ELLAGIC ACID: A NOVEL POLYPHENOLIC ANTIOXIDANT AND THEIR THERAPEUTIC APPLICATIONS

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Summary

Polyphenolic compounds are widely distributed in plants and are known to be an excellent antioxidant. Ellagic acid (EA), a phenolic phytonutrient commonly found in fruits and nuts such as raspberries, strawberries, walnuts, pecans and pomegranate. It is well recognized as a chemopreventive agent. It plays a beneficial role in the prevention and treatment of various human diseases. Some of the therapeutic applications of EA are anticancer, antioxidant, antimutagenic, anti-inflammatory and antibacterial activities. The possible beneficial health effect of EA is due to its strong antioxidant activity. This review summarizes a vast literature pertaining the therapeutic actions of Ellagic acid in various experimental diseased conditions.

key words: Ellagic acid, Polyphenol, antioxidant, anticancer, anti-inflammatory.

Introduction

In recent years, researchers have begun focusing on phytochemicals a ubiquitous bioactive compound found in plant foods. Among various phytochemicals, polyphenols have attracted considerable interest in the past few years due to their potential health benefits. Generally, polyphenolic compounds are widely distributed in plant foods such as fruits, vegetables, cereals and beverages (1). Particularly polyphenols are considered as potential therapeutic agents against a wide range of ailments including neurodegenerative diseases, cancer, diabetes, antibacterial, antiallergic, hepatoprotective, antithrombotic, antiviral, vasodilatory actions, cardiovascular dysfunctions, inflammatory diseases and in ageing (2).

Ellagic acid

Ellagic acid is a naturally occurring phenolic phytonutrient found in varieties of fruits and nuts such as raspberries, strawberries, walnuts, pecans, cranberries, blackberries, grapes and other plant foods (3). Generally, it is present in plants in the form of hydrolysable tannins called ellagitannins. Ellagitannins are esters of glucose with hexahydroxydiphenic acid, when hydrolysed they yield ellagic acid (EA). It is a dimeric derivative of gallic acid. When two gallic acid groups link side by side within a tannin molecule, a hexahydroxydiphenoyl (HHDP) group is formed. When the HHDP group is then bioactively separated from the tannin molecule it spontaneously rearranges itself in to subunits and then forms ellagic acid (4). EA was first discovered by Braconnot in 1831. Where, EA was isolated from algarobilla and certain other fruits (Nierenstein). Ellagic acid was first synthesised by heating gallic acid with arsenic acid (Lowen).

Table 1. Major constituent of EA present in Dietary sources

Sources	Content	Reference
Rasp berry	263-30mg / 100g	(5)
Straw berry	25mg/100g	(6)
Black berry	1.5-2.0 mg/100	(7)
Walnut	802 mg/50g (8 nuts)	(8)
Peacan	20.96-86.2 mg/g	(9)
Chestnut	1.61-24.9 mg/kg	(10)
Pomegranate juice	2020-2660 mg/L	(11)

Structure

EA (4,4′,5,5′,6,6′-Hexahydroxydiphenic acid 2,6,2′,6′-dilactone), is a complex planner molecule with the molecular weight of 338.2g/mol. It is highly thermostable due to the 4 rings present in the molecule (Figure .1) which represents lipophilic dominance and the 4 phenolic groups and the 2 lactones representing the hydrophilic zone. These properties of EA result in high water insolubility. However it is soluble in acidified methanol, ethanol and DMSO (12).

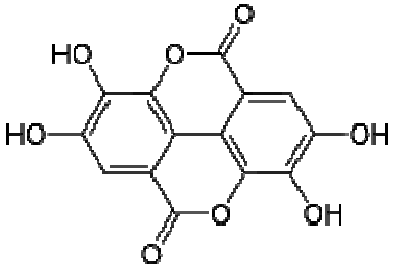

Figure 1: Structure of EA

Pharmacokinetic properties

In order to define the physiological relevance of EA, it is important to understand the natural dietary or diet supplemented EA intake and to briefly summarize knowledge on the bioavailability *in vivo*. Generally, ellagitannins from berries and nuts are hydrolysed in the intestine into ellagic acid. The extent of absorption and metabolism of EA is mainly due to the microflora in the intestine. On oral ingestion, EA and its metabolites have been isolated from blood, bile, urine and feaces (13). Studies on experimental animals show that on oral administration, the level of ^{3}H- EA was found to be high in blood for 30 min, in urine and bile for 120 min and in liver, lung and kidney for 15 min (14). During intravenal administration, EA was rapidly eliminated and almost 90% was eliminated from blood within 15min after administration (15). Transcellular absorption and epithelial cell accumulation of EA was investigated in the human intestinal Caco-2 cells, where they found a limited transcellular absorption and EA appeared to accumulate selectively in the epithelial cells of the aerodigestive tract (16). From these studies it is suggested that only a limited fraction of EA is orally bioavailable (14). This low bioavailability of ellagic acid is due to the hydrophobic nature of the molecule.

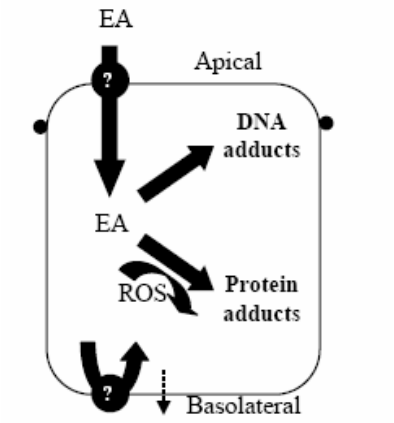

Figure 2: Proposed model of EA disposition in epithelial Caco-2 cells (16).

THERAPEUTICAL APPLICATIONS

Antioxidant activity

Antioxidants are substances that neutralize free radicals by delaying or inhibiting oxidation of substrate on which free radicals attack (17). The phenolic nature of ellagic acid makes itself a powerful antioxidant. Among all polyphenolic compounds, EA has the potential to scavenge reactive oxygen species (ROS) (18). Their protective efficacy depends on the type of ROS that is generated, the place of generation and the severity of damage. It also possess scavenging action against both superoxide and hydroxyl radicals and inhibits lipid peroxidation and 8-ohdG formations both *in vitro* and *in vivo* (19, 20, 21, 22). This is brought about by maintaining the activities of antioxidant enzymes like SOD, CAT & GPx. Since ROS have been implicated in the development of various pathological conditions, EA has the ability to control these diseases through its potential antioxidant activity.

Anticancer activity

Several nutritional and epidemiological studies indicate that consuming diets rich in vegetables and fruits may help to reduce the incidence of a broad range of malignancies (23, 24). In this regard, EA has attracted much attention as an anticancer agent. Numerous experimental and cell culture studies have been documented to show the chemopreventive potential of EA. Generally, the anticancer activity of EA was observed against various chemical carcinogens including nitrosamines, azoxymethane, mycotoxins and polycyclic aromatic hydrocarbons (25). EA has been clinically shown to cause apoptosis in certain cancer cells and has exhibited anticarcinogenic effects against a wide range of carcinogenesis in several tissues such as colon, esophageal, liver, lung, tongue and skin. These studies were carried out on both *in vitro* and *in vivo*. In early 1980s, EA was shown to inhibit skin tumors, where Lesc et al. in 1983 demonstrated that topical application of EA to NMRI mice afforded substantial protection against 7,12-dimethylbenz(a)anthracene induced skin tumorigenesis (26). Meanwhile, Mukhtar et al. showed an age related changes in benzo(a)pyrene metabolism and epoxide metabolising activities in rat skin (27). Further, they also investigated that application of EA to the skin which exerts strong protective effect against PAHs-induced skin carcinogenesis in BALB/c mice (28).

Likewise, Chang et al. reported that skin application of EA resulted in reduction of 7,8-BP-diol-9, 10-epoxide-2-induced skin tumorigenesis in CD-1 mice (29). In addition, chronic p.o. feeding of EA to mice resulted in delayed tumour onset and affords substantial protection against PAH-induced skin tumorigenecity (30). Lesca has demonstrated i.p. administration in sunflower oil or parentral administration in the diet of EA to A/J mice protects against BP-induced lung tumor formation (26). In a report, EA was compared with quercetin against N-nitrosodiethylamine-induced lung tumorigenesis in mice. It is evident from their result that, EA was found to be a better chemopreventer than quercetin (31). Recently, EA also reduces the incidence of N-nitrosomethyl benzylamine (NMBA) induced tumours in the rat oesophagus (32). There was a investigation on two polyphenolic compounds, EA and quercetin in MOLT-4 human leukemia cells which showed an enhanced anticarcinogenic potential through its synergestic biochemical interactions (33). Another study was demonstrated on colon cancer cells (SW 480), where their observation suggested that,

the growth inhibition of EA is mediated by signalling pathways that mediate DNA damage, triggers p^{53}, which in turn activates p^{21} and at the same time alters the growth factor expression, resulting in the down regulation of IGF-II (34). In addition, an investigation was made on human bladder cell line, where EA affects human bladder tumor cell NAT activity (35). Moreover, EA was also investigated on DNA adduction of the potent mammary carcinogen dibenzo(a)pyrene (DBP) using human breast cancer cells (36). Reports also show that the effect of EA on cell cycle events and apoptosis were studied in cervical carcinoma cells (37). Further, structure function relationship of EA was investigated by synthesizing different chemical analogs of EA and their findings showed that, different portions of EA molecule are responsible for its different putative anticarcinogenic activities. (38). The effect of EA by oral administration on *in vitro* and *in vivo* N-acetylation and metabolism of 2-aminofluorene was investigated in brain tissues from male Sprague dawley rats and their findings showed that EA decreases the carcinogen in brain tissues (39). EA is a powerful phenolic compound with proapoptotic and antiproliferative activity. Moud et al. have reported that EA stimulates mitochondrial depolarization, cytochrome C release and caspase activation (40).

Antimutagenic effect

The initial step in the formation of cancer is to damage the genome of a somatic cell producing a mutation in an oncogene or a tumor suppressor gene. This mutation largely results by the action of environmental factors. These environmental factors include chemical carcinogen and radiation originating almost exclusively from human activities. Numerous literatures confirm the antimutagenic effect of ellagic acid. However Francis observed only a small reduction of AFB1 mutagencity although the mutagenecity of N-methyl-N^1-nitro-N-nitrosoguanidine was reduced markedly. Similarly, the mutagenic effect of IQ was markedly reduced by the action of ellagic acid (41). Moreover EA was found to be effective in inhibiting the mutagenecity of MNU in the reaction of double-strand DNA with MNU (42). Smerak1 et al. also showed that EA similarly inhibited the mutagenic effects of AFB1, IQ & MNU in mice after a three-day exposure a revealed by a significant reduction of the number of micronuclei in the bone marrow in the groups of mice treated with EA & mutagen in comparison with the mice treated with mutagen only (43).

Mechanism of EA in cancer

Several mechanisms has been proposed to explain antimutagenic and anticarcinogenic effects of EA inhibits polycyclic aromatic hydrocarbon-induced tumorigenesis by inhibition of the CYP1 A1-dependent activation of benzo(a)pyrene adducts (44, 45, 46, 47, 48) by detoxifying the activated benzo(a)pyrene diolepoxide through 2 additional mechanisms. It reduces the expression of the phase II detoxification enzyme glutathione S-transferase (48). EA has also been shown to induce the expression of the phase II detoxification enzyme NAD (P) H quinine reductase (49). In addition, EA has been shown to bind DNA and inhibit the formation of O^6-methylguanine by methylating carcinogens (42, 50). Masjid et al. also demonstrated that dietary administration of EA to mice increased the levels of reduced glutathione and glutathione reductase in liver (51). In addition, Ahn et al. reported that EA inhibits hepatic cytochrome P450 1A1 and 2E1 in a non-competitive

manner. Further, it stimulates the mitochondrial death pathway through inhibiting the transcription factor NF-kB. Important mechanisms for the anticarcinogenic effects of polyphenols include the reduction of proliferation activity and the induction of apoptosis in cancer cells (52). EA has been reported to cause a Go/G1-phase arrest, reduction in proliferation and induction of apoptosis at 100μmol/I.

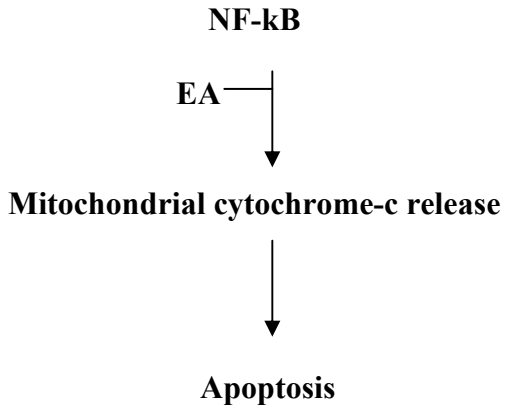

Figure 3: Proposed mechanism of induction in apoptosis by EA (40)

Table 2: Investigation of EA on different cancer tissues in various models

Tissues	Model	Reference
Skin	BALB/c mice	(30)
Lung	Mice, Hamster	(31) (53)
Oesophageal	Rat	(52)
Colon cancer	SW480 Rat	(54) (55)
Bladder	T24 & TSGH 8301	(39)
Breast	MCF-7	(36)
Cervical	Caski	(39)
Pancreatic	PANC-1, MIA PaCa-2	(40)
Neuroblastoma	SH-SY5Y	(56)
Leukemia	MOLT-4	(33)
Liver	Rat	(52)

Anti-inflammatory and antinociceptive activity

Many human ailments commonly occur through inflammation which is the central part of various pathological diseases such as arthritis, crohn's disease and psoriasis which can also lead to the development of both cardiovascular diseases and cancer. The cyclooxygenase enzymes are crucial in the production of pro-inflammatory molecules by both the cyclooxygenase and 5 lipooxygenase pathways (57) and inhibitors are commonly used as anti-inflammatory drugs. The anti-inflammatory effects of ellagic acid have been demonstrated in various animal model studies. Rogerio et al. examined the anti-inflammatory effect of EA by suggesting that

ellagic acid significantly decreases paw edema as measured by callipers after an injection of 1% carageenan and decreases the number of acid-induced writhing periods in mice. Similarly, the antinociceptive effect of EA in Sprague dawley rats was examined using the modified hot plate, and their findings proved that, EA possess both anti-inflammatory and antinociceptive activity (58). A very recent study was done on EA in combination with anesthetic adjuvants which showed effective against inflammation (59). In addition, the anti-inflamatory activity of EA was also investigated by using a standardized pomegranate rind extract containing 13% EA and finally proved to possess anti-inflammatory activity (60).

Hepatoprotective effect

Liver being the major organ to metabolise all foreign compounds is susceptible to various diseases such as hepatitis, cirrhosis and alcohol related disorders. The major causes of these diseases are due to exposure to different environmental pollutants and xenobiotics eg: paracetamol, CCL_4, thioacetamide, alcohol etc. various food additives play a major role in protecting liver. Experiments were carried on EA in rats which significantly reduced the number of gamma glutamyl transpeptidase positive foci induced by AFB 1 which is considered as the precursor of hepatocellular neoplasm. Moreover, EA was shown to inhibit liver fibrosis against CCL_4 in rats. Their results suggested that EA on oral administration can circumvent the CCL toxicity and subsequent fibrosis (61). In addition, dietary EA was shown to reduce the incidence of N-2-fluronenylacetamide induced hepatocarcinogenesis in rats. EA was also investigated on 8-hydroxydeoxyguanosineformation in liver nuclear DNA of rats treated with 2-nitropropane. On treatment with EA, it significantly inhibited 8-OH-dG formation in the liver nuclear DNA of male F-344 rats (20). In addition, oral administration of EA was shown to exert preventive effects against chronic alcohol-induced liver damage in rats (62). Consequently the antioxidant and cytoprotective property of EA was also reported to prevent liver damage induced by various type of oxidative stress (63).

Cataractogenic activity

Cataract is a multifunctional disease associated with several risk factors such as aging, diabetes, malnutrition, diarrhoea, poverty, sunlight, smoking, hypertension and renal failure (64). Free radical induced oxidative stress is postulated to be perhaps the major factor leading to senile cataract formation (65). A variety of natural compounds have been reported to prevent cataract formation (66, 67, 68). A recent study was investigated to evaluate the prevention of selenite induced cataractogenisis in wistar rats. On administration of EA (200 mg/kg) intraperitonially prevented the experimental selenite by maintaining the antioxidant defence systems and by inhibiting lipid peroxidation (69).

Antibacterial effects

The antibacterial activity of EA was supported by numerous investigations. Prashanth et al. investigated the antibacterial activity of EA, which slows down the detoriation operated by microorganisms (70). Report also shows that EA and other tannic acid were investigated against *Staphylococcus aureus* which may be a useful

adjuvant for the treatment of *S.aureaus* infections (71). EA in alone or in combination with L-ascorbic acid, successfully reduced the microbial growth by extending shef life of fish during storage at 0°C (72). A very recent report by Loo et al. evaluated the anticariogenic activities of EA against some cariogenic microorganisms *in vitro* in which EA showed a promising antimicrobial agent against oral pathogens in humans, thereby reducing the incidence of dental carries (73).

Antiplasmodial activity

In extent to antibacterial activity it also showed antiplasmodial activity. Sturm et al. reported the antiplasmodial activity of EA in the upper nanomolar range, which has been linked to the inhibition of plasmepsin II and an important of beta-hematin formation in the parasite (74).

Future prospects and conclusion

An overwhelming number of both *in vivo* and *in vitro* studies suggest that EA has immense potential for the prevention and treatment of numerous diseases. It may act individually, additively and synergistically to exert its beneficial role. Despite its lower bioavailability, their potential therapeutical values against various human diseases are not up to the mark. Therefore enhanced bioavailability and possible mechanism of action is necessary in future which will likely bring this promising phytonutrient to the forefront of therapeutic agents for the treatment of human diseases. Since most of the therapeutic effects of EA are based on *in vivo* and *in vitro* studies, clinical trails are needed to fully realize it's potential.

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