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Review on Ehrlich Ascites Carcinoma in Mice and Cancer Treatment with Special Reference to The Potential Protective and Therapeutic Effects of Hesperidin Versus Cisplatin

Nahed Saleh[1], Tamer Allam[1], Abdelfattah M. Abdelfattah[1*] and Nermeen El-Borai[2]

(1) Department of Clinical Pathology, Faculty of Veterinary Medicine, University of Sadat City, Minufiya, Egypt.

(2) Department of Forensic Medicine & Toxicology, Faculty of veterinary medicine, University of Sadat City, Minufiya, Egypt.

**Corresponding author:* abdelfattah.mohamed@vet.usc.edu.eg *Received: 13/2/2022 Accepted: 11/3/2022*

ABSTRACT

Ehrlich Ascites Carcinoma (EAC) is an experimental transplantable neoplasm, which discovered firstly as a mammary tumor in mouse. Like other cancers growing in body cavities, EAC cells fill the mouse peritoneum by rapid cells division causing a local inflammatory reaction, with increasing vascular permeability leading to intense edema formation, cellular migration and progressive ascitic fluid formation. Cisplatin (Cis) is one of the most frequently used antineoplastic agents for various types of cancer. Cisplatin exhibited its antitumor effect via generating DNA adducts, which in turn leads to cell cycle arrest and initiating apoptotic signaling pathway. Unfortunately, the use of Cis in cancer patients is now strictly regulated due to the fact that it is linked to a variety of adverse effects, including nephrotoxicity, severe nausea and vomiting, myelosuppression, ototoxicity, and neurotoxicity. Furthermore, many cisplatin-treated patients who relapsed after a period of recovery have developed drug resistance. Natural products have played an essential role in health promotion and illness prevention for many years. There is a lot of data that is linked to the creation of natural product-based medicines that have been utilized to inhibit, reverse, or slow down the carcinogenesis process. Hesperidin (Hesp) is a bioflavonoid found primarily in citrus fruits such as clementine, lemons, mandarins, grapefruit, and oranges. Hesperidin is proposed to provide a wide range of health benefits including anti-inflammatory and antimicrobial properties, as well as cancer treatment.

Keywords: Ehrlich Ascites Carcinoma, EAC, chemotherapy, cisplatin, hesperidin.

INTRODUCTION

Cancer is a disease in which cells in a specific section of the body uncontrollably proliferate and replicate. Cancerous cells have the ability to penetrate and kill healthy tissue, including organs. In outbred mice, Ehrlich Ascites Carcinoma (EAC) is a spontaneous mammary adenocarcinoma that has adapted to generate ascites and is transmitted by successive intraperitoneal (i/p) passages (Kaleoğlu and İşli, 1977 and Frajacomo *et al*., 2016). Chemotherapy is a type of cancer treatment that employs the use of chemicals to kill cancer cells. Unfortunately, most anticancer medicines have cytotoxic effects on normal cells, resulting in unwanted side effects (Rani *et al*., 2012). As a result, it is critical to seek for compounds with anti-tumor capabilities or to optimize the anti-tumor effects of standard anti-cancer treatments at low concentrations in order to limit the negative side effects of these drugs in normal tissues. Many anti-carcinogenic natural substances originating from herbs, vegetables, plant extracts, and fruits

are now available (Cragg and Pezzuto, 2016). Flavonoids are a class of polyphenolic chemicals present in a wide range of fruits and vegetables that have antibacterial, antiviral, anticancer, immunostimulatory, and antioxidant characteristics (Arafa *et al*., 2009 and Firuzi *et al*., 2011). According to data gained from several in vitro and in vivo research, hesperidin is a bioflavonoid, a type of plant pigment found largely in citrus fruit that has been recognized as a potent anti-inflammatory, antibacterial, anti-carcinogenic, and antioxidant agent (Hosseinimehr *et al*., 2012). The aim of this review was to highlight the present and up-to-date protective and therapeutic impacts of hesperidin as a potential anti-tumor agent with special emphasis on EAC in comparison to the well-known chemotherapeutic agent cisplatin in rat to explore its clinical importance and application.

Ehrlich Ascites Carcinoma (EAC)

Firstly, Ehrlich Ascites Carcinoma (EAC) appeared in a female mouse as a spontaneous mammary glands cancer and then used as a trial tumor for experimental studies by transplanting tumor tissues from mouse to mouse (Ehrlich and Apolant, 1905; Aktaş, 1996 and Taşkin, 2002).

Loewenthal and Jahn (1932) named the liquid obtained from the peritoneum of the mouse as "Ehrlich Ascites Carcinoma" referring to the ascites fluid and Ehrlich carcinoma cells. EAC is a type of undifferentiated carcinoma that has a high transplantable capacity, no regression, rapid proliferation, a shorter life span, and is 100 percent malignant with no tumor specific transplantation antigen (TSTA) (Kaleoğlu and İşli, 1977).

EAC is typically applied in ascites form or in solid form; if ascites fluid containing tumor cells is injected intraperitoneally (i.p), the ascites form is obtained, while if it is injected subcutaneously (s.c.), the solid form is created (Zeybek, 1996 and Okay, 1998).

In solid form of Ehrlich tumor, fibrinogen is considered a regular component of solid tumors stroma. Fibrinogen extravasated from plasma is rapidly clotted to fibrin by the help of tumor cell-associated and perhaps by other tissue procoagulants. The deposited fibrin gel matrix organizes solid tumors into discrete nests of malignant cells and serves as a provisional matrix that facilitates the ingrowth of macrophages, new blood vessels, and fibroblasts with generation of mature stroma. Finally, within a week, a tumor of 1 cm in diameter is produced (Yea and Dvorak, 1994).

This very aggressive tumor kills nearly all of the experimental animals in a short time (Sakai *et al*., 2010). Ascites tumor cells, unlike solid tumors, proliferate largely as a cell suspension in body cavities. EAC cells grow in two phases after being injected intraperitoneally into mice: a proliferation phase in which the number of cells grows exponentially, and a plateau phase followed by a resting phase in which the number of cells remains nearly constant (Siems *et al*., 1993).

Morphological and metabolic changes occur as the EAC transitions from the proliferating to the plateau phase, for example: degradation of structure (Segura *et al*., 2001), decreased number of mitochondria (Schwendel *et al*., 1994), decreased DNA and RNA biosynthesis (Aktaş, 1996), loss of intracellular purine and pyrimidine nucleotides, nucleosides and bases (Schwendel *et al*., 1994), a decline of the ATP concentration and turnover (Siems *et al*., 1993), decreased protein synthesis (Siems *et al*., 1993), increased thymidine concentration with a decrease of thymidine kinase activity (Aktaş, 1996), decreased glutathione (GSH) concentration (Lobo *et al*., 2000) and increased triglycerides, cholesterol esters and free fatty acids (Aktaş, 1996).

During the proliferating phase, EAC increased through rapid cell division and in the load peritoneal cavity. The host animal died after a period of time due to the pressure exerted by the tumor volume and/or the harm caused by the tumor (Aktaş, 1996 and Altun, 1996). Moreover, tumor cells secrete a vascular permeability factor and so the blood vessels in the peritoneal cavity of mice with EAC exhibit microvascular permeability that rose dramatically in comparison to those in the control group and help in accumulation of ascites fluids (Senger *et al*., 1983).

Moreover, Altun (1996) discovered that the rate of cell proliferation in the bone marrow was suppressed in mice depending on the age of the tumor. This proved that the inhibitory substances in ascites fluid had an effect on the host animal's normal cell population. Furthermore, tumor development can produce antioxidant disruptions

in tumor host tissues as well as a loss of cellular redox equilibrium. Tumor cells may also generate massive quantities of hydrogen peroxide, which may contribute to their capacity to mutate, kill normal tissues, and assault other tissues. This indicates that there is a direct relationship between changes in tumor cell proliferation rate and changes in the antioxidant system (Gupta, 2004).

The main standard modalities of cancer treatment

There are numerous methods and medications available to treat cancer, with many more under investigation. Some therapies are "local," such as surgery and radiation therapy, and are used to treat a specific tumor or body part. Chemotherapy, immunotherapy, and targeted therapy are examples of "systemic" treatments since they have an impact on the entire body. The most prevalent types of cancer treatment include:

1. **Surgery**

Surgery was found to be the main modality that has been used to treat cancer for many years which targets to remove the tumor either partially or completely. However, surgery also plays a key role in diagnosing cancer through exploration of a particular area to obtain a sample for the diagnosis of a suspicious mass and finding out how far it may have spread **(Eyre *et al*., 2002)**.

2. **Radiotherapy**

Radiation therapy is a cancer treatment that involves the use of high doses of radiation to kill cancer cells through direct damage to cancer cells and/or triggering activation of $CD8^+$ T cells **(Lee *et al*., 2009)**. However, because of the undesired biological repercussions, which are not restricted to malignant tissues but also spread to nearby normal tissues, its use is still accompanied with harmful side effects and potential risks. Furthermore, the generation of free radicals and increased levels of lipid peroxides in tissues, particularly cell membranes, is one of the most prominent causes of cellular damage after radiation **(Hospers *et al*., 1999 and Oliinyk *et al*., 2001)**.

3. **Chemotherapy**

Chemotherapy is a cancer treatment in which medicines are used to destroy cancer cells. Chemotherapy, which is a common cancer treatment, works on the idea of stopping tumor cells from growing and spreading or killing them. Chemotherapy is also utilized if tumor spread occurs and surgical treatment is not possible. Chemotherapy is more effective against cancers that are poorly differentiated and grow quickly **(Mycek *et al*., 1998)**.

Chemotherapy is also the primary treatment option for many kinds of cancers, whether or not they require surgery. This includes many solid and hematological malignancies. It can also be used before surgery (i.e., neo-adjuvant therapy) to decrease a tumor so that it becomes easier to remove especially in large tumors or in those that are strongly attached to the surrounding tissues **(Coffey *et al*., 2009)**. All chemotherapeutic drugs used to treat cancer are not cancer-specific, meaning they impact both proliferating neoplastic cells and healthy cells (some chemotherapeutic agents are hepatoxic, nephrotoxic, cardiotoxic, etc.) **(Mycek *et al*., 1998)**. As a result, novel drugs that can have anti-tumor actions while having minimal side effects on normal tissues are needed **(Soini *et al*., 1998)**.

Cisplatin (Cis)

The search for anti-cancer drugs, with few notable exceptions, has proved to be a long, arduous, and frustrating exercise. Cisplatin considers one of these few exceptionally successful chemotherapeutic drugs **(Siddik, 2003 and Wang and Lippard, 2005)**. Since its fortuitous discovery in 1965, it has been widely used alone or in combination for treatment of various solid tumors **(Rosenberg *et al*., 1965 and Siddik, 2003)**.

Cisplatin has been known for over a century. **Dr. Michel Peyrone** first made cisplatin in 1845 and the structure was determined in the 1890 by **Dr. Albert Werner (Trzaska, 2005)**. However, it was more than a half a century after **Werner's** work before **Rosenberg** and his coworkers recognized the potential anti-tumor activity of platinum complexes during experiments examining the relationship between bacterial cell division and the potential influence of electrical current **(Rosenberg *et al*., 1965 and Rosenber *et al*., 2006)**.

Cisplatin was first approved by the FDA (Food and Drug Administration) in 1978 for the treatment of testicular and bladder cancer. Furthermore, the National Cancer Institute (NCI) has approved cisplatin for the following cancers: testicular cancer, bladder cancer, cervical cancer, non-small cell lung cancer (NSCLC), malignant mesothelioma, ovarian cancer, and squamous

cell carcinoma of the head and neck **(Siddik, 2003 and Reed, 2006)**.

Cisplatin is also combined with radiotherapy and has been used effectively for many tumor types especially for treating NSCLC and some squamous cell carcinoma. The effectiveness of all these treatment options depends especially on the cancer type and the treatment protocol **(Hazuka *et al*., 1994)**.

A. Chemical composition and pharmacokinetics

Cisplatin has a molecular structure, with a core platinum atom surrounded by two chlorine atoms and two ammonia groups **(Page *et al*., 1985)**. After cisplatin is injected intravenously, it attaches to plasma proteins and about 30-70% of it undergoes renal excretion **(Abu-Surrah, 2007)**. The remaining fraction reaches the bloodstream unaltered. Once it reaches the bloodstream, it diffuses through the cell membrane into the cell **(Ishida *et al*., 2002)**.

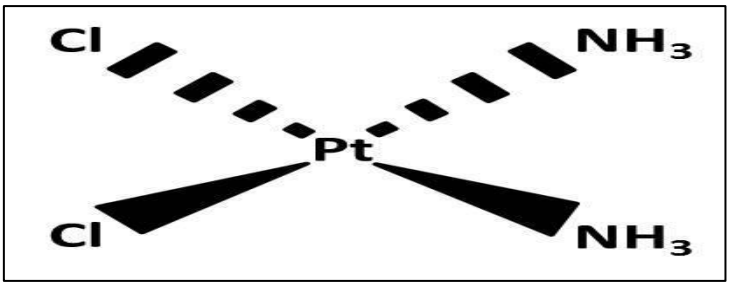

Figure 1. Structure of cisplatin: cis-diamminedichloroplatinum II **(CDDP) (Page *et al*., 1985)**.

Cisplatin is spread throughout the body at first, although it likes to store in the liver, kidney, skin, and muscle tissues. Cis stays in the liver and kidney tissues for a long time, with high quantities in the kidney tissue as long as 12 days following treatment in the dog. The largest amounts of tissue cisplatin are found in tissues where the medication has a strong anticancer effect, such as the uterus and ovary **(Barabas *et al*., 2008)**.

Cisplatin is activated by an aquation process in which the two chloride leaving groups are exchanged for water or hydroxyl ligands. Cisplatin enters cells primarily through passive diffusion, which involves the loss of chloride groups from the cisplatin molecule, or active absorption, which involves the cell's copper transporter (CTR1) **(Ishida *et al*., 2002)**. Cisplatin's clearance is biphasic, and only trace quantities of platinum were found in bile, implying that fecal excretion is low. On the other hand, according to **Barabas *et al*. (2008)**, free platinum clearance is 156 percent higher than creatinine clearance, implying that cisplatin or a metabolite is secreted by the kidney.

B. Mechanism of action of cisplatin

Inside the cell, cisplatin molecule undergoes hydrolysis. This hydrolysis is facilitated by a lower level of chloride inside the cell than the extracellular fluid. A chlorine ligand of cisplatin is substituted by a molecule of water inside the cell, producing a positively charged species. The mono-aqua chloroplatinum (II) species formed during hydrolysis is the active species which binds to various cellular targets causing cell killing **(Bose, 2002)**.

Approximately 1% of administered cisplatin binds to genomic DNA **(Eastman, 1990)**. Different platinum DNA adducts lead to distortion of DNA in a distinctive manner, leading to various cellular responses in the tumor cells **(Wang and Lippard, 2005)**. Finally, cisplatin DNA adducts lead to inhibition of replication **(Duman *et al*., 1999)** and transcription **(Corda *et al*., 1993)** causing cell cycle arrest **(Sorenson and Eastman, 1988)**. Also, cisplatin binds to a variety of RNA polymerases, leading to their arrest **(Wang and Lippard, 2005 and Jung and Lippard, 2007)**.

C. Side effects of cisplatin treatment

Cisplatin has a limited therapeutic index, which means that the amount of medicine required to achieve a meaningful reduction in tumor burden frequently causes nephrotoxicity. With repeated administrations of the medicine and its accumulation in the renal tubular fluid, nephrotoxicity becomes more persistent and severe **(Fillastre and Raguenez-Viotte, 1989)**. The most frequent manifestation associated with nephrotoxicity is hypomagnesemia, the incidence is expected to be within 40 to 100% of people **(Maxwell *et al*., 1994)**.

Other symptoms like Fanconi-like syndrome **(Wangila *et al*., 2006)**, distal renal tubular acidosis **(Swainson *et al*., 1985)**, renal concentrating defect **(Seguro *et al*., 1989)** and thrombotic microangiopathy **(Jackson *et al*., 1984)** are among a few of the well-known complications. Cisplatin also causes intracellular injury, which results in the production of damage

associated molecular pattern molecules (DAMPS), often known as "alarmins". Toll-like receptors (TLRs) are known to be influenced by DAMPs (family of receptors that play an important role in the immune system) attracts inflammatory cells via numerous routes, including the release of chemokines and other cytokines such as TNFα **(Gluba *et al*., 2010)**.

The pathophysiology of cisplatin-induced kidney damage is thought to be caused by oxidative stress, which damages the lipid components of the cell membrane and denatures proteins, resulting in enzyme inactivation and mitochondrial malfunction. Furthermore, cisplatin inhibits antioxidant enzymes, resulting in lower levels of glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) in the kidneys **(Yilmaz *et al*., 2004; Badary *et al*., 2005 and Kawai *et al*., 2006)**.

McKeage (1995) discovered that cisplatin-induced gastrointestinal toxicity is linked to the death of rapidly dividing cells lining the gastrointestinal tract and in the bone marrow. Cisplatin also excites the chemoreceptor trigger zone, causing vomiting two to three hours after taking the medicine. Myelosuppression occurs in 25 to 30 percent of people who undergo treatment with cisplatin which is indicated by thrombocytopenia and leukopenia that are more pronounced with higher doses **(McKeage, 1995)**. Anemia was also reported with the use of cisplatin **(Nguyen *et al*., 1981)**.

Cisplatin can lead to electrolyte disturbance by causing hypomagnesemia, hypokalemia, and hypocalcemia **(McKeage, 1995)**. Ototoxicity has been found in humans more frequently than in dogs. Hearing loss in the high-frequency range is dose-dependent, cumulative, and often irreversible **(Schell *et al*., 1989)**.

Drug resistance is a serious problem with cisplatin, in addition to the previously listed adverse effects. Reduced drug accumulation, enhanced drug detoxification, improved DNA damage repair, and higher cell survival despite DNA damage are all mechanisms driving the development of platinum-based drug resistance **(Wang and Lippard, 2005 and Hall *et al*., 2008)**.

4. Cancer chemoprevention

Chemoprevention, which is defined as the use of synthetic or natural medicines (alone or in combination) to prevent the progression of cancer in humans, is currently the most promising technique for cancer prevention. Plants, vegetables, herbs, and spices have long been recognized as one of the most important sources of cancer chemopreventive medication research **(Abdullaev, 2001)**. An ideal cancer chemopreventive drug is one that occurs naturally and may promote apoptosis in tumor cells without causing significant side effects **(Surh, 1999)**.

Hesperidin (Hesp)

Hesperidin is the main flavonoid in citrus fruits and can be isolated in large amounts from the rinds of some citrus species e.g., *Citrus aurantium L*. (Bitter orange), *Citrus sinensis L*. (Sweet orange), and *Citrus unshiu Marcov*. (Satsuma mandarin) **(Wilmsen *et al*., 2005)**. Hesperidin (3,5,7-trihydroxyflavanone7-rhamnoglucoside) is the food-bound form of hesperetin and one of two compounds incorrectly labelled as ‘Vitamin P' **(Garg *et al*., 2001)**.

Hesperidin was discovered in lemons and other citrus fruits after being extracted from the albedo (the spongy inner portion of the peel) of oranges by French chemist **LeBreton** in 1828 **(Manthey and Grohmann, 1998)**. Due to its antioxidant and anti-inflammatory properties, hesperidin possesses a variety of biological effects in models of cardiovascular disease **(Roohbakhsh *et al*., 2015)** and diabetes **(Homayouni *et al*., 2018)** as well for the prevention of cancer **(Roohbakhsh *et al*., 2015)**. Additionally, hesperidin can cross the blood-brain barrier and possesses neuroprotective actions **(Garg *et al*., 2001)**.

In animal models, hesperidin's antioxidant and anti-inflammatory effects were found to alleviate symptoms of Alzheimer's disease **(Sawikr *et al*., 2017)**, Parkinson's disease **(Jung and Kim, 2018)**, Huntington's disease **(Menze *et al*., 2012)**, depression **(Antunes *et al*., 2016)**, neuroimmunological multiple sclerosis (MS) **(Haghmorad *et al*., 2017)**, brain ischemia reperfusion injury **(Gaur and Kumar, 2010)**, and traumatic injury in central nervous system (CNS) tissues **(Kosari-Nasab *et al*., 2018)**.

A. Chemical composition and pharmacokinetics of hesperidin

In the chemical formula of hesperidin, glucose is linked to aglycone based structure (hesperetin) and rhamnose is attached to this structure from glucose moiety **(Garg *et al*., 2001)**. Hesperidin is most typically found in nature in the rutinoside form, which is non-bitter (such as orange). Meanwhile, hesperidin is found in grapefruit as neohesperidosides. The presence of hydroxyl moieties in both aromatic and heterocyclic rings is widely recognized to be responsible for hesperidin's biological actions **(Garg *et al*., 2001)**.

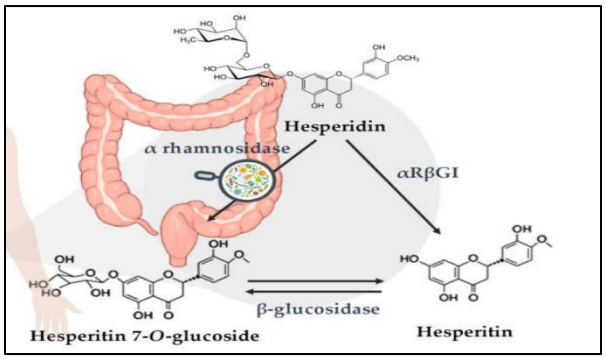

Figure 2. Chemical structures of hesperidin and its aglycone, hesperetin **(Garg *et al*., 2001)**.

Hesperidin's antioxidant capabilities are closely linked to the presence and number of hydroxyl moieties. Dietary flavonoids are primarily consumed orally, and their absorption is influenced by physicochemical factors such as molecular weight, lipophilicity, structural configuration, solubility, and acid dissociation constant **(Kumar *et al*., 2013)**.

The gastrointestinal tract (small intestine and colon) is the primary site for flavonoid absorption, and it is regulated by the nature of flavonoids, whether glycoside or aglycone. Glycosides must be transformed into aglycones before they can be absorbed, whereas aglycones can be absorbed directly **(Kumar *et al*., 2013)**. To be absorbed, conjugated, metabolized, and/or excreted, flavonoids must be broken into heterocyclic rings and reduced into phenyl acids (mostly by intestinal bacteria) **(Yao *et al*., 2004)**. Moreover, the small intestine is where flavonoid conjugation takes place, followed by the liver for further metabolism. Sulphates and glucuronide derivatives are produced in the liver, and they are excreted through urine or bile. Flavonoids that are not digested in the gut make their way to the colon, where they are structurally altered by colonic bacteria before being reabsorbed **(Yao *et al*., 2004)**. Flavonoids are bio-transformed in the intestine, resulting in metabolites. The portal vein transports these metabolites to the liver, where they are then delivered to target tissues, discharged to bile for enterohepatic recirculation, or removed through feces or urine **(Thilakarathna and Vasantha Rupasinghe, 2013)**.

B. Pharmacological effects of hesperidin

a. Hesperidin as a potent antioxidant

In 2011, Hussein and Othman reported that hesperidin had a strong reducing power, chelating action on Fe2C, and scavenging activities for free radicals, hydrogen peroxide, hydroxyl radicals, and superoxide. **Rice-Evan *et al*. (1996)** clarified that hesperidin significantly protects DNA, lipids, and proteins against free radical damage. **In 2008, Choi** reported that in 7,12-dimethylbenz(a)anthracene (DMBA)-treated mice, hesperidin effectively reduced protein oxidation, which is known to cause significant oxidative damage in organs such as the liver and mammary glands. Furthermore, hesperidin significantly improved the reduction in SOD and CAT levels seen in the DMBA-treated group, indicating hesperidin's potent antioxidant properties.

b. Beneficial effects of hesperidin on cancers

Colon cancer was reduced by 22% in rats treated with orange juice, and lung cancer was reduced by 29% in rats treated with mandarin juice. The presence of high quantities of flavonoids like hesperidin in juices has been linked to these effects **(Wilmsen *et al*., 2005)**. Also, **Park *et al*. (2008) and Bartoszewski *et al*. (2014)** reported that hesperidin induced apoptotic cell death in a variety of tumor cells via both extrinsic and intrinsic mechanisms. Hesperidin, for example, activates particular intracellular death-receptor pathways in colon cancer cells by causing DNA fragmentation and the production of perinuclear apoptotic bodies. Apoptosis was generally triggered by upregulation of Bax and Caspase-3 **(Park *et al*., 2007)**.

In human gastric cancer cells treated with hesperidin (100 M), apoptotic alterations such as chromatin condensation, apoptotic morphology of cellular bodies, modification of Bcl-2, and activation of Caspase 3 were seen, suggesting

that hesperidin could be used to treat gastric cancer patients **(Park *et al*., 2008)**.

Hesperidin (80 M) significantly stimulated cell shrinkage, vacuolation, production of plasma membrane blebs, and cell detachment in human breast carcinoma cell line (Michigan cancer foundation-7 MCF-7) when tested for apoptotic activity. Other apoptotic properties such as an increase in LDH (lactate dehydrogenase) level, depletion of GSH, DNA fragmentation, accumulation of p53 protein, and stimulation of caspase 3 protein were also showed **(Natarajan *et al*., 2011)**.

c. Influence of hesperidin on cancer-related inflammation

Parhiz *et al*. (2015) claimed that Hesp is an effective anti-inflammatory drug that can be used to treat a variety of inflammatory-mediated illnesses, including cancer. It targets several inflammatory components (Interleukin-6 (IL-6), Cyclooxygenase-2 (COX-2), Tumor necrosis factor-α (TNF-α), Inducible nitric oxide synthase (iNOS)) involved in tumor development **(Parhiz *et al*., 2015)**. Treatment with Citrus Juices (rich in Hesperidin) in N′-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) causing lung and colon carcinogenesis in mouse and rat reported decreasing of mRNA expression of many cytokines (IL-1β, TNF-α, IL-6) and inflammatory enzymes (COX-2 and iNOS) and increasing of mRNA expression of Nrf2 (Nuclear factor-2), quinine reductase and glutathione S-transferase which obviously clarified the anti-inflammatory role of hesperidin against NNK causing cancer **(Tanaka *et al*., 2011)**. Furthermore, in persons with metabolic syndrome, therapy with 500 mg hesperidin dramatically lowered plasma levels of two inflammatory biomarkers, C-reactive protein (CRP) and serum amyloid A (SAA) **(Rizza *et al*., 2011)**.

Hesperidin was found to inhibit epinephrine and adenosine diphosphate (ADP)-induced blood cell aggregation, including erythrocytes, leukocytes, and platelets, in human and animal studies, which could explain its beneficial effects on abnormal capillary permeability and fragility, as well as its protection against various traumas and stresses **(Garg *et al*., 2001)**.

Conclusions and future prospects

In conclusion, this review can provide important insights into hesperidin's potential as a promising agent for cancer prevention and therapy, and it may provide major support for the clinical implementation of this product in the future. Nevertheless, further preclinical, and clinical studies are warranted to increase the translation applicability of hesperidin to confirm the full potential of this bioflavonoid in cancer prevention and intervention.

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