Review

Effectiveness of Silymarin, Sulforaphane, Lycopene, Green Tea, Tryptophan, Glutathione and Escin on human health: results of a narrative review

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Abstract: *Background*: Recently the role of nutraceutical compounds in the prevention of human diseases is rapidly increasing. Here, we aim to evaluate the beneficial effect of dietary supplementation with seven active principles, lycopene, sulphoraphane, silymarin, glutathione, escin, tryptophan, green tea catechins, on human health. *Methods*: An extensive search of PubMed and Medline database was performed with the following keywords: "silymarin", "sulforaphane", "lycopene", "green tea catechins", "tryptophan", "glutathione" and "escin" accompanied by the keywords "supplement", "supplementation", "nutraceutics". All pre-clinical and clinical trials have been considered for this review. *Results*: One-hundred and eighteen full-text articles were eligible for inclusion in this review. The papers examined presented considerable variability due to the wide heterogeneity of dosages administered, population involved and outcomes pursued. *Conclusion*: Nutritional supplementation with lycopene, sulphoraphane, silymarin, glutathione, escin, tryptophan and green tea catechins appears to exert a wide range of benefits on human health, ranging from mood and cognition to cardiovascular health, fertility, metabolism, antioxidant and anti-inflammatory capabilities, potential anti-cancer effects. Further studies are required to better define the potential synergic effect, optimal dosage, mechanism of action and tolerability profile of these substances.

Keywords: sulphoraphane; silymarin; lycopene; glutathione; escin; tryptophan; green tea; men health; prevention.

1. Introduction

Nowadays, dietary supplementation is a widespread practice which involves not only patients affected by actual diseases, but also healthy population. Over 50% of adults in the US regularly include some form of supplement intake in their daily routine [1]. Reasons for supplementation may range from a general intention to "stay healthy" and prevent diseases – cancer in particular – to addressing specific needs tied to known medical conditions or deficits [2]. Furthermore, the user almost always chooses the supplement as a "self-prescription", rarely consulting a doctor before purchase [3].

There is an enormous amount of substances and actives of natural origin that are known for their purported health benefits, and the aim of this review is to narratively

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summarize the properties of seven particular principles which commonly recur in commercially available supplements and present some interest in the fields of not only urology and andrology, but also general well-being and prevention of oxidative-stress damage consequent to pollutants environmental exposure.

1.1 Research Questions

We put forth this research query:

Is dietary supplementation with silymarin, sulforaphane, lycopene, green tea catechins, tryptophan, glutathione and escin able to protect the human health, increase fertility and prevent aging disease?

In order to respond to these research questions, we performed a systematic review of all available studies performed with the aim to evaluate the efficacy of a dietary supplementation with silymarin, sulforaphane, lycopene, green tea catechins, tryptophan, glutathione and escin in the human health benefit.

2. Materials and Methods

2.1 Research Strategy and Literature Search

From January to March 2023, three independent reviewers (C.d.A., F.S., C.V.) performed the research in PubMed database, Cochrane CENTRAL and Scopus. All disagreements between the two reviewers were resolved by three experienced supervisors (F.P., T.C., A.P.). All references cited in relevant articles were also reviewed and analyzed. Considering the extent of the literature published on the subject in general and on each of the active substances in particular, the authors saw fit to present the results of this review in a narrative fashion. Systematic or meta-analytical comparison of so heterogeneous outcomes in measurements, population and methodology is beyond the scope of this work. The reaserch strategy includes the following keywords: “silymarin”, “sulforaphane”, “lycopene”, “green tea catechins”, “tryptophan”, “glutathione” and “escin” accompanied by the keywords “supplement”, “supplementation”, “nutraceutics”. Only papers in English language were included. Randomized controlled trials (RCTs), quasi-RCTs, non-randomized trials were included as priority, whereas prospective and retrospective cohort studies, case-control studies were included in case of significant results or population numbers. Case reports and case series were excluded. Pre-clinical and in-vitro evidence was examined and presented in the case of lack of significant clinical evidence for certain substances.

3. Results

A total of 98 papers were included in this review: 7 for silymarin, 21 for sulforaphane, 9 for lycopene, 33 for green tea catechins, 12 for tryptophan, 4 for glutathione and 12 for escin. Of these, 39 were pre-clinical evidence papers and the remaining 59 were clinical trials. Amongst clinical trials papers, 50 were RCTs, 7 were non-RCTs and 2 were case-control studies.

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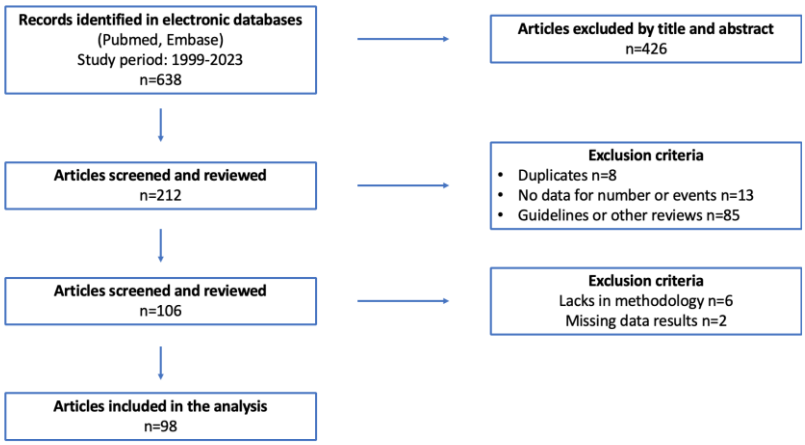

Figure 1. The figure shows the papers' selection process according to PRISMA recommendations.

Substance	Reference	Specie	Level of Evidence	Dose & Duration	Main Conclusions	Clinical field of significance
Green tea	(47)	Human	RCT	50 or 100 or 200 mg, single dose	Highest dose significantly increases excretion of cancerogen acrylamide	Antioxidant activity
	(48)	Human	Non-RCT	300 mg/day for 14 days	Slight decrease in plasma leukocyte count, significant increase in antioxidant status	
	(49)	Human	RCT	1 cup single dose or 2 cups/day for 7 days	Increase in heme oxygenase-1 activity, decrease in lymphocytic DNA damage	
	(50)	Human	Non-RCT	254 mg, single dose	Plasma PCOOH levels decreased with an inverse correlation to the increase in plasma EGCG levels	
	(51)	Human	RCT	4 cups/day or 2 capsules/day for 8 weeks	Decrease in SAA levels	
	(52)	Human	RCT	4 cups/day for 16 weeks	Decrease in urinary 8-OHdG levels in heavy smokers with	

					mutations of glutathione-S-transferase	
	(53)	Human	RCT	800 mg/day for 6 weeks	Beneficial period x treatment interaction in terms of body weight control in overweight subjects	Metabolism & cardiovascular health
	(54)	Human	RCT	4 cups/day or 2 capsules/day for 8 weeks	Significant decrease in body weight and BMI	
	(55)	Human	RCT	456 mg/day for 8 weeks	Mild changes in insulin level	
	(56)	Human	RCT	630 mg/day for 14 weeks	Reduction in cholesterol levels	
	(57)	Human	RCT	400 mg/day or 800 mg/day for 8 weeks	Reduction in LDL cholesterol and glucose-related markers	
	(58)	Human	RCT	100 mg/day for 4 weeks	Improvements in insulin resistance	
	(59)	Human	RCT	1450 mg, single dose	Reduction in some circulating catecholamines	
	(60)	Human	RCT	1500 mg/day for 16 weeks	Within-group reduction in waist circumference, HOMA-IR index, insulin level; increase in ghrelin level	
	(61)	Human	RCT	456 mg/day for 8 weeks	Reduction in HbA1c levels, borderline significant reduction in blood diastolic pressure	
	(62)	Human	RCT	350 mg/day for 7 days	Reduction in insulin levels	
	(63)	Human	RCT	800 mg/day for 8 weeks	Reduction in blood diastolic pressure	

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	(64)	Human	RCT	540 mg/day for 24 weeks	Improvements in skeletal muscle mass in sarcopenic subjects	Physical performance
	(65)	Human	RCT	250 mg/day for 4 weeks	No negative effects on endurance-training adaptation	
	(66)	Human	RCT	570 mg/day for 8 weeks	Improvements in aerobic capacity during training	
	(67)	Human	RCT	1500 mg/day for 10 weeks	Improvements in metabolic and antioxidant status during physical exercise	
	(68)	Human	RCT	900 mg/day for 52 weeks	Reduction in incidence of relapsing metachronous colorectal adenomas	Anti-cancer activity
	(69)	Human	Case-control	>2 cups/day for >20 years	Significant decrease in incidence of de novo myelodysplastic syndromes	
	(70)	Human	RCT	600 mg/day for 24 weeks	No effect in preventing PCa incidence	
	(71)	Human	RCT	600 mg/day for up to 20 weeks	No effect in preventing PCa incidence	
	(72)	Human	Non-RCT	6000 mg/day for a median of 4 weeks	No anti-neoplastic activity in PCa patients	
	(73)	Human	RCT	600 mg/day for 52 weeks	No effect in preventing PCa incidence in HGPIN patients	
	(74)	Human	RCT	400 mg/day for 52 weeks	No effect in preventing PCa incidence in HGPIN and ASAP patients	
	(75)	Human	RCT	843 mg/day for 52 weeks	Reduction of mammographic density	

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					in women aged 50-55	
	(78)	Human	RCT	800 mg/day for 16 weeks	Absence of recurrence in 1/3 of treated women with ovarian cancer	
	(79)	Human	RCT	800 mg/day for 16 weeks	No protective effect on CIN	
	(76)	Human	RCT	200 mg/day for up to 12 weeks	Lower recurrence of CIN	
	(80)	Human	RCT	N/A	Higher clinical response in uVIN	
Sylmarin	(9)	Human	RCT	160 mg 4 tablets/day for 10 weeks	The dietary supplement utilized in this study was shown to delay PSA progression after potentially curative treatment in a significant fashion	Anti-cancer activity
	(10)	Human	RCT	570 mg/day for 24 weeks	The combination of this study significantly reduced two markers of lipid metabolism known to be associated with PCa progression	
	(11)	Human	RCT	570 mg daily for 24 weeks	Improvement of IPSS score, urodynamic parameters: maximal rate of urine flow (Qmax), average flow (Qave), V and RV, total PSA value	Antioxidant
Sulforaphane	(27)	Human	RCT	Two tablets containing 10 mg sulforaphane each, three times/day for 24 weeks	Median log PSA slopes were consistently lower in sulforaphane-treated men	Anti-cancer activity

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	(26)	Human	RCT	Two 100-µmol/day taken 12 h apart. Mean intervention period was 4.4 wk	The supplement was associated with significant interactions in gene expression among some genes that are related to PCa development	
	(20)	Human	RCT	A weekly 300 mL portion of soup made from a standard broccoli or from an experimental broccoli genotypes with enhanced concentrations of glucoraphanin	Changes in gene expression and associated oncogenic pathways were attenuated in men on the glucoraphanin-rich broccoli soup in a dose-dependent manner.	
Lycopene	(39)	Human	Non-RCT	10 mg/day	A significant and maintained effect on PSA velocity over 1 year was demostrated	Anti-cancer activity
	(41)	Human	RCT	4 mg twice a day for 52 weeks	Lycopene delay or prevent HGPIN from developing into occult prostate cancer	
	(43)	Human	RCT	30 mg/day for 3 weeks	Three weeks supplementation lowers PSA in patients with non-metastatic prostate cancer	

The **table 1** summarize the relevant findings according to the compounds.

4. Discussion

4.1 Silymarin

Silymarin is a flavonolignan complex derived from the seeds of milk thistle (Silybum marianum). It has been shown to have antioxidant, anti-inflammatory, anticancer, cancer

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protective and also hypocholesterolemic and hepatoprotective properties [4]. In a study on prostate cancer (PCa) cells, silymarin was able to inhibit cell growth and induce differentiation of these cells [5]. In different studies Silymarin has been shown to have anti-cancer effects particularly in preventing the growth of PCa cells [6–8]. In addition, silymarin may delay progression after a radical treatment for PCa [9,10]. Another study found that silymarin in combination with selenium was able to reduce the lower urinary tract symptoms (LUTS) and PSA level in BPH patients [11]. The most common adverse effects reported are gastrointestinal symptoms. The frequency ranges from 2% to 10% in controlled trials and it is comparable to those of placebo. Other adverse effects are dermatological symptoms and headache, which are similar in frequency to placebo [12].

4.2 Sulforaphane

Sulforaphane is a sulphur-containing compound found in cruciferous vegetables such as broccoli, Brussels sprouts and cabbage. Sulforaphane has several health benefits, including antioxidant and anti-inflammatory effects, as well as the ability to inhibit the growth of cancer cells (13-35). Different studies showed a protective effect on different target in both in vitro [16,21–24] and in vivo models associated to oxidative stress damages [15–19]. Several researches highlighted the antiproliferative features of sulforaphane in bladder [28,33–35], prostate [30,31] and kidney cancer [29]. Sulforaphane showed to have potential in preventing prostate cancer progression in men treated with radical prostatectomy [27]. Sulforaphane is able to change the gene expression in prostatic tissue [26,32] and this mechanism may explain the reduction of the cancer risk progression [20].

4.3 Lycopene

Lycopene is a natural compound that belongs to the carotenoid family of phytochemicals, which are found in various fruits and vegetables. It is a bright red pigment that gives tomatoes its distinct colour [36]. Lycopene has been extensively studied over the past few decades for its ability to attenuate inflammation and oxidative stress [37]. In vitro and in vivo models also highlighted its suppressive ability against cancer cells growth [38,39].
Research suggests that it may have a large number of potential health benefits, particularly in the field of cancer prevention [40–42] and cardiovascular health promotion [36]. Lycopene has been studied for its potential in preventing prostate cancer [40]. In a study on men with prostate cancer, lycopene supplementation was able to reduce prostate-specific antigen (PSA) levels [43]. Lycopene's anti-inflammatory and antioxidant properties are believed to contribute to its potential protective effects against prostate cancer [44].
In addition, lycopene may be a novel approach for the treatment of benign prostatic hyperplasia [45].

4.4 Green Tea

Green tea is a well-known common beverage obtained with the infusion of camellia sinensis leaves and buds, the daily consumption of which is particularly widespread in eastern countries. Its purported health benefits have been attributed to its rich content in plant polyphenols, especially epigallocatechin-3-gallate (EGCG), which is the most represented catechin in green tea and the most investigated one [46]. Experimental evidence on humans exists on a wide range of beneficial effects by either consumption of green tea as a beverage as well as supplementation with green tea extracts in the form or EGCG capsules or powder. The most reported health benefit of green tea polyphenols is, by far, its antioxidant activity. There is a wealth of studies investigating the mechanism and magnitude of this effect in human subjects. One such study investigated the antioxidant activity of tea polyphenols by attempting to measure their capacity to protect against the toxicity of probable human cancerogens such as acrylamide, which is commonly present in carbohydrate-rich heat-processed food. In a randomized single-blind trial, 78 adults were randomly assigned to receive corn starch capsules containing either placebo or 50 mg, 100 or 200 mg tea polyphenols after ingesting a bag of potato chips containing an estimated level of acrylamide. Blood samples were taken before, after 2 days and after 10 days, which

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showed that tea polyphenol supplementation with the highest dose (200 mg) significantly increased the excretion of acrylamide via its oxidative metabolism pathway [47]. More commonly, antioxidant activity of green tea has been investigated by measuring variations in the blood levels of cells or enzymes involved in antioxidant status. For instance, leukocyte activity and total plasma antioxidant status was measured by chemiluminescence methods measuring the capacity of plasma to scavenge superoxide in a recent study. Volunteers were asked to take 300 mg of green tea extract daily for 14 days; whole blood samples from participants were stimulated with a bacterial peptide. The intervention caused a slight but irrelevant decrease in circulating leukocytes count, but the total antioxidant status significantly increased ($p=0.05$) [48]. Antioxidant activity of green tea was also observed in a study on 16 subjects ingesting either a single dose of green tea, 7 days of 2-cups-per-day green tea, or water as control treatment. Both the single-dose and the 7-days group showed significant increase ($p<0.0005$) in the activity of human oxoguanine glycosilase 1 (hOGG1), a DNA repair enzyme, and in the activity of heme oxygenase-1, a protein with antioxidant and anti-inflammatory effects. Lymphocytic DNA damage was also significantly lower ($p<0.001$) in the intervention groups [49]. Furthermore, a trial in which 18 healthy male volunteers ingested green tea extract (254 mg of catechins) highlighted that plasma phosphatidylcholine hydroperoxide (PCOOH) levels decreased with an inverse correlation with the increase of epigallocatechin plasma levels, suggesting an effective antioxidant capability of the substance[50]. Supplementation of 4 cups per day of green tea, or 2 capsules per day of green tea extract, compared to placebo, were also associated in reduction of Serum Amyloid Alpha (SAA), an inflammation marker, in 35 obese subjects with metabolic syndrome during the course of an 8-week study [51]. Finally, a 4-month phase II randomized controlled three-arm trial studied the effect of drinking 4 cups daily of either green or black tea or water on oxidative DNA damage among a population of 133 heavy smokers by measurement of urinary 8-hydroxydeoxyguanosine (8-OHdG). This marker decreased significantly in green tea consumers after 4 months of the intervention in subjects with mutated genotypes of glutathione-S-transferase which are associated with many different types of tumors [52].

Abundant experimental literature exists on the purported effects of green tea catechins on metabolism and cardiovascular health. The general consensus seems to be that the regular intake of green tea and/or green tea extracts produces positive effects on body weight control, lipid and general hormonal metabolism, glucose level control and blood pressure control. However, those results do not always reach statistical significance. The main issue with evaluating this body of work comes from the extreme heterogeneity of outcomes investigated and methods used to express those outcomes. For instance, green tea consumption has been observed to either be protective against weight gain or be associated with body weight reduction in studies mostly conducted on overweight or obese individuals, even if some of these results were significant only when compared against the baseline and not against the placebo arm in some cases [53–55]. Lipid metabolism has been shown to be positively impacted by long-term green tea supplementation (12 to 14 weeks) in terms of lowering of LDL cholesterol in some studies [56,57], while one other study showed no acute benefit on total or LDL cholesterol after a single assumption of a high-dose EGCG supplement, even if a significant attenuation of postprandial triglycerides was observed [46]. Findings on blood glucose level control, insulin and insulin-resistance are mostly inconsistent between the various studies, with some showing positive effects, others concluding for either limited or no benefit [46,55,57–63]. In terms of blood pressure control, green tea supplementation has been associated with a positive lowering effect, but the results were significant against placebo only in a single study on 88 overweight or obese males, while another study showed only a within-group significance when comparing to baseline levels [55,63].

Green tea integration has also been investigated in terms of possible benefits on physical performance in the context of regular physical exercise or training programs. Improvements in muscle mass have been observed in sarcopenic elderly adults supplemented with green tea extract and essential amino acids during the course of 24 weeks

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[64]. Positive modifications in enzymes involved in physical endurance capacity have been found in previously untrained men and overweight women [65–67].

Green tea has a long history of having been associated with anti-cancer or at least chemo-preventive properties. While this seems to be confirmed by human experimentation on supplementation in relation to certain tumours, such as relapsing colorectal adenoma [68] or incidence of de novo myelodysplastic syndromes [69], evidence is not as solid for neoplasms of uro-andrological interest and no clear conclusions on safety were drawn [70–74]. Evidence for a clear benefit of green tea supplementation in human gynaecologic neoplasms is not totally conclusive either, despite very strong basic science, animal and human epidemiological evidence. EGCG supplementation for 12 months has been linked to a reduction of mammographic density (MD) – a measurement linked with breast cancer risk – in 1075 post-menopausal women, but only in the very selective group of women aged 50 to 55 years old [75]. Moreover, in a phase II clinical trial on ovarian cancer recurrence, less than one third of women treated with green tea supplementation presented absence of recurrence at 18 months [76–78]. Findings on Cervical Intraepithelial Neoplasia (CIN) and Vulvar Usual type of Intraepithelial Neoplasia (uVIN) were more encouraging, with significant benefits on clinical response or absence of progression in treatment arms. Of note, some of these studies included the application of catechin ointments with or without oral green tea supplements tested against placebo (79-81).

With regards to safety of green tea consumption, adverse effects ranging from gastrointestinal symptoms to liver toxicity have been reported with very high doses but to date, no real consensus on a recommended upper level of intake has been reached [81].

4.5 Tryptophan

Tryptophan (TRP) is an essential amino acid, thus it cannot be synthesized by human and must be obtained through dietary intake, usually by means of plant- and animal-based proteins and most commonly in the quantity or 3.5-5.0 mg per kilogram of body weight, or more depending on nutritional habits. Dietary supplementation with TRP has a long history because of the purported benefits on mood and nervous system health, it being a precursor of the neurotransmitter Serotonin or 5-hydroxytryptamine (5HT) which itself strongly modulates mood and sleep functions [82]. The effects of TRP on mood and nervous system health have been widely examined in interventional trials. Most commonly, improvements were found in depressive symptoms, stress management, sleep patterns, social-cognitive and emotional processing, attention and chronic pain. For instance, notable improvements in depression symptoms, mood and sleep patterns were highlighted in depressed young adults, healthy adult women undergoing a series of cognitive and emotional tests and elderly individuals with depression and sleep disorders when supplemented with TRP [83–85]. Increased recognition of positive emotions and consequent improvements in social interaction was observed in healthy middle-aged and older adults who underwent supplementation with TRP, suggesting a potential role of the substance in preventing age-related decline in social-cognitive processes [86]. Behavior can be positively influenced by TRP supplementation, as a shift towards inclination to charity, improvement in response patterns to negative words and emotion- and task-related impulsivity were observed in several studies [87–90]. Chronic pain seems also positively influenced by TRP supplementation, as observed in 60 females with fibromyalgia syndrome supplemented with a multi-nutraceutical compound [91]. There is also limited clinical evidence of TRP supplementation on gastrointestinal health, although in a few selected medical conditions. In particular, TRP, glutamine, leucine and micronutrient supplementation improved environmental enteropathy in affected Zambian adults [92] and a nutraceutical formulation with TRP and other actives significantly improved gastrointestinal symptoms in subjects with irritable bowel syndrome [93]. Another scarcely explored possible health benefit of TRP seems to pertain to physical exercise and performance. In fact, TRP supplementation has been observed to improve physical performance in aerobic exercise with supramaximal intercalated anaerobic bouts in 20 young healthy man, possibly because of serotoninergic modifications in neural drive [94]. When it comes to the

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safety of TRP supplementation, there are a few considerations to make. Unfortunately, in late 1989 a new syndrome named Eosinophilia Myalgia Syndrome (EMS) presenting with muscle pain and a high eosinophil blood count, appeared and was initially linked with inordinate or excessive intake of TRP supplements. Later investigation concluded that this syndrome was not TRP itself but the presence of contaminants in some of the supplements available. In fact, the incidence of EMS gradually returned to zero after the ban of certain products. Only excessive TRP consumption may actually induce side-effects when taken at very high doses (generally estimated in upwards of 70-200 mg/kg) which consist in tremor, nausea and dizziness. Of course, special caution must be exercised in individuals who are chronically treated with 5HT reuptake inhibitors, as TRP interactions with such drugs may cause Serotonin Syndrome, which manifests with delirium, seizure, fever and rarely coma. Although large-scale dose-related assessment remain to be found, it is nonetheless not likely that modest supplementation with TRP may cause more than mild and occasional side-effects [82].

4.6 Glutathione

Glutathione (GSH) is a tripeptide present in most tissues, especially in the liver, and plays an extremely important role in the protection of cells from damage caused by free radicals and from endogenous and exogenous toxicity [95]. Glutathione is known to have a strong antioxidant power which is expressed because of its intrinsic ability to alternate a reduced form (GSH), predominant and higher than 98%, with an oxidized form (GSSG), lower than 1%. It is distributed primarily in the cytosol and, to a lesser extent, in organelles such as the mitochondrion, nucleus, and endoplasmic reticulum where it participates in many cellular metabolic activities including ROS removal, DNA and protein synthesis, and signal transduction [96]. The anti-oxidant activity being its main distinctive character, glutathione has seen wide pre-clinical and clinical experimentation mainly in the fields of oxidation-reduction balance, cancer prevention and male infertility. It is known that reactive oxygen species (ROS) are essential for cellular metabolism and for various biochemical processes, but when in excess they can generate oxidative stress that can cause cell death. This is why cells possess several enzymes capable of maintaining a healthy balance between the synthesis and transformation of ROS into non-reactive forms. Glutathione is an essential part of a class of antioxidant enzymes called glutathione peroxidase. Glutathione peroxidase converts hydrogen peroxide (H¬¬¬¬2O2) into water with a mechanism that can be represented as follows:

2 G-SH + ROOH ◎ GSSG + ROH + H20

In this process, the reduced glutathione (GSH) is oxidized to GSSG by glutathione peroxidase and then regains its antioxidant capacity through the activity of another enzyme, glutathione reductase, which converts the macromolecule back into the reduced form with a NADPH-dependent process [95]. Glutathione has been investigated, mostly in the pre-clinical setting, because of its theorized anti-cancer activity. Cancer cells require high concentrations of ROS, higher than normal cells, and, therefore, need an equally effective antioxidant system to stem the resulting oxidative stress[95]. In fact, the reduction of GSH is implicated in the induction of various mechanisms of cell death (apoptosis, necrosis, autophagy) which the tumor cell inhibits, to the point that in many cancerous forms the increase in the level of GSH has been associated with resistance to chemotherapy [97]. Therefore, it is legitimate to hypothesize that the modulation of GSH concentration in the tumor population could be a valid therapeutic target to induce cell death by directly interfering with GSH synthesis, by inhibition of glutamate cysteine ligase (GCL) [98], or by exploiting the affinity that GSH shows towards various substrates such as isothiocyanates [97]. The latter are phytochemicals well represented in plant foods such as cruciferous vegetables and avidly bind the sulfhydryl group of the cysteine residue of GSH, demonstrating that they can play an important role in prevention of tumors such as prostate cancer [99]. Glutathione has also been of interest in the field of male infertility. The

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antioxidant property of reduced glutathione is essential for the development and protection of the germinal epithelium from ROS damage. In particular, reduced glutathione reacts with lipid peroxides which, if left active, can induce alterations in membrane permeability and potential, undermining the integrity of spermatozoa, whose cell membrane is rich in polyunsaturated fatty acids [100,101]. As evidence of this, the reduction of GSH, induced or pathological, can generate an oxidative stress such as to activate the autophagy mechanism in germ cells as an adaptive response [102]. From this it can be deduced that adequate levels of GSH can promote the survival and well-being of germ cells. Nonetheless, antioxidant therapy is commonly included as a means of treatment for male infertility. Spermatozoa are aerobic cells and, as such, are equipped with a wealth of different antioxidant enzymes for the various types of ROS to be modulated [103]. Indeed, ROS are natural products of sperm metabolism and are not always harmful. At low concentrations and carefully regulated, they lead to the genesis of the signals necessary for the fertilization processes. Conversely, at high concentrations, they trigger oxidative stress to which spermatozoa are particularly sensitive and which can cause damage to the DNA and the lipid and protein content of the cell [103]. Despite the high risk of incurring in injury due to oxidative stress, however, spermatozoa have a very low concentration (-0.3 mM) of reduced glutathione compared to somatic cells (10 mM), probably due to their inability to synthesize new proteins [103]. Yet in various studies it has been found that glutathione plays an important role in the reactivation of some antioxidant enzymes. In particular, in a study conducted on 112 patients between 28 and 38 years of age, it was possible to compare the concentration of phospholipid-hydroperoxide glutathione peroxidase, a selenoprotein belonging to the glutathione peroxidase family, both in a group of 75 men with infertility of various kinds (varicocele, unilateral orchidopexy, orchitis, testicular trauma, unknown) and in a group of 37 healthy donor men. The results showed that in the infertility group the enzyme activity (93.2 ± 60.1 mU/mg) was much lower than in the control group (187.5 ± 55.3 mU/mg), resulting in a decrease in sperm count ($P < 0.01$) and a percentage increase in morphological ($P < 0.001$) and motility ($P < 0.001$) changes [104].

There is also experimental evidence of the utility of glutathione for the treatment of other pathological conditions linked to male infertility. Leukocytospermia and Varicocele represent two different pathological conditions but both associated with oxidative stress resulting from inflammation [100]. Specifically, varicocele increases oxidant levels and decreases antioxidant levels [105]. In a study conducted on 53 patients, the concentrations of various antioxidant substances were evaluated, including GSH/GSSG ratio in three different groups: a first represented by patients with leukocytospermia, a second including patients with varicocele and a third control group with healthy patients. At the end of the study, it was possible to determine that the GSH/GSSH ratio was significantly higher in the control group than in the leukocytospermia group ($P < 0.05$) and in the varicocele group ($P < 0.001$) and, among the latter, it was higher in the leukocytospermia compared with the varicocele group ($P < 0.05$). Sperm concentration ($P < 0.001$), sperm motility ($P < 0.001$), and the percentage of sperm cells with normal morphology ($P < 0.001$) were also positively correlated with GSH/GSSG ratio [100].

4.7 Escin

Escin is a natural blend of triterpene saponins extracted from the seeds and shell of the seeds of Aesculus ippocastanum. It includes various isoforms of which the most exploitable clinically is β-escin. This form, although endowed with considerable clinical efficacy, is unfortunately impaired by a reduced bioavailability when administered orally, therefore requiring modifications aimed at increasing its water solubility. Nonetheless, from a pharmacological perspective, escin has been shown to possess different and perfectly usable beneficial activities although with not fully clarified mechanisms [106]. In fact, it is present in commercially available supplements and compounds for the management of varicose veins, hematomas, hemorrhoids and venous congestion, but presents potential utility also in the fields of urolithiasis, male infertility and varicocele, prostate and bladder cancer and chronic prostatitis. The purported anti-edema effect has actual mechanistic basis. In

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various preclinical models, it has been found that escin can inhibit the activity of hyaluronidases, hyaluronic acid degradation enzymes, favoring the reconstitution and strengthening of one of the essential components of the extravascular matrix of the capillaries and thus reducing the loss of plasma from the endothelium [106]. Escin may also be beneficial in protecting the endothelium from hypoxic damage. In various studies conducted on the endothelial cells of the umbilical vein incubated in hypoxic conditions, it was possible to appreciate the response of the escin capable of inhibiting the cascade of reactions causing tissue damage. In a study by Arnould et al. it was seen how, in hypoxia, the endothelial cells reduced the amount of ATP by 40% and increased the activity of phospholipase A2, an enzyme involved in the release of precursors of inflammatory mediators, as well as stimulating adhesiveness to neutrophils. In this setting, administration of escin (at a dose of 100-750 ng/mL) inhibited ATP loss, reduced phospholipase A2 activity by 57-72%, and been shown to prevent increased adhesion to neutrophils [107]. To confirm this ability, in another study conducted on the same model, attention was paid to the hypoxia-induced alterations in the expression of PECAM-1, a macromolecule important for the integrity of the junctions in the interendothelial adhesion sites and for the modulation of neutrophil transmigration. Escin, like adhesiveness, has been shown to be able to prevent PECAM-1 alterations as well [108]. Moreover, escin has been shown to prevent hypoxia-induced reorganization of the endothelial cytoskeleton, with consequent reduction of permeability and resolution of edema [106]. Effects of escin on vascular tone have also been investigated. In the in vitro study on a segment of the saphenous vein (obtained by saphenectomy and pretreated with norepinephrine), the administration of escin (at a dose of 5-10 μg/mL) induced an increase in venous tone which was maintained for more than 1 hour after application. This increase has been shown to be suppressed by incubating the segment with indomethacin or other NSAIDs. This suggests that the effect of escin on vascular tone may be dependent on prostaglandin F2α [109,110]. As described in a recent review, escin also seems to have an anti-inflammatory activity comparable to that of glucocorticoids, with the added advantage of triggering less adverse events. In fact, there are many similarities between escin and glucocorticoids, starting from the chemical structures, both belonging to the tetracyclic triterpenoids. Even more surprising are the similarities regarding pharmacological effects. Starting from an animal model, it would seem that escin can not only induce a down-regulation of inflammatory mediators by upregulating the expression of glucocorticoid receptor (GR) but can also inhibit the expression of NFkB and of AP-1. This follows, on the one hand, the marked anti-inflammatory activity of glucocorticoids and, on the other, recalls their anti-edema effect, with the inhibition of the pro-inflammatory pathways in the capillary endothelium which, by reducing permeability, solves consequently also edema. Escin, therefore, induces anti-inflammatory effects through transrepression (reduction of pro-inflammatory protein synthesis) and transactivation (increase of Ikb, lipocortin 1 and superoxide dismutase). Unlike glucocorticoids, however, it does not inhibit the physiological processes of tissue repair, does not increase the endogenous secretion of corticosterone and does not induce cellular apoptosis in the spleen or thymus of mice [111].

Benefits of escin in patients suffering from urolithiasis have been observed in clinical settings. Urolithiasis is a pathological condition characterized by the presence of solid agglomerates of various kinds (calcium, struvite, uric acid, cystine) present in the kidney and urinary tract. It is a very frequent pathology (it is estimated that 3 out of 20 men and 2 women out of 20 experience lithiasis at least once in their life) which can trigger even very intense symptoms with violent abdominal pain, restlessness, nausea, hematuria and dysuria [112]. In a prospective study, in particular, the effects of escin and prednisolone were compared on patients suffering from symptomatic ureteral calculi. A total of 360 patients were randomized into a first group treated with escin, a second group treated with glucocorticoid prednisolone and a third placebo control group. After 10 days of treatment, the reduction in pain, the decrease in the dilatation of the urinary tract, the rate of expulsion of the stones and any adverse events that occurred during the treatment were evaluated. The group treated with escin showed better outcomes than the placebo group

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(P < 0.00001) and superior efficacy compared to the prednisolone group (P < 0.05). Regarding stone expulsion, significant differences were found between the treatment groups and the control group (P < 0.05) but not between the escin group and the prednisolone group (P > 0.05), while adverse events were recorded only in the group taking prednisolone [113].

A promising role of escin can be found in the field of male infertility. A first potential mechanism might be in the anti-oxidant properties of this substance. As known, reactive oxygen species (ROS), although indispensable at low concentrations for fertilization processes, at high concentrations can damage the protein and lipid component and the DNA of spermatozoa [103]. In an animal model of a gastric ulcer induced by the administration of indomethacin, it was found that escin (at a dose of 0.45, 0.9 or 1.8 mg/Kg), in addition to reducing the concentration of malondialdehyde, TNF-alpha, P -selectin and VCAM-1, also promoted the activity of myeloperoxidases, superoxide dismutases, catalases and glutathione peroxidases, suggesting a relevant anti-oxidant effect [114]. Moreover, there are evidences of efficacy of escin in ameliorating seminal alterations linked with varicocele. In an animal model (rats), the pathological venous dilatation due to varicocele was reproduced with partial ligation of the left renal vein. After 4 weeks of daily administration of escin, the density of polymorphonuclear leukocytes, the number and motility of spermatozoa in the epididymis, the concentration of follicle-stimulating hormone, luteinizing hormone and testosterone were evaluated. It was seen that, in the group of rats treated with escin, the testicular blood flow was significantly reduced, the density of polymorphonuclear leukocytes underwent a significant decrease and, conversely, the sperm count increased [115]. Findings in human subjects were also encouranging. In a randomized placebo-controlled trial, three groups of patients with varicocele infertility were compared, including a control group, a group treated with surgery and one treated with orally administered escin at a dose of 60 mg/day continuously for 2 months. At the end of the treatment, compared to the control group, patients treated with surgery and escin showed an improvement both in sperm density (68.8% and 57.5% respectively compared to 38.5% in the control group) and in sperm motility (77.1% and 55.7% respectively versus 46.2% of the control group) [116].

Of note, escin is being investigated for potential cytotoxic effect and anti-tumor activity. In a recent study on both in vivo and in vitro models, the response of escin on castration-resistant cells of prostate cancer was evaluated. At the end of the treatment, it was found that escin can induce a cytotoxic effect in resistant cancer cells by inducing a chain mechanism. In fact, escin has been shown to be able to stimulate cell cycle arrest in G2/M leading to a marked reduction of cyclinβ1 expression and cyclin-dependent kinase 1 activation, with concomitant induction of p21 [117]. Potential cytotoxic properties were also investigated in bladder cancer cells. In the in vitro model, the ability of Escin to inhibit cell growth and induce the apoptosis seems to be mediated by modulation of the FAS receptor. Escin can also induce a cytotoxic effect also by reducing the mitochondrial membrane potential and increasing the activity of cytochrome C with consequent release of ROS [118].

Escin could also be of interest in the management of chronic prostatitis (CP) and chronic pelvic pain syndrome (CPPS). CP/CPPS is a pathological clinical condition characterized by pain in the perineum, pelvis, suprapubic area and external genitalia, urinary disturbances and ejaculation disorders, without evidence of bacterial infection. It is a condition that significantly reduces the quality of life of patients and which has been the subject of many studies. Among these, a recent study investigated the potential and efficacy of the combined treatment of extracorporeal shock wave therapy (ESWT) and administration of bromelain and escin. A total of 100 CP/CPPS patients were randomized into two groups, one treated with ESWT alone and another treated with ESWT plus bromelain and escin. Pain intensity, urinary and prostatitis symptoms and quality of life were assessed after 4, 12 and 24 weeks showing a significant reduction, and even disappearance, of pain in a significantly higher percentage of patients in the treated group with combined therapy compared to the group treated with ESWT alone, as well as slight improvements that

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were also found with regard to prostatic and urinary symptoms [119]. This can be explained by going back to the anti-inflammatory activity of both escin and bromelain (inhibition of mediators such as NF-kB, IL-1β, IL-6, TNF-α, PGE2), which inhibit different inflammatory pathways by cooperating with the ESWT in addressing the complex pathogenesis of CP/CPPS.

As for tolerability, escin has not yet produced drug interactions and is, in general, well tolerated. Even where it has produced adverse events, they have been mild and transient, commonly represented by gastrointestinal disorders (constipation, diarrhea, vomiting and nausea), headache, dizziness, hot flushes, itching and fatigue [120,121].

5. Conclusions and limitations

Although limited by the extreme heterogeneity of the studies included in this review in terms of methodology, measurements and outcomes, current evidence available on the substances investigated frame them as potential tools in the hands of the informed physician. Silymarin, sulforaphane, lycopene, green tea catechins, tryptophan, glutathione and escin, each demonstrated a potential benefit in offsetting the negative effects of oxidative stress and inflammation induced by environmental pollution on human health; an improvement ranging from mood and cognition to cardiovascular health and metabolism has also been observed. These substances have also shown a possible promising anti-aging and anti-cancer effect. Finally, a wide range of medical conditions, especially interesting for the urologist and andrologist, can be addressed by proper utilization of such supplements. Due to the different pathways involved in their mechanism of action, it is also unlikely for these substances to generate negative interactions in the case of simultaneous intake. Thus, further studies are required to better define the potential synergic effect, optimal dosage, mechanism of action and tolerability profile of these substances.

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