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Review Article

A review on Ellagic acid as a natural antioxidant

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ABSTRACT

Ellagic acid(EA) is a naturally occurring polyphenolic compound. Recently there is growing interest in understanding the role and mechanism of the phytochemicals; such as polyphenolics, flavonoids and phenylpropanoids as inhibitors of oxidative stress. EA has wide array of biological properties, such as radical scavenging, chemopreventive, antiviral and antibacterial properties. EA is a naturally occurring dietary antimutagen and anticarcinogen with strong antioxidant and anti-inflammatory activities. EA is abundant in berries, walnuts pecans, pomegranate, cranberries and other plant foods in the form of hydrolysable tannins called ellagitannins. Ellagitannins are mostly found in the plants belonging to families *Myrtaceae*, *Punicaceae* and *Combrataceae*. While focusing on the multifunctional activities of such compounds, this review briefly points out increasing interest in polyphenolic derivatives as an antioxidant, which holds future promises.

Keywords: Ellagic acid, polyphenolic, natural, antioxidant

INTRODUCTION

The term tannin was first applied by Seguin in 1796 to denote substances present in plant extract, which are able to combine with protein of animal hides to prevent their putrefaction and convert them into leather. The tannin-protein co-precipitation is important not only in the leather industry but also in relation to the physiological activity of herbal medicines, taste of foodstuffs and value of feed for herbivores. Two main groups of tannins are usually recognized; these are the hydrolysable tannins and the condensed tannins. Acids or enzymes such as tannase may hydrolyze the hydrolysable tannins. They are formed from several molecules of phenolic acids, which are united by ester linkage to a central glucose molecule. Two principal types of hydrolysable tannins are gallitannins and ellagitannin, which are units composed of gallic acid and hexahydroxydiphenic acid respectively. Ellagitannins are mostly found in the plants belonging to families *Myrtaceae*, *Punicaceae* and *Combrataceae*[1].

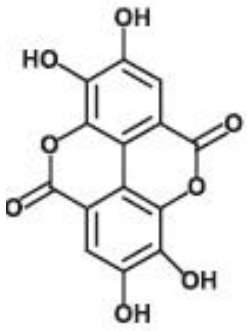
Ellagitannins are a major class of phenolics largely responsible for the astringent and antioxidant properties of raspberries and blackberries. The Rubus ellagitannins constitute a complex mixture of monomeric and oligomeric tannins. Rubus oligomeric ellagitannins contain besides the well-known ellagic acid (EA) and gallic acid moieties, the sanguisorboyl linking ester group. When exposed to acids or bases, ester bonds are hydrolyzed and the hexahydroxydiphenic acid spontaneously cyclizes into EA[2]. On hydrolysis ellagitannins spontaneously rearrange to the dilactone EA[3].

Recently there is growing interest in understanding the role and mechanism of the phytochemicals; such as polyphenolics, flavonoids and phenylpropanoids as inhibitors of oxidative stress [4]. Among all phytochemicals, EA has been receiving the most attention because of its wide array of biological properties, such as radical scavenging,

chemopreventive, antiviral and antibacterial properties[5] .EA is known as a naturally occurring dietary antimutagen and anticarcinogen with strong antioxidant and anti-inflammatory activities[6]. EA is abundant in berries, walnuts, pecans, pomegranate, cranberries and other plant foods in the form of hydrolysable tannins called ellagitannins[7 8].

Isolation of EA

In 1956 scientist Leonard Jurd reported the phytochemical screening of pellicle of the walnut. He had isolated various polyphenolic compounds such as EA, methyl gallate, gallic acid and tannin. The tannin was composed chiefly of four polyphenolic esters or glycosides, and on hydrolysis gave the above compounds. EA has melting point of about 360 ^{o}C and shows absorption maxima at 255 and 366nm. Jurd also synthesized EA by the method of Perkins and Nierenstein from gallic acid[9].



The chemical name of EA is 2,3,7,8,-Tetrahydroxy-[1]benzopyrano[5,4,3-cde] [1]benzopyran-5,10-dione.[10]

In the seeds of five commonly grown cranberry species: red raspberry, black raspberry, boysenberry, marion blackberry and evergreen blackberry they found 6-7% protein and 11-18% oil. The oils contained 53-63% linoleic acid, 15-31% linolenic acid, and 3-8% saturated fatty acids. All five species showed presence of ellagitannins and free EA [11].

Scottish-grown red raspberries are a rich source of vitamin C and phenolics. Most notably, the anthocyanins cyanidin-3-sophoroside, cyanidin-3-(2(G)-glucosylrutinoside), and cyanidin-3-glucoside, and two ellagitannins, sanguiin H-6 and lambertianin C,are present together with trace levels of flavonols, EA, and hydroxycinnamates[12].

Red raspberries contain EA, its 4-arabinoside, its 4' (4"-acetyl) arabinoside, and its 4' (4"-acetyl) xyloside, quercetin and kaempferol 3-glucosides. In addition, two unidentified EA derivatives were detected[13]. Cloudberry, *Rubus chamaemorus L. (Rosaceae)* is a herbaceous plant and it contains vitamin C and ellagitannins with a high level of EA that exhibits biological activities[14]. *Fragaria xananassa*, a strawberry, contains about 40 phenolic compounds including glycosides of quercetin, kaempferol, cyanidin, pelargonidin, and EA, together with flavanols, derivatives of p-coumaric acid and ellagitannins. Quercetin-3-malonylhexoside and a deoxyhexoside of EA were reported for the first time [15].

Anogeissus latifolia also contains leucocyanidins and tannoid principles like EA and its derivatives[16]. *Punica granatum* contains EA, 3,3',4'-tri-O-methyl EA, ethyl brevifolincarboxylate, urolic and maslinic acids, and daucosterol [17]. EA and punicalagin were isolated from pomegranate (*Punica granatum*) and observed as beta-secretase inhibitors[18].

A new ellagitannin, methyl (S)-flavogallonate along with fourteen known compounds, gallic acid, methyl gallate, ethyl gallate, 2,3-di-O- [(S 4,5,6,4', 5', 6'-hexahydroxybiphenyl-2, 2'-diyldicarbonyl]-(α/β)-D-glucopyranose, vitexin, isovitexin, orientin, iso-orientin,

kaempferol 3-O-β-D-rutinoside, rutin, neosaponarin, EA, flavogallonic acid and (α/β)-punicalagin have been isolated from the leaves of *Terminalia myriocarpa Heurck*[19] .

Leaves of *Melaleuca quinquenervia* contain gallic acid, EA, 3-O-methyl EA, 3,4,3'-tri-O-methylEA, 2,3-O-hexahydroxydiphenoyl- (α/β)-D-(4)C(1)-glucopyranose , castalin and grandinin[20]. From the methanolic extract of *Turpinia ternata* stems, new EA derivative 3,4'-di-O-methylEA-4-O-α-L-arabinofuranoside and the known compounds EA, 3-O-methyl EA, 3-O-methylEA-3'-O-α-L-rhamnopyranoside, and 3,3'-di-O-methylEA-4'-O-α-D-glucopyranoside were isolated[21]. EA rhamnosides isolated from the stem bark of *Eucalyptus globulus* were 3-O-methyl EA,3'-O-α-rhamnopyranoside, 3-O-methylEA 3'-O-α-3''-O-acetylrhamnopyranoside, 3-O-methylEA 3'-O-α-2''-O-acetylrhamnopyranoside,3-O-methylEA3'-O-α-4''-O-acetylrhamnopyranoside, respectively[22]. Stem bark of *Pteleopsis hylodendron* gives EA derivatives such as 3, 4-methylenedioxy-3'-O-methyl-4'-O-glucoside EA and the pteleoEA derivative, 3,4-methylenedioxy-3'-O-methyl EA,3,3'-di-O-methylEA and 3,3',4'-tri-O-methyl EA [23].

On phytochemical investigation of *Geranium robertianum* L., herb *Robert (Geraniaceae*), hyperoside, EA, isoquercitrin, quercetrin, kaempferols, caftaric acid and rutoside were identified[24].

The root bark of *Anisophyllea dichostyla* R. Br. is traditionally used in the Democratic Republic of Congo for the treatment of several conditions such as anorexia, fatigue and intestinal infections. Phytochemical screening of methanolic extract of root barks of *Anisophyllea dichostyla* R. Br gave several polyphenol antioxidants.The polyphenol content (3.32g/kg) was predominantly ellagitannins (25%) and polyhydroxyflavan-3-ols (catechins and procyanidins, 75%) with 3'-O-methyl-3,4-methylenedioxo EA 4'-O-β-d-glucopyranoside and (-)-epicatechin.Other main compounds are (+)-catechin, (-)-epicatechin. Other compounds present are 3-O-gallate, 3-O-methyl EA, 3,3'-di-O-methyl EA, 3'-O-methyl-3,4-methylenedioxo EA, 3'-O-methyl-3,4-methylenedioxo EA 4'-O-β-d-glucopyranoside, and 3'-O-methyl EA 4-O-β-d-xylopyranoside,3,4-methylenedioxo EA 4'-O- β-d-glucopyranoside, 3,3'-di-O-methyl EA 4-O-β-d-xylopyranoside [25].

Phenolic acids, flavonols, anthocyanins, EA, and numerous EA derivatives were isolated from Muscadine grapes [26]. The methanolic extract of the stem bark of *Lafoensia pacari (Lythraceae*) showed free radical scavenging activity in the diphenyl picryl hydrazyl radical (DPPH) decoloration assay and inhibited the enzyme xanthine oxidase *'in vitro'*. Bioassay-guided isolation led to EA as the main active compound of Brazilian and Paraguayan collections of the plant[27].

I. Analytical Methods

An SPE RP high-performance liquid chromatography (HPLC) method was optimized and applied for identification and determination of EA in hips of fourteen species of roses, wildly growing in Poland[6] . EA, its derivatives and anthocyanins were characterized and quantified by novel chromatographic conditions in eight muscadine grape (*Vitis rotundifolia*) cultivars and evaluated for antioxidant capacity as influenced by two ripening stages and their location within the fruit (skin, pulp, and juice)[28].

The EA, total phenolic and vitamin C contents in four raspberry cultivars (Heritage, Autumn Bliss, Rubi, and Zeva) grown in Spain were detected and quantified by HPLC in fresh, just frozen, and stored fruits at -20 ^{o}C for a one year period. EA [207-244 mg/ kg of fresh weight (fw)], total phenolic (137-1776 mg/ kgof fw), and vitamin C (221-312 mg /kg of fw) contents in raw materials were higher in the late cultivars Zeva and Rubi than in the early cultivars Autumn Bliss and Heritage. The freezing process slightly affected the values of extracted EA, total phenolic, and vitamin C content[29].

Methanolic extracts of Rosa L. leaves of seventeen rose species were analyzed for content of EA, quercetin and kaempferol using SPE-RP-HPLC methods. Additionally, total phenolic content was determined spectrophotometrically according to the Folin-Ciocalteu procedure and calculated as gallic acid equivalents. Antioxidant activity of methanolic extracts of Rosa L. leaves was evaluated *in vitro* using a spectrophotometric method based on measuring the radical scavenging effect on DPPH radicals, and the leaves showed good antioxidant activity[30].

A sample preparation for EA in several kinds of foodstuffs, solid samples was refluxed with methanol, and then the extract was refined using a solid-phase cartridge. The liquid samples were directly pretreated by solid-phase extraction [31].

Aldona Krawczyk et al. optimized sample preparation procedures and experimental conditions for the fast and simple determination of the free and the total EA content in cloudberry (*Rubus chamaemorus L.*) leaves by means of HPLC. For the first time the mixture of vitamin C and α tocopherol has been used to protect EA from the influence of oxygen and light and this combination of the two agents provided the best effect in comparison to other known antioxidants. The influence of time and temperature on EA yield in the hydrolysed sample has also been analysed. The HPLC conditions applied resulted in a satisfactory separation of EA standard solution as well as the studied sample [32].Newsome AG developed improved quantification of free and ester-bound gallic acid in foods and beverages by UHPLC-MS/MS.[33] Singh A etal did profiling of gallic and EA derivatives in different plant parts of Terminalia arjuna by HPLC-ESI-QTOF-MS/MS.[34] Two spectrofluorimetric methods have been developed by Sádecká J and Tóthová J. for the rapid determination of ellagic acid. The first method is based on the complex formation between ellagic acid and borax in methanol solution. The second method is based on the complex formation between ellagic acid and boric acid in ethanol solution.[35]

Bioavailability of Ellagic Acid

Plant polyphenols play an important role in human nutrition and are implicated with numerous biological properties including antioxidant[36], anti- inflammatory, anticancer and antiatherosclerotic activities. Among all these phytochemicals, EA, a dimeric derivative of gallic acid, occurs in fruits and nuts in either its free form as EA-glycosides, or bound as ellagitannins [31,37]. When the EA was orally administered to rat, 10 % of the dose was excreted and detected as EA metabolites in urine and feces[38]. The low concentration of free EA in plasma has been attributed to its low solubility in water[39] and may also be due to its extensive metabolic transformation and degradation prior to absorption[40]. In addition EA has been reported to bind irreversibly to cellular DNA and proteins, which may also account for its limited transcellular absorption. The poor absorption of EA has been reported to affect its *in vivo* anti-tumor activity since it is possible that sufficient concentrations are not present in plasma or target cells after oral administration[37,39]. It has been proposed that the gut microflora metabolize insoluble EA. Since ellagitannins are more soluble, their absorption or that of their transformation products is facilitated [39].

Also, since ellagitannins are easily hydrolyzed, the *in vivo* action of physiological pH and /or enzymatic action by gut microflora could cause them to break down to release EA units. Navindra etal have studied the absorption, bioavailability and pharmacokinetics of EA administered orally to humans. They found the presence of EA in plasma samples after consumption of ellagitannins from pomegranate juice[41]. When the metabolism study of pomegranate juice containing punicalagin or other food ellagitannins was carried out, the main metabolites observed were the 6H-dibenzo[b,d]pyran-6-one derivatives, as aglycones or glucuronide[42]. When the metabolism study of foodstuffs containing ellagitannins was carried

out, the metabolite 3,8-dihydroxy-6H-dibenzo[b,d]pyran-6-one (urolithin B) conjugated with glucuronic acid was detected [43].Yang J etal showed that premixing soy protein isolate and pomegranate juice did not affect the bioavailability or the metabolism of pomegranate ellagitannins in healthy volunteers.[44] Avachat AM and Patel VG had developed self-nano emulsifying drug delivery system based on the phospholipid complex technique, to improve the oral bioavailability of ellagic acid.[45]

Antioxidant Activity

A chemical reaction that usually takes place at ambient temperature between atmospheric oxygen and organic compound is generally defined as autoxidation. Substances that can suppress autoxidation are termed as inhibitors or antioxidants. Oxidation commonly involves free radical mechanism. Free radical may be broadly defined as a molecule or ion containing unpaired electron. Reactive oxygen species (ROS) is a term, which encompasses all highly reactive oxygen containing molecules, including free radicals. Types of ROS include the hydroxyl radicals, hypochlorite radicals and various lipid peroxides. All are capable of reacting with membrane lipids, proteins and enzymes and other small molecules, resulting in cellular damage[46]. Five types of caneberries evergreen blackberries (*Rubus laciniatus*), marionberries (*Rubus ursinus*), boysenberries (*Rubus ursinus x idaeus*), red raspberries (*Rubus idaeus*), and black raspberries (*Rubus occidentalis*) showed good antioxidant activity and this was measured by their oxygen radical absorbance capacity (ORAC)[47].

EA, which has been shown to be a potent antioxidant[36,48], inhibited the elevation of NO production induced by endrin[49]. EA and vitamin E succinate showed protective effect on 2,3,7,8-tetrachlorodibenzo-p-dioxin induced oxidative stress in different brain regions of rats, such as cerebral cortex, hippocampus, cerebellum, and brain stem. These parts were assayed for production of superoxide anion, lipid peroxidation, and DNA single-strand breaks. The mechanism behind this protection is modulation of antioxidant enzyme activities[50 51].

Addition of EA to liver microsomes of mice resulted in a steady increase in inhibition of NADPH-dependent lipid peroxidation[52] . *In vitro* experiments showed that liver microsomes from animals treated with EA and CCl_4, decreased lipid peroxidation compared to microsome prepared from rats exposed to CCl_4 alone . Lino et al. examined the effect of EA, one of the polyphenols that is abundantly present in whisky as a nonalcoholic component on gastric lesions induced by ammonia plus ischemia or ischemia/reperfusion in rats in relation to the antioxidative system. Results suggest that EA exhibits gastric protective action against gastric lesions induced by NH_4OH or reperfusion in the ischemic stomach, probably due to its anti-oxidative activity [53].

Fish phospholipid liposomes were prepared and used as an artificial membrane system to study factors influencing lipid oxidation. The extent of lipid oxidation was indexed by measuring the amount of thiobarbituric acid reactive substances (TBARS) produced. Fe^{2+},$Fe^{3+}$and Cu^{2+}were potent prooxidants in catalysing lipid oxidation. Morin, luteolin (flavonoids), butein (chalcone), tannic acid, EA (polyphenols), butylated hydroxyanisole and butylated hydroxytoluene (synthetic antioxidants) are potent antioxidants of Fe^{2+}-catalyzed lipid oxidation[54].

EA inhibits gamma-radiation (hydroxyl radical) induced lipid peroxidation in rat liver microsomes in a dose- and concentration-dependent manner[55]. Naturally occurring plant

polyphenols, which include EA, tannic acid, caffeic acid and ferulic acid, were tested for their superoxide anion radical (SOR)-scavenging activities. SOR were produced by interaction of the tumor promoter benzoyl peroxide (BPO) with murine peritoneal macrophages *in vitro*. Ferulic acid was observed to be the most effective and EA the least effective inhibitor of SOR formation[56] **.**

Alcohol, by its property of generating free radicals, causes severe damage to the membrane and affects almost all organs of the human body. Devipriya et al. investigated *in vivo*, the antioxidant potential of EA against oxidative stress induced by alcohol intoxication[4].

II. Antioxidant and cardioprotective activities

The study of Hannum SM shows that strawberries have antioxidant property because they contains EA and certain flavonoids: anthocyanin, catechin, quercetin and kaempferol. Antioxidants help to lower risk of cardiovascular events by inhibition of LDL-cholesterol oxidation, promotion of plaque stability, improved vascular endothelial function and decreased tendency for thrombosis. Furthermore, strawberry extracts have been shown to inhibit COX enzymes *in vitro*, which would modulate the inflammatory process. Individual compounds in strawberries have demonstrated anticancer activity in several different experimental systems, blocking initiation of carcinogenesis and suppressing progression and proliferation of tumors. Preliminary animal studies have indicated that diets rich in strawberries may also have the potential to provide benefits to the aging brain[57]. As EA is a potent antioxidant, it could prevent atherosclerosis via suppression of oxidative stress and apoptosis in hyperlipidemic rabbits[58].

EA gives cardioprotective effect[59] on a model of norepinephrine myocarditis in rats[60]. Expression of cell adhesion molecules by endothelium and the attachment of monocytes to endothelium may play a major role in atherosclerosis. Yu YM et al.. investigated the effects of EA on the formation of intracellular reactive oxygen species, the translocation of NF Kappa B and expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 and endothelial leucocyte adhesion molecule (E-selectin) induced by IL-1beta in human umbilical vein endothelial cells (HUVEC). It was observed that EA significantly reduced the binding of human monocytic cell line, U937, to IL-1beta-treated HUVEC. The production of reactive oxygen species by IL-1beta was dose-dependently suppressed by EA. Supplementation with increasing doses of EA effective in inhibiting the expression of VCAM-1 and E-selectin. Furthermore, the inhibition of IL-1beta-induced adhesion molecule expression by EA was manifested by the suppression of nuclear translocation of p65 and p50. In conclusion, EA inhibits IL-1beta-induced nuclear translocation of p65 and p50 and there by suppressing the expression of VCAM-1 and E-selectin, resulting in decreased monocyte adhesion. Thus, EA has anti-inflammatory properties and also may play an important role in the prevention of atherosclerosis [61].

EA can reduce the infarct size, by regulating apoptotic gene expressions and enhancing the activities of mitochondrial respiratory marker enzymes and cell viability, thereby protecting the myocardium against isoproterenol-induced myocardial infarction. The decreased infarct size associated with inhibited apoptosis and increased respiratory marker enzymes provide insight on the role of ellagic acid in antiapoptotic mechanism, and it may be the reason for its cardioprotective activity.[62]

III. Antioxidant and chemopreventive activities

Certain dietary constituents can protect against chemically induced carcinogenesis in rodents. A principal mechanism by which these compounds exert their protective effects is likely to be via induction of carcinogen detoxification. This can be mediated by conjugation

with glutathione, which is synthesized by the sequential actions of glutamate-cysteine ligase (GLCL) and glutathione synthetase. They found that dietary administration of the naturally occurring chemopreventive agents; EA, coumarin or alpha-angelicalactone caused an increase in GLCL activity of 3- to 5-fold in rat liver[63].

EA is an inhibitor of the *in vitro* mutagenicity of N-nitrosodimethylamine (NDMA) in Salmonella typhimurium strain TA100 using pyrazole-induced rat liver 9000-x g supernatant (S-9)[64]. Effects of dietary supplementation with the antioxidants EA, quercetin and vanillin were examined using a medium term multi-organ carcinogenesis model in rats. Various carcinogens were used like diethylnitrosamine, 1, 2-dimethylhydrazine, N-butyl-N-(4- hydroxybutyl) nitrosamine and 2, 2'-dihydroxy-di-n-propylnitrosamine. EA and quercetin exerted potent chemopreventive action in both the initiation and promotion stages in the experimental system[65].

The plant phenolic antioxidants caffeic acid, EA, chlorogenic acid and ferulic acid showed the inhibition of tongue carcinogenesis induced by 4-nitroquinoline-1-oxide (4-NQO) when they were administered concurrently with the carcinogen[66]. EA acid shows antiproliferative activity.[67]
Ellagitanins, and EA, inhibited the proliferation of the cancer cells[68]. EA caused a slight, but significant cell cycle arrest at the G1 phase, and urolithins caused cell cycle arrest at the G2/M phase and upregulated p21 expression. Apoptotic cells were detected by Annexin V-FITC/PI assay when treated with the compounds. Disruption in mitochondrial membrane potential and activation of caspases 8 and 9 suggest that both extrinsic and intrinsic apoptotic pathways may be involved. Activation of caspase 3 and cleavage of PARP further confirmed the induction of the apoptosis. ET, and EA, showed anti-cancer activity by arresting the cell cycle and inducing apoptosis on HT-29 human colon cancer cells. This study suggests that the BRB seeds could be a potential source of anti-cancer ET.[69]

EA did not show significant inhibitory effect on initiation of hepatocarcinogenesis by the food carcinogen 2-amino-3-methylimidazo [4,5-f] quinoline as compared to other natural antioxidants like β-carotene, α-tocopherol, glutathione, vanillin and quercetin [70].

EA shows suppression of aflatoxin B1 --induced chromosome aberrations in rat bone marrow cells[71]. Food additives such as turmeric (*Curcuma longa*), the active ingredient curcumin (diferuloyl methane), asafoetida (flavouring agent), butylated hydroxyanisole, butylated hydroxytoluene and EA were found to inhibit the mutagenesis induced by aflatoxin B1 in Salmonella tester strains TA 98 and TA 100. Antioxidant food additives help in ameliorating aflatoxin-induced mutagenicity and carcinogenicity [72].

Polyphenols have been shown to induce apoptosis in a variety of tumor cells including leukemia both *in vitro* and *in vivo.* However, their action on normal human peripheral blood mononuclear cells (PBMCs) during oxidative stress remains to be explored. When the evaluation for anti-apoptotic and radical scavenging activities of dietary phenolics namely caffeic acid, EA and ferulic acid were carried out. The phenolics significantly inhibited DNA damage and lipid peroxidation but they could not alter the Bcl-2 expression in PBMCs.This indicated that anti-apoptotic effect of EA, caffeic acid and ferulic acid in PBMCs is through the Bcl-2 independent mechanism [73].

In another study the effects of four isolated polyphenolic extracts from red muscadine grapes (*Vitis rotundifolia*) on vital cell parameters and the induction of apoptosis in Caco-2 colon carcinoma cells were examined. The efficacy of the polyphenolics on vital cell parameters correlated well to the presence of EA glycosides and flavonoids and also to the

antioxidant capacity. This study demonstrated the anticancer properties of EA rich extracts from red muscadine juice[74].

Naturally occurring antimutagenic compounds were extensively analyzed for their capacity to protect cells from induced damage. The ability of taurine and EA to act against damage induced by mitomycin-C and hydrogen peroxide in Chinese hamster ovary cells cultivated *in vitro* was studied. EA proved to have more than one mechanism of action probably as a scavenger of oxygen species produced by H_2O_2 treatment and as a protector of the DNA double helix from alkylating agent injury[75].

In another study ascorbic acid, melatonin and EA, were also evaluated for their ability to modulate DNA damage produced by two strong radical oxygen inducers (H_2O_2 and Bleomycin) in cultured CHO cells. The alkaline comet assay was used to measure DNA damage and a cytofluorimetric analysis was performed to reveal the intracellular oxidative species. There was marked reduction of H_2O_2 and Bleomycin-induced DNA damage by EA [76].

Prenatal exposure to cocaine induces malformations, which are caused by ischemic-reperfusion injury. A study was undertaken to assess a new *in vitro* model which uses a routine rat whole embryo culture system that incorporates a change in oxygen status and to examine the effects of altered oxygen status and pretreatment with EA, an anti-oxidant after cocaine exposure. EA blocked the effects of cocaine on developmental score and GSH (reduced glutathione) level. These data support ischemia-reperfusion injury as the mechanism of cocaine developmental toxicity[77].

Khanduja et al. looked into the anticarcinogenic[78] potential of plant polyphenols EA and quercetin against N-nitrosodiethylamine-induced lung tumorigenesis in mice. Both EA and quercetin caused a significant increase in GSH and decrease in NADPH- and ascorbate-dependent lipid peroxidation. EA was found to be more effective in the lipid peroxidation and increasing the GSH [79].

Induction of glutathione S-transferase (GST) enzymes can increase detoxification of carcinogens and reduce carcinogen-induced mutagenesis and tumorigenesis. The effect of EA was examined on the expression of glutathione S-transferase-Ya. Rats fed EA demonstrated significant increases in total hepatic GST activity, hepatic GST-Ya activity and hepatic GST-Ya mRNA. It was confirmed that observed increase in GST-Ya mRNA was due to EA inducing transcription of the GST-Ya gene[80].

Induction of cellular detoxification enzymes can increase detoxification of carcinogens and reduce carcinogen-induced mutagenesis and tumorigenesis. EA induces transcription of the QR gene, which is responsible for expression of the phase II detoxification enzyme NADPH quinone reductase (QR) and this induction is mediated through the antioxidant responsive element of the QR gene[81].

In another study pomegranate extract (PE) from the rind containing 90% EA was tested for its skin-whitening effect. Results suggest that the skin-whitening effect of PE was probably due to inhibition of the proliferation of melanocytes and melanin synthesis by tyrosinase in melanocytes[82].

Dietary phenolic compounds are known to elicit vital cellular responses such as cell cycle arrest, apoptosis and differentiation by activating a cascade of molecular events. Narayanan et al. used human cDNA microarrays with 2400 clones consisting of 17 prosite motifs to characterize alterations in gene expression pattern in response to the phenolic

antioxidants EA and resveratrol (RE). Over a 48-hr exposure of androgen - sensitive LNCaP cells to EA and RE, a total of 593 and 555 genes respectively showed more than a twofold difference in expression [83].

In a recent study it has been reported that berry extracts rich in polyphenols and EA can induce gamma-glutamylcysteine synthetase heavy subunit (GCS(h)) activity *in vivo* using transgenic mice[84].

EA, tannic acid, caffeic acid and ferulic acid offer considerable promise as anticarcinogens. Topical application of polyphenols simultaneously with phorbol-12-myristate-13-acetate (PMA) or mezerein resulted in significant protection against 7,12-dimethyl-benz [a] anthracene-induced skin tumors in mice [85].

Pomegranate (*Punica granatum* L.) fruits are widely consumed as juice (PJ). The potent antioxidant and anti-atherosclerotic activities of PJ are attributed to its polyphenols including punicalagin, the major fruit ellagitannin, and EA. Punicalagin is the major antioxidant polyphenol ingredient in PJ. Punicalagin, EA, a standardized total pomegranate tannin (TPT) extract and PJ were evaluated for *in vitro* antiproliferative, apoptotic and antioxidant activities. Apoptotic effects were evaluated against the HT-29 and HCT116 colon cancer cell lines. Antioxidant effects were evaluated using inhibition of lipid peroxidation and Trolox equivalent antioxidant capacity assays. The superior bioactivity of PJ compared to its purified polyphenols illustrated the multifactorial effects and chemical synergy of the action of multiple compounds compared to single purified active ingredients[86] .

EA, green tea, and diallyl sulfide showed preventive property against N-nitrosomethylbenzylamine induced mutagenicity in the esophagus of the rat[87]. EA acts as antimutagenic agent and has been shown to inhibit chemically induced cancer in the lung, liver, skin and esophagus of rodents[88].

Failure of treatment of cancer in clinic by radio/chemotherapy is generally attributed to tumor resistance. It is, therefore, important to develop strategies to increase the cytotoxicity of tumor cells by radiation in combination with new tumor selective cytotoxic agents. Bhosle et al. describe the role of EA and gamma radiation on the oxidative stress and subsequent cytotoxicity of tumor cells in vitro as well as in vivo and their sparing effects on normal cells. Combined treatment of tumor with EA and radiation enhances oxidative stress and cytotoxicity in tumor cells. EA protects normal cells against radiation damage. This may offer potential therapeutic benefit, which warrants clinical study for application in cancer radiotherapy[89].

The effects of five naturally occurring antioxidants; beta-carotene, vitamin C, vitamin E, EA and epigallocatechin gallate on 8-hydroxydeoxyguanosine (8-OH-dG) formation by 2-nitropropane, a hepatocarcinogen in rats, were studied. Vitamin E and EA have showed promising anticarcinogenic effect towards 2-nitropropane [90].

The potential of ellagic acid to down-regulate the 17beta-estradiol-induced hTERT alpha+beta+ mRNA expression may be a mechanism via which ellagic acid exerts, at least in part, its chemopreventive effects in breast cancer.[91]

Miscellaneous

As cranberry fruits contains phenolic phytochemicals such as phenolic acids, flavonoids and EA, consumption of cranberry fruits shows lower incidences of urinary tract infections and inhibition of peptic ulcer-associated bacterium; Helicobacter pylori [92]. Methoxyellagic acid glucoside from Feijoa sellowiana leaf extract showed bone mineralization enhancing activity.[93]

EA plays a role to modulate dichloroacetic acid -induced developmental toxicity in zebrafish embryos. As in development of toxicity by dichloroacetic acid, oxidative stress plays major role[94]. EA has a protective effect against cisplatin-induced nephrotoxicity and oxidative stress in rat but not enough to inhibit cisplatin-induced renal dysfunction[95]. EA administration orally can circumvent the carbon tetrachloride toxicity and subsequent fibrosis[96] . EA was shown to be potent *in vitro* inhibitor of GSH-transferase(s) activity. Other plant phenols such as ferulic acid, caffeic acid and chlorogenic acid also showed a concentration dependent inhibition of GSH-transferase(s) activity[97]. Growth inhibition of cell proliferation by EA is mediated by signaling pathways that mediate DNA damage, triggers p53, which in turn activates p21 and at the same time alters the growth factor expression, resulting in the down regulation of insulin like growth factor IGF-II[98]. EA shows equal protective ability as N-acetylcysteine against nicotine-induced toxicity in rat peripheral blood lymphocytes [99].

EA possesses antihepatotoxic, antisteatosic, anticholestatic, antifibrogenic, antihepatocarcinogenic and antiviral properties that improves the hepatic architectural and functions against toxic and pathological conditions. EA possesses potent neuroprotective effects through its free radical scavenging properties, iron chelation, activation of different cell signaling pathways, and mitigation of mitochondrial dysfunction[100]

EA, one of the polyphenols that are abundantly contained in whisky as a nonalcoholic component, has antioxidant and anti-inflammatory activities. In the recent study, the action of whisky and pure ethanol on the rat gastric mucosa, and examined the role of EA in reducing the damaging effect of whisky in the stomach was studied. Results of the study suggest that whisky is less irritating to the gastric mucosa, as compared with pure ethanol, and this property of whisky may be explained by EA, and its radical scavenging action[101].

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