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Review Article

Pharmacological and Analytical Aspects of Esculetin: A Comprehensive Review

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Abstract

Plants have been used as a source of food material and natural remedies for the treatment of various ailments. Medicinal plants exhibit various pharmacological activities due to the presence of one or more phytoconstituents such as alkaloids, glycosides, flavonoids, terpenes etc. Coumarins (1, 2 benzopyrones) are a large group of naturally occurring secondary metabolites present in higher plants. Esculetin (6, 7-dihydroxycoumarin) is a coumarin derivative found in a plant, *Cichorium intybus* which is widely distributed in Europe and other parts of the world. It is also present in a Chinese plant, *Bougainllra spectabillis* and used as folk medicines. Although it is found in herbal medicines, exhibit various pharmacological actions such as antioxidant, hepatoprotective, anti-inflammatory, anticancer, hypoglycemic and chemopreventive action. Only limited scientific research has been published. The aim of this review is to collect all available scientific information which will provide valuable information to researchers in further development and screening methods for the treatment of various diseases by using esculetin as a potential molecule.

Keywords: Esculetin; Anti-inflammatory; Coumarins; Proliferative; Alkaloids; Saponinis

Introduction

Coumarins (1, 2 benzopyrones) were first isolated from the plant *Dipteryx odorata* Willd (Fabaceae) in 1820 [1]. They are a large group of naturally occurring secondary metabolites present in higher plants and more than 1000 such compounds have been already described and used in various diseases like multiple sclerosis, T cell lymphoma, multi drug resistant tumor organ transplant and in the treatment of nicotine addiction. Coumarins are further sub divided in to four major groups: the simple coumarins, furano coumarins, pyrano coumarins and pyrone substituted coumarins. The simple coumarines are (coumarin, umbelliferone, herniarin, scopoletin and esculetin) the hydroxylated, alkoxylated and alkylated derivatives of the parent compound coumarin along with their glycosides. Furanocoumarins consist of a five-membered furan ring attached to the coumarin nucleus, divided into linear or angular types with substituents at one or both of the remaining benzoid positions. Pyranocoumarins are the analogous of furanocoumarins, but contain a six-membered ring. pyrone substituted coumarins include 4-hydroxycoumarin. The oral anticoagulant Warfarin, belongs to this coumarin subtype.

Coumarins comprise a very large group of naturally occurring compounds (Figure 1) found throughout the plant kingdom [2-4]. Highest proportion of coumarines are found in some essential oils and fruits such as in cinnamon bark oil, cassia leaf oil lavender oil, bilberry, cloudberry, green tea respectively. It also found in other foods such as in chicory [5]. *Rutaceae* and *umbelliferae* family plants contain the highest proportion of coumarins. It is distributed throughout all parts of the plant, but highest levels in the fruits,

followed by the roots, stems and leaves. Recently six new minor coumarins have been isolated from the fruits and the stem bark of *Calophyllum dispar* (*Clusiaceae*). The genus *Calophyllum* is widely distributed in the tropical rain forest which comprised of 200 species and several of them are used in folk medicine [6].

The important coumarins isolated from microbial sources i.e. from *Streptomyces* species include novobiocin, coumermycin respectively. Another coumarin isolated from *Aspergillus* species include aflatoxins, a group of highly toxic fungal metabolites and the most commonly occurring member of the group is aflatoxin B1. Coumarin group of antibiotics isolated from various streptomyces species are the potent inhibitors of bacterial DNA gyrase and all possess a 3-amino-4-hydroxy-coumarin moiety and a substituted deoxysugar; noviose, as their structural core that is essential for their biological activity. Eg novobiocin, coumermycin A1 and clorobiocin,

Biological activity of coumarins

Coumarins shows various pharmacological and physiological activity such as anticoagulant, antibacterial, antihelminitic, hypothermal and vasodilatory action [7] depicted in (Figure 1 and 2).

Overview of esculetin

Esculetin (6,7-dihydroxycoumarin) is a coumarin derivative found in various plants that are used as folk medicines, such as *Artemisia scoparia, Artemisia capillaries, Ceratostigma willmottianum* and *Citrus limonia* [8]. Few studies have shown the beneficial effect of esculetin such as free radical scavenging activity [9], analgesic [10], anti-inflammatory, antioxidant [11,12], antiproliferative, antitumour [13,14], immunomodulatory [15] and hypoglycemic activity [16,17] depicted in figure 3.

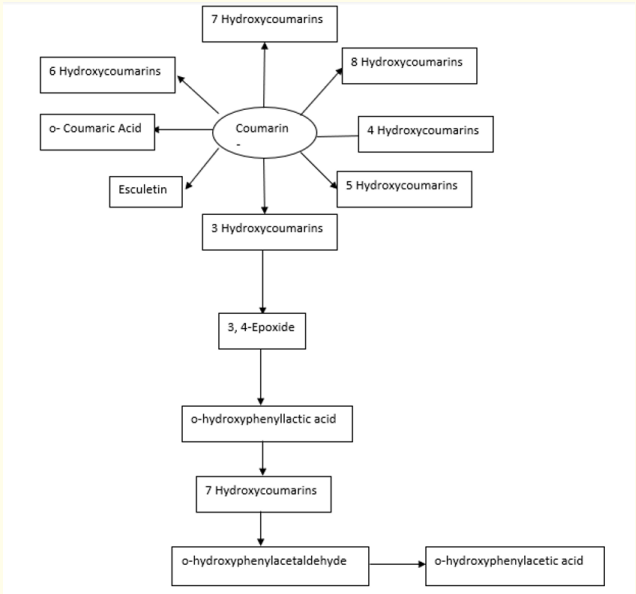

Figure 2: Biotransformation of Coumarin's.

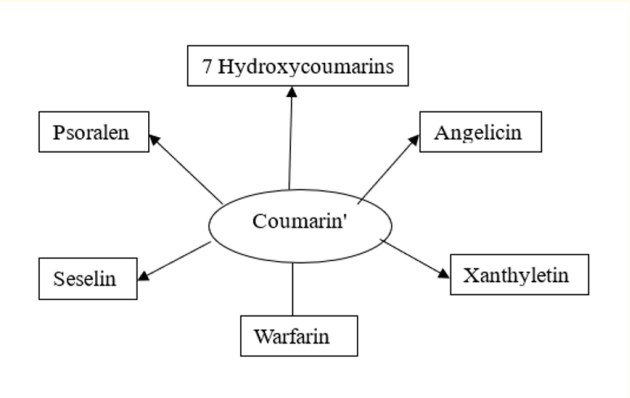

Figure 1: Overview of Coumarin's derivatives.

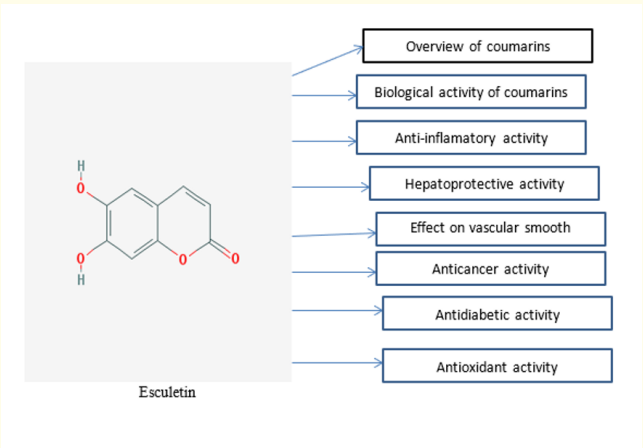

Figure 3: Pharmacological actions of Esculetin.

Analytical techniques

For the determination of Esculetin and its metabolites in rat plasma a newly developed, simple, sensitive, accurae, reproducible and precise new liquid chromatography tandem mass spectrometry (LC–MS/MS) method operated in the negative electrospray ionization (ESI) switching mode was validated. Plasma samples were pretreated by solid phase extraction and separated on reverse phase C18 column maintained at 40^0 C with mobile phase of 0.01% formic acid in water and methanol (20:80, v/v) at a flow rate of 0.3 mL/min and the cycle time of the method was 2.5 min per injection using isocratic elution method [19]. In another study interaction between esculetin and human serum albumin (HAS) was evaluated by FT-IR, circular dichorism (CD) and UV spectroscopy and was found that the interaction was spontaneous and esculetin induces conformational changes in HAS [20].

Simultaneous estimation of esculetin isolated from various plants including Artemisiae capillaris Flos, Cichorium intybus L, Sedum kamtschaticum Fisch, Citrus limonia Osbeck, Digitalis purpurea L and Euphorbia lathyris L, in blood and bile was quantified by HPLC-coupled to UV For simultaneous estimation of esculetin form complicated medicinal preparation a specific and highly reproducible non-aqueous capillary electrophoresis (NACE) with UV detection method was developed and validated which can be used for determination of various components with high recovery rate [21].

Pharmacological activities of esculetin

Anticancer activity

Effects of esculetin on benzo [a] pyrene (BaP) induced lung carcinogenesis in mice was investigated by measuring the antioxidant parameters such as lipid peroxidation, reduced glutathione, superoxide dismutase and levels of Bcl-2 and NF-kB protein in lung tissues. Treatment with esculetin (50mg/kg BW) shown significant reduction in oxidative stress as compared to BaP treated group. Western blot analysis in esculetin treated groups in comparison with B[a]P treated group shown decreased levels Bcl-2 and NF-kB (found to possess oncogenic roles in B[a]P induced lung carcinogenesis).These results suggest that esculetin modulates the expression of Bcl-2 and NF-kB and prevents carcinogenesis thus showing anti-apoptotic, anti-proliferative and therapeutic activity in B[a]P induced lung carcinogenesis in mice [22].

In another study esculetin significantly suppressed the growth of oral cancer in SAS cells in a dose and time dependent manner by inhibiting the growth of oral cancer cells through the induction of cell cycle arrest and apoptosis [23].

In another study the effect of esculetin on human leukemia cells was evaluated and was found to inhibit the survival of human promyelocytic leukemia HL-60 cells in a concentration and time dependent manner. The antiproliferative and cytotoxic effect of esculetin is mainly due to induction of apoptosis that is associated with translocation of cytochrome c and caspase activation in HL-60 cells [21].

Hypoglycemic activity

Antidiabetic activity of esculetin was evaluated in streptozocin induced diabetic rats. Oral administration of esculetin treated group (10, 20 and 40 mg/kg bw) for 45 days significantly reduced the levels of plasma glucose, glycosylated heamoglobin (HbA1c) and increased the levels of heamoglobin (Hb) and insulin which was due to improved glycemic control. Esculetin had further significantly increased the activities of the key enzymes involved in carbohydrate metabolism such as glucokinase and glucose-6-phosphate dehydrogenase whereas, glucose-6-phosphatase and fructose-1,6-bisphosphatase had significantly decreased due to normalization of enzyme activity, esculetin promotes the potentiation of insulin release from b-cells of the islets which is responsible for normal glucose utilization. Esculetin treated group exerted a more pronounced antidiabetic effect at a dose of 40 mg/kg of body weight when treated for 45 days. Treatment with esculetin (40 mg/kg BW) exerts a protective effect in diabetes by attenuating hyperglycemia-mediated oxidative stress and antioxidant competence in hepatic and renal tissues [24].

Anti-inflammatory activity

Esculetin and 4-methylesculetin shown potent anti-inflammatory activities which could be due to their inhibition of the cyclooxygenase and lipoxygenase pathways [18]. These coumarins have been reported to reduce eicosanoid generation by acting on 5-lipoxygenase and cyclooxygenase rather than by affecting phospholipase A2.

In another study the intestinal anti-inflammatory activity of esculetin and methy esculetin was studied in experimental model of rat colitis induced by trinitrobenzenesulphonic acid (TNBS) and

found that esculetin shown significant anti-inflammatory activity by promoting the reduction of the lesion accompanied by a reduction in the incidence of diarrhoea and restoration of the glutathione content. Similar effects were produced by 4-methylesculetin which inhibited the myeloperoxidase and alkaline phosphatase activities in the acute intestinal inflammatory process and in the model of colitis in rats.

Oestrogenic activity

Extracts of several herbs and phytoconstituents including esculetin was screened for *In vivo* and *In vitro* oestrogenic activity and found to possess proliferative action on MCF-7 (an oestrogen-sensitive breast cancer cell line). *In vivo* study shown that esculetin possesses uterotropic activity at an dose of 50 and 100mg/kg body weight,estrogenic activity of esculetin in MCF-7 cells is mediated by the inhibition of MAP kinase signals and tyrosine kinase receptors, a process which is conducive to arrest in the S phase of the cell cycle.

Effect on vascular smooth muscles

Effect of esculetin on vascular smooth muscle cells (VSMC) was evaluated in both in vitro and animal models and found that Esculetin treatment decreased both cell growth and DNA synthesis in dose-dependent and a time-dependent manner. Treatment of VSMC with esculetin induced cell-cycle arrest in the G1-phase and decreased cyclinD1/CDK4 and cyclinE/CDK2, which are associated with G1- to S-phase cell-cycle progression. Furthermore, esculetin arrests G1-phase cell-cycle due to significant up-regulation of p21WAF1.Esculetin inhibit the proliferation of VSMC which is finally responsible for inhibition and the progression of atherosclerosis the leading cause of coronary artery diseases.

Hepatoprotective activity

Effect of esculetin against paracetamol and CCl4-induced hepatic damage in mice was evaluated and found that esculetin significantly lowered the level of both ALAT and ASAT as compared to paracetamol and CCl_4 treated group at dose of 6mg/kg.

In another study the effect of esculetin on pentobarbital sleeping time as well as on CCl4-induced prolongation of pentobarbital sleeping time was studied in mice and found that pretreatment with esculetin prolonged the pentobarbital sleeping time as compare to both paracetamol and CCl4 treated group confirming hepatoprotectivity [25].

Antioxidant activity

Esculetin (6,7-dihydroxy coumarin) present in several plant species, shown potent antioxidant effect in human hepatoma HepG2 cells against reactive oxygen species (ROS) induced by hydrogen peroxide. Following exposure to hydrogen peroxide pretreatment of HepG2 cells with esculetin prevented cell death and maintained cell integrity. Esculetin significantly decreased the production of ROS and intracellular glutathione caused by hydrogen peroxide exposure. Esculetin up-regulates expression of NQO1 at the mRNA protein level, leading to the increased expression of enzyme activity responsible for chemoprotection.

In another study oral supplementation of esculetin to diabetic rats for 45 days significantly lowered lipid peroxidation markers such as thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides (HP) and conjugated dienes (CD); reduction in the enzymic antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST); nonenzymic antioxidants Vitamin C, E and reduced glutathione (GSH) of diabetic rats as compare to normal. Thus treatment with esculetin (40 mg/kg BW) exerts a protective effect in diabetes by attenuating hyperglycemia-mediated oxidative stress and antioxidant competence in hepatic and renal tissues [24].

Discussion

About 25% of the drugs prescribed worldwide are derived from plants and 121 such active compounds are in use of the total 252 drugs in World Health Organization (WHO)'s essential medicine list, 11% is exclusively of plant origin [26].

Coumarins are low molecular weight secondary plant metabolites of benzopyrone family of consist of a benzene ring joined to a pyrone ring. Coumarins are found in the plants, volatile essential oils (cinnamon bark, cassia leaf) and in fruits (bilberry, cloudberry).They are of great interest due to their biological activity. Particularly bacteriostatic, protective and anticancer action makes these compound attractive for further screening. The most important groups are 7 hydroxy coumarins have antitumour activity against several human tumors cell lines. Both coumarin and coumarin derivatives have shown promising effect as potential inhibitors of cellular proliferation in various cancer cell lines [27].

The present review provides relevant and updated information on analytical and pharmacological activity and of esculetin. Coumarins plays a significant role in the human health, so more investigation should be performed regarding general health beneficial property including its uses as nutraceutical and food supplement in the future. For the production of the high level of esculetin, tissue culture techniques could be the right option in the future. From the literature search, it was found that esculetin has a huge biological potential. However, parameters like toxicity studies should be scientifically investigated in order to support the pharmacological uses of esculetin.

Conclusion

This review provides valuable information regarding the pharmacological and analytical aspects of esculetin, which may be useful to the researcher who wants to explore the hidden potential of this phytoconstituents for further research and development in the treatment of various diseases.

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Conflict of Interest

None.

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