10] Throughout gestation, metabolic and physiological characteristics of the mother undergo variations to pivot solely on ensuring the growth of the fetus in her womb, and there are a lot of substances including natural drugs which can cause distress in the growing fetus, particularly when taken in the first trimester of pregnancy. Taking the case of Senna, a known anthraquinone laxative utilised in the mitigation of constipation, a very familiar problem in pregnant women. It has been discovered that the usage of anthraquinone laxative is hazardous (Clarke et al., 2007), due to contractions in uterus induced by constituents of the drug (Conover, 2003). [11]

Valproic acid is in the market as an anticonvulsant since 1974, and is used in many countries because of its efficiency against several types of epilepsy and as a mood stabilizer. One of its main actions is the increase in the level of gamma

amino butyric acid (GABA) in the brain. However, many of these drugs are teratogenic as their use by the pregnant mother has been associated with an increased risk of major congenital abnormalities in the embryo and fetus. These anomalies are anatomical and/or functional and may also have neurological, behavioral and cognitive effects. [12] Valproic acid (2-propylvaleric acid, 2-propylpentanoic acid or n-dipropylacetic acid) [13] is a branched short-chain fatty acid deriving from valeric acid, a low molecular weight carboxylic acid [14] naturally produced by valerian, *Valeriana officinalis* was first synthesized in 1882 by Burton**.** [13] However, VPA antiepileptic property was discovered after more than 50 years, in 1962, when it was tested as a solvent for other molecules being checked for potential anticonvulsant activity. After laboratory studies demonstrated its anticonvulsant activity, the first clinical trial using VPA in epilepsy was performed in 1964**.** The drug made its way to the European market under the brand Depakine in France in1967 brand followed by the UK in 1973 and other European countries in the following decade. Being liquid, it is used as a coniugated sodium salt, constituting a water-soluble powder. After FDA approval in 1978, it also became available in the US. [14] It is used as well as in migraine, bipolar, mood, and anxiety disorders. Recent work has also demonstrated its efficacy as adjuvant therapy in HIV, cancer, and neurodegenerative diseases as its HDAC inhibition property. [15] The most commonly reported fetal anomaly due to VPA administration is neural tube defects. Previous studies suggested that VP administration during pregnancy increased the ROS production. These ROS damages the DNA double-stranded structure. [16]

Vitamin C is a water-soluble vitamin and is an essential nutrient for humans. Vitamin C neutralizes the ROS production in oxidative stress. Its antioxidant mechanisms involve a direct reaction with aqueous peroxyl radicals or by indirectly restoring the fat-soluble antioxidant vitamins. These properties of vitamin C are most probably due to its chemical properties as a chelating and reducing agent. [17]

Vitamin E is also called the “protecting vitamin”. Due to excessive production of ROS and/or insufficient consumption of antioxidants, oxidative stress arises. When the generation of ROS and other radical species exceeds then scavenging ability of antioxidants fail, injury to cells can occur. The mitochondrial respiratory chain produces the majority of ROS, although they may also be generated through exogenous exposures such as alcohol, cigarette smoke, and environmental pollutants. Antioxidants (such as vitamins C and E) and antioxidant cofactors (such as selenium, zinc, and copper) can dispose of, scavenge, or suppress the formation of ROS. [18] Vitamin E functioning as a chain-breaking antioxidant was reported to protect cellular membranes against ROS, for example through defending PUFAs from auto-oxidation. Antioxidants such as vitamin C and vitamin E have been reported to be efficient, in pregnancy and neonatal health. [19]

## MATERIALS AND METHODS

### Plant Collection and extraction of phytochemicals

*Momordica dioica* fruits were collected from markets of Bangalore in the month of August. The fruits were identified by a botanist Dr. Geetanjali, (HOD of Botany Department, Sree Siddaganga College Tumkur University, India) and reference No. 141/17-18 was provided for future reference. The fruits were washed thoroughly with water to remove the adhering soil, mud, and debris. All old insect damage or fungus infected

fruits were removed. The fruits were cut and dried in the shade at room temperature to a constant mass. The plant material was coarsely powdered using blender. The powder was stored in an airtight container and was protected from light. The course ground powder of fruits of MD was transferred in to the extraction glass and the plant material was loaded into the main chamber of the soxhlet extractor. Then this part of the extractor is connected into the round bottom flask containing extraction solvent. The grinded course powder was packed in the tightly in the soxhlet extractor and methanol solvent was used for the extraction of fruits of MD powder. In this extraction process 250ml solvent was used and was carried for about 6hr. The extract was again re-extracted under the same conditions to ensure complete extraction. The methanol was filled into the solvent vessel and extracted at a temperature of 750c for 6h. The solvent was drained into a beaker by opening the spigot on the soxhlet extractor. The solvent was removed from the extractor and dried. The extract was then stored in dry airtight bottles for the pharmacological studies. The portion of the extract which is non- soluble remains in the thimble and it was discarded [20].

**Phytochemical test**

The phytochemical screening of the methanolic extract of fruits of *Momordcia dioica* was already carried out in order to ascertain the presence of its constituents. [21]

**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. **REG. No: KCP-IAEC/09/21-22/04/18/12/2**1

**Acute toxicity study**

Acute toxicity studies were already conducted according to OECD guidelines (425) [22] 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 $1/5^{th}$, 1/10th and 1/20th of 5000mg/kg as high dose, mid dose and low dose respectively. [22]

**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 (1ml/kg/day/oral) orally from day 6-17. **Group II**; Positive control- Animals received Valproic acid [23] (1000 mg/kg. b.w. I.V) orally from day 6-17. **Group III**; Treatment control- Animals received Valproic acid [23] (1000 mg/kg./I.V) + Vit.C (8.3mg/kg/p.o) + Vit E (100mg/kg/p.o)[24] from day 6-17. **Group IV;** Test group I- Animals received MEFMD (250mg/kg/p.o). **Group V**; Test group II -Animals received MEFMD (500mg/kg/p.o). **Group VI**; Test group III– Animals received MEFMD (250mg/kg/p.o) orally from day 6-17 respectively. The pregnancies were interrupted just prior to the calculated date of delivery at day 20 of

gestation and all groups were subjected to laparotomy under euthanization using pentobarbital and then were sacrificed. [25]

**Antioxidant activity**

**Procedure:** The blood was collected in a covered test tube and allowed to clot by leaving it undisturbed at room temperature. This usually takes 15-30minutes. Remove the clot by centrifuging at 1000-2000 x g for 10 minutes in a refrigerated centrifuge. The resultant supernatant is the serum. The obtained serum was used for the purpose of different biochemical parameters estimation.

Lipid Hydroperoxide Assay [26]

Glutathione Peroxidase Assay [27]

4.6.3 Superoxide Dismutase Assay [27]

**Estimation of Lipid Peroxidation**

**Preparation of reagents: Preparation of MDA Standard:**

Reconstitute a bottle of TBA with 7.5 ml glacial acetic acid followed by adjusting with $ddH_2O$ to make 25 ml TBA solution. Dilute 10 µl of 4.17 M MDA standard in 407 µl of $ddH_2O$ to make 0.1 M MDA standard. Dilute 10 µl of 0.1 M MDA standard in 490 µl of $ddH_2O$ to make 2 mM MDA standard. Use 2 mM MDA standard to generate MDA standard curve dilutions.

**Procedure**

Mix 20 µl of serum with 500 µl of 42 mM sulfuric acid. Add 125 µl of PTA followed by vortexing. Centrifuge at 13,000 × *g* at room temperature for 5 min. Add 200 µl of BHT to 10 ml of $ddH_2O$ in a separate tube followed by vortexing. Collect and resuspend serum pellet with 102 µl of BHT/$ddH_2O$ on ice. Adjust the final volume to 200 µl with $ddH_2O$ followed by vortexing. Incubate at 37°C for 2 h. Mix 600 µl of TBA solution with 200 µl MDA standard/serum sample to generate 800 µl of MDA-TBA adduct. Incubate at 95 °C for 1 h and then place on ice bath for 15 min. Pipette 200 µl of MDA-TBA adduct into a 96-well microplate in duplicate. Measure the absorbance at a wavelength of 532 nm on the microplate reader. Establish the standard curve using the serial dilutions of MDA standard. Calculate MDA concentrations in serum samples. [28]

**Formula to calculate Lipid peroxidation:** (OD 532 x 100/1.56) x TV / [dwt x 1000]

OD: optical density

TV: total volume of the extract (ml)

Dwt: weight of dry tissue (g)

**Estimation of Glutathione:** Known amount of enzyme preparation were allowed to react with hydrogen peroxide in the presence of GSH for a specified time period. Then the remaining GSH were measured by the method of Ellman.

Reagents

1) 0.4MTris buffer, pH 7.0

2) 10 mM Sodium azide

3) 2.5 mM Hydrogen peroxide

4) 4-mM Reduced glutathione

5.) 10% TCA

6) 0.3 M Phosphate solution

7.) 4mMEDTA

8.) Ellman's reagent: 0.04% DTNB in 1% sodium citrate

9.)Reduced glutathione standard: 20 mg reduced glutathione/100 ml of water

**Procedure:** To 0.2 ml of tris buffer, 0.2 ml of EDTA, 0.1 ml of sodium azide and 0.5 ml of tissue homogenate were added. To this mixture, 0.2 ml of glutathione and 0.1 ml of hydrogen peroxide were added. The contents were mixed well and incubated at 37°C for 10 minutes along with a tube containing all the reagents except sample. After 10 minutes the reaction were arrested with the addition of 0.5 ml of 10 % TCA, centrifuged and the supernatant were assayed for glutathione by Ellman's method. To 2.0 ml of the supernatant, 3.0 ml disodium hydrogen phosphate solution and 1.0 ml of DTNB reagent were added. The colours developed were read at 412 nm. Standards in the range of 200- 1000 jag were taken and treated in the similar manner. The activity was expressed in term of fig of glutathione consumed/min/mg protein.

**Formula to calculate Glutathione Peroxidase:**

Gpx = 2(mRateS - mRateB). VRm. DF/6.22 x Vs

mRateS = 1000 x ΔA 340/min of sample

mRateB = 1000 x Δ A 340/min of blank

6.22 = NADPH 340nm milli molar absorption co efficient at 1cm path length VRXM= volume of reaction mixture

VS = Volume of sample

2 = correction for 2 moles GSH oxidized to 1 mole GSSG per mole NADPH oxidized DF = dilution factor

**Estimation of Superoxide dismutase (SOD)**

**Reagents used:**

1. 1M sodium carbonated buffer (pH 10.2)
2. Epinephrine

**Procedure:** 0.8 ml of 0.1 M sodium carbonated buffer (pH 10.2), 0.1ml of supernatant, 0.1 ml of epinephrine was added in quartz cuvette. The absorbance at 295 nm by using spectrophotometer was measured. The absorbance change for 0 min and 1 min was recorded.

**Formula to calculate SOD:** %Inhibition of pyrogallol auto oxidation: ΔA test X 100 ΔA control [29].

**Statistical analysis.**

The results were expressed as Mean ± S.E.M from n=10 rats in each group. Data was analyzed using statistical software Graph Pad Prism version 9. One way annova was used to ascertain the significance of variations between the numbers of abnormal fetuses in different groups followed by Tukey's test. Differences are considered significant at $P \leq 0.001$.

## RESULTS

**A. Presence of phytoconstituents**

The presence of phytoconstitutents were already established and found to contain alkaloids, steroids,

triterpenoids, flavonoids, glycosides, saponins, triterpenes of ursolic acid, saturated fatty acids, ascorbic acids, proteins, carbohydrates, lectins, carotenes, vitamin A, thiamine, riboflavins, niacin, bitter principles oleanoic acid, stearic acid, and gypsogenin. [10]

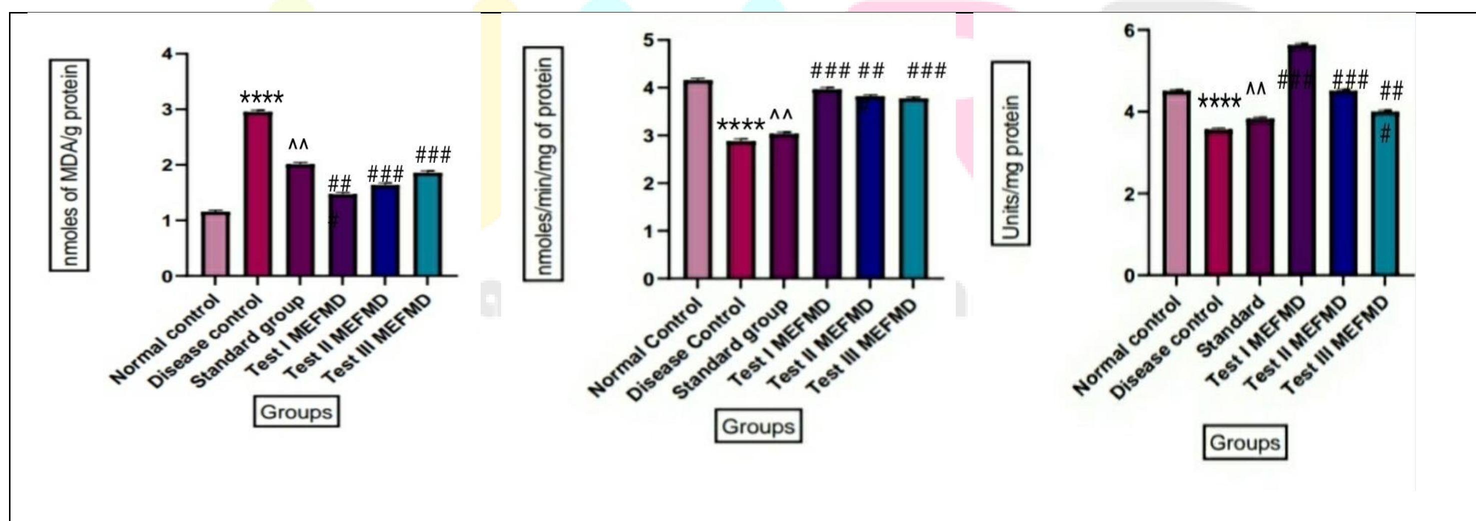
**B. Assessment of serum antioxidant tests of the samples**

The effect of graded doses of MEFMD on lipid peroxidation, glutathione levels, and SOD are listed out in table 1.1.

Table 1.1: Assessment of serum antioxidant tests of the samples

| Group | Treatment | LPO (nmoles of MDA/g of protein) | Glutathione levels (mmol/min/mg/protein) | SOD (Units/mg protein) |
|---|---|---|---|---|
| I | Normal control | 1.157±0.0091 | 4.160±0.0124 | 4.505±0.0125 |
| II | Disease control | 2.955±0.0095$^{****}$ | 2.882±0.0142$^{****}$ | 3.571±0.0108$^{****}$ |
| III | Positive control | 2.013±0.0105^^ | 3.036±0.010^^ | 3.837±0.0100^^ |
| IV | Test group I- MEFMD | 1.471±0.0110$^{\#\#\#\#}$ | 3.965±0.0124$^{\#\#\#\#}$ | 5.635±0.00957$^{\#\#\#\#}$ |
| V | Test group II- MEFMD | 1.639±0.0110$^{\#\#\#\#}$ | 3.821±0.009$^{\#\#\#\#}$ | 4.515±0.00991$^{\#\#\#\#}$ |
| VI | Test group III- MEFMD | 1.857±0.0105$^{\#\#\#\#}$ | 3.777±0.009$^{\#\#\#\#}$ | 4.010±0.00930$^{\#\#\#\#}$ |

The data are expressed as Mean ± S.E.M (n=10) rats in each group, $^{****}$P<0.0001 when compared with Normal Control, ^^P<0.001 when compared with positive control, $^{\#\#\#\#}$P<0.0001 when compared with positive control using one way Anova followed by Tukey‟s test.




Note: Disease control here refers to positive control and standard group here refers to positive control.

## CONCLUSION

Through the ages, plants have always offered huge prospects toward the betterment of human health either by ameliorating disease conditions or enhancing normal physiological activity. Every drug possesses toxic effects but a valuable pharmacologically active compound should have an acceptable balance between therapeutic

effects and toxic /untoward effects. To ensure the safety and efficacy of natural products, a battery of genotoxic and/or mutagenicity assays as well as teratogenecity studies are required to be performed to screen the toxicity mechanism.[71]

The target of this study was to evaluate the antioxidant activity of methanolic extract of fruits of *Momordica dioica* against valproic acid-induced teratogenecity in rats. 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* were proven to contain antioxidant properties as far as this study is concerned 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 which was clearly illustrated by comparing the disease control group (valproic acid) with the test groups (MEFMD) which showed an increase in superoxide dismutase and glutathione peroxidase level, whereas decrease in lipid peroxidation. Therefore it can be concluded that the methanolic extract of fruits of *Momordica dioica* has potent antioxidant and protective activity against valproic acid-induced teratogenicity in rats.

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**REFERENCES**

1. Evaluation of Teratogenic Effect of Methanolic Extract of Fruits of Momordica dioica In Rat Model. Blessy Elizabeth Babukutty, Deepak Kumar Jha, Ali Salman Al-Shami, Jeevan Khadka - IJFMR Volume 5, Issue 4, July-August 2023.

2. Alliance G: Teratogens/prenatal substance abuse. A district of Columbia guide for patients and health professionals. *Genetic Alliance*. 2010.

3. Vargesson Neil, Ramesh C. Thalidomide, Reproductive and Developmental Toxicology. *Academic Press*. 2022; 3: 423-437.

4. Donnai D, Sydney B, Jefferey H. Congenital Disorders. Encyclopedia of Genetics. *Academic press*. 2001; 448-449.

5. In Vitro Antioxidant Activities of Methanolic Extracts of *Caesalpinia volkensii* Harms., *Vernonia lasiopus* O. Hoffm., and *Acacia hockii* De Wild Beatrice Muthoni Guchu, Alex King'ori Machocho, Stephen Kiruthi Mwihia, and Mathew Piero Ngugi. Oxidative stress Volume 2020 Article ID 3586268.

6. Mahdi-Pour B, Jothy SL, Latha LY, Chen Y, Sasidharan S. Antioxidant activity of methanol extracts of different parts of Lantana camara. Asian Pac J Trop Biomed. 2012 Dec;2(12):960-5.

7. Talukdar SN, Hossain MN. Phytochemical, phytotherapeutical and pharmacological study of Momordica dioica. *Evidence-Based Complementary and Alternative Medicine*. 2014 (11).

8. Anjana M, Swathi V, Ramya Sai A, Divya N, Sunisha Y. A Review on Momordica dioica fruits. *J Adv Plant Sci*. 2019; 2:201.

9. 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).

10. 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.

11. Costa KC, Bezerra SB, Norte CM, Nunes LM, Olinda TM. Medicinal plants with teratogenic potential: current considerations. *Brazilian Journal of Pharmaceutical Sciences.* 2012; 48(3):427-33.

12. Valproic Acid- Asher Ornoy, Valproic acid in pregnancy: How much are we endangering the embryo and fetus, Reproductive Toxicology, Volume 28, Issue 1, 2009, Pages 1-10, ISSN 0890-6238.

13. Chateauvieux S, Morceau F, Dicato M, Diederich M. Molecular and therapeutic potential and toxicity of valproic acid. *Journal of Biomedicine and Biotechnology*. 2010.

14. Romoli M, Mazzocchetti P, D'Alonzo R, Siliquini S, Rinaldi VE, Verrotti A, et al Valproic Acid and Epilepsy: From Molecular Mechanisms to Clinical Evidences. *Curr Neuropharmacol.* 2019; 17(10): 926-946.

15. Rahman M, Nguyen H. Valproic Acid. In: StatPearls. Treasure Island (FL): StatPearls.2022.

16. Saeed M, Saleem U, Anwar F, Ahmad B, Anwar A. Inhibition of Valproic Acid-Induced Prenatal Developmental Abnormalities with Antioxidants in Rats. ACS Omega. 2020 Mar 2;5(10):4953-4961. doi: 10.1021/acsomega.9b03792. PMID: 32201781; PMCID: PMC7081441.

17. Batool S. Protective role of vitamin c against teratogenic effects of lead acetate on mouse kidney. *Pakistan Journal of Medical and Health Sciences.* 2011; 425-429.

18. Lykkesfeldt J, Michels AJ, Frei B. Vitamin C. *Biochemical, Physiological, and Molecular Aspects of Human Nutrition.* 2014; 5(1):16-8.

19. Ungurianu A, Zanfirescu A, Nițulescu G, Margină D. Vitamin E beyond Its Antioxidant Label. Antioxidants. 2021;10(5):634.

20. 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.

21. 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.

22. OECD test No 425: Acute oral toxicity: Up-and-down procedure. OECD guidelines for the testing of chemicals 2008.

23. Khera KS. Valproic acid induced placental and teratogenic effects in rats. *Teratology*. 1992; 45(6): 603-10.

24. Amrani A, Zama D, Boubekri N, Benaissa O, Meraihi ZB, Benayache S, Bettuzzi S. The protective effect of Chrysanthemum fantanesii extract, vitamin E and C on sodium valproate-induced embryotoxicity in pregnant mice. *Journal of Medicinal Plants Research.* 2012; 6(19): 3535-44.

25. Sukandar EY, Safitri D. Evaluation of teratogenic effect of tempuyung (Sonchus arvensis) extract on Wistar Rats. *International journal of farmakognosy and phytochemical research.* 2016; 8(5): 761-6.

26. Saeed M, Saleem U, Anwar F, Ahmad B, Anwar A. Inhibition of valproic acid-induced prenatal developmental abnormalities with antioxidants in rats. *ACS omega*. 2020; 5(10): 4953-61.

27. Alcala M, Bolado VE, Sánchez-Vera I, Clapés S, Dasí F, Sáez G et al. Prevention of Teratogenesis in Pregnancies of Obese Rats by Vitamin E Supplementation. *Antioxidants.* 2021;10(8):1173.

28. Tang Q, Su YW, Xian CJ. Determining Oxidative Damage by Lipid Peroxidation Assay in Rat Serum. *Bio Protoc.* 2019; 20;9(12):e3263.

29. Kakkar P.D, Viswanathan P.N. A modified spectrophotometric assay of superoxide dismutase. *Indian Journal of Biochemistry and Biophysics.* 1984; 21(2):130-132.