Therapeutic and toxicological effects of natural compounds: Data from HPV16-transgenic and ICR mice (Review)

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Abstract. The aim of present mini-review was to highlight the effects of certain natural plant food compounds in animal models, analyzing their therapeutic effects and toxicological levels. Several natural compounds have been shown to promote health and to reduce the risk of disease due to their functional and nutraceutical properties. However, it has been observed that some diseases can still progress even in the presence of natural compounds. Thus, there is a need to conduct further research in order to elucidate the mechanisms underlying the functions and effects of natural compounds and to determine which products exert positive effects and which products have pharmacological potential to be applied in therapies.

Contents

1. Introduction

Natural compounds are chemical substances produced by living organisms (1). These compounds have been used since ancient times as methods of traditional medicine for the treatment of various diseases (2). Thus, bioactive ingredients present in natural products are important for the human diet, allowing them to maintain bodily functions and prevent diseases (3). Vitamins, fibers, amino acids, fatty acids and phenolic compounds are some examples of these bioactive products (4).

In addition, nutraceuticals and functional foods, which are based on very active natural compounds, have become very relevant in the human diet and in the preparation of animal feed, since their preventive effects on several diseases and their health-promoting effects have been recognized. Such findings have prompted the growing interest of the scientific community for these natural compounds and in the development of further knowledge in order to better understand the mechanisms underlying their effects and their role in the diet. Functional foods can be defined as foods that promote health and/or reduce the risk of disease in addition to their nutritional effects (4). On the other hand, the term nutraceutical is used to describe substances that are not recognized as nutrients, but which have health benefits (4).

However, not all natural products have beneficial effects for humans. Animals and plants produce toxic substances for their own protection (5). Several plant toxins can act as promoters of carcinogenesis when ingested.

Animal models pose important ethical issues to researchers, and have been partially replaced by alternative experimental models (6). Reducing the number of experimental animals in each experiment and refining the experimental design in order to minimize animal distress, while optimizing data collection remains an essential challenge. On the other hand, translating the findings from animal models into human populations also requires careful evaluation. In this regard, the models selected for these experiments present an important translational value. The N-butyl-N-(4-hydroxybutyl)-nitrosamine (BBN) mouse model accurately represents the basal subtype of human muscle-invasive bladder cancer (7) and human papillomavirus (HPV)16-transgenic mice are genetically modified to reproduce the lesions induced by HPV in human patients (8-10).

2. Natural plant food compounds in animal models

HPV16-transgenic mouse model. An HPV16-transgenic mouse model was previously used by the authors of this review to test certain natural compounds, including ptaquiloside from bracken (*Pteridium* spp) (11), curcumin and rutin (12), *Laurus nobilis* (*L. nobilis*; laurel) (13), the red seaweed *Porphyra*

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umbilicalis (*P. umbilicalis*) (14) and dimethylaminoparthenolide (DMAPT) (15).

Previous studies have reported the protective effects of curcumin against cardiovascular and neurogenerative diseases (16,17). Rutin has several pharmacological properties, such as anti-inflammatory, antioxidant, antidiabetic and anticancer activities that render it advantageous against various diseases (18). *In vitro* studies have demonstrated that *L. nobilis* exerts positive effects against breast and colorectal cancer (19,20). DMAPT has shown promising antitumor activities against certain types of leukemia and solid tumors (21,22). Data for *P. umbilicalis* therapy in the literature are limited. However, several lines of research have demonstrated promising results with the seaweed displaying anticancer properties (23).

The HPV16-transgenic mouse model expresses genes of the early region of HPV16 under the influence of the human keratin 14 (K14) enhancer/promoter. The early region expresses oncogenes *E6* and *E7* that cause lesions in the squamous epithelium of these animals namely on the face, ears, chest and anus (8). K14HPV16 is associated with the development of multiple stages of pre-malignant lesions, such as hyperplasia, dysplasia and papillomatosis that may result in invasive carcinoma; these lesions exhibit similar molecular and morphological characteristics with those of patients infected with HPV16 (8-10). Bracken (*Pteridium* spp) is a fern belonging to the Pteridaceae family and is part of the human diet, particularly in China, Japan and Brazil (24,25). However, bracken can infect wastelands and pastures, and bracken toxins, such as ptaquiloside, contaminates the milk and meat of exposed animals (26,27). In addition, bracken can be considered hazardous to animal health by facilitating the persistence of bovine papillomavirus (BPV) infection in cattle and has also been associated with an increased risk of digestive malignancies in the human population (28). The hypothesis that ptaquiloside exerts immunosuppressive effects by counteracting the action of $CD8^+$ T cells against papillomavirus-induced lesions has been tested using HPV16-trangenic mice (11). Mice were treated with 0.5 mg ptaquiloside orally per week for 10 consecutive weeks. The results revealed that ptaquiloside decreased the activation and the degranulatory function of $CD8^+$ T cells infiltrating papillomavirus-induced lesions, revealed by a decrease in the expression of CD44 and CD107a; this confirmed the role of ptaquiloside as an immunosuppressive toxin (11).

Based on evidence that suggests that cyclooxygenase-2 (COX2) may be a therapeutic target in HPV-induced cancer (29) and nutraceutical compounds may have influence on HPV-induced lesions (30), the effects of curcumin (a polyphenol) and rutin (a quercetin glycoside) on the expression of COX2 and tumor-associated inflammation in HPV16-trangenic mice were evaluated (12). Diet was supplemented with curcumin or rutin for 24 weeks. COX2 expression was found to be reduced in the dermis and epidermis by rutin, and both compounds reduced leukocyte infiltration; however, neither compound prevented epidermal dysplasia. These results indicated that COX2 expression in the HPV16 mouse model can be modulated by the compounds evaluated, reducing tumor-associated inflammation. However, curcumin and rutin were not sufficient to terminate cancer progression (12).

Laurel (*L. nobilis*) is widely used as a spice and flavoring compound in the culinary and food industry (31). It is also used for the treatment of several health issues, such as gastric problems (32). *In vitro* studies had demonstrated the activity of Laurel (*L. nobilis*) on HPV-transformed cell lines (33). In addition, the *in vivo* efficacy and hepatic toxicity of a laurel extract (20 mg/animal/day) was previously evaluated using HPV16-induced cancer mice. The assay lasted three weeks and the results revealed that laurel extract did not prevent the progression of HPV-induced cutaneous lesions; however, the extract was well tolerated by the animals, since no changes concerning hematological, histological, biochemical and hepatic oxidative stress were observed (13).

P. umbilicalis is an intertidal red seaweed used as a food and has a high protein content, vitamins and fibers (34). *P. umbilicalis* can also be used to improve the nutritional profile of meat preparations, increasing its antioxidant properties (35). The potential of *P. umbilicalis* as a chemopreventive agent against HPV16-induced lesions was previously evaluated (14). For that purpose, the seaweed was incorporated into the base diet of HPV16-trangenic mice (10% seaweed) for 22 days (14). The results revealed a significant reduction in the incidence of pre-malignant dysplastic lesions and anti-genotoxic activity against HPV-induced DNA damage (14). The results suggested that diet supplementation with this red seaweed may exert chemopreventive effects and the concentration used was safe and did not induce toxicity in the animals (14).

DMAPT (15) is an analogue of the natural sesquiterpene, lactone parthenolide, isolated from the plant *Tanacetum parthenium* (feverfew). DMAPT is water-soluble with nuclear factor (NF)-κB inhibitory activity. In a previous study, it was administered orally (dissolved in water) at 100 mg/kg/day for 6 consecutive weeks. It was demonstrated that DMPAT reduced the incidence of epidermal dysplasia in transgenic mice, as well as the expression of the *Bcl2* and *Blc2/1* genes. It was also suggested that DMPAT prevented cachexia, since it preserved body weight and strength and no differences were observed in muscle mass or the expression of NF-κB subunits in skeletal muscle tissue. The results indicated that the NF-κB inhibitor, DMPAT, prevented wasting syndrome and exerted chemopreventive effects against HPV-induced lesions (15).

ICR mouse model. Olive leaf extract (OLE) and green tea have demonstrated promising results, namely in prostate and breast cancer, respectively (36,37).

Additionally, the authors of this review have previously used a different mouse model in order to examine the effects of ingesting high concentrations of OLE on the livers of ICR mice for a 14-week administration workflow. The choice of the olive leaf was justified by its use as a food supplement, as a natural therapy, and it medical properties, such as its anti-hypotensive (38), antioxidant (39), antitumoral (40) and hypocholosterolemic properties (41). The results of a previous study indicated that OLE induced changes in the liver biochemistry and histology, as well as hepatic mitochondrial bioenergetics of mice (42). The excessive intake of certain phytochemicals in OLE products may be associated with health issues and attention should be paid when considering the use of commercial products.

As previously mentioned, natural compounds can be used as chemopreventive agents against cancer (43). Green tea (*Camellia sinensis; C. sinensis*) is one of the most popular beverages worldwide and the chemopreventive effects of its constituents, such as polyphenolic compounds, have already been reported in several animal models (44). Based on these findings, the authors of this review previously evaluated the chemopreventive effects of whole green tea (*C. sinensis*) (0.5%) on urinary bladder cancer induced by BBN in ICR mice for 20 consecutive weeks (45). This urothelial chemical carcinogen induces bladder tumors in laboratory animals, which are similar to those observed in humans (46).

The results revealed that the BBN-exposed groups treated with *C. sinensis* only developed preneoplastic lesions and the number of inflammatory aggregates was lower in the animals treated with green tea (*C. sinensis*) compared with the untreated animals. Green tea infusion administration also influenced urothelial inflammation (45).

Taken together, these studies highlight the roles of polyphenols in disease prevention and management, due to their anti-inflammatory and cancer chemopreventive activities.

3. Concluding remarks

The results mentioned in the present review bring together some of our research team's experience in using natural compounds in animal models. Overall, the results contributed to increasing the knowledge and the influence of the compounds tested in the animal models used. Currently, the authors of the present review are working on several projects in order to assess the pharmacological potential of several natural compounds in different animal models. Researchers in the health sciences can take several advantages from the natural compounds, including in the field of cancer therapy and prevention.

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Availability of data and materials

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Authors' contributions

TF, ENG, RMGDC, ER and PAO contributed to the conception and design of the manuscript, literature search, drafting the work and revising the manuscript. All authors have read and approved the final manuscript. All authors have read and approved the final manuscript.

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Not applicable.

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Competing interests

The authors declare that they have no competing interests.

References

1. Cutler SJ and Cutler HG: Biologically active natural products: Pharmaceuticals. CRC Press, Boca Raton, FL, 2000.
2. Pereira C, Barros L and Ferreira ICFR: Dietary supplements: Foods, medicines, or both? a controversial designation with unspecific legislation. Curr Pharm Des 23: 2722-2730, 2017.
3. Shahidi F and Ambigaipalan P: Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects-A review. J Functional Foods 18: 820-897, 2015.
4. Caleja C, Ribeiro A, Barreiro MF and Ferreira ICFR: Phenolic compounds as nutraceuticals or functional food ingredients. Curr Pharm Des 23: 2787-2806, 2017.
5. Schmidt U, Struck S, Gruening B, Hossbach J, Jaeger IS, Parol R, Lindequist U, Teuscher E and Preissner R: SuperToxic: A comprehensive database of toxic compounds. Nucleic Acids Res 37: D295-D299, 2009.
6. Oliveira PA, Gil da Costa RM, Vasconcelos-Nóbrega C, Arantes-Rodrigues R and Pinto-Leite R: Challenges with in vitro and in vivo experimental models of urinary bladder cancer for novel drug discovery. Expert Opin Drug Discov 11: 599-607, 2016.
7. Fantini D, Glaser AP, Rimar KJ, Wang Y, Schipma M, Varghese N, Rademaker A, Behdad A, Yellapa A, Yu Y, *et al*: A carcinogen-induced mouse model recapitulates the molecular alterations of human muscle invasive bladder cancer. Oncogene 37: 1911-1925, 2018.
8. Arbeit J, Münger K, Howley P and Hanahan D: Progressive squamous epithelial neoplasia in K14-human papillomavirus type 16 transgenic mice. J Virol 68: 4358-4368, 1994.
9. Smith-McCune K, Zhu YH, Hanahan D and Arbeit J: Cross-Species comparison of angiogenesis during the premalignant stages of squamous carcinogenesis in the human cervix and K14-HPV16 transgenic mice. Cancer Res 57: 1294-1300, 1997.
10. Coussens LM, Hanahan D and Arbeit JM: Genetic predisposition and parameters of malignant progression in K14-HPV16 transgenic mice. Am J Pathol 149: 1899-1917, 1996.
11. Santos C, Ferreirinha P, Sousa H, Ribeiro J, Bastos MM, Neto T, Oliveira PA, Medeiros R, Vilanova M and Gil da Costa RM: Ptaquiloside from bracken (*Pteridium* spp.) inhibits tumour-infiltrating CD8+ T cells in HPV-16 transgenic mice. Food Chem Toxicol 97: 277-285, 2016.
12. Moutinho MSS, Aragão S, Carmo D, Casaca F, Silva S, Ribeiro J, Sousa H, Pires I, Queiroga F, Colaço B, *et al*: Curcumin and rutin down-regulate cyclooxygenase-2 and reduce tumor-associated inflammation in HPV16-Transgenic Mice. Anticancer Res 38: 1461-1466, 2018.
13. Medeiros-Fonseca B, Mestre VF, Colaço B, Pires MJ, Martins T, Gil da Costa RM, Neuparth MJ, Medeiros R, Moutinho MSS, Dias MI, *et al*: *Laurus nobilis* (laurel) aqueous leaf extract's toxicological and anti-tumor activities in HPV16-transgenic mice. Food Funct 9: 4419-4428, 2018.
14. Santos S, Ferreira T, Almeida J, Pires MJ, Colaço A, Lemos S, Gil da Costa RM, Medeiros R, Bastos MMSM, Neuparth MJ, *et al*: Dietary supplementation with the red seaweed *porphyra umbilicalis* protects against DNA damage and pre-malignant dysplastic skin lesions in HPV-Transgenic mice. Mar Drugs 17: 615, 2019.
15. Santos JMO, Moreira-Pais A, Neto T, Peixoto da Silva S, Oliveira PA, Ferreira R, Mendes J, Bastos MMSM, Lopes C, Casaca F, *et al*: Dimethylaminoparthenolide reduces the incidence of dysplasia and ameliorates a wasting syndrome in HPV16-transgenic mice. Drug Dev Res 80: 824-830, 2019.

16. Chen M, Du ZY, Zheng X, Li DL, Zhou RP and Zhang K: Use of curcumin in diagnosis, prevention, and treatment of Alzheimer's disease. Neural Regen Res 13: 742-752, 2018.
17. Jiang S, Han J, Li T, Xin Z, Ma Z, Di W, Hu W, Gong B, Di S, Wang D and Yang Y: Curcumin as a potential protective compound against cardiac diseases. Pharmacol Res 119: 373-383, 2017.
18. Ganeshpurkar A and Saluja AK: The pharmacological potential of rutin. Saudi Pharm J 25: 149-164, 2017.
19. Al-Kalaldeh JZ, Abu-Dahab R and Afifi FU: Volatile oil composition and antiproliferative activity of *laurus nobilis*, origanum syriacum, origanum vulgare, and salvia triloba against human breast adenocarcinoma cells. Nutr Res 30: 271-278, 2010.
20. Bennett L, Abeywardena M, Burnard S, Forsyth S, Head R, King K, Patten G, Watkins P, Williams R, Zabaras D and Lockett T: Molecular size fractions of bay leaf (*Laurus nobilis*) exhibit differentiated regulation of colorectal cancer cell growth in vitro. Nutr Cancer 65: 746-764, 2013.
21. Neelakantan S, Nasim S, Guzman ML, Jordan CT and Crooks PA: Aminoparthenolides as novel anti-leukemic agents: Discovery of the NF-κB inhibitor, DMAPT (LC-1). Bioorg Med Chem Lett 19: 4346-4349, 2009.
22. Guzman ML, Rossi RM, Neelakantan S, Li X, Corbett CA, Hassane DC, Becker MW, Bennett JM, Sullivan E, Lachowicz JL, *et al*: An orally bioavailable parthenolide analog selectively eradicates acute myelogenous leukemia stem and progenitor cells. Blood 110: 4427-4435, 2007.
23. Namvar F, Tahir PM, Mohamad R, Mahdavi M, Abedi P, Najafi TF, Rahmanand HS and Jawaid M: Biomedical properties of edible seaweed in cancer therapy and chemoprevention trials: A review. Nat Prod Commun 8: 1811-1820, 2013.
24. Liu Y, Wujisguleng W and Long C: Food uses of ferns in China: A review. Acta Soc Bot Pol 81: 263-270, 2012.
25. Alonso-Amelot ME and Avendaño M: Human carcinogenesis and bracken fern: A review of the evidence. Curr Med Chem 9: 675-686, 2002.
26. Clauson-Kaas F, Jensen PH, Jacobsen OS, Juhler RK and Hansen HC: The naturally occurring carcinogen ptaquiloside is present in groundwater below bracken vegetation. Environ Toxicol Chem 33: 1030-1034, 2014.
27. Virgilio A, Sinisi A, Russo V, Gerardo S, Santoro A, Galeone A, Taglialatela-Scafati O and Roperto F: Ptaquiloside, the major carcinogen of bracken fern, in the pooled raw milk of healthy sheep and goats: An underestimated, global concern of food safety. J Agric Food Chem 63: 4886-4892, 2015.
28. Gil da Costa RM, Bastos MM, Oliveira PA and Lopes C: Bracken-associated human and animal health hazards: Chemical, biological and pathological evidence. J Hazard Mater 15: 1-12, 2012.
29. Santos C, Neto T, Ferreirinha P, Sousa H, Ribeiro J, Bastos MMSM, Faustino-Rocha AI, Oliveira PA, Medeiros R, Vilanova M and da Costa RMG: Celecoxib promotes degranulation of CD8(+) T cells in HPV-induced lesions of K14-HPV16 transgenic mice. Life Sci 157: 67-73, 2016.
30. Gil da Costa RM, Aragão S, Moutinho M, Alvarado A, Carmo D, Casaca F, Silva S, Ribeiro J, Sousa H, Ferreira R, *et al*: HPV16 induces a wasting syndrome in transgenic mice: Amelioration by dietary polyphenols via NF-κB inhibition. Life Sci 169: 11-19, 2017.
31. Caputo L, Nazzaro F, Souza LF, Aliberti L, De Martino L, Fratianni F, Coppola R and De Feo V: *Laurus nobilis*: Composition of essential oil and its biological activities. Molecules 22: 930, 2017.
32. Qnais EY, Abdulla FA, Kaddumi EG and Abdalla SS: Antidiarrheal activity of *laurus nobilis* L. Leaf extract in rats. J Med Food 15: 51-57, 2011.
33. Dias MI, Barreira JC, Calhelha RC, Queiroz MJ, Oliveira MB, Soković M and Ferreira IC: Two-dimensional PCA highlights the differentiated antitumor and antimicrobial activity of methanolic and aqueous extracts of *Laurus nobilis* L. From different origins. Biomed Res Int 2014: 520464, 2014.
34. Cofrades S, López-López I, Bravo L, Ruiz-Capillas C, Bastida S, Larrea MT and Jiménez-Colmenero F: Nutritional and antioxidant properties of different brown and red spanish edible seaweeds. Food Sci Technol Int 16: 361-370, 2010.
35. López-López I, Bastida S, Ruiz-Capillas C, Bravo L, Larrea MT, Sánchez-Muniz F, Cofrades S and Jiménez-Colmenero F: Composition and antioxidant capacity of low-salt meat emulsion model systems containing edible seaweeds. Meat Sci 83: 492-498, 2009.
36. Li MJ, Yin YC, Wang J and Jiang YF: Green tea compounds in breast cancer prevention and treatment. World J Clin Oncol 5: 520-528, 2014.
37. Boss A, Bishop KS, Marlow G, Barnett MP and Ferguson LR: Evidence to support the anti-cancer effect of olive leaf extract and future directions. Nutrients 19: 513, 2016.
38. Khayyal MT, el-Ghazaly MA, Abdallah DM, Nassar NN, Okpanyi SN and Kreuter MH: Blood pressure lowering effect of an olive leaf extract (Olea europaea) in L-NAME induced hypertension in rats. Arzneimittelforschung 52: 797-802, 2002.
39. Bouaziz M and Sayadi S: Isolation and evaluation of antioxidants from leaves of a Tunisian cultivar olive tree. Eur J Lipid Sci Technol 107: 497-504, 2005.
40. Grawish ME, Zyada MM and Zaher AR: Inhibition of 4-NQO-induced F433 rat tongue carcinogenesis by oleuropein-rich extract. Med Oncol 28: 1163-1168, 2011.
41. Poudyal H, Campbell F and Brown L: Olive leaf extract attenuates cardiac, hepatic, and metabolic changes in high carbohydrate-, high fat-fed rats. J Nutr 140: 946-953, 2010.
42. Arantes-Rodrigues R, Henriques A, Pires MJ, Colaço B, Calado AM, Rema P, Colaço A, Fernandes T, De la Cruz PL, Lopes C, *et al*: High doses of olive leaf extract induce liver changes in mice. Food Chem Toxicol 49: 1989-1997, 2011.
43. Cabrera C, Artacho R and Giménez R: Beneficial effects of green tea-a review. J Am Coll Nutr 25: 79-99, 2006.
44. Lambert JD, Hong J, Yang GY, Liao J and Yang CS: Inhibition of carcinogenesis by polyphenols: Evidence from laboratory investigations. Am J Clin Nutr 81 (1 Suppl): 284S-291S, 2005.
45. Henriques A, Arantes-Rodrigues R, R, Faustino-Rocha A I, Teixeira-Guedes C, Pinho-Oliveira J, Talhada D, Teixeira J H, Andrade A, Colaço B, Paiva-Cardoso M N, *et al*: The effects of whole green tea infusion on mouse urinary bladder chemical carcinogenesis. Iran J Basic Med Sci 17: 145-148, 2014.
46. Vasconcelos-Nóbrega C, Colaço A, Lopes C and Oliveira PA: Review: BBN as an urothelial carcinogen. In Vivo 26: 727-739, 2012.

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