Research Article

Anti-mitotic effect of Lumerax and Curcumin on onion root tip system

Amit Jharia¹,², Saurabh Pagare¹,⁴*, Asha Khanna¹,², Manila Bhatia¹, Varsha Aglawe², Mubashir Azam Mir³

¹AVIKA Biological Science Research Foundation, Jabalpur (M.P.) India
²Department of Zoology and Biotechnology, Govt. Science College, Jabalpur (M.P.) India
³Department of Zoology and Biotechnology, Govt. M. H. College of Home Science & Science for Women, Jabalpur (M.P.) India
⁴Department of Botany and Biotechnology, Sarojini Naidu Govt. Girls P.G. College, Bhopal (M.P.) India

Received: 27 June 2020 Revised: 19 August 2020 Accepted: 21 August 2020

Abstract

Objective: Artemether is a common anti-malarial drug used in combination with Lumefantrine for the treatment of uncomplicated falciparum malaria. It is also reported to be used as anti-cancer drug; hence it was thought worthwhile to explore its anti-mitotic effects on the onion root tip system. Material and methods: Onion bulbs were grown in tap water where they sprouted 2-3 mm roots. Their mitotic index reading at 0 hour was noted then they were transferred to the test solutions. The plasma concentration of the drug is reported to be 184ng/ml; so for testing its effect the drug was added to the water in which onion tips were grown with 92ng/ml (½ plasma concentration), 184ng/ml (plasma concentration) and 368ng/ml (2× plasma concentration). Results and conclusion: Readings of % mitotic index (MI) were noted at 0 hr and 24 & 48 hr after putting the onions in the experimental solutions. It was found that there was depression of the MI% at all the three concentrations when compared to their respective 0hr readings and most of the time the depressions were statistically significant. Regarding the effect of curcumin, it was found that at a concentration 0.33% and 0.66% curcumin induces MI% depression after 24 & 48 hr, but at 1.1%, the depression is less and actually there is a slight increase. Thus it acts as both anti-mitotic and an immunomodulator.

Keywords: Artemether, curcumin, onion root tip, mitotic index

Introduction

Artemether is a popular anti-malarial drug; it is an artemisinin derivative in which the lactone has been converted to the corresponding lactol methyl ether (figure 1A). It is used in combination with lumefantrine as an antimalarial for the treatment of multi-drug resistant strains of falciparum malaria (for treatment of acute uncomplicated malaria) (Kayla et al., 2012).

Artemisinin is extracted from Artemisia annua which is a common type of wormwood native to temperate Asia. In traditional Chinese medicine Artemisia annua is traditionally used to treat fever. The proposed mechanism of action of artemisin involves cleavage of endoperoxide bridges by iron, producing free radicals which damage biological macromolecules causing oxidative stress in the cells of the parasite. Anti-malarial drug artemisinin and its derivatives have several other important properties which include anti-cancerous properties. This class of drug can be a therapeutic alternative in highly aggressive cancer with rapid dissemination, without developing drug resistance. They also exhibit synergism with other anticancer drugs with no increased toxicity towards normal cells. They arrest cell cycle at G0/G1, and regulate several important factors that control several pathways that effect drug response, drug interaction etc. Newly developed synthetic artemesminis have been reported to show considerable anti-neoplastic activity, but information is still scarce (Das, 2015a). Considerable anti-tumour activity has been observed in animal models when artemisin derivative

*Address for Corresponding Author:
Dr. Saurabh Pagare
Department of Botany and Biotechnology, Sarojini Naidu Govt. Girls P.G. College, Bhopal (M.P.) India
Email: saurabhpagare4@gmail.com

DOI: https://doi.org/10.31024/apj.2020.5.4.1
2456-1436/2020, N.S. Memorial Scientific Research and Education Society. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

www.apjonline.in
(DHA) is used in combination with various anticancer agents such as Cyclophosphamide and Cisplatin in animal models (Das, 2015b).

Curcumin (Diferuloylmethane) is a component of turmeric which is orange-yellow in colour (Figure 1B). It is used as a spice in Indian cooking in very small amounts. Its anti-inflammatory effects are well known traditionally, but it also shows immunomodulatory affects that can modulate T cells, B cells, macrophages, neutrophils to boost immunity and disease resistance. It has beneficial effects in arthritis, allergy, asthma, heart disease etc, it is also used as an anti-cancerous agent. The onion plant (Allium cepa) is a suitable indicator plant for several environmental agents (Firbas and Tomaz, 2014). Onion root tip cells are also very suitable for study of anti-mitotic agents as the bulb can be grown in the lab in desired conditions, the mitotic squash preparation are easy to prepare and the chromosome (2n = 16) are large so that morphological changes can be easily observed under a light microscope at 40X and 100X magnification.

Materials and Methods

Drug preparation

The tablets of Artemether-Lumefantrine or lumeraX 40 was bought from local chemist, lumeraX 40 is a schedule ‘H’ drug whose uncoated tablet contains Artemether IP- 40 mg, Lumefantrine Ph. Int-240mg. Therefore the total dose of lumeraX 40 tablet is 280mg which includes 40mg of Artemether. Since the plasma concentration of Artemether is 184ng/ml (Ali et al., 2010) and it is difficult to measure nano-grams (ng) thus the stock solution was made in a concentrated form and diluted 100 times.

Curcumin extraction: 20% of ethanol extract was prepared by adding 20g of turmeric powder (made from dried turmeric rhizome) in 70% ethanol to make 100ml solution. Then the solution was shaken for 24 hours at room temperature. Then the solution was filtered through whatman no. 1 filter paper to get an amber colour liquid. Then this liquid was evaporated in a water bath at 50°C until 2/3rd liquid was left (Tanvir et al., 2017). It is reported that the curcumin content of crude turmeric is 3% (Gunners, 2018). So the curcumin concentration of the solution obtained is about 3.3%.

Preparation of test material

For mitotic studies three healthy medium sized onion bulbs weighing 25-28g were taken with 3-5mm root length. Their outermost brownish scaly skin and dead roots were scraped off near the disc. They were left in tubes filled with tap water to grow for 3 days so that their discs were submerged in water. They were left to grow at room temperature (average temp. 24-25°C and 46.6% avg. humidity) and partial exposure to sunlight until their roots were nearly 1cm long. For each bulb the zero hour or stat Mitotic Index was determined.

Squash preparation

The terminal 2cm of the root meristems was cut and heated in a mixture of aceticarmine: N/10 HCl in a 9:1 ratio. The watch glass containing the root tips was heated until the tips were soft and darkly stained. A tip was then taken and squashed in a drop of fresh aceticarmine on a clean slide after a cover slip was put. The slide was wrapped in layers of filter paper and squashed by the application of direct vertical pressure of the thumb. The slides of mitosis thus prepared were scanned under the microscope at 40X in various fields. Cells showing various stages of mitosis and non dividing cells were counted. 500-800 cells per onion bulb were counted. Mitotic index (MI) was calculated by using the formula:

\[ \text{MI} \% = \frac{\text{Total No. of dividing cells}}{\text{Total no. of cells counted}} \times 100 \]

The 3 onion bulbs were then put in 3 containers containing 1/2P, P, 2P concentrations of the Artemether drug and other 3 onions was put in 3 different containers containing 2.5ml (1.1%), 1.25ml (0.66%), 0.625ml (0.33%) concentrations of curcumin and the readings of MI were taken at 24 and 48 hour respectively after exposure. Each
test was run in triplicate. The data of MI was recorded; the Mean and Standard Deviation (SD) were calculated. The significance of the difference in MI at various times of exposure was calculated by Student’s test.

Results

According to the observations and readings it was found that even at 1/2p concentration of Artemether there was a significant depression of mitotic index % (13.1±8.0) after 24 hours of exposure as compared to (17.5±6.85) at 0 hours which is significant at P<0.01, there is a further depression of MI% after 48 hours as it falls to (12.29±3.66) which is significant at P<0.01 (Figure 2A, B and C).

In exposure of onion root tips to plasma concentration p of the drug, after 24 hours the MI% falls to 7.85±1.01 for an initial reading of 15.85±6.1 at 0 hours; this fall is significant at P<0.01. At 48 hours of exposure there is a further fall of MI%; now it was 7.08±1.01 which is highly significant at P<0.001. At 2p concentration, there was a drastic fall in the MI% as compared to the initial reading; in 24 hours it fell to 6.83±2.58 from 16.55±7.72 which is highly significant at P<0.001 and after 48 hours it fell further to 6.30±1.74 which is also highly significant at P<0.001. However, if the readings of MI% for 24 hours and 48 hours are compared there is a further depression of MI% with the time of exposure but these differences are not significant (Figure 2D).

In the case of curcumin the result shows that at 0.33% concentration of curcumin after 24 hour exposure the mitotic index was 11.48±4.63 which shows high significant depression at P<0.001 because it was 14.78±4.44 at 0 hour. The MI% is further depressed when the exposure time is increased to 48 hours it falls to 8.36±4.46 which is significant at P<0.01 (Figure 3 A, B and C).

A similar trend is observed at 0.66% concentration of curcumin. The MI% falls from 14.06±6.17 at 0 hours to 10.03±2.48 after 24 hours of exposure which is a significant depression of mitotic index. The MI% shows a further decrease when the roots are exposed for 48 hours, it now drops to 9.27±3.69 which is significant at P<0.05 as compared to the 0 hour reading. Thus the observations confirm the finding that curcumin has an anti-mitogenic effects at these concentrations. However, when the concentration is increased to 1.1%, at 24 hours there is a slight depression of MI% (13.96±2.46) as compared to that of 0 hour (14.46±3.07) but the depression is non-significant. After 48 hours of exposure the MI% was found to increase to 17.42±10.09, which significantly higher than the 0 hour reading (Figure 3D).

![Figure 2 (A, B and C). Percentage of dividing cells in different Artemether concentrations at 0, 24 and 48 hrs respectively and (D). Mitotic index of onion root tip in different Artemether concentrations](www.apjonline.in)
Figure 3. Percentage of dividing cells in different Curcumin concentrations at (A) 0, (B) 24 and (C) 48 hrs and (D) Mitotic index of onion root tip in different Curcumin concentrations.

Figure 4. Effect of different concentrations of Artemether and Curcumin on various stages of mitotic cell division in onion root tip system: (A) Early Prophase (EP), Telophase (T) and Late Prophase (LP); (B) 2n=16 chromosomes of onion; (C) cell plate formation (CP); (D) Irregular anaphase; (E) sticky anaphase (A) and metaphase (M); (F) Cells arrested in phophase in curcumin; (G) All cells arrested in Prophase in 0.66% concentration of curcumin; (H) Prophase (P), Telophase (T) and Metaphase (M) in 1.1% concentration of curcumin.
Discussion
Recent studies have shown that artemisinin drug exhibited significant cytotoxicity and inhibitory effects on cancer cells including leukaemia, stomach cancer, breast cancer and pancreatic cancer cells (Zhao et al., 2017). As per the above observations and results it is clear that Artemisinin derivative i.e. Artemether is capable of inhibiting the cell growth by arresting these cells in G0/G1 phase.

However in case of curcumin it seems that curcumin in smaller dose is inhibitory to cell division but in large concentration it can stimulate cell division also. The findings agree with our traditional knowledge also; in India we take low concentration of turmeric daily in diet which perhaps serves the purpose of surveillance and control unwanted cell divisions. We are familiar with the ideas of traditional Indian medicine, that turmeric is an immune booster also, infact Hardira capsules(turmeric) are marketed by Himalaya which is a popular prescription for cough, cold, flu, arthritis etc (Anupama, 2017).

Mitotic arrest induced by curcumin has been reported by Blakemoor et al., 2012 who studied its effect on 8 colorectal cancer lines. They reported G2/M arrest after 12hr treatments with 5-10µm concentration of curcumin. They reported aberrant mitosis due to spindle abnormalities resulting from conformational changes in tubulin and mitotic arrest was also evident as depression in mitotic index percentage in this study.

The anti-mutagenic potential of curcumin on chromosomal aberrations in *Allium cepa* was investigated by Raghunathan et al. (2007). They treated the root tips with sodium azide at 200 and 300µg/ml for 3 hours and curcumin was given at 5,10,20µg/ml for 16hr prior to sodium azide treatment. They found that the total number of aberrations was significantly reduced in root tip cells pre-treated with curcumin (showing anti-mutagenic potential of curcumin). They also report the reduction of MI% in all curcumin treated groups. This agrees with the findings of this investigation.

The antimitotic effect of Artesunate, which is another antimalarial drug, was also demonstrated by Shrivastava et al. (2020) who worked out its inhibitory effects on onion root tip mitosis. The ameliorative effect of tulsi (*Ocimum sanctum*) extract was also worked out by them.

Conclusion
The work demonstrates that onion root tips show a significant fall in MI% if exposed to plasma concentration (P) of the drug for 24 hrs. After 48 hrs the MI% values show a further fall. At exposure to 2P concentrations the MI% shows a more drastic reduction. Exposures to 0.33% of curcumin for 24 and 48hrs also show a significant reduction of MI%. Similar trends were also observed for 0.66% of curcumin treatment. But interestingly, at 1.1% concentration exposures for 48 hrs the MI% was found to increase.

Thus curcumin was found to be a depressant of mitotic activity at lower concentrations, but it acts as a mitotic stimulator at higher concentration.

Acknowledgements
The authors are grateful to Dr. A.L Mahobia, Principal, Government Science College, Jabalpur (M.P.) and wish to acknowledge AVIKA Biological Science Research Foundation, Jabalpur (M.P.) for providing necessary facilities to carry out research work.

References
Tanvir EM, Hossen MS, Hossain MF, Adroz R, Gan SH,