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Hesperidin-Sources, chemistry, extraction, measurement and biologic effects on reproduction in animals: A review

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Abstract
This paper reviewed the polyphenolic compound, hesperidin, found mostly in citrus fruit peels and examined its use in animal reproduction. Hesperidin, as a polyphenolic bioflavonoid flavanone glycoside, has wide range of biological and therapeutic benefits. Its application in the enhancement of reproductive indices in animals is limited due to its low water solubility, poor absorption and bioavailability. Synthetic variants of hesperidin such as alpha-glycosyl-hesperidin, hesperidin-7, 3'-O-dimethylether (HDME) and hesperidin methyl chalcone have been developed to enhance its solubility in water vis-a-vis its absorption and bioavailability. In the body, it is *metabolism* into hesperitin, which is responsible for most of its biological and therapeutic actions. The biological and therapeutic benefits include: antioxidant properties through scavenging for free radicals; anti-inflammatory actions, analgesic, metal-chelating and antitoxic properties, cardioprotective and neuroprotective effects, antibacterial, prevention of bone resorption and glucose homeostasis. Also, hesperidin modulates cellular and intracellular molecules signaling. The effects of hesperidin, its extraction, metabolites and toxicology are discussed in the review. It was concluded that hesperidin is a potent antioxidant that can be used to ameliorate oxidative stress in animals exposed to severe environmental conditions.

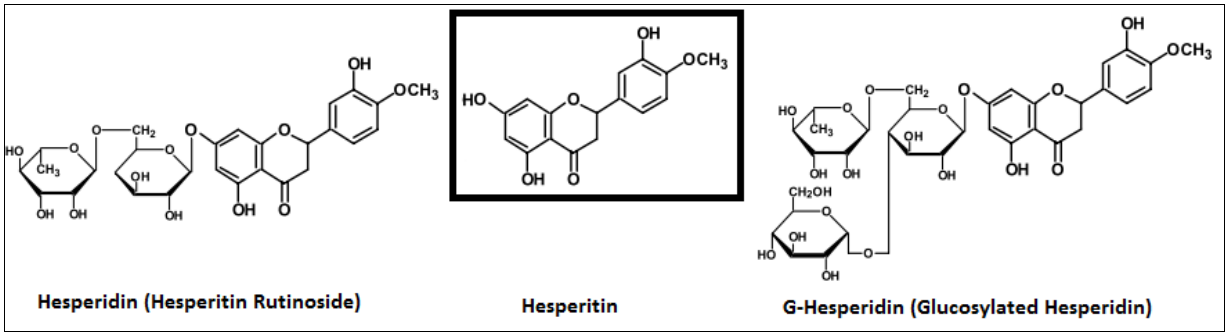
Keywords: Oxidative stress, severe environmental conditions, hesperidin, animal reproduction, free radical, biological, therapeutic, citrus.

1. Introduction
Extreme environmental conditions adversely interfere with reproductive performances in ewes and other animals. Severe environmental conditions such as thermal stress, water scarcity, under nutrition, transhumance and nomadism and the presence of endocrine disrupting substances predispose the animals to oxidative stress through excess production of free radical species that undermines their reproductive performance and overall productivity [1, 2, 9, 31, 33, 84, 97, 104, 116]. Antioxidants have been employed in the amelioration of the adverse effects of severe environmental conditions [112, 114]. The current review is focused on hesperidin as a phytochemical and its role in improving reproductive performance in ewes through its antioxidant ameliorative capability.

2. Hesperidin
Hesperidin is a polyphenolic bioflavonoid flavanone glycoside that is predominantly found in orange peels and other citrus fruits like tangerine and grape fruits [81]. Smashed dried tangerine contains 5-10% of hesperidin [61]. It was first isolated in 1828 by Leverton from the spongy inner portion of orange peel of the family *Hesperides* [21]. It was also latter detected in lemons by Pheffer in 1874 and has also been isolated with neohesperidin, an isomer of hesperidin, from citrus fruits and other plants [41]. It was given the name hesperidin, which comes from the term 'Hesperidium', meaning citrus fruits [56, 78, 115]. Also, it is referred to as hesperetin-7-rutinoside. Hesperidin is the food-bound form of hesperitin, and was earlier referred to as Vitamin P due to its vitamin-like properties of wound healing and was used in combination with Vitamin C for the treatment of scurvy [50, 73].

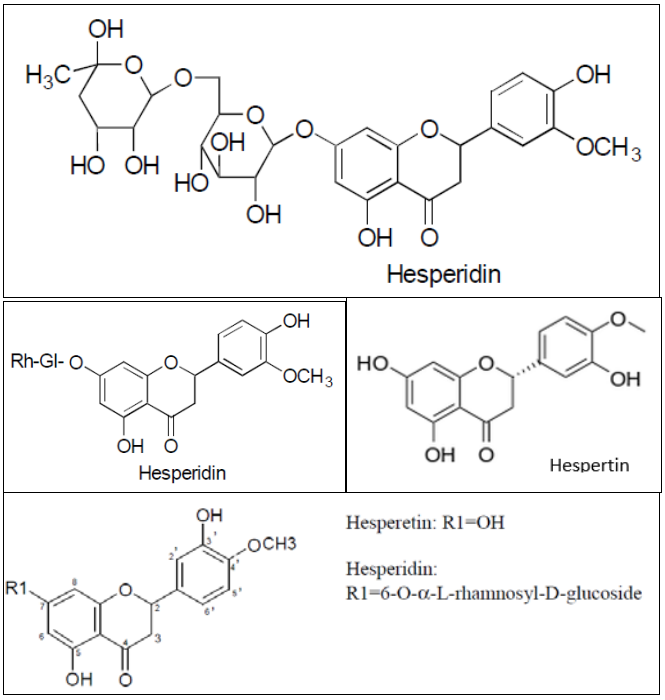
3. Chemical Structure

Hesperidin is a glycoside containing the flavanone hesperitin and the disaccharide rutinose, which chemically is referred to as hesperitin-7-O-rutinoside or hesperitin-7-O-rhamnosyl (1-6) glucoside [82, 106]. Hesperitin is the aglycone form that has no sugar moiety, while rutinose, as the glycone form, contains sugar moiety and is sometimes referred to as 5,7,3'-trihydroxy-4'-methoxyflavanone [29, 92].



Source: Jadeja and Devkar (2014).

Hesperidin is a prod rug for hesperitin, which is the main molecule in the hesperidin structure, just as G-hesperidin is a prod rug of hesperidin. Hesperitin and hesperidin can both exist as S and R isomers [58].



Source: Jadeja and Devkar (2014).

Pure hesperidin is white needle-like powder upon recrystallization with a melting point ranging between 252-254°C, a molecular weight of 610.6 and the chemical formula**:** $C_{28}H_{34}O_{15}$ [57, 115].

Variants and Formulations

There are several variants of hesperidin and some of the variants have been developed in order to improve its solubility in water and thereby improve its absorption from the gut. Glucosyl-hesperidin (G-Hesperidin), which is also referred to as alpha gycolsyl hesperidin, is a synthetically modified and prodrug of hesperidin where the diglycoside group has been modified into a triglycoside, while the hesperitin structure remained unchanged [72]. G-hesperidin has high solubility in water [105]. While in the body, G-hesperidin is converted into hesperidin, which then releases free hesperitin that is responsible for its biological and therapeutic properties. G-hesperidin fed to rats was reported to have more rapid appearance (3.7 fold) in blood plasma and had greater bioavailability (4 µM) of hesperitin in plasma when compared to rats fed hesperidin [55, 105]. G-hesperidin is commonly used as a skin tonic and has been shown to help blood circulation when applied to the skin and thus reduces negative physical effects of stress, tiredness and cold in humans.

Another synthetic variant of hesperidin is the lipid soluble form called hesperidin-7,3'-O-dimethylether (HDME), which contains one modified hydroxyl into methoxyl group on the B ring [108]. HDME possesses phosphodiesterase inhibitory potential than hesperitin [54].

Hesperidin methyl chalcone is another water-soluble hesperidin variant that is commonly used in medicinal preparations. It is a semi-synthetic form of hesperidin that has an open ring with several methyl group substitutions, which contributes to its higher water solubility and improved metabolic stability [14, 102].

Daflon is a patented brand name of a combination of 10% hesperidin and 90% Diosmin, which is used for the treatment of chronic venous insufficiency (CVI) and other venous system disorders such as restless leg syndrome, leg oedema and varicose veins [16, 32, 40].

The salt variant of hesperidin, phosphorylated hesperidin has contraceptives properties in rodents [71] and in rabbits [46]. Phosphorylated hesperidin inhibits the actions of acrosin and hyaluronidase enzymes, which prevents sperm head capacitation, and thereby inhibiting fertilization [35].

Dosage, Absorption, Metabolism, Bioavailability and Elimination

5.1 *Dosage*

The supplemental dose of hesperidin used in most studies is 500 mg/day for humans, given orally as preventive treatment against oxidative stress and associated disorders. The lowest beneficial dose of hesperidin in rodents is around 25 mg/kg/day through oral administration and doses in rodents ranged between 5 mg/kg to 450 mg/kg body weight [8, 83]. An adult human weighing 68 kg will require to consume 1800 mL of orange juice or 1800 g of fresh orange to meet the daily oral dose of hesperidin. A total of 500 mg supplemental dose of hesperidin can be achieved by consuming 5 – 10 g of the dried tangerine peel. Hesperidin has also been given as injectable form intraperitoneally to rats and mice at the dose of 0.1-1 mg/kg [24].

Hesperidin is characterized by low water solubility, poor absorption and limited bioavailability, which limits its biological and therapeutic benefit [38]. The absorption of hesperidin can be improved through treatment of orange juice

with the enzyme α-rhamnosidase, increasing its water solubility, its hydrolisation to hesperitin by gut microflora and its absorption into blood circulation and the par cellular pathway [11, 53, 75, 100, 105]. The moderate to high oral ingestion of hesperidin results in micromolar concentrations of hesperitin in blood circulation and maximum blood values could be attained in about 5-8 hours after ingestion, and this level may be maintained for 24 hours [12, 55]. The application of hesperidin in therapeutic activities and other uses are highly enhanced through a better understanding of its mechanism of absorption, which requires further investigation especially in domestic animals.

5.2 Metabolism and elimination

Hesperidin is hydrolysed by the enzyme, hesperidin 6-O-alpha-L-rhamnosyl-beta-D-glucosidase to produce rutinose and hesperitin in the gut, which is absorbed into blood circulation to mediate the actions of hesperidin in the body [12, 76]. Microbial fermentation of hesperidin may also produce small phenolic compounds such as *p*-hydroxyphenylpropionic acid, p-coumaric acid, p-hydroxybenzoic acid, and phenylpropionic acid [26].

After absorption, hesperitin is glucuronidated to hesperitin-7-O-glucuronide, which is responsible for its anti-inflammatory and antioxidant actions. The meta bolisation of hesperitin to its glucuronideforms by uridine 5'diphospho-glucuronosyltransferase (UGT or UDP-glucuronosyl transferase) enzyme produces 87% as monoglucuronide and 13% as sulphoglucuronides [19, 20, 65]. Oral hesperidin has a delayed half-life of 5-8 hours due to gut microbial action and is eliminated through urine as monoglucuronide, within 24 hours after ingestion [5, 65].

5.3 Mode of action, biological and therapeutic properties

Hesperidin has several biological and therapeutic properties through its direct effects and its agycone metabolite, hesperitin [42, 51, 117]. Hesperidin has potent analgesic effect as part of its direct effect due to its oestrogenic and opioidergic effects, especially when taken orally and in low concentrations [64, 24]. Hesperidin is reported to have sedative effect when injected intraperitoneally to mice as a result of its opioidergic properties, which decreases locomotor and exploratory activities [64, 66, 103].

Hesperidin, as an antioxidant, acts by scavenging ROS and chelating metallic agents in the body [6, 79, 96]. It also enhances phagocytosis of disease-causing agents such as microbial [42] and viral organisms [87]. Hesperidin catalyses the metabolism of endo- and exo-biotics through aromatisation and sulphonation of xenobiotics, drugs and endogenous compounds [39, 45]. It has neuro-protective properties through negative nitric oxide signaling and reduction of neurodegenerative changes [101], and protects against cisplatin toxicity and its destructive effects on the liver [47]. It improves cardiac blood flow [7, 25, 91]; protects against ischaemia-reperfusion injury [30, 80, 86]; and protects middle cerebral occlusion-induced stroke [85]. It stimulates opioidergic signalling causing weak sedative and analgesic effects [64]; and has an anti-allergic effects [44]. Hesperidin and its metabolite hesperitin, potently suppress synoviocyte proliferation in joints and limit alteration of cytokine secretion [59]. Hesperidin attenuates acute renal injury, inflammation and DNA damage in rats [88, 107].

In reproduction, hesperitin influences the secretion and activities of hormones within the body. It has both oestrogenic (at lower concentrations) and anti-oestrogenic (at higher concentrations) effects, depending on its concentrations in the body [62]. It modulates the actions of hormones like oestrogens, androgens and thyroid hormone [74]. Oral administration of hesperidin at 50 mg/kg for at least eight weeks, reduces urinary DNA damage [107]. Hesperidin attenuated oxidative changes induced by doxorubicin toxicity in rat testes [99]. Oral administration of hesperidin to rats at varying dosages reduced circulating lipids, serum and hepatic superoxide dimutatse and monaldehyde and ensures cellular membranous integrity [43].

Several mechanisms of action have been proposed to explain biologic effects of hesperidin. Hesperidin inhibits a number of enzymes such as aldose reductase, xanthine oxidase, phosphodiesterase, Ca2+-ATPase, lipoxygenase and cyclooxygenase [70]. It is *metabolism* into hesperitin, which is responsible for its vascular actions through improving capillary endothelial cell integrity. Its deficiency in the diet is linked with abnormal capillary leakiness as well as pain in the extremities, causing aches, weakness and night leg cramps in men [28].

6. Extraction, Isolation Quantification of Hesperidin

Various methods have been advanced for the purposes of extraction, isolation and quantification of hesperidin in biological systems. The methods range from the primitive Soxhlet extraction technique to the more modern highly sensitive high-performance liquid chromatography techniques. The primitive methods were cumbersome. It involves the use of large quantities of solvents, which lack sensitivity, accuracy and were laborious and time consuming. Newer methods were developed with higher degrees of automation to address an increasing need for greater productivity as well as faster, more sensitive and accurate assays. These newer methods are more convenient even though more expensive in terms of the initial purchase price of the equipment. The newer methods are based on the principles of using solid extractions at higher temperatures and pressures. The extraction, isolation and quantification of flavonoids are based on the temperature, solvent extracting power and extraction time [23].

The soxhlet extraction, maceration, ultrasonic and the chromatographic techniques are among the methods used in the extraction of polyphenols including hesperidin from plants. Maceration and soxhlet extraction are considered as conventional extraction methods, though have been modernised and have low efficiency and potential environmental pollution effects due to large volumes of organic solvent used and long extraction time required [17]. A number of newer methods have been developed in recent years such as microwave, ultrasound-assisted extraction and techniques based on use of compressed fluids as extracting agents, such as subcritical water extraction, supercritical fluid extraction, pressurized fluid extraction or accelerated solvent extraction [17]. More advanced sensitive methods have also been developed that are more accurate, rapid and more efficient and include the liquid chromatography-mass spectrometry and high performance liquid chromatography [18, 34, 48, 49]. The liquid chromatography and the high liquid chromatography are also used in the isolate and quantify hesperidin in living biological tissues such as human plasma, rat serum and human urine and plants [2, 49, 60, 67].

7. Toxicology and Side Effects of Hesperidin

The median lethal dose (LD_{50}) (mouse) for hesperidin given intraperitoneally is 1 g/kg body weight [13]. The LD_{50} of

hesperidin oral acute toxicity is over 2000 mg/kg, while sub-achute and chronic toxicity levels at doses above 2000 mg/kg were observed [111]. There is no known documented toxicity or side-effects of hesperidin. However, the injection of hesperidin results in sedation in mice [118] and causes slight irritation, when in contact with the eyes [13, 15]. It is also reported to cause irritation of the respiratory system, when the powder is inhaled [13, 15]. The use of hesperidin has also been reported to retard blood clotting and increase blood flow, and may complicate bleeding disorders [63].

8. Hesperidin as a Potential Antioxidant in Enhancing Reproduction in Livestock

There is a dearth of information regarding the use and role of hesperidin in animal reproduction. However, hesperidin, being a bio-flavonone glycoside, may enhance reproductive performance through its ROS scavenging properties, thereby ameliorating the negative effects of excess ROS in body tissues. Furthermore, hesperidin is reported to act as an anti-inflammatory agent and may therefore wade off inflammation due the presence of microbial agents in body tissues. Recent studies focused on hesperidin supplementation to ameliorate ovarian toxicity-induced oxidative stress and infertility in rat models [3, 22, 52]. A study found that hesperidin alleviated the reproductive and foetal weight defects, ameliorated some hematological parameters, decreased cortisol levels and increased 17β-estradiol rates in pregnant rats subjected to formal-dehyde toxicity [69]. Hesperidin also improved the follicular development in 3D culture of isolated preantral ovarian follicles and increased the concentration of progesterone and oestradiol in mice [93]. It has been reported that hesperidin supplementation in 7,12-Dimethylbenz (a) anthracene (DMBA)- and acrylamide-exposed adult Sprague Dawley female rats resulted in abrogation of toxicity and significant increase in serum progesterone and oestradiol levels, significantly lower malondialdehyde concentration, higher glutathione levels and superoxide dismutase activity [36, 37]. Hesperidin supplementation also attenuated ovarian oxidative damage caused by ischaemia-reperfusion in Wistar female rats through decreasing the malondialdehyde levels and increase in the superoxide dismutase activity [4].

Hesperidin also resulted in significant regression of surgically-induced endometriotic foci in rats, reduced malondialdehyde, higher glutathione levels and increased superoxide dismutase activity while also improving the histological parameters [68]. The high administration of hesperidin of between 1000 – 5000 ppm to ovariectomized athymic mice resulted in the aromatase inhibition and thus counteracting oestrogenic activity through down-regulation of oestrogen production [109] Hesperidin inhibits ovarian cancer cell viability through endoplasmic reticulum stress signaling pathways; increased cytotoxicity in a dose-and time-dependent manner; induced apoptosis; increased cleaved caspase-3 protein expression levels in A2780 cells; and markedly increased the protein expression of anti-growth arrest-and DNA damage-inducible gene 153 [113]. Phosphorylated hesperidin has been reported to possess anti-fertility properties when tested in both male and female mice as well as in human subjects [27, 94, 98]. In both male and female mice used for the experiment, the administration of phosphorylated hesperidin at 20 mg/kg body weight, given intraperitoneally, resulted in reduced pregnancies. The human subjects given oral phosphorylated hesperidin for 3 months at a dose of 100 mg/kg body, showed contraception, which was reversible upon withdrawal of the hesperidin salt. Studies on the reproductive effects of hesperidin are limited. This necessitated the current review to shed light on the effect of hesperidin on reproduction and to explore its potentials for improving reproductive parameters in Yankasa ewes.

9. Conclusion

Hesperidin is a potentially important antioxidant with a lot of biological and therapeutic benefits. As a potent free radical scavenger, hesperidin has an extensive and beneficial role in animal reproduction including amelioration of adverse effects of stress conditions, enhance pathogens phagocytosis and amelioration of other body damaging conditions in animals. Though it is poorly absorbed, synthetic prodrugs of hesperidin like G-hesperidin, which are highly soluble have higher bioavailability, and this may improve further studies to elucidate its mechanism of action and roles in the management of oxidative stress and other body damaging conditions in both man and animals.

Stemming from the old conventional phytochemical extraction methods through the use of Soxhlet extraction, newer and more advanced methods of extraction have evolved with more accuracy, cost effective, sensitivity and less cumbersome. From the old soxhlet to a more advanced and automated soxhlet extraction techniques, ultrasonic and chromatographic methods have emerged as more sophisticated extraction approaches. The chromatographic methods started with paper chromatography to thin layer chromatography to liquid chromatographic mass spectrometric methods; and now more advanced and sophisticated methods referred to as the high performance liquid chromatography (HPLC). HPLC has more advanced variants which are more accurate, sensitive, cost effective, less cumbersome, with ability to detect samples/analytes in nano quantities including in biological systems. This has changed the landscape in analytical chemistry and biotechnology. HPLC and LC/MS are also used to detect, measure and analyze metabolites of hesperidin in the body and other biological samples. To optimize the data obtained from these analyses, it is essential to understand and deal with mass spectral interferences arising from the sample itself, the mobile phase, or the environment.

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11. Conflict of Interest

The authors declare that there is no conflict of interest associated with this review article.

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