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EFFECT OF POORANACHANDIRODAYAM ON COLON CANCER CELL LINES

***Dr. Preetha S.P, Ph.D[1]., Dr. Karthikeyan Ramaiyan, M.V.Sc[2]., Dr. Vasudevan Ramakrishnan, M.D (Siddha)[3], Dr. SathyaRajeswaran M.D (Siddha)[4]**

[1]Assistant Professor, Department of Veterinary Pharmacology and Toxicology, Madras Veterinary College, Chennai -07.

[2]Junior Research Fellow- AYUSH, Department of Veterinary Pharmacology and Toxicology, Madras Veterinary College, Chennai -07.

[3]Senior Research Fellow,-AYUSH, Department of Veterinary Pharmacology and Toxicology, Madras Veterinary College, Chennai -07.

[4]Assistant Director General, Siddha Central Research Institute, Arumbakkam, Chennai – 106.

Abstract:

Colon cancer is the major cause of death worldwide. Pooranachandirodayam is a herbo-mineral siddha preparation used to treat chronic ailments. This study was conducted to elucidate the efficacy and probable underlying mechanism of Pooranachandirodayam in colon cancer using in vitro studies. But Pooranachandirodayam had solubility issues with the methods earlier described for in vitro studies and with different concentrations of other varying solvents. From the results of the in vitro studies, PC shows no effect on the colon cancer cells, which may be due to the inappropriate vehicle used.

Keywords: *Colorectal cancer - Pooranachandirodayam – solubilisation – in vitro studies*

Corresponding author:

Dr. Preetha S.P, Ph.D,

Assistant Professor,
Department of Veterinary Pharmacology and Toxicology,
Madras Veterinary College,
Chennai -07.
E-Mail: drsppreetha@gmail.com

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INTRODUCTION:

Colorectal cancer (CRC), the third leading cause of death worldwide (1) is expected to increase alarmingly by 60% with over 2.2 million new cases and 1.1 million deaths by the year 2030 (2). 5- Fluro uracil (5-FU) continues to be the drug of choice for CRC (3), but its anticancer effect in the course of chemotherapy is frequently restrained by the development of drug resistance or non-selective cytotoxicity (4) causing several serious side effects. These limitations highlight the urgent need to develop alternative, safe therapeutic strategies for the treatment of malignant diseases.

Many people especially in the developing countries resort to traditional medicines that are not part of the conventional chemotherapy for chronic illness since they believe that traditional medicines are "natural" and "natural" means "always safe". In India, there is a separate department for Indian Systems of Medicine known as AYUSH (Ayurveda, Yoga, Unani, Siddha, Homoeopathy) which was established in March 1995 to promote indigenous systems (5)

Poorana Chandirodayam (PC) is one such herbo-mineral Siddha preparation which consists of Gold, Mercury, Sulphur along with Hibiscus or Aloe juice (6, 7), used to treat chronic ailments such as tuberculosis, jaundice, cancerous ulcer, male sterility (8). Mercurial and Gold preparations are routinely used in Siddha medicine for the treatment of debilitating diseases (9). There are several reports that claim that repeated incineration of these metals and its salts with herbal juices not only eliminates its harmful effects (10) but also reduces the particle size even up to nano level and increases its potency (11).

So the beneficial effects reported to be associated with this traditional medicine PC should not be ignored without any scientific validation. Hence, this present study was designed to investigate the *in vitro* efficacy of PC on colon cancer cells.

MATERIALS AND METHODS:

Colon cancer line (HT-29) was purchased from the Centre for Cellular and Molecular Biology (CCMB), Hyderabad. PC was purchased from SKM Siddha and Ayurvedha Company (India) Private Limited, Tamilnadu. All other chemicals and solvents used in this study were of analytical grade.

Cell Culture:

DMEM containing high glucose (Hi-media) along with 10% Fetal Bovine Serum (Gibco) and commercial antibiotic solution (Antibiotic – antimycotic – Gibco) were used to culture the cells 0.25% Trypsin EDTA (Hi-media) was used to trypsinize the cells. The cells took 36-48 hours to attain 80% confluency.

Solubilization of PC:

PC is water insoluble. So a suspension of PC was prepared using the procedure described by Hazeena Begum and Muthukumaran (11). Further the solubility of PC was tested using different concentrations of dimethyl sulfoxide (DMSO), gum acacia, ethanol, hydrochloric acid (HCl), nitric acid (HNO_3) and aqua regia as per the standard protocol (Figure 1).

Cell Viability and counting:-

The cells were stained with Trypan blue dye (12). Cell viability and counting was done manually using Haemocytometer.

MTT assay:-

3-(4,5-dimethylthiazol-2-yl) and 2,5diphenyl tetrazolium bromide dye (Sigma) were used for this assay. MTT dye reduction test was done as per ATCC protocol (13). The plates were read at 570 nm using Elisa reader (Figure 2).

RESULTS AND DISCUSSION:

Solubilization of PC:

The dissolution properties of water-insoluble herbo-mineral preparations remain a great challenge in the *in vitro* trials. The effectiveness of a formulation depends on its ability to make the drug available at the site of action (14).

PC is a herbo-mineral preparation insoluble in water. Hazeena Begum and Muthukumaran (11) had earlier reported that a suspension of PC can be prepared by gradually triturating 10 g of gum acacia in 100 ml of distilled water to which PC was added at the dose of 3mg/ml/100g. Hence in the present study, PC suspension was prepared using the above protocol (11). But the suspension could not solubilize PC.

The above suspension was further sonicated (vibra cell) in an attempt to solubilise PC. But PC remained insoluble. Hence the prepared suspension was not suitable for further *in vitro* studies.

Further, different solvents of varying concentrations were used to solubilize PC.10% and 30% concentrations of ethanol, DMSO, HCl, HNO_3 and aqua regia were used to address the solubility issues of PC. PC was found insoluble in all solvents and precipitated except with 10 % of HNO_3 and aqua regia.10% HNO_3 had better ability to dissolve PC compared to aqua regia. Hence PC dissolved in 10%

HNO_3 was used for further *in vitro* studies.
However the acidic environment created by HNO_3 must be neutralized to make it suitable for *in vitro* trials. Hence DMEM – High glucose with HEPES buffer was used to neutralize the acidic environment.

MTT:
This assay revealed that the IC_{50} values of 5-FU was 170 ± 0.0055 µg. There was significant difference ($P<0.001$) between the IC_{50} values of various concentrations of 5-FU. 5-FU markedly ($P<0.001$) destroyed the colon cancer cells, whereas PC had minimal effect on the cancer cells.

PC contains a mixture of compounds that work synergistically (15) and proven to be effective even in very low concentration (16). It is already reported that the purification process of this drug reduces the toxicity (17) and increases its bioavailability through the cells of the body (18).

But from the results of the present study, PC had minimal effect on the HT-29
colon cancer cells. This may be due to the effect of the acid (HNO_3) used to solubilize PC that would have changed the actual nature of the drug.

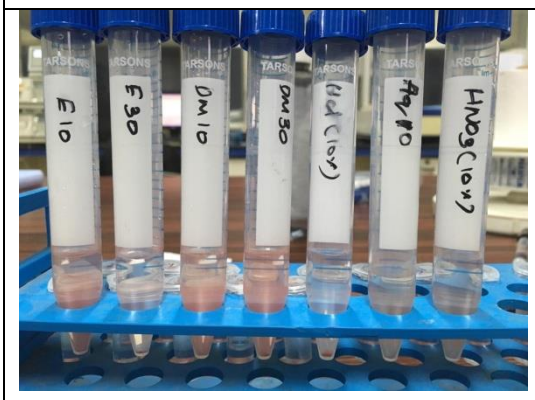
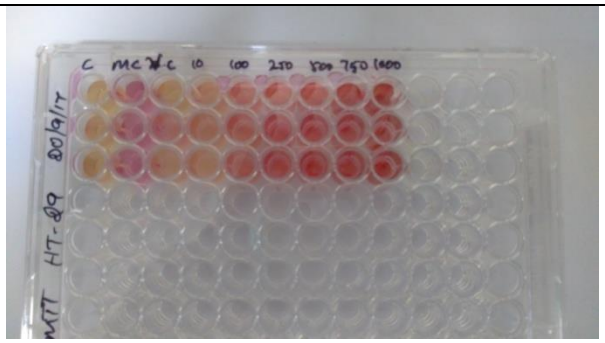
Image showing the various solvents used to solubilise PC. Only in aqua regia and 10% HNO_3, PC solubilised completely (no precipitation).	Image showing the 96 well plates used to perform MTT. Cells treated with various concentration of 5- fluoro uracil and PC
	

Figure 1 **Figure 2**

CONCLUSION:

From the results of the *in vitro* studies, PC shows no effect on the colon cancer cells, which may be due to the inappropriate vehicle used. Though the Indian Siddha medicine PC is reported to be widely used, clinically successful effective immuno-modulatory agent used to treat patients with several chronic diseases such as cancer, conclusive evidences demonstrating its effect on colon cancer cells under *in vitro* trials could not be obtained. This may be due to the use of inappropriate vehicle (HNO_3) used to solubilize PC for the *in vitro* studies that would have changed the actual nature of the drug. To elucidate the exact underlying multiple mechanisms by which PC can act on the cancer cells in the human body, further *in vivo* studies are required.

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