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In Vitro Cytotoxic and Gene toxicity Effects of Flavonoid of Portulaca Oleracea L. on HepG2 and K562 Malignant Cell Lines

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Abstract: **Cancer is a deadly disease. It is characterized by unrestrained growth and reproduction of cells, loss of contact inhibition, and eventually metastasis. All of these changes represent underlying mutation or in appropriate expression of genes involved in the control of the cell cycle and related processes. Objective: The present study was carried out to evaluate the cytogenetic toxicity and gene toxicity effect of Flavonoids. Method: Flavonoids isolated from P. oleracea L.was tested for gene and cytotoxicity effect against two malignant cell lines viz. Hepatocellular Carcinoma, human (Hepg2) with ATCC No. HB-8065 and Erythroleuckemia, ATCC No.CCL-243, in different concentration varying from 10µm to 200µm, the study included the anti-proliferative effects of Apigenin flavonoid of P. oleracea L on the mitotic index (cell division) and gene toxicity effect on same cell lines in vitro. Among these two cell lines, HepG2 shown growth reduction at 200µm by 20 % but where in K562 was not shown significant reduction in cell growth at tested dose level. The gene toxicity by comet assay has revealed that, hepG2 cells DNA has shown more comet tail formation and K562 DNA showed moderate comet tail formation when compared to control at tested dose level.**

Conclusion: the flavonoids tested were most effective on gene toxicity and moderate effect on Cytotoxicity.

Keywords: Portulaca oleracea L, Cytotoxicity, hepG2 cell and K562 cell

1. INTRODUCTION

Cancer is a deadly disease. It is characterized by unrestrained growth and reproduction of cells, loss of contact inhibition, and eventually metastasis. All of these changes represent underlying mutation or in appropriate expression of genes involved in the control of the cell cycle and related processes.[4]The bombardment of Halabja city in Iraqi Kurdistan region in 1988 with chemical weapons, caused more than 5000 martyrs and 10000 injury victim survivors are still suffering from a variety of health complains, which include cancers, and other health complains like respiratory, ophthalmological, dermatological, reproductive, and immunological complains.[2] It is widely held that (80-90%), of human cancers may be attributable to environmental, lifestyle factors such as tobacco, alcohol, and dietary habits.[3, 5]the application of ethno-pharmacology and complimentary alternative medicine in combating current health issues is eminent. Numerous studies have evaluated the biological activities of various phytochemicals produced by plants, particularly the anti-proliferative and cell cycle regulatory effects, in relation to cancer prevention.[9, 10, 11]

P. oleracea L. (Purslane) has been used as a kind of food and medicinal plant for thousands of years in China. *P. oleracea L*is very important because of its special medical function and all its therapeutic values are attributed to the presence of many biologically active compounds which include; flavonoids (Apigenin, Kaempferol, Quercetin, Luteolin, Myricetin, Genistein, and Genistin), Alkaloids, Coumarins, Anthraquinone Glycoside, cardiac glycoside, and high content of ω-3 fatty acids.[6]*P. oleracea L* is a fascinating plant recognized in most cultures for its extensive nutritional benefits. It has been used traditionally as a vegetable for human consumption. On the other hand, traditional medicinal systems of China, India, Europe and Middle Eastern countries have used *P. oleracea L* to treat various human ailments such as hemorrhoids, burns and wounds, pain, headache, scurvy, fever and urinary disorder. Therefore, this edible vegetable is dubbed the global panacea.[12]Extensive modern pharmacological studies have attested its wide range of biological effects. it was reported to contain a high antioxidant

property[13], which is mainly attributed to the rich source of omega-3 polyunsaturated fatty acids[14] and flavonoid compounds; particularlykaempferol, apigenin, myricetin, quercetin, luteolin, carotene and alkaloids[15, 16]. Studies have also shown that the plant possesses significant analgesic and anti-inflammatory activities when compared with synthetic drugs.[17] Nevertheless, the anti-proliferative effect of *P. oleracea L* has rarely been reported. However,[18] reported that water soluble polysaccharides isolated from this plant possesses mild cytotoxic activity against cervical cancer HeLa cell line and the sulphated form of these polysaccharides enhances the anti-tumour effect.[18]In addition Luteolin which can be found from *P. oleracea L* was previously proved to induce cell cycle arrest and apoptosis of colon cancer hT-29 cell via decreased of IGF-II production and down regulated insulin-like growth factor-receptor signaling.[19] Many of these researches have verified the importance of *P. oleracea L*. Furthermore, with its high ethno pharmacological values and the present of cytotoxic polysaccharides and flavonoid especially luteolin, it is a promising plant in the investigation of cancer prevention. The aim of this study was to investigate the cytotoxicity and Gene toxicity effect of *P. oleracea L* towards malignant cell lines viz. Hepatocellular Carcinoma, human (Hepg2) with ATCC No. HB-8065 and Erythroleuchemia, ATCC No.CCL-243,

2. MATERIALS AND METHODS

2.1 Plant material

Healthy aerial part of the plant of *P. oleracea L*as shown in Fig 1 was collected from around Gulbarga university campus during the month of June 2012. The plant material was identified and authenticated from the Department of Botany Gulbarga University, Gulbarga, Karnataka (India), voucher specimen (No. HGUG-5013) has deposited. The plant was classified by botanist in the biology department of college of education, scientific departments.[7]

Plant Classification

Kingdom- Plantae (Plants)
Sub kingdom- Tracheobionta
Division- Spermatophyta
Sub Division- Angiospermae
Class- Dicotyledoneae
Order- Caryophyllales
Family- Portulacaceae
Genus- Portulaca
Species- *P. oleracea* L.

Fig: 1. Showing *P. oleracea L* with Classification

2. 2. Chemicals

Ethanol, Petroleum ether, H_2SO_4, Hexane, DMSO, Phosphate Buffer Saline(PBS), PMS, XTT, KCl, DMEM, RPMI, FCS, Agarose, Ethidium bromide, Tris HCl, EDTA, Trypsin, Titron-100, all the Chemical, Solvents and Reagents used were Analytical grade obtained from Hi media and Sigma Aldrich.

2.3. Extraction of Flavonoids by Soxhlet method

Before extraction, *P. oleracea* L was crushed into powder by versatile plant pulverizer. The powder of the sample was degreased by Soxhlet extractor with Petroleum ether until the color of elute become colorless. a). For extraction of Apigenin, The same powder sample was accurately weighed and placed in Soxhlet extractor by adding with a ratio of 80 ml of ethanol: water (70:30) solvent, followed by the extraction for 5 hrs. The extracted solution was concentrated. The extract was centrifuged at 11000 rpm for 30 min; supernatant was taken for further use, where in case of extraction of Rutin, 10 g of sample was extraction in 300 ml of Methanol: Ethanol (9:1, v/v) for 20 min the extract was centrifuged at 8,000 rpm for 10 min and the supernatant diluted to 25 mL in a volumetric flask (25 mL) using methanol–ethanol (90:10, v/v) as solvent, and filtered through 0.45 μm. The filtrate was taken for future use.[8. 20]

2.4. Preparation of Drug

The Apigenin and Rutin Flavonoids isolated from *P.oleracea* L were prepared in required Concentration in PBS for both Cytotoxicity and Gene toxicity studies.

2.5. Cell lines and Culture media

The human Hepatoma cell line (HepG2) and Human Leukemia Cell lines (K562) were collected from Indian Institute of Science Bengaluru and maintained with DMEM and RPMI 1640 media containing 10% Fetal cough Serum and 100ng/ml, each of penicillin and streptomycin at 37^OC in a humidified atmosphere with 5 % CO_2.

2.6. Cell growth study by XTT Assay

The tetrazolium salt (XTT) assay was carried out to assay for cytotoxicity. HepG2 and K562 cells were seed into 96-wells micro titer plate using 200μl /well of a cell suspension containing 10^3 cell/well DMEM and RPMI media with 10% FCS respectively. The drug dissolved in water, so as to get the concentration varying from 0.01mM to 0.2mM and added in a volume of 20μl. After 44 h of drug treatment the wells were pulsed with 50μg of XTT and 5μm PMS (as electron coupling agent) after 4 h of incubation, absorbance was read at 450 and 630 nm in the well was read on a dual wavelength Tecan i-control plate reader. In all experiment triplicates were used for each concentration.

2.7 Gene toxicity study by Comet Assay

The status of individual ovary cells was determined using a modified alkaline single cell gel electrophoresis (SCGL)/ Comet assay.[21] The ovary was collected by removing excessive fats and homogenized in PBS with the help of homogenizer at 37^{O}C. the homogenate so obtained was diluted in PBS. Clear window frosted microscopic slide was coated with 1% normal melting agarose. The well was punched at one side of the slide the aliquot of 10 µl cell suspension was added and to this 20 µl of lysis solution was added followed by another coat of 0.5 % of low melting agarose at 37^{O}C. the slide was placed in moisture chamber for overnight. After lysis , the slide was immersed in the freshly prepared electrophoresis buffer (10mM Tris, 0.08mM Boric acid, 0.5M EDTA, pH 8.2) and electrophoresed for 1 h at 50 V. after electrophoresis slide was dehydrated by immersing in absolute alcohol for 3-5 times. Then slide was stained with ethidium bromide followed by observation under trans-illuminator for comet formation and image was photographed.

2.8 Statistical analysis

Results are expressed as Mean ± SEM. The statistical analysis was carried out using one way ANOVA analysis. The p-value of 0.05 or less was considered significant for all experiment.

3. RESULTS AND DISCUSSION

3.1. Cell Growth study by XTT Assay

Recently, experts in cancer prevention, detection and treatment have reviewed the need of more research in chemoprevention.[22] Thus, relevant approaches particularly food-based entities remain essential in reducing the risk of cancer. *P. oleracea L*, a ubiquitous garden weed has shown to provide a rich plant source of nutritional benefits. With that, the potential Cytotoxicity and Gene toxicity of standardized Apigenin and Rutin of *P. oleracea L* towards both of cancerous cells were explored. These were determined through XTT assay and Comate assay respectively.

XTT assay that is commonly used to screen for the cell proliferation, viability and cytotoxic effects was done to determine the cell viability by assessing healthy cells.[23] Under the current experimental conditions, no IC50 was obtained within the range of the tested concentration (10 – 200 µM/mL) on both the cell lines. However, there was a significant decline in cell viability at concentration of 200 µg/mL to hepG2 cells (Figure 2), The reduction of cell viability as compared with untreated control cell may be contributed by apoptosis or anti-proliferation. On the other hand, the Apigenin and Rutin were showed selective inhibition on the both Cancerous cells, which indicates the nontoxic effect of the plant and is safe for daily consumption as represented in Table 1.

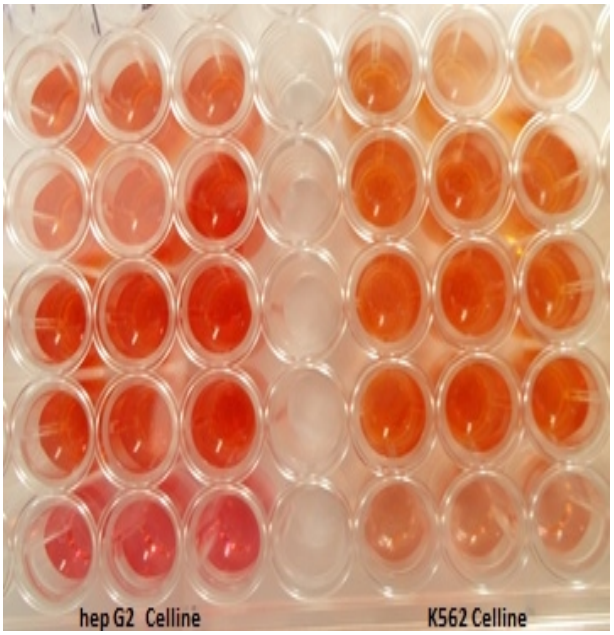

Fig 2: Evaluation of Cytotoxicity by XTT Assay

Table: 1 Cytotoxicity of Apigenin and Rutin on Human Cell lines

Concentration Of drug (µM)	O D of Hepg2 at 450 nm (Mean± SD)	O D of K562 at 450 nm (Mean± SD)	O D of Hepg2 at 450 nm (Mean± SD)	O D of K562 at 450 nm (Mean± SD)
	Apigenin		Rutin	
200	2.91±0.20	1.17±0.23	1.34±0.57	1.36±0.33
150	1.83±0.31	1.02±0.39	1.82±0.21	1.38±0.34
100	1.29±0.10	1.22±0.14	1.46±0.19	1.40±0.30
50	1.40±0.04	1.04±0.16	1.53±0.24	1.58±0.24
25	1.24±0.19	1.06±0.33	1.38±0.09	1.18±0.08
10	1.11±0.18	0.96±0.28	1.67±0.25	1.14±0.28
Control	0.82±0.20	0.92±0.05	0.82±0.30	0.92±0.05

3.2. Gene toxicity study

The length of comet tail for treated and control groups were shown in table 2**.** The cells treated with 50mg/ml concentration have shown significant increase in Comet tail length formation, when compared with controlled group, where in case of Rutin treatment have shown moderate Comet tail length formation when compared with control as

represented in Fig 3**.**Evaluation of gene toxicity by Apigenin and Rutin of *P. oleracea L* was made by comet assay on human carcinoma cell lines has shown significant increase ($p \leq 0.05$) DNA damage and tail formation compared to control where K562 have shown moderate DNA damage and comet tail formation. Felix (2001) reported that DNA topoisomerase II causes Chromosomal breakage and translocation in leukemia. Direct testing for drug resistance patterns in DNA directed drug moieties by SCGE/CLSM reveal individual variability of human malignant cell lines warranting comparison with results of MTT testing and in vivo patient response.[1]

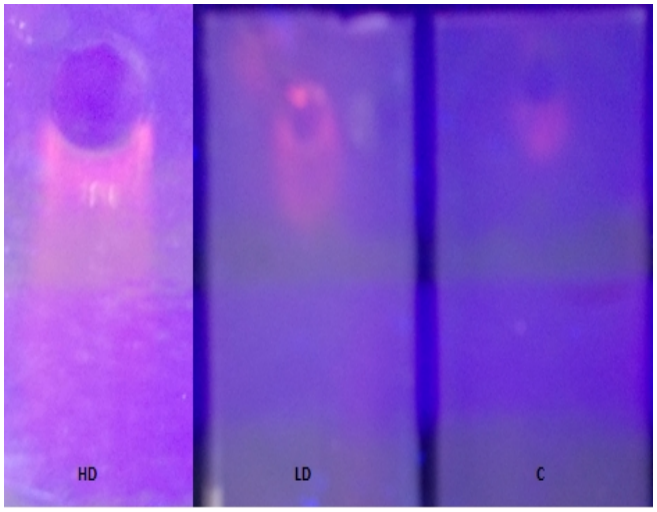

Fig 3: Showing Gene Toxicity

Table 2: The Gene toxicity study of Apigenin and Rutin by comate assay method

Cell line	Dose µg/ml	Apigenin Comate tail length (mm) (Mean ± SD)	Rutin Comate tail length (mm) (Mean ± SD)
HepG2	50	30.1 ± 2.89	23.1 ± 2.75
K562	50	20.6 ± 1.95	16.6 ± 3.57
Control	50	0.00 ±0.00	0.0 0.00

4. CONCLUSION

In conclusion, our data indicated that the Apigenin of *P. oleracea L* specifically reduced viability of various cancerous cell lines either through G0/G1 or S phase arrest or via induction of sub-G0/G1 DNA fragmentation (on hepG2 cells). However, the mechanism of the action is still unclear. Thus, further investigation of the molecular mechanisms involved is needed to fully understand the *P. oleracea L* as a chemo preventive food.

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Conflicts of Interest

The authors have indicated that they have no conflicts of interest regarding this article.

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