Evaluation of Antimitotic Activity of Momordica Dioica Fruits on Allium Cepa Root Meristematic Cells

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ARTICLE INFORMATION

Received: July 03, 2019
Revised: August 21, 2019
Accepted: October 16, 2019
Published online: November 05, 2019

OBJECTIVE: Natural occurring phenolic compounds play an important role in cancer prevention and shows antimitotic activity. Number of active constituents like phenolic acid, curcuminoids, coumarine, ligans, quinones, etc. is showing antimitotic activity of Momordica dioica. The present work is on phytochemical investigation and examines antimitotic activity of aqueous extract of fruits Momordica dioica at concentration of 15 mg/ml on Allium cepa root meristematic cells.

METHODS: The fruits are air dried and extracted with solvents like water by maceration method. The evaluation of antimitotic activity is done by using Allium cepa root meristematic cells parameters where and methotrexate was used as a standard drugs.

RESULT AND DISCUSSION: In Allium assay, aqueous extract of fruits of Momordica dioica(15 mg/ml) and methotrexate act against cells of allium roots and lesser the growth of root and mitotic index when compared with distilled water as control group. The result indicated that cytotoxic property is due to presence of phenolic, alkaloids and flavonoids compounds in 15 mg/ml concentration of aqueous extract of Momordica dioica fruits extract.

CONCLUSION: On the basis of result, we concluded that, 15 mg/ml concentration of Momordica dioica fruits shows good antimitotic activity on the Allium cepa root tip assay.

Keywords: Momordica dioica, Allium cepa, Antimitotic, Maceration and Methotrexate

DOI: 10.15415/jptrm.2019.72009

1. Introduction

In today’s world, peoples are suffered from lots of dangerous diseases. Cancer is one of that disease and second leading cause of death in the world. (Cragg and Newman, 2005) Now a day, various new anticancer agents are isolated from the natural sources of drugs. Natural sources of drugs are herbal based drugs consists of about 60-80 % of all other drugs and used as medicine from 1990. (Lee, 2010; Li and Vederas, 2009) The active constituent which is isolated from plants playa vital role in treatment of various diseases and gives special attention such herbal based plants due to its pharmacological activities. (Newman, et al., 2003) There are certain plants which are used in treatment of diseases like cancer. It is found that most of isolated compounds from plants show good anticancer activity (Gonzales and Valerio, 2006; Chopra, 1996). The Momordica dioica is recurring, having distinct male female plants consisting of family cucurbitaceae, which is commonly known as small bitter gourd or spiny gourd throughout the world where as in India it is called as jangle Karela, Kantola or Karola. The plant Momordica dioica shows various pharmacological or medicinal properties like analgesics, anti-tumorogenic, anti-inflammatory and anti-diabetic activity (The useful plants of India, 1992; The wealth of India 1962; Kartika and Basu 1987).

The fruit of 10 mg/ml concentration of aqueous extract of Momordica dioica shows good antimitotic activity. (Patil, et al., 2018) From the dichloromethane extract of Momordica dioica roots, five active constituents are isolated which gives good anti-cancer activity on cancerous cell like L1210. At 4 mg/ml it shows good growth inhibitory index about 50 %. (Luo- L, et al., 1998) Based on review of literature that no work has to be done on antimitotic activity at concentration of 15 mg/ml of aqueous extracts of fruits of Momordica dioica. Hence in this study aqueous extracts of fruits of Momordica dioica in 15 mg/ml concentration was assessed by using Allium cepa root meristematic cells. The 15 mg/ml concentration of aqueous extracts of fruits Momordica dioica was taken because to see the effect of fruits at 15 mg/ml concentration.
2. Materials and Methods

A. Plant Material & Reagents

Momordica dioica fruits were agglomerated from Nandurbar (MS) region and authenticated by Professor S. K. Tayade, Botany Department, College of Arts, Commerce and Science, Shahada, Lonkheda-425409, (MS). Chemicals and reagents are acquired from Rajesh Chemicals, Mumbai.

B. Preparation of Aqueous Extract of Fruits

Collected fruits were washed with distilled water, shade dried and pulverized to a rough form and extraction is done by maceration method using water-chloroform solvent. The filtrate was evaporated by using rotary-evaporator which removed water solvent in vacuum at 40°C. Take small amount of water extract of Momordica dioica fruits and dissolved in to small amount of distilled water to produce test solution. This test solution was used to study different chemical tests such as alkaloids, flavonoids, tannins, carbohydrates and proteins for detection of different chemical constituents present in the aqueous extract (Harborne, 1988; Kokate, et al., 1999).

C. Determination of Mitotic Index

(Abhang, et al., 1999; Willams and Omoh, 1996) Red qualities of onions (Allium cepa) are purchased from near market area and are stored for further study. The red onion bulbs are place in tap water at room temperature for 48 hours. On the other hand prepared different solutions at concentration of aqueous extract of Momordica dioica fruits at of 15 mg/ml, standard drug methotrexate (Zydus Cadila, Ahmedabad) at 0.1 mg/ml and distilled water which served as control respectively. The developed roots then dipped into solutions of aqueous, methotrexate and distilled water for two hours. Then removed the roots then cut to separate tip part of roots and placed in to fixing solution consists of acetic acid 45% v/v and ethanol 95% v/v in ratio of 1:3 v/v for near about 10-15 hours. Then roots are reacted with 1N HCL and place in to oven for 15 min at 50°C for warm of roots. Removed it then washed with distilled water and stain with staining agent like carmine. The roots are then placed on the slides and crushed it and observed under microscope. Count the number of cells present in each stages of mitosis and calculate mitotic index which is as

Mitotic Index = Number of dividing cells $\times 100$

D. Statistical Analysis

(Kulkarni, 1993) The experiments were carried out in triplicates and the data were expressed as mean $\pm$ SEM. The significance of difference among the various treated cells and control cells were analyzed by means of one-way ANOVA.

3. Results

From Table No. 1 showed the presence of phytoconstituents such as alkaloids, flavonoids, tannins, carbohydrates and proteins in aqueous extracts of fruits Momordica dioica.

Table 1: Preliminary Phytochemical screening of 15 mg/ml aqueous extracts of fruits Momordica dioica.

| Sr. No. | Phytochemical Test | Test | Inference |
|--------|-------------------|------|-----------|
| 1.     | Test for carbohydrate | Molish test | + |
| 2.     | Test for proteins | Biuret test | + |
| 3.     | Test for flavonoids | Lead acetate test | + |
| 4.     | Test for alkaloids | Dragondroff's test: | + |
| 5.     | Test for tannins and phenolic compounds | Lead Acetate test | + |

Table 2: Antimitotic activity after treatment of Allium cepa roots with 15 mg/ml aqueous extracts of fruits Momordica dioica, methotrexate and control water.

| Sr. No. | Different Solutions used for treatment | Total No. of cells | No. of Dividing cells | Mitotic Index |
|--------|----------------------------------------|--------------------|----------------------|--------------|
| 1.     | Water(Control)                         | 145                | 15 4 2 4 102          | 17.25±1.15   |
| 2.     | Methotrexate (0.1mg/ml Positive Control) | 103                | 6 4 1 1 91           | 11.65±0.35** |
| 3.     | Aqueous Extracts (15mg/ml)             | 120                | 10 3 2 1 104         | 13.34±1.05*  |

P-Prophase, M-Metaphase, A-Anaphase, T-Telophase

Table No. 2 showed that fruits Momordica dioica (15 mg/ml) and methotrexate gives antimitotic in Allium cepa root meristematic cells. The total number of non-dividing cells in controlled group (distilled water), standard drug group (methotrexate 0.1 mg/ml) and aqueous extract (15 mg/ml) was found to be 120, 91 and 104 respectively. The total number of dividing cells in different stages of mitosis such as prophase, metaphase, anaphase and telophase in controlled group (distilled water) was found to be 15, 4, 2 and 4, in
standard group (methotrexate) were found to be 6, 4, 1 and 1 and in 15 mg/ml concentration of aqueous extracts was found to be 10, 3, 2 and 1 respectively. The average mitotic index in water (control), methotrexate (standard) and 15 mg/ml concentration of aqueous extracts of *Momordica dioica* fruits was found to be 17, 11 and 13 respectively.

4. Discussion

The fruits of *Momordica dioica* shows wide spectrum of medicinal activities. The plant contains various phytochemical compounds having diverse chemical structure and nature. The main active constituents are found in plants as phenolic, flavonoids and tannins compound. In existing study, *Allium cepa* root meristamatic cell model is used to study antimitotic property of fruits *Momordica dioica* in 15 mg/ml concentration. (Abhang, et al., 1991; Latha, 1988) In this parameters, division of cells is same as that of division of cancerous cells in human being. Therefore these cells are used to study antimitotic activity in different parts of plants. *Allium cepa* meristamatic cells assay is fast, quick to detect toxicity in cells and toxicity in cells genetic material. The inhibition of root growth and antimitotic property gives indication of destruction of cells genetic material. The destruction of cells of genetic material assay action on *Allium cepa* as a plant helpful in studying karyotype of plant and skill to connect with those of mammalian cells in toxic evaluation.

The good genotoxic assay performance of *Allium cepa* as a plant system has been attributed to the easily studied karyotype of plant and the ability to correlate outcomes of assays with those of mammalian cells in the course toxic evaluations. (Fiskesjo, 1985) In *Allium cepa* assay, aqueous extracts of fruits of *Momordica dioica* (15 mg/ml) was found to inhibit root growth in *Allium cepa* root meristamatic cells and decreased mitotic index after treatment. This suggests that the aqueous extract of fruits of *Momordica dioica* has fair antimitotic potential in concentration of 15 mg/ml. In *Allium cepa* assay, MI (mitotic index) is considered as an indication of biomarkers cells proliferation in metaphase cell cycle. (Bhattacharya and Haldar, 2012; Periyanayagam, et al., 2013) Earlier it was proved that fruits of *Momordica dioica* shows good anticancer activity in different cell lines such as PA-I and Hela cell line by using MTT assay (Ahirrao, 2019).

Conclusion

Our finding support that 15 mg/ml concentration of aqueous extracts of fruits *Momordica dioica* inhibits cell division in *Allium cepa* meristamatic cells and may helpful in inhibiting abnormal cell growth like cancerous cells. Further experiments are needed, both in vitro and in vivo to obtain detail mechanisms of action. Positive outcomes from study introduce this plant as a new anticancer drug.

Acknowledgements

Authors are thankful to Principal of College of Pharmacy, Shahada-425409, Maharashtra for their help and support to complete the research work.
References

Abhang, R. Y., Joglekar, P. P. and Kulkarni, P. H. (1991). Preliminary study on the effect of T. cordifolia on mitosis. Anc. Sci. Life., 11(1-2), 7-8.

Abhang, R. Y., Joglekar, P. P. and Kulkarni, P. H. (1999). Preliminary study on the effect of T. Cordifolia on mitosis. Ancient Science, 1(7), PMC3336579.

Ahirrao, R. A. (2019). Anticancer activity of Fruits of Momordica Dioica by using MTT assay. Madridge J. Immunol. 3(2), 89-92.

https://doi.org/10.18689/mjim-1000120

Bhattacharya, S. and Haldar, P. K. (2012). Evaluation of antimitotic and genotoxic effects of the triterpenoid enriched extract from Trichosanthes dioica root. American-Eurasian Journal of Toxicological Sciences, 4(1), 20-23.

https://doi.org/10.5829/idosi.ajots.2012.4.1.56310

Chopra, R. N., Nayar, S. L. and Chopra, I. C. (1996). In Glossary of Indian medicinal plants, National Institute of Science Communication, New Delhi, India, 169

Cragg, G. M. and Newman, D. J. (2005). Plants as a source of anticancer agents. J Ethnopharmacology, 100(1-2), 72-79. https://doi.org/10.1016/j.jep.2005.05.011

Fiskejo, G. (1985). The Allium test as a standard in environmental monitoring. Hereditas, 102(1), 99-112.

https://doi.org/10.1111/j.1601-5223.1985.tb00471.x

Gonzales, G. F. and Valerio, L. G. (2006). Medicinal plants from Peru: a review of plants as potential agents against cancer. Antican. Agents Med. Chem., 6(5), 429-444. https://doi.org/10.2174/187152006778226486

Harborne, J. B. (1988). Phytochemical methods: A guide to modern techniques of plant analysis. London: Chapman and Hall Publishing House.

https://doi.org/10.1016/0031-9422(88)84139-6

Kartika, K. R. and Basu, B. D. (1987). In; Indian medicinal plants II, Ed: International book distributors, Dehradun, India, 1133.

Kokate, C. K., Purohit, A. P. and Gokhale, S. B. (1999). Textbook of Pharmacognosy. 11th Edition, NiraliPrakashan, Pune, 225-226.

Kulkarni, S. K. (1993). Handbook of experimental pharmacology, 2nd Edition, Vallabh Prakashan, Mumbai, 172-189.

Latha, P. G., Chandralekha, C. T., Vilasini, G. and Panikkar, K. R. (1998). Effects of the flower extract of Ixora coccinea Linn. on the meristematic cells Allium cepa. Anc. Sci. Life, 17(4), 262-267.

Lee, K. H. (2010). Discovery and development of natural product derived chemotherapeutic agents based on a medicinal chemistry approach. J. Nat. Prod. 73(3), 500-516. https://doi.org/10.1021/np900821e

Li, J. W. and Vederas, J. C. (2009). Drug discovery and natural products: end of an era or an endless frontier? Science, 325(5937), 161-165.

https://doi.org/10.1126/science.1168243

Luo- L, Li- Z and Zhang- Y, Huang- R,(1998). Triterpenes and steroidal compounds from Momordica dioica. Yao-Xue-Xue-Bao., 33(11), 839-842.

Newman, D. J., Cragg, G. M. and Snader, K. M. (2003). Natural products as sources of new drugs over the period 1981-2002. J. Nat Prod., 66(7), 1022-1037. https://doi.org/10.1021/np030096l

Patil, N. S., Patil, K. B., Patil, M. R. and Ahirrao, R. A.(2018). Antimitotic activity of fruits of momordica dioica by using Allium cepa root tip assay. Asian J. Pharm. Res., 8(4), 221-224.

https://doi.org/10.5958/2231-5691.2018.00037.0

Periyanayagam, K., Kasirajan, B., Karthikeyan, V., Indumathi, R. and Kumuda, T. (2013). Vitisvinifera. L (Vitaceae) fruits towards antimitotic and antiproliferative activity in anticancer drug discovery. Innovare J. Health Sci., 1(3), 32-35.

The useful plants of India (1992), Ed: Publication and Information Directorate, CSIR, New Delhi, India, 379.

The wealth of India (1962), A dictionary of Indian Raw materials and Industrial products VI, Ed: Publication and Information Directorate, CSIR, New Delhi, India, 408.

Williams, G. O. and Omoh, L. E. (1996). Mitotic effects of aqueous leaf extract of Cymbopogoncitrus in Allium cepa roots. Cytobios, 87, 161-164.
