Review

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Phytochemical, ethanomedicinal and pharmacological applications of escin from *Aesculus hippocastanum* L. towards future medicine

<https://doi.org/10.1515/jbcpp-2019-0115>

Received July 31, 2019; accepted March 19, 2020; published online July 11, 2020

Abstract: Medicinal plants are used from ancient times for treatment of various ailments. *Aesculus hippocastanum* (Horse chestnut), is the popular and most valuable tree native to the South East Europe. It's seed extracts and their concentrates contain phytochemicals like flavonoids, polyphenols, triterpenoid saponin glycosides (escin), epicatechin, tannins, kaempferol, esculin, fraxin, carbohydrate, essential fatty acids (linoleic acid), oleic acid and purine bases (adenine and guanine). Due to these vital phyto-constituents, horse chestnut is used in phytomedicine for the prevention and treatment of diverse disorders as in venous congestion in leg ulcers, bruises, arthritis, rheumatism, diarrhoea, phlebitis etc. We collected the pharmacological applications of *Aesculus hippocastanum* L. extracts and escin as the chief bioactive compound and their uses in traditionally and clinically for the management of various disorders. This review describes the efficacy of *A. hippocastanum* L. extracts and their bioactive compounds. So in the future this plant may be useful for the alternative treatment measure for various ailments via incorporating either extract or escin into novel delivery systems for improving the social health in future and would provide improved quality of life.

Keywords: *Aesculus hippocastanum*; bioactive compound; escin; pharmacological properties.

Introduction

According to WHO (world health organization) about 80% population utilize only traditional medication for the treatment of various ailments or as health promoting agent. Chief bioactive constituents or whole plants are used for the preparation of herbal extract or medicine which may utilize for the different purpose [1]. Plants are the important part of green urban and rural areas with many positive economic, social, and climatic effects [2, 3]. Plants are the valuable source of natural products such as fruits, bark, leaves, stems, wood, food, dietary supplements, cosmetic and most important herbal medicines [4]. Phytotherapy is considered owing to their minimal or no side effect as reported by scientist for Pyroolidine alkaloids presenting plant extracts, when evaluated for any potential toxicity at 250 mg/kg for 28 days didn't show any toxicity in compared to Pyrazolodine molecule. Further herbal bioactives are cost-effective for any therapy in compared to Allopathic medications as they are much expensive and have higher toxicity risks [5, 6, 73]. *Aesculus hippocastanum* L. commonly known as horse chestnut and buckeyes [7], is a rapidly growing ornamental tree belonging to the family hippocastanaceae, used for the prevention of many diseases and highly adapted in polluted environment [8]. Trees are grown up to 30 m in height and 1 m thick with short stem having a rounded crown [9]. Escin is a chief bioactive compound of horse chestnut seed, which is chemically related to triterpenic glycoside. Seed cotyledons are the main source of escin, but small quantity is also found in leaves, bark and immature fruit pericarp [10]. Escin has different pharmacological activities such as anti-inflammatory, vasoprotective in chronic venous insufficiency. On the basis of scientific research about 20 individual compounds have been identified including escin out of which the most active component is escin Ia, due to their role as antidiabetic, anti-inflammatory and gastroprotective [11]. The

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Table 1: Phytoconstituents with their chemical structures.

S.No.	Chemical constituent	Chemical structure	References												
1	Beta-escin	<p>R= Tiglic acid or angelic acid</p> <p>Chemical structure of aescin</p>	[18]												
2	Esculetin, Scopoletin and Fraxetin	<table><tr><th></th><th>R1</th><th>R2</th></tr><tr><td>Esculetin (I)</td><td>OH</td><td>H</td></tr><tr><td>Scopoletin (II)</td><td>OCH3</td><td>H</td></tr><tr><td>Fraxetin (III)</td><td>OCH3</td><td>OH</td></tr></table> <p>Structure of esculetin, scopoletin and fraxetin</p>		R1	R2	Esculetin (I)	OH	H	Scopoletin (II)	OCH3	H	Fraxetin (III)	OCH3	OH	[23]
	R1	R2													
Esculetin (I)	OH	H													
Scopoletin (II)	OCH3	H													
Fraxetin (III)	OCH3	OH													
3	Escin Ia		[39]												
4	Escin I b (beta)		[27]												
5	Isoescin Ia (alpha)	<p>Ang</p>	[27]												

Table 2: Active phytoconstituent and their pharmacological activities.

S.No.	Active constituent	Pharmacological activities	References
1	Aescin	Anti-inflammatory, anti-edematous, anti-secretory, anti-oxidative, veno-tonic activity	[24, 44]
2	Esculin	Used for treatment of CVI, burn skin inflammation and hemorrhoids	[23]
3	Flavonoids	Radical scavining, antiviral, antibacterial activity and used in capillary fragility	[7]

leaves of the plants are attacked by leaf mining insect *Cameraria ohridella* which is known as horse chestnut leaf-miner [12]. The main host of the leaf miner is white flowering horse chestnut (*Aesculus hippocastanum* L.) but caterpillars of this species have also been observed on other trees of the same genus [13]. The larvae of the moth feed on the leaf parenchyma. Some time whole leaf is infected and get brown, dry up and fall off while, margins of the leaf blade were fold-in [14]. The *Cameraria ohridella* minig affects the quality of seeds but has no effect on the quantity of seed [15]. There are some discriminating methods to prevent the plant from pests by the segregation of leaves of the plants, microinjection of a special gel (endotherapy), large sticky traps placed on tree trunks, pheromone traps, soil injections but these are very expensive and harmful for human, so parasitoids are the natural enemies of leafminer [16]. Around 100 varieties or species of *Aesculus* have been discovered and from them 210 compounds were isolated from the seeds are of different classes [17].

Phytoconstituents

The most valuable constituents of *Aesculus hippocastanum* seed extract are escin and prosapogenin. Escin is a group of

triterpenoid saponin glycoside including glycosides of protoescigenin barringtogenol C [7]. Escin is categorized into two classes α - and β -escin and they can be differentiated on the basis of its melting point, hemolytic index, solubility in water and by specific rotation [18]. Leaf extracts are rich in carbohydrate and anthocyanin [19]. Flowers contain coumarin such as esculin, esculetin, scopoletin and fraxetin. Esculetin, scopoletin and fraxetin are inhibitors of the proinflammatory lipoxygenase (LOX) and cyclooxygenase (COX) pathways of arachidonate metabolism, thus, act as an anti-inflammatory agent. *A. hippocastanum* also contains flavonoids, tannins, amines, amino acids, uric acid, phytosterol, resins, citric acid epicatechin, leucocyanidine, fatty acids such as oleic and linoleic acid [20–22], kaempferol derivative like kaempferol: 3-*O*- α -arabinofuranoside, 3-*O*- β -glucopyranoside, 3-*O*- α -rhamnopyranoside, 3-*O*- α -rhamnopyranosyl (1→6)-*O*- β -glucopyranoside and quercetin: 3-*O*- α -arabinofuranoside, 3-*O*- β -glucopyranoside, 3-*O*- α -rhamnopyranosyl (1→6)-*O*- β -glucopyranoside [23]. The chemical structures of phytoconstituents present in horse chestnut are shown in Table 1.

Various research results confirmed that *Aesculus hippocastanum* and its bioactive compound possess different pharmacological properties which are as shown in Table 2

Traditional uses

In folk medicine, *A. hippocastanum* extract and their preparations are applied for the management of peripheral and vascular disorders like varicose veins, venous and hematoma [17, 24], cardiac venous insufficiency, hemorrhoids, pain, swelling and heaviness of lower limb [25], burns, epidermis abrasion, diarrhoea, phlebitis, fever and for the treatment of enlargement of prostate gland [26, 27]. In China, Beta-escin is traditionally used as for flatulence, anorexia,

Figure 1: Specific pharmacological effects.

Table 3: Pharmacological findings of *Aesculus* and escin.

S.No.	<i>Aesculus hippocastanum</i> extract/Escin	Efficacy tested against	Types of observations	Models used	Mechanism	References
1	leaves extract ALH-L1005	In dentistry	Preclinical	Beagle dogs	Suppress the activity of gelatinase and collagenase, which could detach periodontal ligaments from alveolar bone	[24]
2	Ethanollic of bark extract of A.	Breast cancer	In vitro	Human breast cancer cells (MCF-7) and healthy cells (3T3)	The extract posses antioxidant potential	[32]
3	Escin	Anti-inflammatory	Preclinical	Mice and rats	Able to reduce TNF-alpha IL-1 beta and PGE2,	[38]
4	Escin	Anti-inflammatory	In vitro	Synovial cells isolated from synovial membrane of the radiocarpal joint cartilage of an 8-month-old Holstein cow	Reduces the amount of COX-2, iNOS, IL-1 β , IL-18 and TNF- α in synovioocyte cells and production of NO and PGE2 in monocyte/macrophage cells	[39]
5	Escins Ia, Ib, IIa and IIb	Anti-inflammatory	Preclinical	Male wistar rats and ddY mice	Decreased hind paw edema	[40]
6	Escin	Anti-inflammatory	Preclinical	Rat and mice	Decreased PGE2 and (COX-) 2	[41]
7	ZnO nanoparticles using the fruit shell extract of horse chestnut	Antibacterial	In vitro	<i>Bacillus thuringiensis</i> .	Bactericidal effect of the ZnO nanoparticles was observed against <i>B. thuringiensis</i> for a concentration of 100 lg mL ⁻¹ in a volume of 20 IL	[42]
8	β -Escin	Hepatoprotective	Preclinical	CCL4- induced hepatotoxicity in Wistar rats	ALT, AST, ALP and GSH by inhibiting the oxidative and nitrosative stress	[43]
9	Escin	Hepato-protective	Preclinical	Mice	Inhibit the immigration of inflammatory cells and liver necrosis by decreasing the serum ALT, AST, TNF- α , IL-1 β , IL-beta-HSD ₂	[44]
10	Escin	Microsomal enzyme function	Preclinical	Rat cytochrome P450 (CYP) enzymes	Inhibit or induce activities of CYP1A2, CYP2C9 and CYP3A4	[45]
11	Escin (lyophilized powder)	Gastroprotective	Preclinical	Mice	Reduce malondialdehyde, TNF- α , P-selectin, VCAM-1 and myeloperoxidase activity.	[46]
12	Escin	Gastroprotective	Clinical study	72 post-operative colorectal cancer patients	Escin has anti-inflammatory and anti infiltration activity so it inhibit postoperative adhesions and improve gastrointestinal motility in post-operative patients	[47]
13	Escin	Gastroprotective effect	Preclinical	Rat	On-prostaglandin dependent mechanism involving its anti-inflammatory and vasoactive properties.	[48]
14	Escins Ia, Ib, IIa and IIb	Gastroprotective effect	Preclinical	Male ddY mice,	Involve capsaicin-sensitive sensory nerves and central nervous system for inhibitory activity of escins Ia-IIb for gastric emptying.	[49]
15	Escin	Neuroprotective action	Preclinical	Male C57BL/6 mice	Attenuation of oxidative stress, prevention of mitochondrial dysfunction and apoptosis via inhibition of the cleaved caspases generation, and maintenance of Bax/Bcl-2	[50]

Table 3: (continued)

S.No.	<i>Aesculus hippocastanum</i> extract/Escin	Efficacy tested against	Types of observations	Models used	Mechanism	References
16	Escin	Neuroprotective action	Preclinical	Male C57BL/6 mice (Chronic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine/pro-benecid (MPTP/p) induced mouse model of PD)	Reduction in TH immune response in startium with marked inhibition of oxidative by mitochondrial complex-I and thus reduced the level of TNF- α , IL-6, IL-10, GPAP, IBA-1 protein expression	[51]
17	β -Escin	Human Colon cancer	Preclinical	Colonic ACF, 7-week-old male F344 rats	Inhibit HT29 colon cancer cell proliferation at G1-S phase	[52]
18	β -Escin	Human lung cancer	Preclinical	NNK induced lung cancer in A/J mice	Inhibits, ALDH1A1, and p-Akt and was able to induce p21 expression in lung tumor cells, suggesting growth arrest of tumor cells	[53]
19	Escin	Lung cancer	<i>In vitro</i>	C6 glioma and A549 lung adenocarcinoma cell lines	Cause cell cycle arrest on G0/G1 phase and induction of apoptosis by caspase-3 activation and bax protein expression on A549 cells.	[54]
20	Escin	Human renal cancer	<i>In vitro</i>	Human renal cancer cells (786-O and Caki-1)	Cause G2/M arrest, and then increased the sub-G1 population, Annexin V binding, activation of caspase-9/-3, cleavage of poly(ADP-ribose) polymerase (PARP) and Bax protein. Escin also decreased the anti-apoptotic protein levels of Bcl-2, X-linked inhibitor of apoptosis protein and survivin	[55]
21	Escin	Breast cancer	<i>In vitro</i>	Human CCA cells (QBC939, Sk-ChA-1, and MZ-ChA-1)	Inhibit P-gp expression through the inhibition of the GSK3 β / β -catenin pathway	[56]
22	Escin la	Breast cancer	<i>In vitro</i>	MDA-MB-231 cells and MCF-7 cells	Inhibition of EMT process by down-regulating LOXL2 expression.	[57]
23	β -escin	Human hepato carcinoma	<i>In vitro</i>	HepG2, PLC/PRF5	Block STAT3 activation, c-SRC, JAK2, Cyclin D1, Bcl-xL, Survivin and VEGF	[58]
24	Escin	Human hepato carcinoma	<i>In vitro</i>	Mice hepatoma, H22 model and human hepatocellular carcinoma using HepG2 cell line	Caspase-independent cell death via AIF/ cytochrome-c translocation from the mitochondria to the nucleus and block G1/S cell cycle	[59]
25	Escin	Pancreatic cancer	<i>In vitro</i>	Human pancreatic cancer cell lines Panc-1, COLO 357, and MIA-Paca	Decreased pancreatic cancer cell survival, induce apoptosis and reduce NF- κ B signal transduction pathway	[60]

Table 3: (continued)

S.No.	<i>Aesculus hippocastanum</i> extract/Escin	Efficacy tested against	Types of observations	Models used	Mechanism	References
26	Escin	Thyroid cancer	Clinical	Patient with advanced thyroid cancer	Reduce the serum levels of TSH, TgAb, Tg and calcitonin and prolonged the PFS and OS	[62]
27	Escin powder	Osteosarcoma	<i>In vitro</i>	Human osteosarcoma cell lines MNNG/HOS (CRL-1547TM, ATCC), Saos-2 (HTB-85TM, ATCC), MG-63 (CRL-1427TM, ATCC), U-2OS (HTB-96TM, ATCC), HUVEC (CRL-1730TM, ATCC) and Male BALB/c-nu mice	Inhibit the osteosarcoma growth by inducing apoptosis and autophagy through the ROS/p38 MAPK pathway	[63]
28	β -escin	Osteosarcoma	<i>in vitro</i>	Human osteosarcoma cell lines MG-63, OS732, U-2OS, HOS and SAOS-2	Inhibit inhibits OS proliferation. β -escin blocked the PI3K/Akt pathway in MG-63 cells and MG-63 xenograft OS model	[64]
29	Escin	Dermatological application	<i>In vitro</i>	Skin	Inhibit hyaluronidase activity and collagenase, elastase and β -glucuronidase	[65]
30	Gel (mixture of dried methanol extract of <i>A. hippocastanum</i> and dried flowers of <i>M. chamomilla</i> extracts)	Dermatological application	<i>In vitro</i>	Pig skin using vertical Franz diffusion cells	Better permeation permeability	[66]
31	Ethanolic extract of <i>Aesculus hippocastanum</i> L seeds	Anti-obesity action	Preclinical	Male Swiss albino mice	Inhibit the elevation of blood glucose levels and pancreatic lipase activity in mice. Reduce leptin, insulin, T3, T4 and LDL-C	[67]
32	Ethanolic extract from stem bark of Japanese horse chestnut	Anti-obesity action	<i>In vitro</i>	3T3-L1 preadipocytes	Reduce PPAR γ and C/EBP α adipogenic marker	[68]
33	beta-escin sodium	Endothelial cells proliferation, migration and apoptosis	<i>In vitro</i>	Human umbilical vein endothelial cells (HUECs) and ECV304 cells	Inhibits angiogenesis by reducing ECs proliferation and migration, and by inducing ECs apoptosis. Western blot also implicate that TSP-1 and the signal proteins: PKC- α , ERK, p38 may be involved in the effects of β -escin sodium on ECs.	[69]
34	<i>Aesculus hippocastanum</i> (AH) seeds (Venotrex tablet)	Diabetic neuropathy	Preclinical	Sprague Dawley rats	Decrease in the glomerular area, fibronectin immunorepression, MDA, TGF- β , BUN, creatinine and urine proteinuria levels.	[70]

antiseptic, antioxidant, anti-pyretic, analgesic and antiaging agent [28], Further it is topically useful in cosmetic industry and in treatment of infections of ear, nose and throat regions [29]. While in other than pharmacological applications horse chestnut (*A. hippocastanum*) was used as a source of non-edible starch and timber [30, 31]. Evidences confirmed pharmacological applications in treatment of gastric ulcer [32], brain trauma, stroke, venous congestion, hemorrhoids, thrombo-phlebitis [33], cardiac vein insufficiency, burns, epidermis abrasion, skin inflammation and frost bite [34, 35, 74]. Further it causes the reduction of cellulite and hematoma, act as an astringent, for diarrhoea, in post-operative edema, in urolithiasis, and enhances oxidative defense system [36]. It also posses the anti-inflammatory, antiedematous [18], hypoglycemic, antithrombotic [33], anti-venotonic, anti-edemigenic activity [37], and antibacterial activity towards the *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus mutans*. Antibacterial activity is particularly against *E. coli* [38].

Pharmacological findings

Literature studies revealed *Aesculus hippocastanum* L. has many traditional uses but followings are specific Pharmacological activities which shown markedly significant results, detailed in Figure 1 and the finding from different researchers are tabulated in Table3.

Anti-obesity effect

Escin is a mixture of triterpene glycoside which is isolated from seeds of *Aesculus hippocastanum*. A study was made to investigate the anti-obesity activity of escin in mice fed with high fat diet for 5 weeks. After treatment with escin results showed the decrease level in leptin, FT level, increase HDL-C concentration but show no effect on LDL-C concentration [68], while in other study it has been found that the ethanolic extract of stem bark of the Japanese horse chestnut is able to inhibit the lipid accumulation in 3T3-L1 adipocytes. The result found that the JHC extract significantly decrease the PPAR γ and C/EBP α which are considered as most important adipogenic key markers, so JHC extract might be useful as anti-obesity agent [69].

Effect on endothelial cells proliferation, migration and apoptosis

Beta-escin was observed for the proliferation, migration and apoptosis in the human umbilical vein endothelial cells

(HUVECs) and ECV304 cells by sulforhodamine B (SRB) assay and result showed that Beta-escin sodium at the dose of 10, 20, 40 $\mu\text{g/mL}$ inhibit the endothelial cells proliferation and induced ECs apoptosis at dose of 40 $\mu\text{g/mL}$. Cell migration was identified by the improved wound assay, while Western blot implicate that Beta-escin sodium increased TSP-1 expression, decreasing PKC- α , phosphor-P38 (mitogen-activated protein kinase) and phosphor- ERK expression and involved in the effects of β -escin sodium on ECs [70].

Effect on diabetic neuropathy

A study was performed to describe the preventive effect of *A. hippocastanum* seed extract on oxidative stress and TGF-beta related diabetic nephropathy on streptozotocin induced diabetic rats. It has been found that escin, is responsible for preventing the negative effect of streptozotocin induced diabetic neuropathy by decreasing fibronectin in glomerular area, reduced immune expression, and reduced MDA, TGF-beta, creatinine, urine proteinuria level and improve the functional properties of kidney by preventing development of diabetic neuropathy but blood glucose level was unaltered [71].

Control of environmental pollution

A. hippocastanum is not only an ornamental tree but it is also an important biomonitor for the polluted environment. A study describes that *A. hippocastanum* tree is very useful for quantifying some heavy metals and toxic elements like cadmium, chromium, copper, lead, vanadium and uranium from urban areas of Plovdiv Town, Bulgaria with different anthropogenic impact. *Aesculus hippocastanum* leaves are effective and trustworthy plant species which helps in controlling the environmental pollution [72].

Role in dentistry

Preclinical research was carried out in beagle dogs to assess the effect of horse chestnut leaves extract ALH-L1005 on periodontitis as a MMP inhibitor. Result showed that the ALH-L1005 horse chestnut leaves extract would be effective in periodontitis due to its ability to suppress the activity of gelatinase and collagenase in inflammation [24].

Anti-inflammatory activity

Research was carried out for evaluation of anti-inflammatory effect of escin, a triterpene saponin obtained

from *A. hippocastanum* against carrageenan induced paw edema and histamine induced capillary permeability in rats using topical gel. Edema was evaluated in paraxylene induced ear swelling in mice and cotton pellet induced granuloma in rat and results describe the decreased level of TNF- α IL-1 beta and PGE2. Further, results confirmed the reduction of enhanced vascular permeability at doses of 50 mg/kg and 200 mg/kg of escin. It reduces the hind paw edema induced by both Carrageenan and acetic acid in all doses [39]. Other experiment confirmed the anti inflammatory effect of escin in compared to ibuprofen and dexamethasone in synoviocyte model for osteoarthritis. The focus of this experiment was to reduce the level of COX-2, iNOS, IL-1 β , IL-18, and TNF- α in synoviocyte cells and production of NO and PGE2 in monocyte/macrophage cells in osteoarthritis disorder and findings showed that, Escin had great ability of decreasing the production of COX-2, iNOS, IL-1 β , IL-18, and TNF- α in synoviocyte cells and production of NO and PGE2 in monocyte/macrophage cells and also reduce pain and inflammation in osteoarthritis [40]. Other anti-inflammatory study showed that escins Ia, Ib, IIa, IIb and desacylescins I and II are effective to treat acute inflammation in rat and mice model. 50-200 mg/kg dose of escins Ia, Ib, IIa, IIb is sufficient to inhibit the increase of vascular permeability while 200 mg/kg dose of escins Ia, Ib, IIa, IIb reduced the hind paw edema induced by carrageenin in rats, so it is confirmed that escin has anti-inflammatory property [41]. Further research was undertaken to evaluate the effects of it on oral administration on acute inflammation and intestinal mucosal injury in animal models. The effect on acute inflammation was observed by carrageenan-induced paw edema in rat and cecal ligation and puncture (CLP) was made to cause intestinal mucosal injury in the mouse model. Results revealed that oral administration of escin inhibited carrageenan-induced paw edema and decreases the production of prostaglandin E2 (PGE2) and cyclooxygenase- (COX-) 2. In CLP induced endotoxemic model escin showed the reduction in intestinal mucosal injury [42].

Antibacterial activity

Aesculus hippocastanum seeds was screened for its antibacterial properties and findings represent that the seeds possess antibacterial activity using various microbes like *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus* mutants and the reports showed more potent

action against the *E. coli* species in compared to others [38]. In another pharmacological screening, antimicrobial activity was performed by the formation of ZnO nanoparticles using the extract of the shells of *Aesculus hippocastanum* seeds of different concentrations and the bactericidal effect of ZnO nanoparticles was observed against *B. thuringiensis* [43].

Hepatoprotective effect

An investigation was performed to know the hepatoprotective role of escin in carbon tetrachloride (CCl₄) induced hepatotoxicity in rats and result showed that it is very effective to decrease the elevated marker enzymes like ALT, AST, ALP and GSH by inhibiting the oxidative and nitrosative stress [44]. Another study was carried out to investigate the protective effect of escin against hepatic injury induced by injecting the LPS. After treatment results explain that escin potentially inhibit the immigration of inflammatory cells and inhibit liver necrosis with reducing serum ALT, AST, TNF-alpha, IL-1beta, II-beta-HSD₂ expression in liver and up regulation of GR expression [45].

Altered microsomal enzymes

Findings from researchers reveal that escin could modify the effect on cytochrome P450 enzyme. A cocktail solution at a dose of 5 mL/kg, containing tolbutamide (5 mg/kg), phenacetin (20 mg/kg), chlorzoxazone (20 mg/kg) and midazolam (10 mg/kg) was given as oral administration to rats then the rats were treated with a single dose or multiple doses of intravenous escin via the caudal vein and results concluded that the single or multiple dose of escin can inhibit the CYP2C9 and CYP3A4 enzyme activity while induces the activities of CYP1A2 but has no inductive or inhibited effect on CYP2E1 [46].

Gastro protective

A gastro protective research was carried out to explain the effect of escin on gastrointestinal tract, and result showed that escin is capable to inhibit the gastric ulceration induced by indomethacin at the dose of 18 mg/kg by intragastric administration. Escin also protect the gastric tissue and decrease level of ulcer index and inhibit the neutrophil infiltration [47]. Other study assesses the gastroprotective activity of escin and results confirmed that escin is clinically well tolerated and has efficiency on improving the

gastrointestinal motility in patients with colorectal cancer as well [48]. A research has been conducted to observe the anti-secretory and anti-ulcerogenic effect of escin in absolute ethanol induced gastric mucosal lesions in rat and results reveals that escin posses gastroprotective effect due to its anti-oxidant and anti-inflammatoy activity [49]. A study was made to confirm that saponin fraction from horse chestnuts and its principal saponins, escins Ia–IIb inhibited the gastric emptying in mice dose dependently either they are fed with a non-nutrient meal (1.5% CMC-Na) or with nutrient meal (40% glucose and milk) and 30% ethanol [50].

Neuroprotective

The current study demonstrate that escin is potentially effective for Parkinson's disease due to its anti-inflammatory and antioxidant property. Experiments suggest that Oral treatment with escin significantly attenuated MPTP/p induced dopaminergic markers and useful as a neuro-protective agent. Neuroprevention of escin may be associated with the reduction of oxidative stress (increased nitrite and deplet NPSH levels), prevention of mitochondrial dysfunction and apoptosis via inhibition of the cleaved caspases generation, and maintenance of Bax/Bcl-2 ratio in MPTP/p model [51]. Other study reported that escin possess neuroprotection against chronic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine/probenecid (MPTP/p)-induced toxicity. Escin causes reduction in TH immune reasponse in startium with marked inhibition of oxidative by mitochondrial complex-I and thus reduced the level of TNF-ALPHA, IL-6, IL-10, GPAP, IBA-1 protein expression in rat model [52].

Anticancer activity

A number of studies indicated that extract of horse chestnut may possess significant cancer chemopreventive capacities.

Effect on colon cancer

Escin is evaluated for anticancer activity on azoxy-methane-induced colonic aberrant crypt foci. In, *in vivo* studies beta-escin inhibits colon carcinogenesis in rats while *in vitro* analysis suggest that Beta-escin exhibit cytotoxic effect in colon cancer cell lines and results showed that Beta-escin significantly inhibit HT29 colon cancer cell proliferation at low concentration by induction of p^{21WAF1/CIP1} and reduction of the level of Cdk₂ and cyclin A

and E composite, and causes cell cycle arresting at G1 phase and phosphorylation of ribosomes [53].

Lung cancer

Beta-escin is the major bioactive compound of *Aesculus hipposactanum* and proved as the potential chemotherapeutic agent. Beta-escin has ability to inhibit the lung adenoma and adenocarcinoma induced by tobacco carcinogen 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in female A/J mice and result showed that Beta-escin reduced the lung adenocarcinoma multiplicity and tumor size whether mice were received short- or long-term exposure. Further, Beta-Escin inhibits ALDH1A1, and p-Akt and induce p21 expression in lung cancer cells, cause growth arrest of tumor cells and CSCs as one of the possible mechanism for the same [53]. Other study demonstrate that escin also inhibit glioma in A549 cell line in dose and time dependent manner. Escin induces apoptosis by caspase-3 activation and expression of bax protein and causes the cell cycle arrest in G0/G1 phase in cancer cell line. Apoptosis is occurs highly in A549 cell as compare to C6 glioma cells by mitochondrial cell death signalling pathway. So we can use escin as a potent antitumor agent [55].

Human renal cancer

A study was carried out to demonstrate the cytotoxic effects of escin on human renal cancer cells (786 O and Caki-1) by MTT assay and result explain that escin causes cell death or apoptosis in human renal cancer cells by inducing the intrinsic-mitochondrial apoptosis pathway and arrest G2/M phase. Also increase the sub-G1 stage, annexin V binding, with activation of caspase protein, cleavage of poly adenosine diphosphate-ribose polymerase (PARP), bax protein and formation of reactive oxygen species (ROS). Escin reduces the anti-apoptotic protein levels of Bcl-2, X-linked inhibitor of apoptosis protein [56].

Breast cancer

An observation was made for investigation of the antioxidant and antitumor activity of bark, seeds, leaves and flowers of *A. hippocastanum* and it was found that all the parts of *A. hippocastanum* extracts has significant antioxidant properties, but bark extract exhibit the highest potential due to its high phenolic content, also it is potentially effective to diminish the cell viability of MCF-7 cell line by about 70% [33]. In another research it was observed that escin suppress the metastasis of triple-negative breast

cancer cells through inhibiting epithelial–mesenchymal transition. Furthermore, study also showed escin can reverse multidrug resistance through inhibition of the GSK3 β / β –catenin pathway in cholangiocarcinoma cells [57]. Another study demonstrated the potential effect of escin Ia on triple negative breast cancer. Result of this study showed that escin Ia reduced the expression of LOXL2 mRNA and stimulate E-cadherin mRNA expression and prevented the EMT process of MDA-MB-231 cells and TNF- α /TGF- β -stimulated MCF-7 cell lines. Stimulated cell lines were transfected with LOXL2 or treated with hypoxia, and found escin Ia down-regulated the level of LOXL2 level. In the TNBC xenograft mice model, the metastasis and EMT of MDA-MB-231 cells were suppressed by escin Ia and cause metastasis of TNBC via the inhibition of epithelial–mesenchymal transition by down regulation of LOXL2 expression [58].

Hepatocellular carcinoma

Many studies proven that escin is well tolerated and has potential ability against inflammatory diseases and cancers. A study was performed to investigate the hepatoprotective role of Beta-escin in hepatocellular carcinoma (HCC). Results of this study confirm that escin inhibited both constitutive and interleukin-6-inducible STAT 3 activation in HCC cells and induced the expression of tyrosine phosphatase Src homology phosphatase 1, which is related with down-regulation of constitutive STAT 3 activation. Finally Beta-escin inhibited the proliferation and potentiated the apoptotic effects of paclitaxel and doxorubicin in HCC cell lines and confirmed it as a novel blocker of STAT 3 which may have potential in the suppression of proliferation [59]. A study was carried out to find the antitumor efficacy of escin on mice hepatocellular carcinoma *in vivo* and human hepatocellular carcinoma using HepG2 cell line *in vitro* and shows positive results, which confirms its potency against hepatocellular carcinoma [61]. Escin cause caspase-independent cell death via AIF/cytochrome-c translocation from the mitochondria to the nucleus and block the G1/S cell cycle checkpoints. Escin also encourage the termination of treated HepG2 cells, possibly by a combination of mechanisms that includes the mitochondria-dependent apoptotic cascade encompassing a caspase-independent mode of cell death mediated via the translocation of AIF/cytochrome-c [60].

Pancreatic cancer

Escin is potentially able to decrease pancreatic cancer cell survival, reduce NF- κ B signal transduction pathway, induced apoptosis and responsible for chemosensitization

of pancreatic tumor cells. Chemosensitization of pancreatic cells arbitrate through the ability of escin to modify cell-signaling molecules i. e. cell proliferating proteins, such as cyclin D and members of the NF- κ B signaling pathways. It is believed that cisplatin and gemcitabine reduces the cell survival rate in a dose-dependent manner. Researcher confirmed the combined effect of both these drugs and found cisplatin have a synergistic effect on pancreatic cell survival, while escin combined with gemcitabine showed only additive effect. High concentrations of escin induced apoptosis and decreased NF- κ B-related proteins and cyclin D expression. So we can said that escin has significant role in the reduction of pancreatic cell survival [62].

Thyroid cancer

A clinical research was made to prove the efficacy of escin that it may reduce the thyroid cancer cells and results reveals that, escin significantly reduced the serum levels of TSH, TgAb, Tg and calcitonin while prolonged the PFS (progression-free survival) and OS (overall survival) at the dose of 0.6 mg/kg/day intravenously for patients with advanced thyroid cancer. Escin is well tolerated and safe agent for thyroid cancer [63].

Osteosarcoma

Many clinical studies prove that escin is a potential chemotherapeutic agent. A research conducted to investigate the therapeutic effect of escin on osteosarcoma in an orthotopic model and result confirmed that escin is able to inhibit the osteosarcoma proliferation, induce apoptosis and autophagy. Apoptosis was confirmed by increased expression of caspase-related proteins and the formation of apoptotic bodies while autophagy was identified by the increased level of LC3, ATG5, ATG12, Beclin expression and autophagosome formation. Escin activates the A p38 mitogen-activated protein kinases (MAPKs) and reactive oxygen species (ROS). A p38 MAPK inhibitor partially regulate the autophagy and apoptosis induce by escin, but ROS scavenger showed inhibitory effect. Finally, study confirmed escin potentially reduced the osteosarcoma via inducing autophagy and apoptosis by the activation of the ROS/p38 MAPK signalling pathway [64]. In other study it was confirmed that Beta-escin is effective to inhibit the cell proliferation of human OS cells *in vitro* and *in vivo* by blocking the PI3K/Akt signalling pathway in MG-63 cells. Beta-escin induces activation of caspase-3, caspase-8 and caspase-9 in MG-63 cells and effective against MG-63, OS732, U-2OS, HOS and SAOS-2 cell proliferation in time and dose dependent manner. The results showed that

Beta-escin significantly act as anti-proliferative and pro-apoptotic agent in an established OS xenograft model. β -escin produce synergistic effect with other chemotherapeutic agents [65].

Dermatological application

According to Wilkinson and Brown, escin inhibits hyaluronidase enzyme, however, decrease level of hyaluronidase leads to be insufficient to produce powerful venotonic effect while the effect of extract of *A. hippocastanum* on the microvasculature was more in compared to only escin, it may be due to presence of other enzymes like collagenase, elastase and β -glucuronidase that's why extracts of *A. hippocastanum* were distinctly more efficient than escin alone. In other study the gel containing the mixture of *A. hippocastanum* and *M. chamomilla* extracts imports better permeability and stability for topical delivery in compared to the gels containing barely escin or *A. hippocastanum* extract alone [66, 67].

Conclusions

From the literature reports, it is confirmed that the active components of horse chestnut tree are responsible for the pharmacological effects. The major component of *A. hippocastanum* is escin, esculetin, esculin, scopoletin and fraxetin out of which some are reported for their marked efficacy like escin, useful for their anti-cancer efficacy against various cancers [54, 55, 57, 72], whereas other secondary metabolites are useful for protective effect either alone or in extract as combination. The recent research indicates anti-inflammatory and antibacterial efficacy of escin [40, 39]. So further researches has to be carried out on other active components to find out some novel medicinal applications of these existing herbal drug, Herbal medicines are safe and better efficacy then synthetic products. Plants are being considered as a valuable source of natural medical products which are helpful in preventing and treating various diseases either by enhancing antioxidant activity, bacterial growth inhibition or by modulation of genetic pathways. Today medicinal herbs are still viewed as essential source of biomolecule for the making of new medicines. The therapeutic appliance of *Aesculus* is due to the presence of important bioactive, some are summarized in this review, so we found out this plant has wider medicinal applications against various conditions including environmental and dental condition as explained by some researchers, still further research is needed for newly

derived phytoconstituents and their application in multidisciplinary area.

Acknowledgments: The authors offer their sincere thanks, Dean R&D, Integral University for providing technical support and assigning communication reference no: IU/R&D/2018-MCN000445, for further communication.

Research funding: None declared.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

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