Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(5): 2450-2452
Received: 19-07-2018
Accepted: 21-08-2018

MJ Raja
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal, Tamilnadu, India

LN Mathuram
Professor and Head (Retd.), Department of Veterinary Pharmacology and Toxicology, Madras Veterinary College, Chennai, Tamilnadu, India

K Kumanan
Professor and Head, Department of Bioinformatics and ARIS Cell, Madras Veterinary College, Chennai, Tamilnadu, India

Correspondence
MJ Raja
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal, Tamilnadu, India

Antiproliferative effect of apigenin: A flavonoid in sarcoma 180 cell line

MJ Raja, LN Mathuram and K Kumanan

Abstract
Apigenin is a common dietary flavonoid that has been used as a traditional medicine for centuries for their promising pharmacological properties as antioxidant, antibacterial, antiviral, anti-inflammatory etc. Currently, investigation and recognition of apigenin as a cancer chemopreventive agent has increased. Hence the present study was aimed to investigate the ability of apigenin to inhibit cell proliferation in sarcoma 180 cell line. Effect on cell viability, changes in the total cellular glutathione, lipid peroxides and sialic acid levels were observed to study the antiproliferative effect of apigenin at three different dose levels with plumbagin as a positive control and DMSO as a negative control. In this study, the flavonoid apigenin significantly inhibited the cellular proliferation in a dose dependent manner. Effective inhibition of cell viability, decrease in glutathione, sialic acid levels and increase in lipid peroxides supported the antiproliferative effect of apigenin and proved that, the apigenin could be used as a natural cancer chemopreventive agent to control cancer cell proliferation.

Keywords: Flavonoids, apigenin, sarcoma 180 cell line and antiproliferative

Introduction
In communities where malnutrition, undernutrition and infectious diseases are no longer a major problem, neoplastic disease comes second to cardio vascular disease as a cause of death [1]. Cancer, a dreadful disease has become the potential threat to life in this century and the world cancer report alarms that cancer patients are expected to rise by 50% resulting in 50 million new cancer cases by 2020 [2]. The National Center for Complementary and Alternative Medicine (NCCAM) estimated that, 30–75% of cancer patients worldwide are using complementary and alternative medicine therapies that include dietary approaches, herbals and other biologically based treatments [3]. Treatment of cancer by use of natural products and traditional medicine by applying the concepts of ayurveda is attaining great significance in the scope of cancer research [4]. The secondary metabolites of medicinal herbs were proved for their antimutagenic and anticancer properties [5].
Bioflavonoids are an ubiquitous group of active, non-toxic polyphenolic compounds widely distributed throughout the plant kingdom with numerous pharmacological actions [6]. The flavonoids have a variety of biological effects on various mammalian cell systems like inhibition of platelet activity [7], anti-inflammatory [8], anti-oxidant activity [9], anticarcinogenic [10] etc. These phytochemicals are naturally occurring, cost effective, and easily available with least or no side effects.
Apigenin is a non-toxic and non-mutagenic plant flavone has gained interest in recent years as a beneficial and health promoting agent because of its striking effects on normal versus cancer cells. Interest in the possible cancer preventive of apigenin has increased owing to reports of potent antioxidant and anti-inflammatory activities [11]. Hence, the present study has been formulated to evaluate the effect of apigenin on certain important biomarker enzymes and to ascertain its antiproliferative effect against sarcoma 180 cell line *in-vitro*.

Materials and Methods
Cell line, Reagents and Growth Media
Sarcoma-180 cell line was procured from National Centre for Cell Science (NCCS), Pune, India. The flavone, apigenin was procured from Gibco BRL, USA and chemicals with analytical grade from Sigma-Aldrich were used throughout this study. Sterile tissue culture flasks, containers, pipettes (Corning, USA) were used to propagate the cells. Dulbecco modified Eagle's medium (Gibco BRL, USA) was used as growth media with the pH range of 7.4 – 7.7. To this, 10 ml of 10% fetal calf serum (Hi Media, USA) was added with 1% antibiotic solution (Penicillin 100 units/ml, Streptomycin 100 µg/ml).

This growth media was checked for contamination by incubation at 37 ^{0}C for 24 – 48 h. The sterile growth media was stored at 4 °C and used for culture work with proper thawing procedure.

Culturing of Sarcoma – 180 cells

Culture flask with confluent monolayer was selected for subculturing and maintenance of the cells. The suspended cells were transferred to a sterile centrifuge tube along with the medium and centrifuged at 4 °C at 1500 rpm for 10 minutes. The supernatant media was discarded and the pelleted cells were suspended in fresh growth medium. The cells were seeded into fresh culture flasks and incubated in 5% CO_2 chamber at 37 °C. When the cell concentration was found to be optimum, these cells were collected and used for experimental study.

Experimental Design

The study was conducted in five groups of sarcoma 180 cell culture containing 2 x 10^5 cells /ml with six replicates for each group.

Assessment of Cell Viability

The cell viability was assessed at 24 h and at 48 h after incubation. Viability of the cells was counted by trypan blue dye exclusion test [12]. After 48 h, the cells were harvested and washed three times with normal saline to estimate total cellular glutathione (GSH), lipid peroxides and sialic acid. Total cellular glutathione, lipid peroxides and sialic acid were determined after disruption of the cells by sonication.

Marker Enzymes Estimation

Total cellular glutathione content was estimated by the method [13] based on its reaction with 5, 5' – dithio-bis (2-nitro benzoate) (DTNB or Ellman's reagent) to give a yellow coloured compound that absorbs at 412 nm. Lipid peroxides content was determined by thiobarbituric acid (TBA) reaction [14]. Sialic acid [15] and protein [16] was estimated.

Statistical Analysis

The data were analysed by One-way ANOVA procedure using SPSS ® 20.0 software package for windows after angular transformation of data wherever necessary.

Results

The effect of apigenin at three different dose levels (Table. 1) on Sarcoma-180 cell line proliferation and changes in the marker enzymes were assessed.

Table 1: Effect of apigenin on cell viability, cellular glutathione, lipid peroxide and sialic acid concentrations in sarcoma 180 cell line ((Mean ± SE, n=6).

Treatment Groups		Cell Viability (%)	Glutathione (nm/mg protein)	Lipid peroxide (nm/mg)	Sialic acid (µg/mg)
T_1	Negative Control (DMSO 50%)	$95.13^{d} \pm 0.06$	$40.21^{c} \pm 0.26$	$0.0892^{a} \pm 0.0021$	$20.60^{d} \pm 0.65$
T_2	Positive Control (Plumbagin - 10µg/ ml)	$56.65^{ab} \pm 1.54$	$25.33^{a} \pm 0.45$	$0.1633^{d} \pm 0.0017$	$13.70^{a} \pm 0.27$
T_3	Apigenin 14 µg	$73.55^{c} \pm 1.62$	$31.49^{b} \pm 1.10$	$0.1470^{b} \pm 0.0024$	$17.83^{bc} \pm 0.54$
T_4	Apigenin 21 µg	$60.06^{b} \pm 0.90$	$29.59^{b} \pm 0.65$	$0.1598^{c} \pm 0.0013$	$16.18^{b} \pm 0.33$
T_5	Apigenin 28 µg	$51.03^{a} \pm 0.48$	$26.07^{a} \pm 0.57$	$0.1626^{d} \pm 0.0023$	$13.82^{a} \pm 0.24$

Means bearing different superscripts differ significantly (P<0.01)

On cell viability, there was a highly significant (P<0.01) decrease in sarcoma-180 cell line in all the treatment groups including plumbagin treated group compared to DMSO treated group, of which Apigenin 28 µg/ml showed a better antiproliferative effect (51.03±0.48) than the standard antiproliferative drug – plumbagin (56.65±1.54). The mean cellular glutathione values were estimated in all the five groups with a significant (P<0.01) decrease in glutathione concentration in plumbagin and all doses of apigenin treated groups compared to DMSO treated group. The decrease in glutathione level showed by apigenin at 28 µg/ml was 26.07 ± 0.57, which is not significant with plumbagin (25.33±0.45). The lipid peroxides produced in treatment groups were estimated with a significant (P<0.01) increase in lipid peroxides level in apigenin 21 µg/ml, 28 µg/ml and plumbagin treated group compared to DMSO treated group. Apigenin at 14 µg/ml recorded an apparent increase in lipid peroxide level. A significant (P<0.01) decrease in the sialic acid concentration in sarcoma-180 cell line was recorded in all apigenin and plumbagin treated groups when compared to DMSO treated group.

Discussion

There are several mechanisms that may be involved in the antiproliferative and/or anticarcinogenic effect of apigenin. It was previously demonstrated that apigenin causes cell cycle arrest via the regulation of CDK1 and p21Cip1 and the induction of apoptosis [17], affinity in binding with NBD compounds [18], suppressive effects on kinase activities, intrinsic and extrinsic apoptotic pathways [19] etc. Like apigenin, luteolin a flavone proved for its antiproliferative effect with significant decrease in cell viability, when treated with A-431 cells in a dose dependent manner [20]. The cellular enzyme glutathione promotes cell stability and its functions. Increased cellular GSH concentration protected cells against oxidative damage, toxic compounds and radiation [21]. A reduction in GSH was noticed in apigenin treated sarcoma 180 cell line and a decrease in glutathione level might have contributed for the loss of cell viability and their antiproliferative action. Similar result was observed in human laryngeal carcinoma after *in vitro* treatment with luteolin [22]. Flavonoids showed increase in lipid peroxides concentration in many cancer studies [22]. Apigenin treated sarcoma cell lines showed similar concentration of lipid peroxides than untreated control group. Free radicals can be generated in biological systems either as by-products of partial oxygen reduction or by xenobiotic catabolism. Lipid peroxidation is the characteristic reactivity of free radicals *in vivo* or *in vitro*. This results in deleterious effects on membranes and cause death of affected cells [23]. Increased accumulation of lipid peroxides in sarcoma 180 cells correlated with their ability to bring about a loss of cell viability. Sialic acids are compounds derived from 9-carbon sugar neuraminic acid and they are terminal sugars from the oligosaccharide chains of glycoprotein and glycolipids, many of which are components of the cell membrane [24]. Increase in sialic acid levels [25, 26] in animal studies with drugs proved their antiproliferative

efficacy. Reduction in sialic acid concentration in sarcoma 180 cells treated with apigenin correlates with the changes in cell viability indicating that the drug has antiproliferative effect.

Conclusion

Thus, the flavonoid apigenin significantly inhibited the cellular proliferation in sarcoma 180 cell line in a dose dependent manner. The findings with effective inhibition on cell viability, decrease in glutathione, sialic acid levels and increase in lipid peroxides supported its antiproliferative effect. Also this finding is consistent with previous work showing that apigenin may be an effective agent against cell growth in some cancer cell. Further developments in this study can be suggested as with the advances in molecular biological techniques in cell culture or *in vivo* models. This cancer chemopreventive agent may be used as probes to characterize the subtle interplay between signalling networks and transcription factors that control cell proliferation and death.

Acknowledgement

The authors acknowledge the facilities and financial assistance provided by Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai-5, Tamilnadu to carry out the research.

References

1. Akulapalli Sudhakar. History of cancer, ancient and modern treatment methods. Journal of Cancer Science and Therapy. 2009; 1(2):1-4.
2. Kar M. Oncology - Its relevance in a developing country like ours. Journal of the Indian Medical Association. 2005; 103(09):2.
3. Richardson MA. Bio pharmacological and herbal therapies for cancer: Research update from NCCAM. The Journal of Nutrition. 2001; 131: 3037s-3040s.
4. Lakshmi Priya MK, Bhanu Priya, Venkata Subbaiah Kotakadi, Josthna P. American Journal of Ethnomedicine. 2015; 2(2):136-142.
5. Gupta, Tandon. Reviews on Indian medicinal plants. New Delhi: Indian Council of Medical Research, 2004, 1-3.
6. Raj Narayana K, Sripal Reddy M, Chaluvadi MR, Krishna DR. Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. Indian Journal of Pharmacology. 2001; 33:2-16.
7. Osman HE, Nabil Maalej, Dhanansayan Shanmuganayagam, John D. Folts. Grape juice but not orange or grape fruit juice inhibits platelet activity in dogs and monkeys *(Macaca fascularis*). The Journal of Nutrition. 1998; 128:2307-2312.
8. Wei B, Chai-Ming Lu, Lo-Ti Tsao, Jih-Pyang Wang, Chun-Nan Lin. *In vitro* anti-inflammatory effects of quercetin 3-0 methyl ether and other constituents from Rhamnus species. Planta Medica. 2001; 67:745-747.
9. Yadav, SB, Vyasji Tripathi, Singh RK, Panday HP. Flavonoid glycosides from *Cuscuta reflexa* stems and their antioxidant activity. Indian Drugs. 2001; 38:95-96.
10. Middleton Jr. E, Chithan Kandaswami, Theoharis C. Theoharides. The effects of flavonoids on mammalian cells: Implications for inflammation, heart disease and cancer. Pharmacological Reviews. 2001; 52:673-751.
11. Gupta S, Afaq F, Mukhtar H. Selective growth-inhibitory, cell-cycle deregulatory and apoptotic response of apigenin in normal versus human prostate carcinoma cells. Biochemical and Biophysical Research Communications. 2001; 287:914-920.
12. Moldeus P, Hogberg J, Orrhenius S. In: Methods in Enzymology. Eds. J. Fleischer and L. Parker, Academic Press, New York. 1978; 52:60.
13. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochimica et Biophysica Acta. 1979; 582:67-78.
14. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry. 1979; 95:351-358.
15. Warren L. Assay for sialic acid in tissues by thiobarbituric acid reaction. The Journal of Biological Chemistry. 1959; 234:1971-1976.
16. Lowry OH, Rosebrough NJ, Fare AL, Randall RL. Protein measurement with the folin phenol reagent. The Journal of Biological Chemistry. 1951; 193:265-275.
17. Choi EJ, Kim GH. Apigenin causes G (2)/M arrest associated with the modulation of p21 (Cip1) and Cdc2 and activates p53-dependent apoptosis pathway in human breast cancer SK-BR-3 cells. The Journal of Nutritional Biochemistry. 2009; 20(4):285-290.
18. Reddy MS, Raj Narayana K, Chaluvadi MR, Krishna DR. Bioflavonoids – their pharmacokinetics and interaction with cytochrome P_{450} isozymes and P-glyocprotein. The Indian Journal of Pharmaceutical Sciences. 2001; 63:187-195.
19. Wang B, Zhao XH. Apigenin induces both intrinsic and extrinsic pathways of apoptosis in human colon carcinoma HCT-116 cells. Oncology Reports. 2017; 37(2):1132-1140.
20. Huang YT, Hwang JJ, Lee PP, Ke FC, Haung JH, Haung CJ *et al*. Effects of luteolin and quercetin, inhibitors of tyrosine kinase, on cell growth metastasis – associated properties in A431 cells over/expressing epidermal growth factor receptor. British Journal of Pharmacology. 1999; 128:999-1010.
21. Meister A. Glutathione metabolism and its selective modification. The Journal of Biological Chemistry. 1988; 263:17205-17208.
22. Elangovan V, Nalini Ramamoorthy, Balasubramanian S, Sekar N, Govindasamy S. Studies on the antiproliferative effect of some naturally occurring bioflavonoidal compounds against human carcinoma of larynx and sarcoma-180 cell lines. Indian Journal Pharmacology. 1994; 26:266-269.
23. Maseki M, Nishigaki I, Hagihara N, Tomoda Y, Yagi K. Lipid peroxide levels and lipid content of serum lipoprotein fractions of pregnant subjects with or without pre-eclampsia. Clinica Chimica Acta. 1981; 115:155-163.
24. Yogeswaran G. Cell surface glycolipids and glycoprotein in malignant transformation. Advances in Cancer Research. 1983; 38:289-350.
25. Bernacki RJ, Kim U. Concomitant elevations in serum sialyltransferase activity and sialic acid content in rats with metastasing mammary tumors. Science. 1977; 195:577-580.
26. Manohar BM, Sundaraj A, Nagarajan B, Shanmugam V. Biochemical markers in the diagnosis of ethmoid carcinoma in cattle. The Indian veterinary journal. 1993; 70:14-16.