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Mini Review

The Activity of Ellagic Acid in Male Reproduction: A mini-review

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Abstract

Ellagic Acid (EA), is a naturally occurring phenolic found in some fruits and nuts. It has a great variety of biological activities, including properties antioxidant, anti-inflammatory, anti-thrombotic, anti-atherogenic, neuroprotective, hepatoprotective, anti-mutagenic, and anti-carcinogenic agent recently. This review aims to summarize experimental research carried out in vivo and in vitro that evaluated the EA treatment in male reproduction and discussed the mechanisms of action. For this purpose, PubMed and SCOPUS databases were searched to identify publications in this regard.

Keywords: Ellagic Acid, Polyphenols, Urolithins, Male Reproduction

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Introduction

Polyphenols represent the most abundant antioxidants in human beings diet. They are of great interest due to the association established between the intake of foods rich in these antioxidants and the prevention of various diseases for high diversity in biological properties.[1,2] The EA is present in some fruits, nuts, and seeds, such as pomegranates, blackberry, raspberries, strawberries, peaches, plums, walnuts, and almonds. It is found in different forms in natural products, including its free structure, EA-glycoside, or forming complex polymers called Ellagitannins (ETs).[3] The EA possesses both a hydrophilic moiety with four hydroxyl groups and two lactones, along with a lipophilic moiety with two six-member hydrocarbons rings.[4] This unique structure makes EA the acceptance of electrons from various substrates taking part in antioxidant redox reaction.[5] It possesses multiple pharmacological properties such as anti-inflammatory, anti-thrombotic, anti-atherogenic, neuroprotective, hepatoprotective, anti-mutagenic, anti-carcinogenic, anti-microbial, skin protection, and cardiovascular effect.[6–9]

To date, only one clinical study has been carried out to assess the impact of EA on male reproduction. Feeder *et al.*, (2014), lead a double-blind prospective study in the male population, which had aimed to assess the effect of the combination of a pomegranate extract and gangala rhizome on sperm quality. The treatment for three months of this combination of extracts contained 9.6 mg of EA. The results showed an increased sperm motility without affecting its morphology in healthy volunteers.[10] This review aims to summarize experimental evidence *in vivo* and *in vitro* that highlights the protective effect of EA on male reproduction.

Ellagic Acid (EA)

The EA is present in several forms, including free, glycosylated, acylated, or a hydrolyzable ETs polymer characterized by multiple hexahydroxydiphenyl-glucose.[3,11] The first step in the metabolism of EA and ETs takes place in the stomach. Although ETs are resistant to acid hydrolysis, and their degradation in the stomach, EA is well absorbed in this place.[12] In the gut, ETs have hydrolyzed EA at neutral pH or through the microbiota releasing EA first, and then dibenzopyran derivatives known as urolithins.[13] The uptake process for EA is via a passive diffusion by a concentration gradient,[14] although also the EA can facilitate the uptake by specific transporters such as sodium-glucose linked SGLT1 and the Organic Anion Transporter Polypeptide (OATP) in CaCo.[15] Biological properties of ET, such as free radical scavenging, further depend on their metabolic transformation inside the gut and also to the modulation of the microbiota.[16] The EA catabolism produces urolithin-D upon the opening of a lactone ring and removal of a carboxyl group. Urolithin D loses 1,2 or 3 from different positions, resulting in the formation of urolithins C, A, and B, and leading lipophilicity.[17,18]

Urolithins are considered useful biomarkers of EA and ETs consumption in various mammals.[19–21] However, the high interindividual variability in the metabolic transformation of

phenolic compounds by gut microbiota has allowed the classification of patients into producers or non-producers of the corresponding metabolites. Some studies have stratified human volunteers, considering the urolithin excretion levels in urine as high, low, and very low excretion.[19,22,23] Recently, individuals have been categorized into three phenotypes, depending on the qualitative and quantitative proportions of the urolithin metabolites produced.[24] Some individuals considered non-metabolizers might become urolithin producers after long-term intake/high doses of ETs and EA, while others remain unchanged.[20,23,24] Besides, EA metabolites have antioxidant activity; urolithins inhibit the release and scavenging reactive oxygen species in neutrophils (higher than ascorbic acid).[25]

In vivo Effect of EA on Male Reproduction in Experimental Animals

Several studies confirm the protection of EA against toxicity on male reproduction caused by chemicals or drugs (

Table 1). A group of researchers in Turkey have shown that the administration of EA can protect it (male reproduction) against male toxicity induced by various toxic agents associated with oxidative stress. In one study, the protective effect of several compounds, including EA on sperm morphology, and testicular histology induced by Cyclophosphamide (CP) were analyzed in rats. Animals received EA (2 mg/kg/day) and CP (5 mg/kg once a week) for eight weeks. They observed that treatment with EA in CP-induced testicular toxicity increased its antioxidant capacity in sperm and reduced lipid peroxidation in plasma. Besides, they protected damages caused by sperm morphology and testicular histology.[26] Also, a decrease was observed in the apoptosis markers in the testes without any change in testosterone level.[27] With the same dose schedule and treatment duration for EA, the same group tested its protective effect against testicular toxicity induced by Aroclor. Results showed that EA protects the histopathological damage of testicular tissue besides increasing its antioxidant capacity in testes.[28] A subsequent study tested the protective effect of EA on testicular and sperm toxicity induced by doxorubicin, a drug used against cancer. The animals received EA (2 mg/kg/day) and doxorubicin (2 mg/kg/day/ once a week) for eight weeks. The results showed that treatment with EA in doxorubicin-treated rats increased testosterone concentration and testicular antioxidant capacity; it also decreased markers of testicular apoptosis and protects against lipid peroxidation. However, EA could not enhance the reduction of sperm quality.[29]

Table 1. Experimental studies *in vivo* and *in vitro* concerning the protection of pure EA on toxicity in male reproduction induced by different chemicals and drugs.

Type of Study	Testicular Toxicity Model	Dose (EA content)	Biological Effects	Ref
In vivo	Cyclophosphamide-induced rats	2 mg/kg for 8 weeks	Improved the induced lipid peroxidation, normalized sperm morphology, and testicular histopathology.	26
In vivo	Cyclophosphamide-induced rats	10 mg/kg for 8 weeks	Decreased lipid peroxidation, sperm, and testicular damage.	27
In vivo	Polychlorinated biphenyl (Aroclor)-induced rats	2 mg/kg for 8 weeks	Decreased abnormal spermatozoa, and the testicular MDA concentration. Increased the glutathione level, and GPx and catalase activities, and epididymal sperm concentration.	28
In vivo	Doxorubicin	2 mg/kg for 8 weeks	Improved impaired oxidant/antioxidant balance, sperm motility, sperm concentration, testosterone levels, and histopathological alteration of testicular tissue accompanied by a decrease in testicular apoptosis.	29
In vivo	Doxorubicin	10 mg/kg for 14 days	Increased sperm quality, serum testosterone, and GSH levels. Decrease MDA concentration and TNF-α.	30
In vivo	Tetrachorodibenzo-p-dioxin	2 mg/kg for 8 weeks	Improved damages in sperm quality, testicular histology, and Johnsen's scoring. Decreased testicular lipoperoxidation.	31
In vivo	Cyclosporine	10 mg/kg for 21 days	Improved sperm quality, increased antioxidant capacity (CAT, GPx, GSH), reduced MDA concentration, and improved testicular abnormalities.	32
In vivo	Cisplatin	10 mg/kg for 10 days	Improved sperm quality, increased antioxidant enzyme activity (CAT, GPx), preventing lipid peroxidation, and decreasing apoptosis.	33
In vivo	Cisplatin	2 mg/kg for 8 weeks	Decreased the MDA concentration, increased the antioxidant activity of SOD, reduced the increased number of apoptotic cells (Bax), and increased the anti-apoptotic cells (Bcl-2) in testes.	34
In vivo	Valproic Acid	10, 25 y 50 mg/kg for 10 days	Increased sperm count, motility, and testicular Johnsen's score.	35
In vivo	Arsenic	10 and 30 mg/kg For 21 days	Decreased testicular Arsenic accumulation. Improved serum testosterone levels, testicular antioxidant markers, and histological parameters.	36
In vivo	Arsenic	50 mg/kg for 40 days	Increased sperm quality and mitochondrial membrane potential. Decreased lipid peroxidation, carbonyl protein, and Arsenic accumulation.	37
In vitro	Ram Semen	1-2 mM 24, 48 and 72 h	Increased sperm motility and viability, antioxidant potential, and GSH concentration.	38
In vitro	Rooster Semen	1-2 mM for 1 week	Improved motility, membrane functionality, and viability. Increased GPx and total antioxidant capacity.	39

Recently, another group of researchers assessed the protective effect of EA at a dose of 10 mg/kg on testicular injury induced by doxorubicin (5 mg/kg twice a week for two weeks). After fourteen days of treatment with EA in doxorubicin-treated rats, the concentration of glutathione increased, and both lipid peroxidation and the level of TNF-α decreased in the testes, it also improved the sperm quality and level of testosterone.[30] Also, Sönmez et al., (2011) tested the effect of EA (2 mg/kg/day) on other compounds such as Tetrachorodibenzo-p-dioxin (TCDD), a known environmental pollutant that alters endocrine functions in male rats. After eight weeks of co-exposure with TCDD and EA, they observed a significant change in sperm quality and antioxidant capacity in testicular tissue. This is while there was no change in testosterone concentration.[31] Another study, using EA (10 mg/kg) for 21 days in cyclosporine-induced testicular toxicity in rats, showed improvement in sperm quality, histology, and testicular antioxidant capacity. Also, protected against oxidative damage caused in the testes.[32] In the same line, the treatment with a dose of EA (10 mg/kg), improved cisplatin-induced testicular damage by improving quality sperm and increasing antioxidant enzyme activity resulting in the prevention of lipid peroxidation.[33] Also, with a lower dose of EA (2 mg/kg) the protection against testicular damage was similar to the previous one by inhibiting apoptosis and increasing superoxide dismutase activity.[34]

In another study, EA at 50 mg/kg dose, protected against sperm abnormalities induced by valproic acid, an anti-epileptic drug.[35] Recently, a group of researchers assessed the antioxidant protective effect of EA in mice exposed to arsenic, a known environmental pollutant.[36,37] Doses in a range of 30-50 mg/kg of EA decreased the accumulation of testicular arsenic, preventing lipid-peroxidation and protein carbonylation via regulating the expression of Nfe212, Ppargca1, and StAR genes, besides to reducing the inflammatory response.[36,37]

In vitro Effect EA in Spermatozoa

Some studies have evaluated the antioxidant effect of EA *in vitro* on sperm quality in different species. Accurately, in 2019, the antioxidant effect of EA was assessed during the preservation of seminal ram fluid. The results showed that EA at concentrations of 1 and 2 mM improves sperm quality after 0, 24 and 48 h of preservation and increases antioxidant potential after 24 to 72 h, accompanied by an increase in the concentration of GSH with EA 1 mM at 72 h, although there were no significant changes in mitochondrial activity and DNA integrity. These results suggest that EA supplementation has a potential effect on sperm quality and oxidative stress during the preservation of semen from ram.[38] In another recent study, the antioxidant capacity of EA and liposomes loaded with EA on post-thawed spermatozoa of roosters was assessed. The results showed that liposomes loaded with EA significantly improved membrane functionality, decreased the appearance of early apoptosis and lipid peroxidation compared to 1 mM ellagic acid. The mitochondrial activity was similar to the one of EA. At 1 mM in both forms, EA significantly increased GPx activity and antioxidant capacity after freezing and thawing. The results suggest that the use of liposomes loaded with EA could further improve the effects of EA.[39] Similar results were found using nanoliposomes supplemented with pomegranate extract in the parameters of sperm quality and antioxidant capacity in ram semen.[40]

Conclusion

The EA exhibits antioxidant activity by increased total antioxidant capacity, eliminating species reactive of oxygen, chelating ferrous ions, reducing ferric ions, and forming complexes with ions contributing to the reduction to free radical formation by inhibiting the Fenton reaction.[41,42] This review shows the protective effect of EA on male reproduction in *vivo* and *in vitro* studies. The majority of the studies presented in this study suggest that the most frequent mechanism of EA is related to its antioxidant activity, protecting from oxidative damage caused by various toxic agents through the increase in the antioxidant capacity of the testes, increasing the activity of antioxidant enzymes. This protective activity improves sperm quality, testicular histology, and decreases lipid peroxidation and oxidative stress. Furthermore, future research on a better understanding of the molecular and biochemical mechanisms of EA on male reproduction is required.

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Conflicts of Interest

The authors declare no conflict of interest.

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