Articles :: Cancer Treatment

Foreword:
This article is a report into a study of the use of rye grass extract (as found in Oralmat) for the treatment of various forms of cancer. The study was carried out in controlled conditions, with the rye grass extract being supplied by Schumacher Pharmaceuticals, the manufacturers of Oralmat.

In all cases, the rye grass extract was seen to be active against cancer, inhibiting the proliferation of cancer cells (slowing the spread of cancer cells).

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Evaluation of Rye Extract for Anti-neoplastic Activity

SUBJECT
Evaluation of anti-neoplastic properties of Rye Extract.

SUMMARY
The anti-neoplastic properties of the Rye Extract were examined using MTT assay.

CONCLUSION
The Rye extract inhibited cell proliferation in all the cancer cell lines tested. The inhibitory response to the Rye extract differed according to the cell line tested. The inhibitory response was marked in the PLC, MCF7 and K562 lines. The Rye extract inhibited cell proliferation maximally in the different cell lines at the concentrations indicated as shown below.

Cell Line	% Inhibition	Concentration of Rye extract
PLC	89.30	0.10%
MCF7	89.00	1.00%
A498	52.30	1.00%
K562	78.13	0.10%
Hep 3B	55.00	0.10%

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INTRODUCTION
A sample of Rye extract was supplied to the Department of Pharmacy by Schumacher Pharmaceuticals. The extract was tested for anti-neoplastic activity by the MTT assay in a number of cell lines. The activity of the Rye extract was compared to vincristine, vinblastine and cycloheximide.

MATERIALS AND METHODS

Reagents and chemicals

Rye extract	Schumacher Pharmaceuticals
MTT	Sigma-Aldrich
Dimethyl Sulphoxide (DMSO)	BDH
RPMI 1640	Highveld Biologicals
Fetal Calf Serum	Highveld Biologicals
PLC Cell Line (Liver Carcinoma)	National Institutes of Health
K562 Cell Line (CML)	National Institutes of Health
A498 Cell Line (Renal Carcinoma)	National Institutes of Health
MCF7 Cell Line (Breast Adenocarcinoma)	National Institutes of Health
Vincristine	Pharmacia and Upjohn
Vinblastine	Pharmachemic
Cycloheximide	Sigma-Aldrich

Equipment and Apparatus

Beckman Refrigerated Centrifuge Model GS15R

Nikon Microscope

Nueber Counting Chamber

Multiskan RC Microplate Reader

Methods

The MTT microculture teterazolium assay by Mossmann (1983) was adapted to study the anti-neoplastic activity of the Rye extract.

1.1.1 Assay Procedure

1.1.1.1 Cell Preparation

2.3.1.1 a) **Liver Carcinoma (PLC)**
PLC cells from a culture flask were trypisinised and centrifuged for 10 minutes at 1100RPM in a Beckman GS15R centrifuge. The cell pellet was resuspended in 2ml of culture medium (RPMI 1640 medium with 5% Fetal Calf Serum with Antibiotics). A cell count was performed using a Nueber hemocytometer and the cell suspension was diluted in the culture medium to give a cell number of $1X10^5$ cells/ml. The cell suspension (100µl) was transferred into a 96 well microlitre plate using a multichannel pipette.

2.3.1.1. b) **Chronic Myclogenous Leukaemia (K562)**
K562 cells from a culture flask were trypinised and centrifuged for 10 minutes at 1100RPM in a Beckman GS15R centrifuge. The cell pellet was resuspended in 2ml of culture medium (RPMI 1640 medium with 10% Fetal Calf Serum with Antibiotics). A cell count was performed using a Nueber hemocytometer and the cell suspension was diluted in the culture medium to give a cell number of $1X10^5$ cells/ml. The cell suspension (100µl) was transferred into a 96 well microlitre plate using a multichannel pipette.

2.3.1.1 c) **Renal Cancer (A498)**
A498 cells from a culture flask were trypinised and centrifuged for 10 minutes at 1100RPM in a Beckman GS15R centrifuge. The cell pellet was resuspended in 2ml of culture medium (EMEM medium with 10% Fetal Calf Serum without Antibiotics). A cell count was performed using a Nueber hemocytometer and the cell suspension was diluted in the culture medium to give a cell number of 1X105 cells/ml. The cell suspension (100 µl) was transferred into a 96 well microlitre plate using a multichannel pipette.

2.3.1.1. d) **Breast Cancer (MCF7)**

MCF7 cells from a culture flask were trypinised and centrifuged for 10 minutes at 1100RPM in a Beckman GS15R centrifuge. The cell pellet was resuspended in 2ml of culture medium (EMEM medium with 5% Fetal Calf Serum with Insulin and Sodium Pyruvate and Antibiotics). A cell count was performed using a Nueber hemocytometer and the cell suspension was diluted in the culture medium to give a cell number of $1X10^5$ cells/ml. The cell suspension (100 µl) was transferred into a 96 well microlitre plate using a multichannel pipette.

2.3.1.1. e) **Liver Cancer (Hep 3B)**
MCF7 cells from a culture flask were trypinised and centrifuged for 10 minutes at 1100RPM in a Beckman GS15R centrifuge. The cell pellet was resuspended in 2ml of culture medium (EMEM medium with 2.5% Fetal Calf Serum with Antibiotics). A cell count was performed using a Nueber hemocytometer and the cell suspension was diluted in the culture medium to give a cell number of $1X10^5$ cells/ml. The cell suspension (100 µl) was transferred into a 96 well microlitre plate using a multichannel pipette.

The positive controls used consisted of 100 µl cells with 25 µl of a 1µg/ml solution of vincristine (1µg/ml), Vinblastine (1µg/ml) and Cycloheximide (1µg/ml). Further controls were 100µl cells only and 100µl cells with 25µl of a 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.10% mixture of 9.96% and 0.040% of ethanol and ascorbic acid respectively. The test solutions consisted of 100µl cells with 25µl of a 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.10% Rye extract solution supplied.

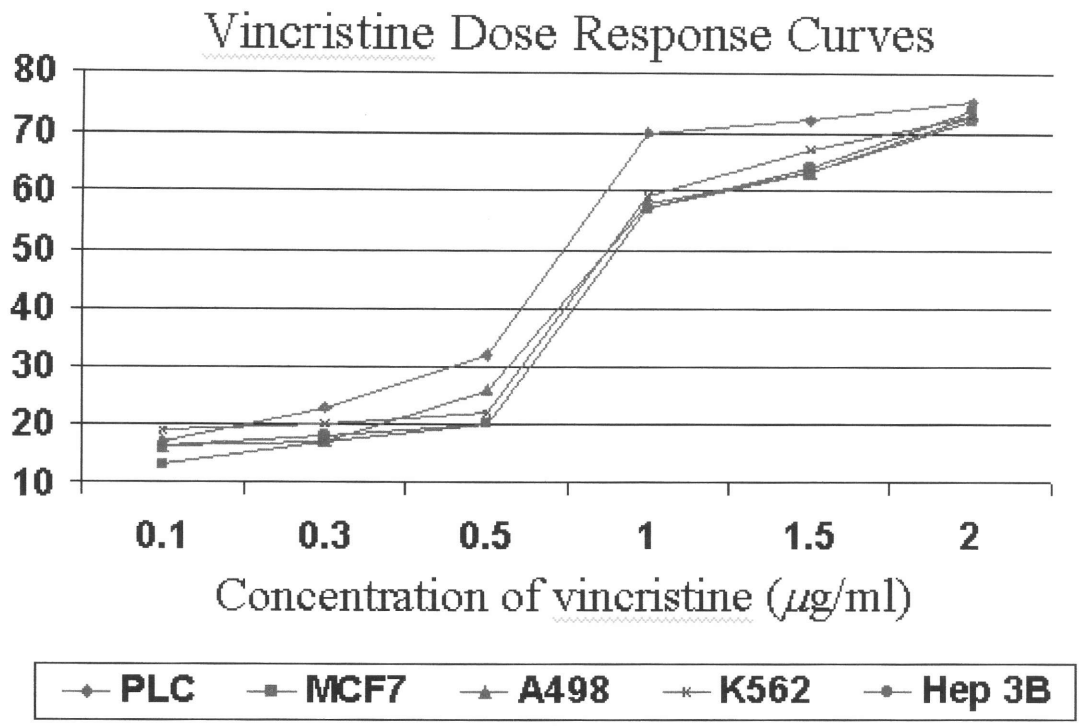
2.3.1.2 ***MTT Assay***
The plates were incubated at 37°C in an atmosphere 5% CO2 in a humidified incubator for 48 hours. At the end of the 48 hours period the plates were centrifuged and the supernatants were aspirated from the wells and 100µl of culture medium with 10µl of a 5mg/ml solution of MTT was added to each well. The plates were incubated for a further 4 hours and then centrifuged for 10 minutes at the end of the four-hour period. The supernatants were removed and 180µl of DMSO was added to each well. After addition of the DMSO the plates were incubated for another 48 hours then the optical density in each well was read at 550nm on a Labsystems Multiskan RC microplate reader.

The inhibition of the proliferation was calculated as follows:

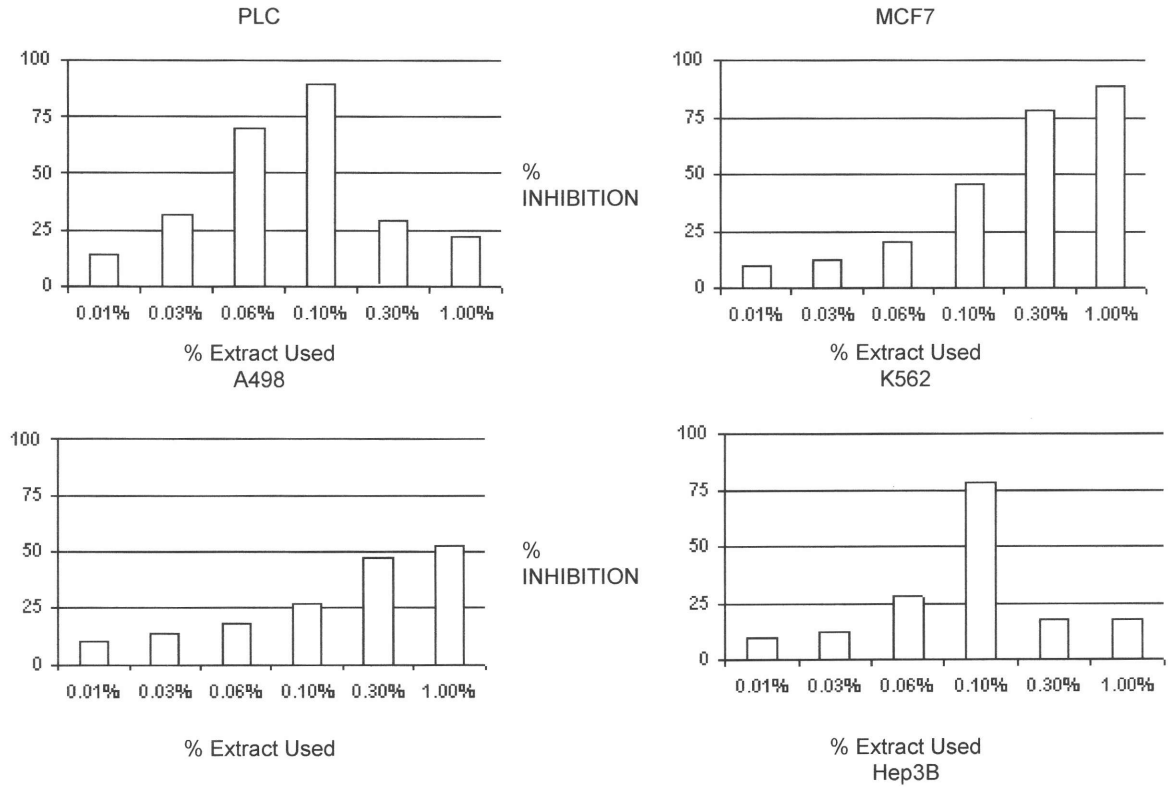
$$\% \text{ Inhibition} = \frac{(\text{mean abs of control} - \text{mean abs of sample})}{\text{mean abs of control}} \times 100$$

RESULTS
Figure 1 shows dose response curves for the activity of vincristine on PLC, MCF7, A498, K562 and Hep 3B cells. Figure 2 shows the inhibition of cell growth by 1µg/ml of cycloheximide, vincristine and vinblastine on PLC, MCF7, A498, K562 and Hep 3B cells. Figure 3 shows the effect of the Rye extract on PLC, MCF7, A498, K562 and Hep 3B cells.

Figure 1. Dose Response Curves for Vincristine on PLC, MCF7, A498, K562 and Hep 3B Cell Lines.



Effect of Rye Extract on Cancer Cells



DISCUSSION

The known anti-neoplastic agent, vincristine, caused a dose-related inhibition of cell proliferation against the PLC, MCF7, A498, K562 and Hep 3B cells over the concentration range 0.1µg/ml to 2µg/ml.

For comparison of inhibition of cell growth at 1µg/ml of cycloheximide, vincristine and vinblastine was tested in all cell lines. All three anti-neoplastic agents appeared to be equally active against all cell lines tested.

The Rye extract exhibited some activity on the cell lines tested. For the liver carcinoma (PLC), the Rye extract caused a bell-shaped response over the concentration range 0.01% to 1.00% with the highest activity of 89.26% inhibition at a concentration of 0.01%. At concentration higher than 0.01% there was a decline in the anti-proliferative activity observed.

On the breast cancer cells (MCF7), the Rye extract exhibited a steady increase of anti-neoplastic activity with increasing concentration with the highest activity of 89.01% observed at a concentration level of 1.0%.

When tested against the renal cancer cells (A498), the Rye extract exhibited a pattern similar to that observed in the breast cancer cells. However, the highest activity observed in the A498 of 52.65% was less than that observed in the breast cancer cells.

For the chronic myclogenous leukaemia cells (K562), the Rye extract caused a bell-shaped response with the highest activity of 78.13% being observed at a concentration of 0.10% of the extract.

The Rye extract was found to be active against the liver cancer cells (Hep 3B). The Rye extract caused bell-shaped response similar to one observed against the PLC cells on the Hep 3B cells. However, the extract had lower activity in the Hep 3B cells when compared to the PLC cells. The extract exhibited the highest activity of 55.01% at a concentration level of 0.10%.

CONCLUSION

The Rye extract caused a dose related inhibition of cell proliferation in MCF7 and A498 cells. However, the response was more marked in the MCF7 cells with near maximum inhibition occurring between 0.30% and 1.00% of the Rye extract. For the A498 cell line a maximum inhibition of approximately 50.00% was observed between 0.300% and 1.00% of the Rye extract.

For the other cell lines, the Rye extract caused a bell-shaped dose related inhibition. The Rye extract was most active against PLC cell line in comparison to the K562 and the Hep 3B cell lines.

REFERENCES

Mosmann, T (1983). Rapid colorimetric assay of cellular growth and survival application to proliferation and cytotoxicity assays. J. Immunol. Meth 65, 55-63.