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Design, Synthesis and *in vitro* Screening of Pyrazolines based compounds as Phytohaemagglutinin (PHA) mimetic

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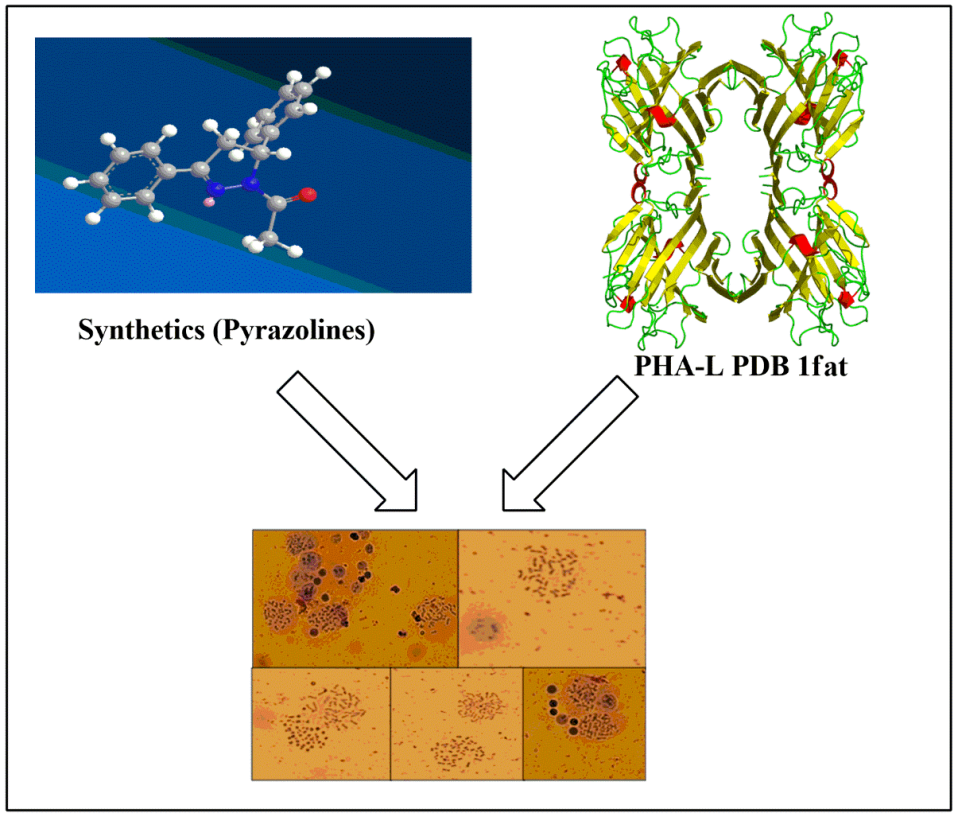
Design, Synthesis and *in vitro* Screening of Pyrazolines based compounds as Phytohaemagglutinin (PHA) mimetic



Abstract: Phytohaemagglutinin (PHA, or phytohemagglutinin) is a lectin found commonly in plants, especially legumes. It has some physiological effects on cell metabolism; it induces mitosis and affects the cell membrane regarding transport and permeability to proteins. It agglutinates most mammalian red blood cell types and have the mitogenic effect. This is the reason that PHA is extensively used in the laboratory as well as clinical set up for karyotyping analysis. The downside of PHA use is its cost and storage (-20^{o}C) resulting into increased cost. We have synthesised acetylated pyrazolines as anticancer agents and during their evaluation for anticancer potential in normal control cells, we were surprised by their cell proliferation activity. We thought of relating our compounds to PHA (PHA mimetic) and performed the basis karyotyping experiment keeping PHA as standard and found our compounds to be PHA mimics. The compounds are thus being evaluated for their further PHA mimetic potential using B/T cell specific cell cycle analysis and karyotyping experiment keeping PHA as standard and found our compounds outstanding PHA in every aspect. The compounds are thus being evaluated for their further PHA mimetic potential.
Keywords: Phytohaemagglutinin; Mitogenic; PHA mimetic

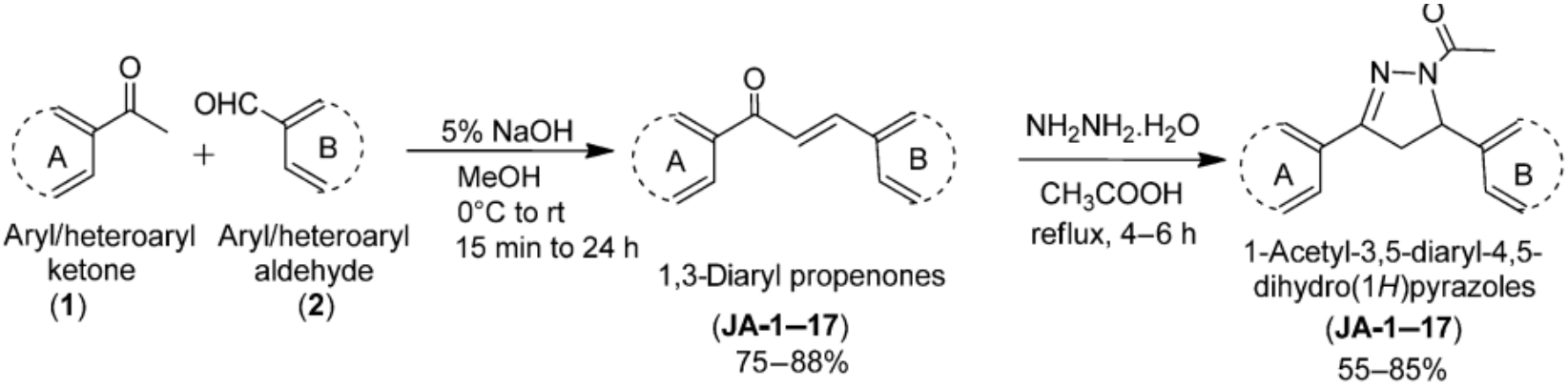
Introduction

- Phytohaemagglutinin (PHA, or phytohemagglutinin) is a lectin found in plants, especially legumes.
- The lectin has a number of effects on cell metabolism; it induces mitosis, and affects the cell membrane in regard to transport and permeability to proteins. It agglutinates most mammalian red blood cell types.
- Lymphocytes cultured with phytohaemagglutinin can be used for karyotype analysis

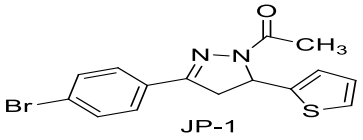
Hypothesis

- While evaluating a series of newly synthesized compounds on human peripheral blood monocytic cells, the synthesized compounds did not exhibit any cytotoxic activity but they showed increased proliferation indicated by the increased intensity of formazan reduction.
- It was, thus, hypothesized that the synthesized compounds may be increasing the cell similar to PHA.

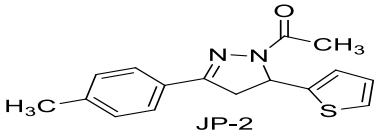
Synthetic Strategy



Chemical structures of the synthetics



JP-1



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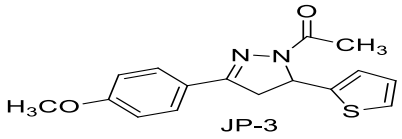

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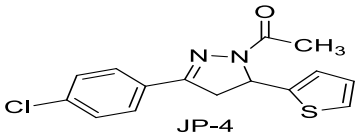

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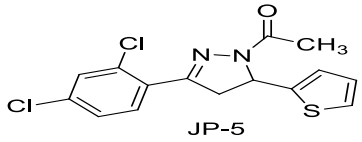

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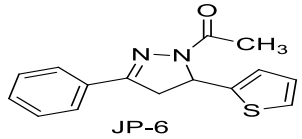

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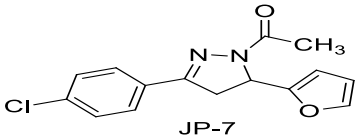

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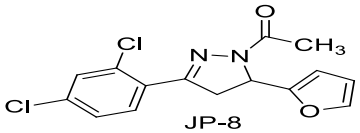

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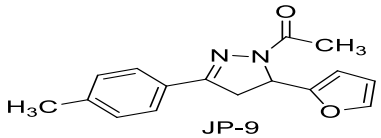

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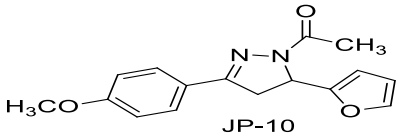

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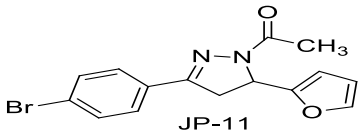

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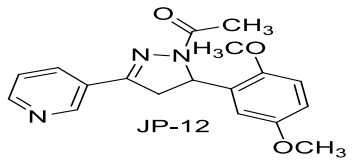

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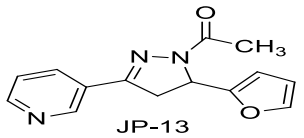

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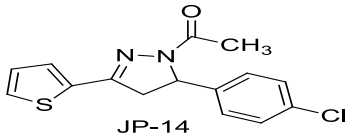

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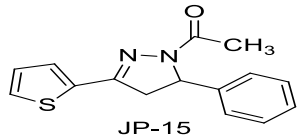

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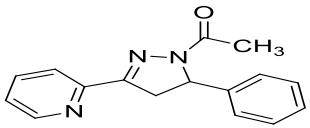
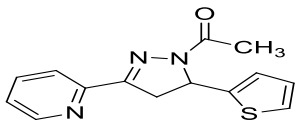


JP-16



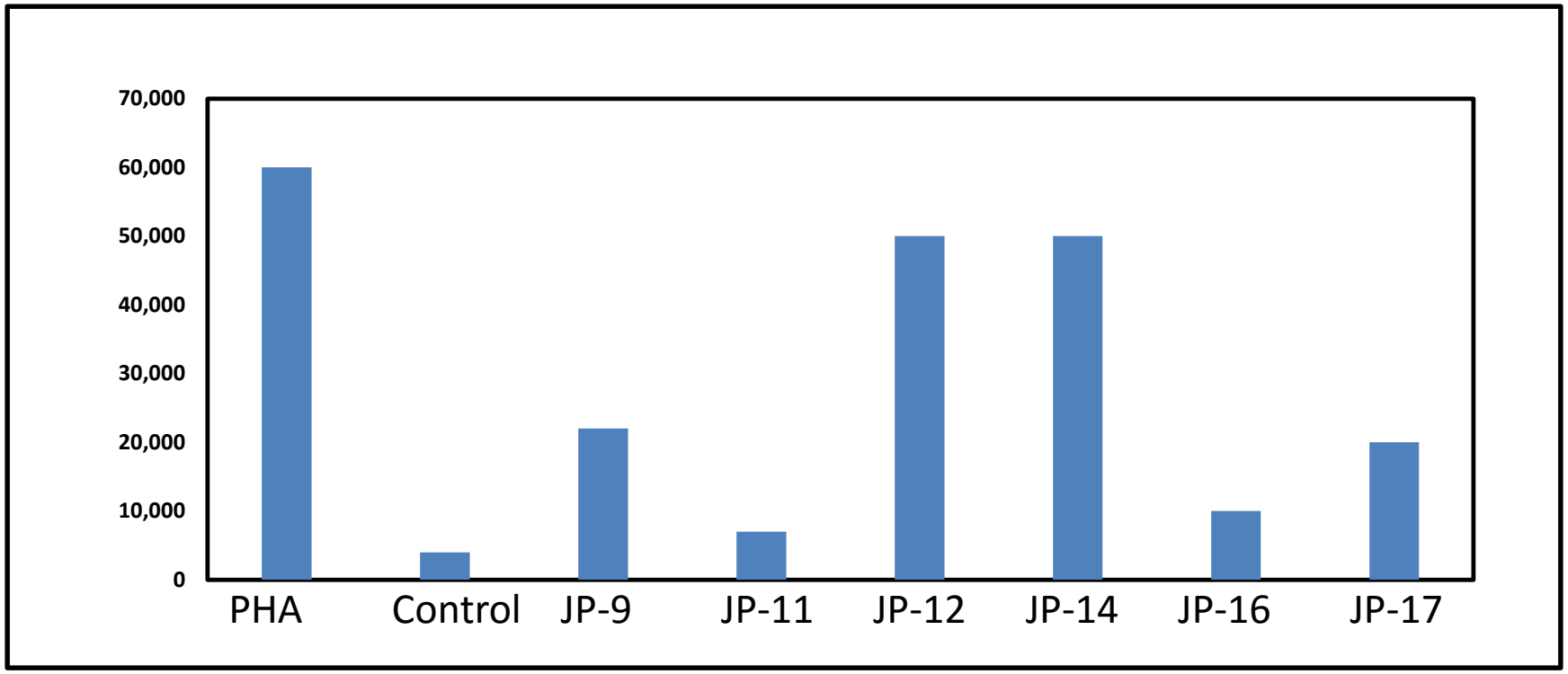
JP-17

Objectives

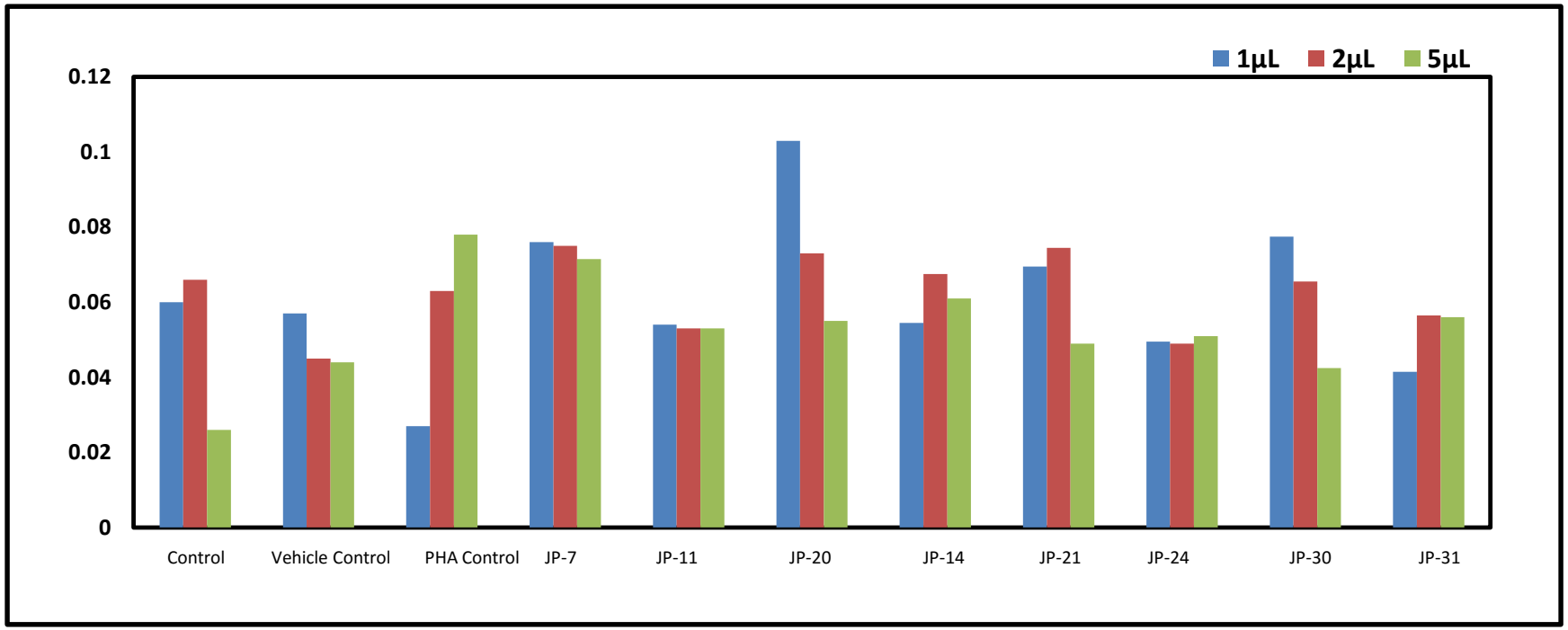
- Determining the absolute cell count in different samples and their comparison with PHA as control.
- Determining the Protein Concentration of the tested samples against PHA.
- Arresting the dividing cells at metaphase and observing them under microscope at 10x and 40x.

Result and Discussions

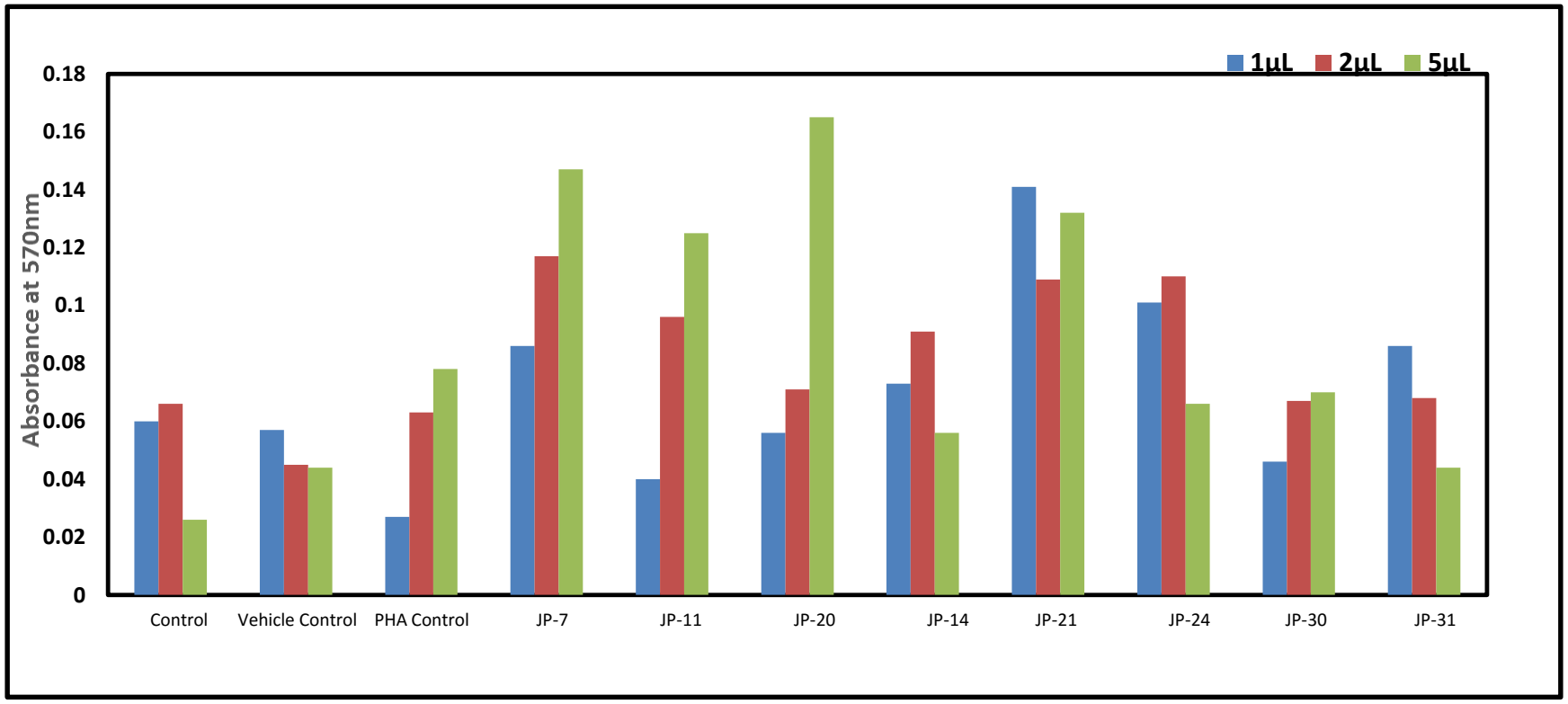
No. of cells were counted on Automated Cell Counter



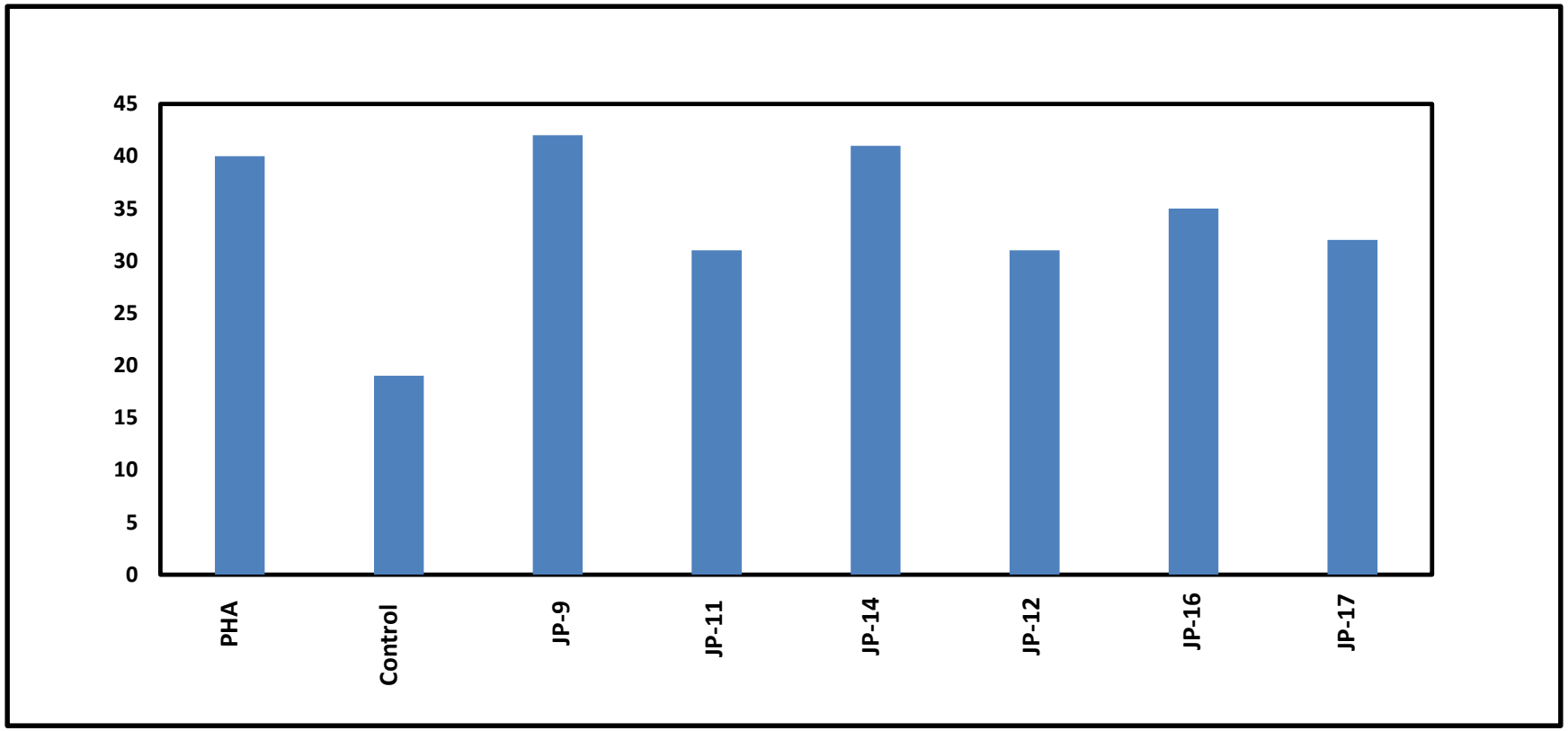
Evaluation of cytotoxic effect on PBC in response to treatment with synthesized compounds at concentrations of 1 µL, 2 µL and 5 µL for a time duration of 72 hrs. Data is expressed as mean values ± S.D. of three independent experiments.



Evaluation of cytotoxic effect on PBC in response to treatment with synthesized compounds at concentrations of 1 µL, 2 µL and 5 µL for a time duration of 96 hrs. Data is expressed as mean values ± S.D. of three independent experiments.



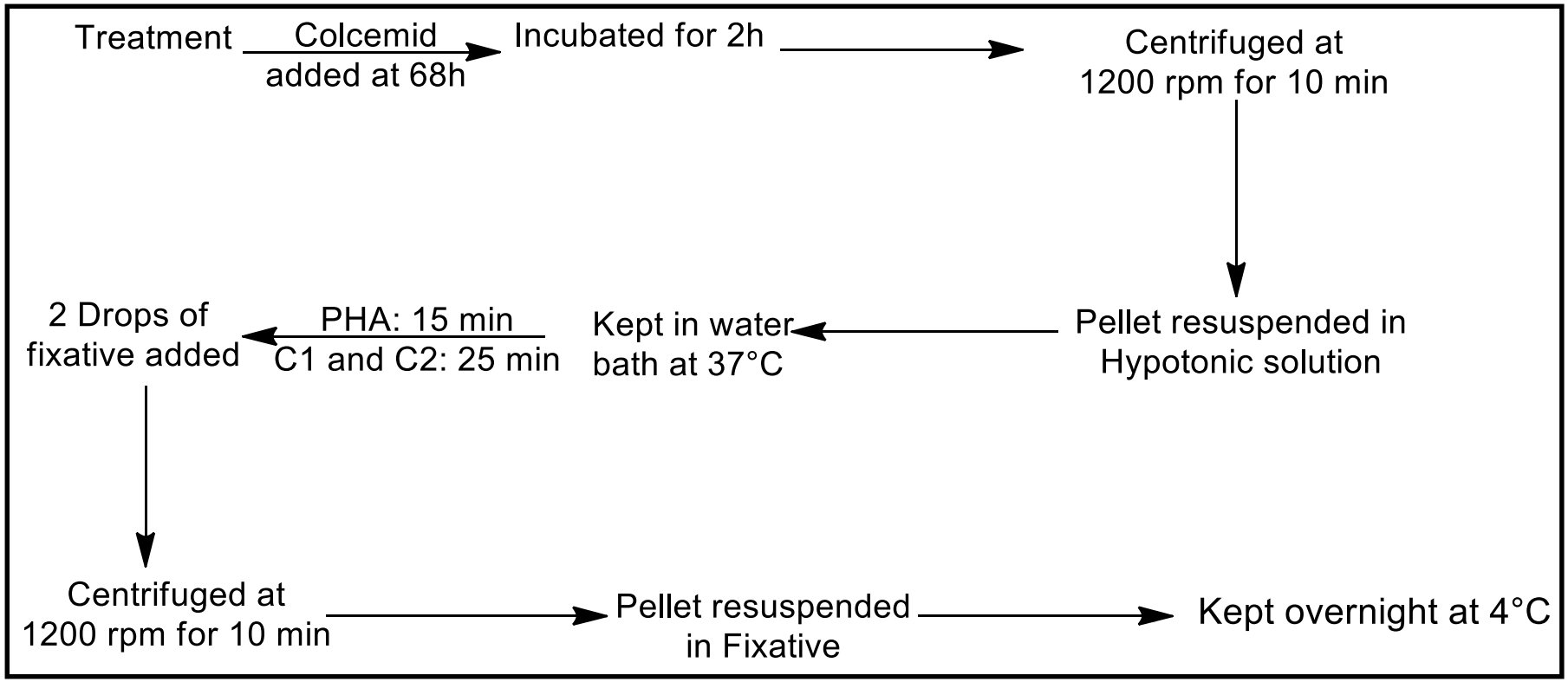
Determination of Protein Concentration



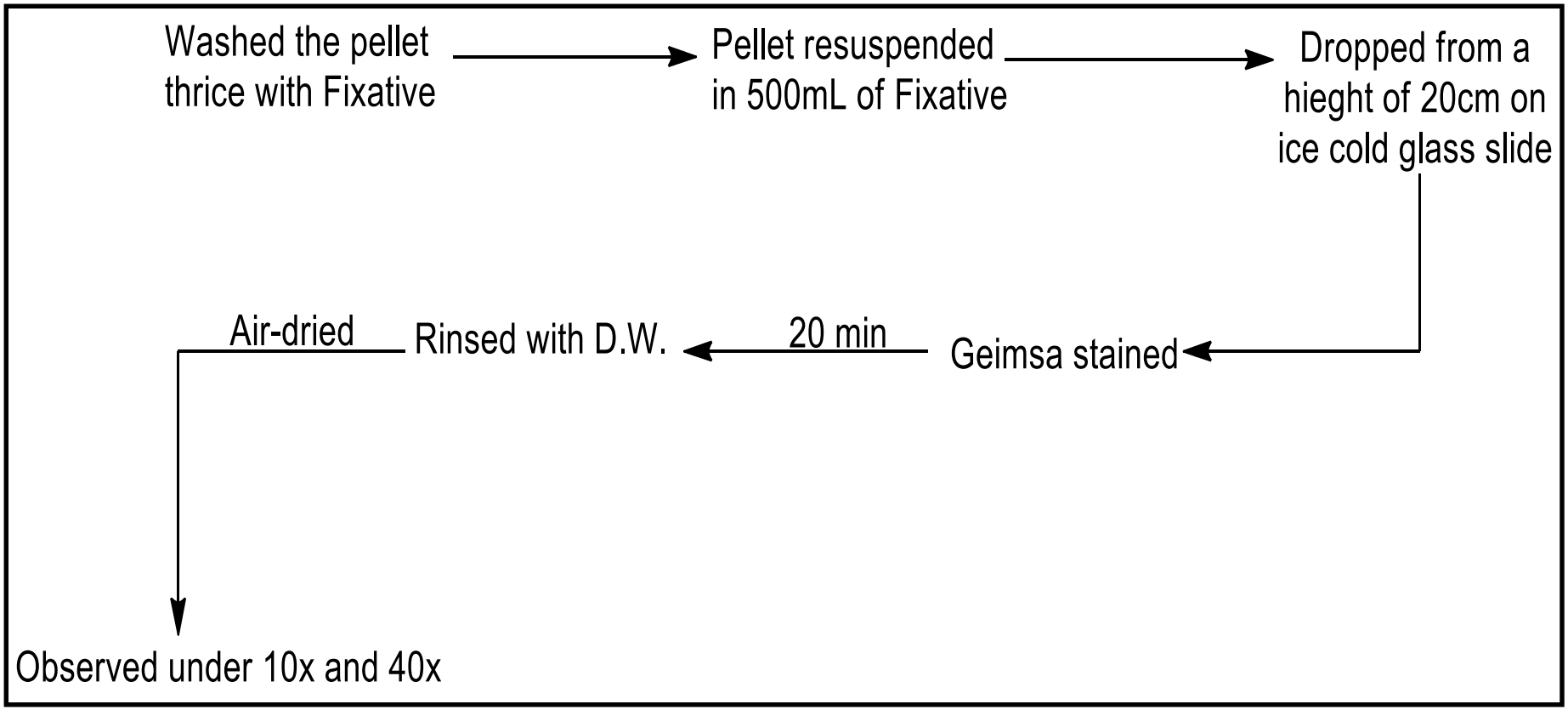
Protocol for Metaphase Arrest

	PHA	C1 (JP-14)	C2 (JP-21)
Media (RPMI)	2mL	2mL	2mL
Blood	150 µL	150 µL	150 µL
PHA	30 µL		
C1		30 µL	
C2			60 µL

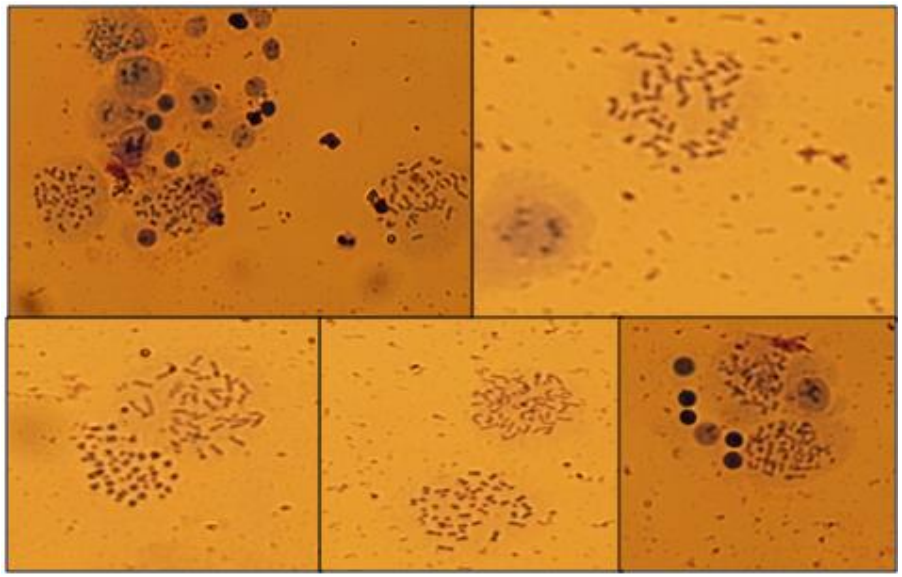
Day1: Treatment Strategy
Incubation for 68h at 37˚C



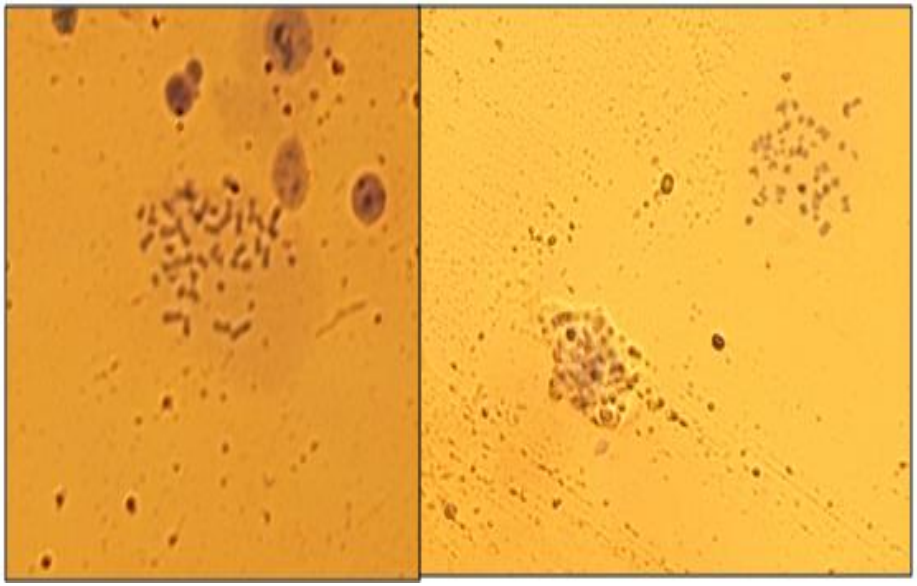
Day2: Metaphase arrest and Harvesting of Cells



Day 3
Slides preparation



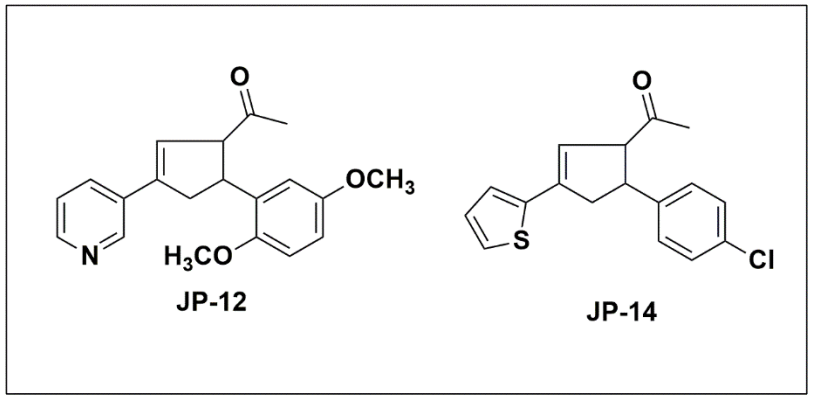
For PHA



For compound C1

Conclusions

- Absolute cell count showed that JP-12 and JP-14 had increased cell count as compared to the control and PHA.
- Protein concentration was also found to be considerably higher in the synthesized compounds in comparison to PHA.
- Similar to PHA, JP-12 was able to bring about the metaphase arrest at the 68th hour.
- From this, it can be concluded that, JP-12 and JP-14, the synthesized compounds show cell proliferation as PHA and have the potential to replace PHA.



Future Prospective

- It would be more helpful to determine the cell type that is more susceptible to cell proliferation
- Studies should be undertaken to delve into the mechanism of how the synthesized compounds bring about this effect.

References

1. Hamelryck, T. W., Dao-Thi, M. H., Poortmans, F., Chrispeels, M. J., Wyns, L., Loris, R. (1996). The crystallographic structure of phytohemagglutinin-L. *The Journal of Biological Chemistry*. *271* (34): 20479–20485.
2. Mire-Sluis,A. R., Wickremasinghe, R. G., Hoffbrand,A. V., Timms, A. M. Francis, G. E., (1987). Human T lymphocytes stimulated by phytohaemagglutinin undergo a single round of cell division without a requirement for interleukin-2 or accessory cells. *Immunology. 60*(1), 7–12.
3. Serke,S., Serke,M., Brudler, O. (1987). Lymphocyte activation by phytohaemagglutinin and pokeweed mitogen: Identification of proliferating cells by monoclonal antibodies. *99* (2), 167-172.

Acknowledgements

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