www.ijrps.pharmascope.org

ISSN: 0975-7538
Research Article

An attempt to reduce the side effects of colchicine

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ABSTRACT

Cancer is basically a disorder characterized by uncontrolled division of cells of any particular tissue or organ. This uncontrolled division of cells occurs by mitosis and one approach to treat cancer is to block this mitotic division of cancer cells by using antimitotic drugs. Among the drugs used so far in this approach, the antimitotic drugs which particularly target microtubules were found to be the most effective. But these drugs cannot cure cancer completely because cancer cells become resistant to these drugs if used for a long time. But colchicine is a drug which irreversibly binds to microtubules and leads to mitotic arrest, thus can overcome the problem of drug resistance because of its irreversible binding to microtubules. Even after having this advantage colchicine is not used in cancer treatment because of its severe side effects which sometimes can even lead to death. These side effects are mainly due to the lack of specificity of action, because of which it causes mitotic arrest in both normal body cells and cancer cells. We can overcome this problem by reducing the concentration of colchicine to such an extent at which it acts mainly on cancer cells with minimal effect on normal body cells. Because microtubules are prominent in rapidly dividing cells when compared to slowly dividing normal body cells, rapidly dividing cells are more susceptible to colchicine in low concentrations. In this study we tried to find out the lowest possible concentration of colchicine which can effectively block the division of rapidly dividing cells with minimal or no effect on slowly dividing cells.

Keywords: Colchicine; Microtubules; Yeast; Colorimeter; Incubation.

1. INTRODUCTION

Colchicine is a powerful antimitotic agent obtained from the plant colchicum autumnale. It blocks mitosis by irreversibly binding to microtubules (Amos et al., 1974) at a specific binding site called as the colchicine site(Joseph Bryan et al., 1972). By binding to microtubules it induces microtubule depolymerization at high concentrations and it suppress microtubule dynamics at low concentrations and blocks the formation of spindle fibers(Campbell et al., 2005) by the union of microtubules. It is a known fact that spindle fibers are responsible for the segregation of chromosomes in mitosis and when spindle fibers are not formed chromosomes cannot be segregated, thus colchicine ultimately brings mitosis to a halt when it is still in metaphase which eventually leads to apoptotic cell death(Jordan et al., 1999) because cell cannot survive in the state of arrested division.

Along with the regular antimitotic actions colchicine is also capable of simulating the cellular changes induced by x rays. Hence called as radiomimetic drug (British Medical Journal., 1950). It can also overcome the problem of drug resistance because of its irreversible binding to microtubules.

Due to all these properties colchicine should prove to be the most effective anti cancer agent. Instead it is not at all used in the treatment of cancer because of its severe side effects which sometimes can be deadly. These side effects can be heamatological (Rowinsky et al., 1997) and neurological (Lobert et al.,1997) and can range from nausea and vomiting to central neurotoxicities and myelosuppression and can sometimes lead to death.

These side effects are mainly because of the action of colchicine on normal cells along with the cancer cells. The effects like myelosuppression are mainly due to the action of colchicine on rapidly dividing haemopoietic cells.

These side effects can be reduced by reducing the dose of colchicine to lowest possible dose at which it is effective mainly on rapidly dividing cells like cancer cells. Colchcine when administered in low doses will mainly target the rapidly dividing cells, because colchicine targets microtubules and microtubules are formed in the cells only during the cell division. So, the rapid the cell divides more it is susceptible to colchicine. Generally the rate of division of the cancer cells is very high

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Received on: 01-06-2010
Revised on: 18-09-2010
Accepted on: 05-10-2010

when compared to any other cells in the body, because of which cancer cells are more susceptible to colchicine in low concentrations than normal body cells.

In this study we have tried to study the effect of colchicine on yeast cells growing in two similar culture mediums of which one was incubated in optimum temperature [26°c] conditions to simulate rapidly dividing cancer cells and other culture was maintained at a low temperature [10°c] to simulate normal body cells because yeast grows slowly at reduced temperatures. We also tried to determine the effect of dose on mitotic arrest when colchicine was administered in low concentrations.

Reasons for choosing yeast as a model organism:

- Yeast is a eukaryotic organism and the cells present in the human body are also eukaryotic in nature.
- The cell cycle regulatory mechanisms found in yeast cells are very similar to that found in human cells.
- It is very easy to culture yeast in the laboratory when compared to other microbes.
- All the stages of mitosis that are seen in humans can be seen in yeasts.
- Because yeast divides very rapidly they can be used to represent cancer cells.

2. MATERIALS AND METHODS

2.1. yeast cells[Saccharomyces cerevisiae]: we bought yeast cells(in dehydrated form) from Hainan Zhonghong Yeast Co., Ltd located at No.2 Shimao East Haikou Hainan China.

2.2. Preparation of culture media (Both potato dextrose agar and potato dextrose broth)

The dextrose required for preparation of media*(*Atlas R.M, Lawrence et al., 1997) was bought from S.G.R Engineers located at 69b/1, Ram Nagar 2nd Main Road, Peravallur, Jawahar Nagar Post, Chennai - 600082, Tamil Nadu, India and agar required for the preparation of media was bought from Marine chemicals located at Deepa Building, Santo Gopalan Road, Chullikal, Kerala India.

Potato dextrose broth was prepared by first preparing a potato infusion by boiling 300gm of sliced (washed but unpeeled) potatoes in water for 30 minutes and then decanting the broth through cheesecloth. Then we added distilled water to it to make up the volume to one litre. Then we have added 20g of dextrose to it and the broth thus obtained was sterilized by autoclaving at 15psi for 15 minutes. This broth was then poured in culture tubes.

Potato dextrose agar was prepared by adding agar to the broth before autoclaving it and this medium containing agar [solidifying agent] was poured into Petri-plates when it was still hot and was allowed to cool down and solidify to form culture plates. These culture plates were later used for counting of yeast cells by plate count method.

2.3. Preparation of colchicine dilutions

Colchicine required for the experiment was bought from Pharmanza India private limited situated at 70/1, Gidc Estate, Kansari, Khambhat - 388630, Gujarat. Then we prepared diluted suspension of colchicine by suspending [it cannot be dissolved to make a solution because 10mg of colchicine is soluble in 1ml of water] 10gm of colchicine in 10ml of distilled water to obtain a colchicine suspension of concentration 1:1. Then we have taken 1ml of this suspension and diluted it to 10ml with distilled water to obtain a suspension of concentration 1:10, in this way we continued the dilution serially and prepared solutions [starting from concentration of 1:100 we can obtain clear solutions] until we obtained a concentration of $1:10^{10}$.

2.4. Inoculation of culture media with yeast cells

The yeast cells obtained in dehydrated form were hydrated by adding water and were allowed to grow in the broth culture. From this broth culture a single yeast cells was isolated using a micro pipette and was inoculated in a different broth culture tube and the same method was followed for inoculating all the culture tubes. These culture tubes were then incubated for the growth of yeast cells.

2.5. Turbidimetric estimation of cell numbers

For the estimation of turbidity we bought colorimeter from Chennai laboratories situated at 4a, Gowri Chitra Garden, Arcat Road, Near Vadapalani Bus Dipo, Chennai, Tamil Nadu. By using colorimeter we first measured the turbidity of potato dextrose broth which was not inoculated with yeast cells. Turbidity was measured in terms of optical density at a wavelength of 420nm. Then we found the optical density of broth cultures which were inoculated with yeast cells and incubated to facilitate the growth of yeast cells . For this we first stirred the broth cultures well for uniform distribution of cells throughout the culture tube and then we have withdrawn a 2ml of the broth and diluted it to about 10 times its volume with distilled water and measured the optical density. As the number of cells in the liquid broth increased the optical density of the liquid broth also increased and vice versa.

It is very important to dilute the liquid broth before turbidimetry because the liquid broth is very turbid if used as it is and because of this, the sensitivity of the instrument may get reduced and it may not detect a small change in the optical density.

2.6. Estimation of cell numbers by plate count method

In this method*(*M W LeChevallier et al., 1980) a small sample of 2ml was withdrawn from 20ml of broth cul-

ture containing the yeast cells and it was diluted in the range of 1:10 to 1:1,00,000 by serial dilution method and 1ml of this diluted solution was spread on the culture plate [known as spread plate technique] prepared in the earlier step and was incubated for 12 hours and during this period each cell developed into a colony and the number of colonies formed is equal to number of cells and these colonies formed were counted,thus we obtained the number of cells present in 1ml of diluted solution which we multiplied with total volume of diluted solution and we then multiplied this value with the dilution factor and obtained the total number of cells present in undiluted potato dextrose broth culture. But generally a single cell may not be spread over the culture plate, thus we cannot say that the colony has formed from a single cell. Hence these are called as colony forming units [CFU'S] instead of cells.

2.7. Treatment of broth culture with colchicine

The diluted solutions of colchicine prepared earlier were used for this purpose and first of all a dilution of 1:1 was added in six different doses of 1ml, 2ml, 4ml, 6ml, 8ml, 10ml and each dose was added to a separate culture tube and mixed well, thus keeping the dilution constant but changing the dose. In the same manner other culture tubes were also treated with remaining dilutions of colchicine. As mentioned earlier a total of 10 different dilutions were prepared and each dilution was administered in six different doses thus the effect of colchicine was studied on sixty six different culture tubes which were incubated at 10°c and these tubes were marked as SET-A.

Same procedure was followed for another sixty six culture tubes which were incubated at 26°c and were marked as SET-B.

3. RESULTS AND DISCUSSIONS

3.1. Plotting of calibration curve

Initially potato dextrose broth was taken in 12 culture tubes numbered from 1-12 and each one of the 12 culture tubes were inoculated with a single Saccharomyces cerevisiae cell isolated under a high power microscope using a micro pipette. These culture tubes were then incubated at 26°c and each test tube was incubated for different time periods ranging from one hour to 12 hours and number of cells present in each culture tube was counted by plate count method using spread plate technique and the same culture tubes were also subjected to turbidimetric analysis [for this the broth containing yeast cells was diluted to 10 times the original value to get proper readings in the colorimeter because the potato dextrose broth as such is

Table 1: Optical density values found at regular intervals during incubation

S.No	Duration of incubation	Number of cells(CFU'S) counted by plate count method	Optical density of liquid broth after diluting it ten times its original volume
1	Culture tube not inoculated with yeast cell	–	0.09
2	1 hour	Could not be estimated even without dilution because of very less cells	0.09
3	2 hours	Could not be estimated even without dilution because of very less cells	0.09
4	3 hours	Could not be estimated even without dilution because of very less cells	0.10
5	4 hours	Number of cells is found to be 300 counted at 1:10 dilution	0.13
6	5 hours	Number of cells is found to be 1,000 at 1:10 dilution	0.19
7	6 hours	Number of cells is found to be 4,000 counted at 1:100 dilution	0.25
8	7 hours	Number of cells is found to be 16,000 counted at 1:100 dilution	0.42
9	8 hours	Number of cells is found to be 10^4×6 counted at 1:1000dilution	0.63
10	9 hours	Number of cells is found to be 10^5×2 counted at 1:10,000 dilution	0.85
11	10 hours	Number of cells is found to be 10^6 counted at 1:10,000dilution	1.21
12	11 hours	Number of cells is found to be 10^6×4 counted at 1:100,000 dilution	1.82
13	12 hours	Number of cells is found to be 10^7 counted at 1:100,000 dilution	2.31

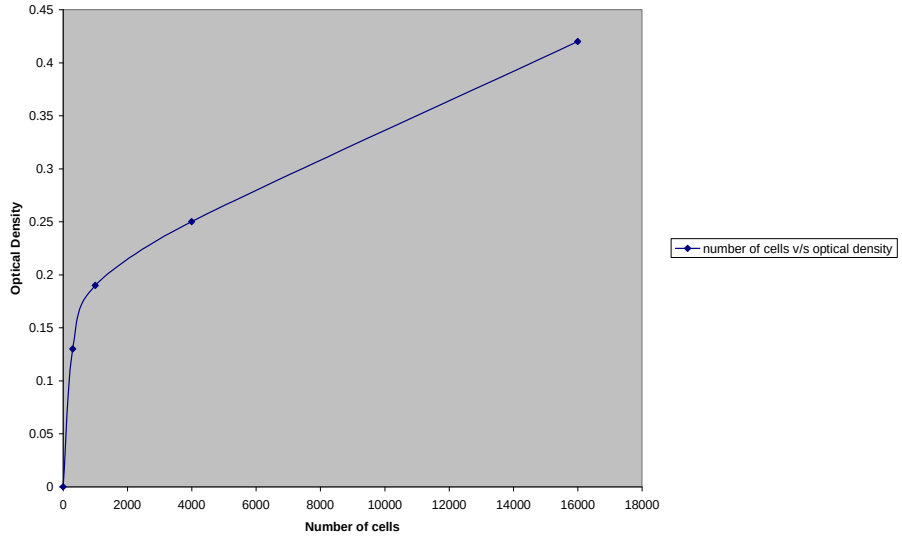

Figure 1: Calibration curve used to find number of cells upto16,000

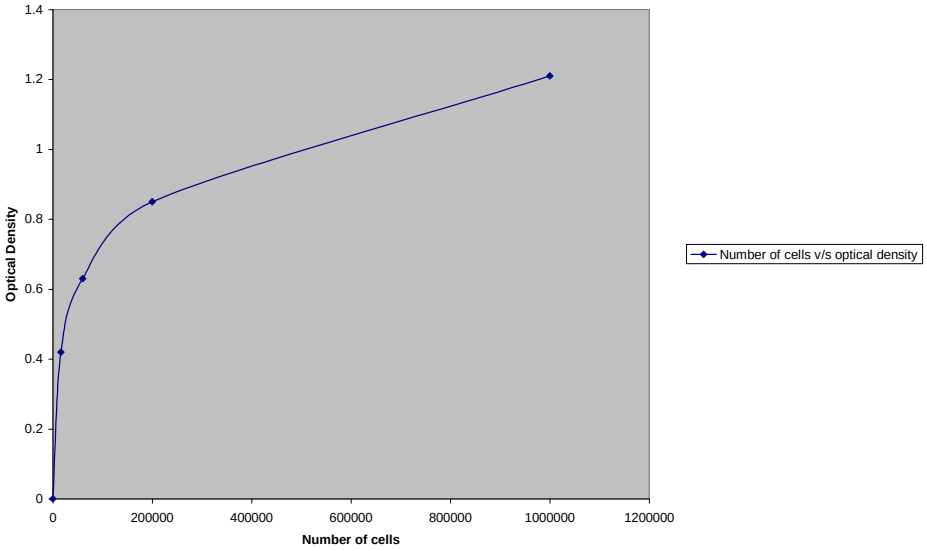

Figure 2: Calibration curve used to find number of cells in the range of 16,000 – 10,00,000

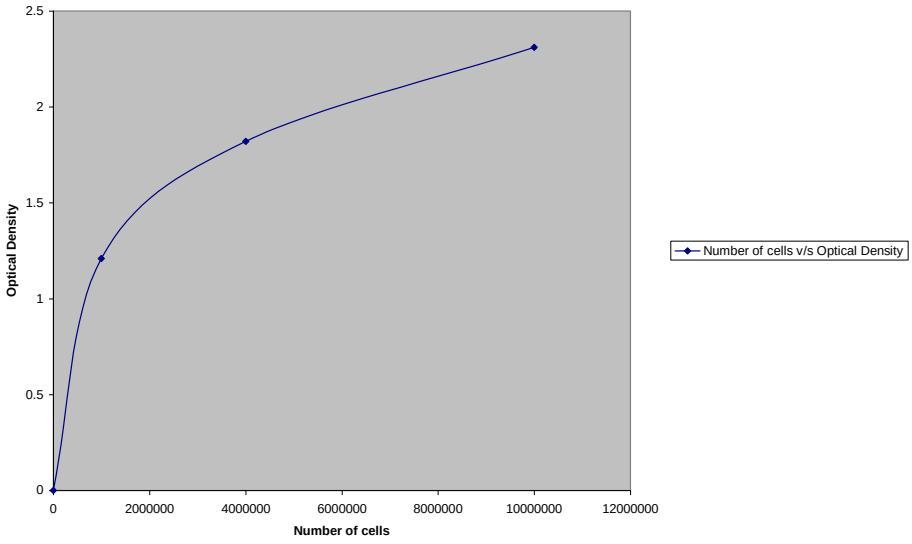

Figure 3: Calibration curve used to find number of cells in the range of 10,00,000 – 1,00,00,00

Table 2: Effect of different dilutions of colchicine on division of yeast cells maintained at 10°c and 26°C

S.No	Set A or B	Dilution of colchicine used	Dose in ml	Optical density	Number of cells before treatment with colchicine	Optical density	Number of cells after treatment with colchicine
1	Set A	1:1	1	0.28	6,000	0.28	6,000
2	Set A	1:1	2	0.28	6,000	0.28	6,000
3	Set A	1:1	4	0.28	6,000	0.28	6,000
4	Set A	1:1	6	0.28	6,000	0.28	6,000
5	Set A	1:1	8	0.28	6,000	0.28	6,000
6	Set A	1:1	10	0.28	6,000	0.28	6,000
7	Set A	1:10	1	0.28	6,000	0.28	6,000
8	Set A	1:10	2	0.28	6,000	0.28	6,000
9	Set A	1:10	4	0.28	6,000	0.28	6,000
10	Set A	1:10	6	0.28	6,000	0.28	6,000
11	Set A	1:10	8	0.28	6,000	0.28	6,000
12	Set A	1:10	10	0.28	6,000	0.28	6,000
13	Set A	1:100	1	0.28	6,000	0.28	6,000
14	Set A	1:100	2	0.28	6,000	0.28	6,000
15	Set A	1:100	4	0.28	6,000	0.28	6,000
16	Set A	1:100	6	0.28	6,000	0.28	6,000
17	Set A	1:100	8	0.28	6,000	0.28	6,000
18	Set A	1:100	10	0.28	6,000	0.28	6,000
19	Set A	1:1000	1	0.28	6,000	0.28	6,000
20	Set A	1:1000	2	0.28	6,000	0.28	6,000
21	Set A	1:1000	4	0.28	6,000	0.28	6,000
22	Set A	1:1000	6	0.28	6,000	0.28	6,000
23	Set A	1:1000	8	0.28	6,000	0.28	6,000
24	Set A	1:1000	10	0.28	6,000	0.28	6,000
25	Set A	$1:10^4$	1	0.28	6,000	0.28	6,000
26	Set A	$1:10^4$	2	0.28	6,000	0.28	6,000
27	Set A	$1:10^4$	4	0.28	6,000	0.28	6,000
28	Set A	$1:10^4$	6	0.28	6,000	0.28	6,000
29	Set A	$1:10^4$	8	0.28	6,000	0.28	6,000
30	Set A	$1:10^4$	10	0.28	6,000	0.28	6,000
31	Set A	$1:10^5$	1	0.28	6,000	0.29	6,000-7000
32	Set A	$1:10^5$	2	0.28	6,000	0.29	6,000-7000
33	Set A	$1:10^5$	4	0.28	6,000	0.29	6,000-7000
34	Set A	$1:10^5$	6	0.28	6,000	0.28	6,000
35	Set A	$1:10^5$	8	0.28	6,000	0.28	6,000
36	Set A	$1:10^5$	10	0.28	6,000	0.28	6,000
37	Set A	$1:10^6$	1	0.28	6,000	0.305	8,000
38	Set A	$1:10^6$	2	0.28	6,000	0.305	8,000
39	Set A	$1:10^6$	4	0.28	6,000	0.29	6000-7000
40	Set A	$1:10^6$	6	0.28	6,000	0.29	6000-7000
41	Set A	$1:10^6$	8	0.28	6,000	0.28	6,000
42	Set A	$1:10^6$	10	0.28	6,000	0.28	6,000
43	Set A	$1:10^7$	1	0.28	6,000	0.305	8,000
44	Set A	$1:10^7$	2	0.28	6,000	0.305	8,000
45	Set A	$1:10^7$	4	0.28	6,000	0.305	8,000
46	Set A	$1:10^7$	6	0.28	6,000	0.3	7,000-8,000
47	Set A	$1:10^7$	8	0.28	6,000	0.3	7000-8,000
48	Set A	$1:10^7$	10	0.28	6,000	0.3	7000-8,000
49	Set A	$1:10^8$	1	0.28	6,000	0.31	8,000-9,000
50	Set A	$1:10^8$	2	0.28	6,000	0.31	8,000-9,000

S.No	Set A or B	Dilution of colchicine used	Dose in ml	Optical density	Number of cells before treatment with colchicine	Optical density	Number of cells after treatment with colchicine
51	Set A	$1:10^8$	4	0.28	6,000	0.31	8,000-9,000
52	Set A	$1:10^8$	6	0.28	6,000	0.31	8,000-9,000
53	Set A	$1:10^8$	8	0.28	6,000	0.305	8,000
54	Set A	$1:10^8$	10	0.28	6,000	0.305	8,000
55	Set A	$1:10^9$	1	0.28	6,000	0.31	8,000-9,000
56	Set A	$1:10^9$	2	0.28	6,000	0.31	8,000-9,000
57	Set A	$1:10^9$	4	0.28	6,000	0.31	8,000-9,000
58	Set A	$1:10^9$	6	0.28	6,000	0.31	8,000-9,000
59	Set A	$1:10^9$	8	0.28	6,000	0.31	8,000-9,000
60	Set A	$1:10^9$	10	0.28	6,000	0.31	8,000-9,000
61	Set A	$1:10^{10}$	1	0.28	6,000	0.325	9,000-10,000
62	Set A	$1:10^{10}$	2	0.28	6,000	0.325	9,000-10,000
63	Set A	$1:10^{10}$	4	0.28	6,000	0.325	9,000-10,000
64	Set A	$1:10^{10}$	6	0.28	6,000	0.325	9,000-10,000
65	Set A	$1:10^{10}$	8	0.28	6,000	0.325	9,000-10,000
66	Set A	$1:10^{10}$	10	0.28	6,000	0.325	9,000-10,000
67	Set B	1:1	1	1.21	10^6	1.21	10^6
68	Set B	1:1	2	1.21	10^6	1.21	10^6
69	Set B	1:1	4	1.21	10^6	1.21	10^6
70	Set B	1:1	6	1.21	10^6	1.21	10^6
71	Set B	1:1	8	1.21	10^6	1.21	10^6
72	Set B	1:1	10	1.21	10^6	1.21	10^6
73	Set B	1:10	1	1.21	10^6	1.21	10^6
74	Set B	1:10	2	1.21	10^6	1.21	10^6
75	Set B	1:10	4	1.21	10^6	1.21	10^6
78	Set B	1:10	6	1.21	10^6	1.21	10^6
79	Set B	1:10	8	1.21	10^6	1.21	10^6
80	Set B	1:10	10	1.21	10^6	1.21	10^6
80	Set B	1:100	1	1.21	10^6	1.21	10^6
81	Set B	1:100	2	1.21	10^6	1.21	10^6
82	Set B	1:100	4	1.21	10^6	1.21	10^6
83	Set B	1:100	6	1.21	10^6	1.21	10^6
84	Set B	1:100	8	1.21	10^6	1.21	10^6
85	Set B	1:100	10	1.21	10^6	1.21	10^6
86	Set B	1:1000	1	1.21	10^6	1.21	10^6
87	Set B	1:1000	2	1.21	10^6	1.21	10^6
88	Set B	1:1000	4	1.21	10^6	1.21	10^6
89	Set B	1:1000	6	1.21	10^6	1.21	10^6
90	Set B	1:1000	8	1.21	10^6	1.21	10^6
91	Set B	1:1000	10	1.21	10^6	1.21	10^6
91	Set B	$1:10^4$	1	1.21	10^6	1.21	10^6
92	Set B	$1:10^4$	2	1.21	10^6	1.21	10^6
93	Set B	$1:10^4$	4	1.21	10^6	1.21	10^6
94	Set B	$1:10^4$	6	1.21	10^6	1.21	10^6
95	Set B	$1:10^4$	8	1.21	10^6	1.21	10^6
96	Set B	$1:10^4$	10	1.21	10^6	1.21	10^6
97	Set B	$1:10^5$	1	1.21	10^6	1.21	10^6
98	Set B	$1:10^5$	2	1.21	10^6	1.21	10^6
99	Set B	$1:10^5$	4	1.21	10^6	1.21	10^6
100	Set B	$1:10^5$	6	1.21	10^6	1.21	10^6
101	Set B	$1:10^5$	8	1.21	10^6	1.21	10^6
102	Set B	$1:10^5$	10	1.21	10^6	1.21	10^6
103	Set B	$1:10^6$	1	1.21	10^6	1.41	$10^6 \times 2$
104	Set B	$1:10^6$	2	1.21	10^6	1.41	$10^6 \times 2$

S.No	Set A or B	Dilution of colchicine used	Dose in ml	Optical density	Number of cells before treatment with colchicine	Optical density	Number of cells after treatment with colchicine
105	Set B	$1:10^6$	4	1.21	10^6	1.21	10^6
106	Set B	$1:10^6$	6	1.21	10^6	1.21	10^6
107	Set B	$1:10^6$	8	1.21	10^6	1.21	10^6
108	Set B	$1:10^6$	10	1.21	10^6	1.21	10^6
109	Set B	$1:10^7$	1	1.21	10^6	1.69	$10^6 \times 3$
110	Set B	$1:10^7$	2	1.21	10^6	1.41	$10^6 \times 2$
111	Set B	$1:10^7$	4	1.21	10^6	1.35	$10^6 + 8 \times 10^5$
112	Set B	$1:10^7$	6	1.21	10^6	1.21	10^6
113	Set B	$1:10^7$	8	1.21	10^6	1.21	10^6
114	Set B	$1:10^7$	10	1.21	10^6	1.21	10^6
115	Set B	$1:10^8$	1	1.21	10^6	1.82	$10^6 \times 4$
116	Set B	$1:10^8$	2	1.21	10^6	1.69	$10^6 \times 3$
117	Set B	$1:10^8$	4	1.21	10^6	1.69	$10^6 \times 3$
118	Set B	$1:10^8$	6	1.21	10^6	1.41	$10^6 \times 2$
119	Set B	$1:10^8$	8	1.21	10^6	1.35	$10^6 + 8 \times 10^5$
120	Set B	$1:10^8$	10	1.21	10^6	1.21	10^6
121	Set B	$1:10^9$	1	1.21	10^6	1.90	$10^6 \times 5$
122	Set B	$1:10^9$	2	1.21	10^6	1.69	$10^6 \times 3$
123	Set B	$1:10^9$	4	1.21	10^6	1.69	$10^6 \times 3$
124	Set B	$1:10^9$	6	1.21	10^6	1.69	$10^6 \times 3$
125	Set B	$1:10^9$	8	1.21	10^6	1.41	$10^6 \times 2$
126	Set B	$1:10^9$	10	1.21	10^6	1.34	$10^6 + 7 \times 10^5$
127	Set B	$1:10^{10}$	1	1.21	10^6	2.05	$10^6 \times 7$
128	Set B	$1:10^{10}$	2	1.21	10^6	1.99	$10^6 \times 6$
129	Set B	$1:10^{10}$	4	1.21	10^6	1.99	$10^6 \times 6$
130	Set B	$1:10^{10}$	6	1.21	10^6	1.90	$10^6 \times 5$
131	Set B	$1:10^{10}$	8	1.21	10^6	1.82	$10^6 \times 4$
132	Set B	$1:10^{10}$	10	1.21	10^6	1.82	$10^6 \times 4$

very turbid and if we use it directly the sensitivity of the instrument is lost] using a colorimeter at a wavelength of 420nm.

Both the number of cells [counted by plate count method] and optical density values varied according to the duration of incubation and these observations are tabulated in table-1 and then the turbidimetric values were plotted against the number of cells and a calibration curve was obtained by using which we could turbidimetrically estimate the number of cells in the broth culture. Due to broad range of data three calibration curves were plotted instead of one, so that the graph will be clear.

Note:- Unlike calibration curve for concentration versus reading in the instrument, we cannot get a linear calibration curve for a graph of number of cells versus optical density. Because the increase in number of cells with increasing duration of incubation is not linear instead it is exponential. Thus we cannot apply linear regression to this graph.

3.2. Estimation of effect of temperature on growth of yeast cells

Saccharomyces cerevisiae was cultured on potato dextrose broth taken in two different culture tubes of 20ml capacity. One culture tube was incubated at a temperature of 26°c and other was incubated at temperature of 10°c for 12 hours. The number of cells in each culture tube were then estimated turbidimetrically [by using calibration curve] after 12 hours and the number of cells in the tube incubated at 10°c was found to be around 10^4 and the number of cells in the culture tube which was incubated at 26°c was found to be around 10^7 thus at reduced temperature the growth rate of yeast cells is found to be less.

3.3. Effect of various concentrations of colchicine on slowly and rapidly dividing cells

The yeast cells which are incubated at 10°c divide slowly and represent normal body cells and the yeast cells which are incubated at 26°c divide at a much faster rate and represent cancer cells.

Potato dextrose broth was taken in 132 culture tubes of 20ml capacity and the culture tubes were numbered from 1-132. All culture tubes were then inoculated with Saccharomyces cerevisiae. Then the culture tubes which are numbered from 1-66 were incubated at 10°c and were considered as set A and the culture tubes which are numbered from 66-132 were incubated at 26°c and were considered as set B. Both the sets of culture tubes were incubated for ten hours after which the number of cells in the culture tubes were esti-

mated turbidimetrically with the help of the calibration curve. Then all of these culture tubes were treated with colchicine dilutions which were in the range of 1:1 to $1:10^{10}$ and each dilution was administered in six different doses of 1ml, 2ml, 4ml, 6ml, 8ml, 10ml and the culture tubes were again incubated for another two hours. The number of cells in the culture tubes was estimated and compared with the number of cells in the culture tube that is not treated with colchicine [the number of cells in the culture tubes not treated with colchicine and incubated at 10°c is around 10^4 and the number of cells in the culture tube which was incubated at 26°c is around 10^7] to study the extent of mitotic arrest caused by different concentrations of colchicine on rapidly dividing cells [set B] and slowly dividing cells [set A]. The results of this test are tabulated in table-2.

From the results we can see that at very high concentrations the extent of mitotic arrest was same in both slowly dividing cells and rapidly dividing cells. But as the concentration of colchicine decreased it was more effective against rapidly dividing cells than slowly dividing cells. This proves the point that the microtubules are more prominent in rapidly dividing cells than slowly dividing cells. Because of this reason, at low concentrations of colchicine mitotic arrest is more in rapidly dividing cells because of which at the end of incubation period the cell numbers were less in case of rapidly dividing cells when compared to slowly dividing cells.

4. CONCLUSIONS

In this experiment we tried to artificially simulate the behaviour of cancer cells and normal body cells and studied the effect of colchicine on these cells, by administering it in different concentrations and tried to find out the lowest concentration of colchicine at which it can effectively cause mitotic arrest in cancer cells without effecting normal cells. We also tried to determine the effect of dose on effectiveness of colchicine even in lowest concentrations.

In this experiment we found that colchicine, if given in high concentration is lethal to both slowly dividing cells and rapidly dividing cells which represent normal body cells and cancer cells respectively.

But when the concentration was reduced to as low as $1:10^{10}$, the overall intensity of action was less, but even in this case the intensity of action was more on rapidly dividing cells when compared to slowly dividing cells. This makes it clear that microtubules are more prominent in rapidly dividing cells than slowly dividing cells, but it is clear from the experiment that even at this concentration there was a considerable impact of dose on the cells because even in this low concentration of $1:10^{10}$ mitotic arrest was more when a high dose [10ml] was administered and mitotic arrest was less when a low dose [1ml] was administered.

But the lowest concentration of colchicine which was found to be most effective on yeast cells was at a dilution of $1:10^9$ at a dose of 10ml , because at this concentration the anti mitotic activity was very high on rapidly dividing cells and effect was very less on slowly dividing cells because the number of rapidly dividing cells after 2 hours incubation post the treatment with colchicine was 1.7 million and the normal value is 10 million [this value is for 12 hours of incubation because the test cultures were incubated for 10 hours before treatment with colchicine and for 2 hours after treatment] which shows a considerable mitotic arrest. Whereas in case of slowly dividing cells the number of cells after 2 hours of incubation post the treatment with colchicine was in between 8000-9000 and the normal value is around 10,000 and this clearly shows that antimitotic activity of colchicine was more on rapidly dividing cells.

Even though these observations cannot be applied directly for dose determination for human beings, these can be used as a base for testing in the animal models. But the main problem with the administration in such low doses in animals is that the volume of distribution is very high in animals and because of this only a very low amount of colchicine actually reaches the cancer cells. If we can find ways to overcome this problem, we will be able to use colchicine in the treatment of cancer.

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