Indian Journal of Biochemistry & Biophysics
Vol. 56, August 2019, pp. 287-300

# The pharmacological potential of hesperidin

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*Received 01 December 2018*; *revised 15 March 2019*

The modern scientific society has presently recognized flavonoids to be a unique class of therapeutic molecules due to their varied therapeutic properties. Of these, hesperidin, found along with vitamin C, has been explored for a number of pharmacological effects. Citrus and oranges possess hesperidin as one of the active constituents. Today, hesperidin has been well recognized for its beneficial effects on health. The present review highlights the current information and health-promoting effects of hesperidin. The review uncovers protective effects of hesperidin on functions and integrity of liver, kidney, heart, and age related memory impairment. Hesperidin demonstrated the antimicrobial, anticancer, antihypertensive and antiulcer effect. The present review focus on current information of hesperidin and its active metabolite hesperetin. Along with this, the chemotherapeutic potential of the same has also discussed.

**Keywords**: Anticancer, Antidiabetic, Antimicrobial, Hesperidin, Organ protection

Medicinal plants and herbal remedies continue to be an important part of the health care system[1]. Herbal remedies are a vital part of the healthcare system in Afro-Asian and European countries. In the present scenario, India, China, Nigeria, and USA as well as WHO have made substantial efforts to explore therapeutic effects of these remedies[2]. Many synthetic molecules share structural homology with various natural products that serve as leads[3,4]. Flavonoids are one of the important classes of phytochemicals that are well accepted for their beneficial effects on human health. In plants, flavonoids play a major role in protecting from 'invading infections' and 'UV radiations[5]. Flavonoids are widely disseminated amongst the 'plant kingdom'. They act as signal molecules during nodulation, promote auxin transport and are responsible for imparting color to flowers which aid in pollination[6]. There are more than 4000 flavonoids present in plant[7]. Fruits, vegetables, nuts, tea, wine, *etc*. are rich sources of flavonoids[8] which form an important part of the diet. The dietary source of flavonoids is the leading domain of the research owing to their health benefits. The use of flavonoids for the promotion of human health finds an interesting past. It was observed that the mediterranean population consuming red wine and a high saturated fat showed low cardiovascular mortality rate made researchers to explore therapeutic effects of flavonoids[9]. Flavonoids have a wide array of bio-spectrum which includes antioxidant, antidiabetic, anticancer and anti-human immunodeficiency virus effects[10]. Continuous efforts are being made to explore the beneficial effects of flavonoids on human

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*Abbreviations*: Akt/Nrf2; ALP, Alkaline phosphatase; ALT, Alanine transaminase; AST, Aspartate transaminase; BACE1, β-secretase 1; BDNF, Brain-derived neurotrophic factor; CAT, Catalase; CFU, Colony-forming units; CNS, Central nervous system; COX-2, Cyclooxygenase-2; DNA, Deoxyribo nucleic acid; EPR, Electro paramagnetic resonance; ERK ½, Extracellular signal-regulated kinases ½; GGT, γ -glutamyl transpeptidase; GPX, Glutathione peroxidase; GR, Glutathione reductase; GSH, Glutathione; GSK-3β, glycogen synthase kinase-3β; Gy, Gray; $H_2O_2$, Hydrogen peroxide; HDL, High-density lipoprotein; HIV, Human immune deficiency virus; HMGB-1, High mobility group box chromosomal protein 1; HMG-CoA, 3-hydroxy-3-methylglutaryl-Coenzyme A; HO-1, Heme oxygenase-1; HSV-2, Herpes simplex virus type 2; $IC_{50}$, Half maximal inhibitory concentration; IgG, Immunoglobulin G; IL, Interleukin; iNOS, Inducible nitric oxide synthase; LDH, Lactate dehydrogenase; LPS, Lipopolysaccharides**;** *l*-T4, L-thyroxine; MPTP, 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine; m-RNA, Messenger ribonucleic acid; MTT, [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]; NO, Nitric oxide; NO-cGMP, Nitric oxide-cyclic adenosine monophosphate; Pgp, P-glycoprotein; PPAR-γ, Peroxisome proliferator-activated receptor γ; RAGE/NF-κB, Receptor for advanced glycation end-products; SOD, Superoxide dismutase; SOD, Superoxide dismutase; SUR1, Sulfonylurea receptor 1; TBARS, Thiobarbituric acid reactive substances; TNF-α, Tumor necrosis factor-α; TrpV1, Transient receptor potential cation channel subfamily V member 1; UV, Ultraviolet; VEGF, Vascular endothelial growth factor; WHO, World health organization

health. Flavonoids are among the important classes of phytoconstituents found in plants. Hesperidin, hesperetin, eriodictyol and naringenin, pelargondin, peoidin, genistein, glycitein, galangin, kaempferol, malvidin, taxifolin, luteolin, apigenin, myricetin, catechin, epicatechin, epigallocatechin, theaflavin, rhamnazin, fisetin *etc* are few of the important flavonoids which are recognized for varied types of biological effects.

Hesperidin, a flavanone, is abundantly found in the rind of citrus fruits. The important sources of hesperidin include *Agathosma serratifolia* (Curtis), *Citrus aurantium* (L.), *Citrus sinensis* (L.), and *Citrus limon* (L.). Gyrogri, a Hungarian researcher, recognised that the intake of 'citrus peel flavonoids' effectively averted 'capillary bleeding'[11]. Hesperidin is a stable compound. However, it gets degraded in the presence of strong oxidizing agents. In general, hesperidin is stable below 75°C, oxygenless and in a neutral or acidic environment. The degradation of hesperidin is promoted in the presence of metal ions ($Cu^{2+}$, $Fe^{3+}$) and in presence of strong light. This review focuses the pharmacological potentials of Hesperidinas studied in various experimental models.

## Pharmacological Effects

### CNS effects

#### *Antiepileptic effects*

Hesperidin has been studied for possible antiepileptic effects. Hesperidin cause attenuation of mitochondrial, biochemical and behavioural alterations inpentylenetetrazole pretreated Laca mice. Further, restoration of reduced glutathione, superoxide dismutase, and catalase levels was also observed. It was observed that the anti-convulsant effect of hesperidin was due to γ-amino butyric acid-benzodiazepine receptor action[12]. In another study by the same research group, it was seen that treatment with *l*-arginine (100 mg/kg) or *l*-$N^G$-nitroarginine methyl ester (10 mg/kg) significantly enhanced neuroprotective effects in combination with hesperidin. Hesperidin through NO-cGMP (Nitric oxide-cyclic adenosine monophosphate) pathway was found to be responsible for neuroprotection[13]. Hesperidin attenuated response towards 4-aminopyridine and bicucullineon rat hippocampal slice preparations. Involvement of large conductance calcium-dependent potassium channel for such effects was postulated[14].

#### *Sedative effect*

Coadministration of alprazolam, bromazepam, midazolam and flunitrazepam with hesperidin, showed overall potentiation of a sedative effect. The selective decrease in the phosphorylation state of extracellular signal-regulated kinases 1/2 (ERK 1/2) was a crucial factor that was responsible for CNS depression[15]. Naltrexone and norbinaltorphimine block the sedative effect of hesperidinthat reveals the involvement of opioid receptors in mediating this effect[16]. Such an effect was enhanced by buspirone and yohimbine and regressed by caffeine and aminophylline. Involvement of adenosine receptors in mediating sedative effects was the key mechanism involved in the effect[17].

#### *Antiparkinson effect*

The intracerebroventricular injection of 6-hydroxydopamine in aged mice developed 'Parkinson' disease-like symptoms. Hesperidin treatment for 28 days in this animal showed improvement in behavioural and biochemical parameters. Treatment with hesperidin averted memory impairment, depression-likebehaviour with restoration of depleted glutathione and catalase in the 'striatum' of aged mice[18]. Hesperidin was capable of providing relief during the episodes of oxidative stress which was possibly due to binding and blockade of sulfonylurea receptor 1 (SUR1) and reverted stroke like condition in animals[19]. Hesperidin administration caused decreased 'reactive oxygen species formation', down regulation ofBax, cyt c, and caspases 3 and 9, with enhancement in the levels of reduced glutathione. There was upregulation of Bcl-2 demonstrating the antiapoptotic effect (against rotenone-induced oxidative stress). In another independent study, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced Parkinson's disease in mice, hesperidin treatment appreciably protected 'microglia activation' and dwindled the release of inflammatory cytokines *viz*. TNF-α, IL-6, IL-4, and IL-10 in the striatum and substantialnigra[20]. Such an effect aided to protect striatum and substantial nigra area in brain. Antiparkinson effects in *Drosophila* model were also observed due to hesperidin[21].

#### *Anti-Alzheimer effect*

In APPswe/PS1dE9 transgenic mice (which demonstrate increase in parenchymal Aβ load, Aβ plaques accumulation commence from 4 months of age with glial activation and deficits in cognitive functions at 6 months of age), hesperidin administration (100 mg/kg per day) for 16 weeks resulted in there plenishment of brain antioxidant levels (SOD, CAT GPX). There was an increase in glycogen synthase kinase-3β (GSK-3β) phosphorylation

and mitochondrial complex I-IV enzymes activities (compared to Donepezil) revealing its beneficial effects[22]. In aluminium chloride treated animals, hesperidin administration (orally) decreased aluminium concentration, brain acetylcholinesterase activity and secretase-related molecules in the brain. Behavioral studies showed increased spontaneous locomotor and exploratory behaviour in 'open field test' along with improved performance in morrismaze. Histological studies of the brain also supported protective effects on the brain. It was postulated that hesperidin chelated aluminium due to wich aforementioned protective effects were seen[23]. In another study, the role of hesperidin against Aβ-induced impairment of glucose utilisationwas studied, whereby, improvement in Aβ-impaired glucose utilisation by inhibition of Aβ-induced autophagy in neuronal cells was observed[24]. Hesperidin also suppressed oxidative stress and inflammation through the commencement of Akt/Nrf2 signalling and embarrassment of RAGE/NF-κB signalling thus offering neuroprotection in APP/PS1 mice[25]. Due to high affinity for β-secretase 1 (BACE1), hesperidin demonstrated complete inhibition of the enzyme at a very low concentration of 500 nM. Amyloid fibril formation was prevented by hesperidin[26]. Antidepressant like effect of hesperidin was due to up-regulation of brain-derived neurotrophic factor (BDNF) levels (in chronic mild stress mice).In a study, hesperidin afford protective effect against cognitive impairment (against ischemic brain damage)[27].

**Analgesic effect**

Hesperidin demonstrated a significant analgesic and anti-inflammatory effectsin experimental animal models[28]. Significant anti-nociceptive effect and absence of motor in-coordination were observed on its oral adminitration to rat. Flumazenil, ketanserin, prazosin, yohimbine, caffeine when given 5 min before hesperidin administration failed to 'revert' its analgesic effects. However, no such effects were demonstrated by its aglycone, hesperetin on animals. Hesperetin was able to inhibit G-protein-activated inwardly rectifying $K^+$ channelsand showed a weak binding affinity towards μ-opioid receptor[29]. Another study revealed thathesperidin also inhibited cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS)[30]. In the case of NOs, there were important docing interactions seen at aspartic acid 476, glutamic acid 710, glutamine 712, glycine 473, tyrosine 711[31]. In the pain induced functional impairment model in the rat, hesperidin administration produced a dose-dependent antinociceptive response. Similarly, a decrease in time spent in paw licking was observed. There was also decrease in capsaicin-induced nociceptive response which suggested the participation of transient receptor potential cation channel subfamily V member 1 (TrpV1) receptor. Co-administration of hesperidin and ketorolac in various combinations produced additive and supra-additive effects. The study suggests that such a combination may be useful to battle arthritic gout type pain (Martínez *et al*., 2011). By preventing the growth of 'methyl methacrylate radicals', it was thought that hesperidin was able to suppressess 'polyunsaturated fatty acid radicals'. The study suggested a significant role of hesperidin as a strong inhibitor of polyunsaturated fatty acid radicals generated from reactive oxygen species. The potent embarrassment of LPS-induced expression of the COX-2 gene in RAW 264.7 cells demonstrated anti-noceceptive activity of hesperidin (Hirata *et al*., 2005). Interaction of hesperidin with COX-2 enzyme has been well studied. Hesperidin treatment caused a noteworthy decrement in the levels of PGE2. Effect was concentration dependent[30].

**Anti-inflammatory effect**

Anti-inflammatory effects of hesperidin and its aglycone hesperetin have been well documented[32]. Hesperidin administration resulted in significant anti-inflammatory effect on rats in paw oedema model (induced by carrageenan). Similar protective effects were observed on animals in dextran-induced oedema model. Paw oedema was decreased by 33% 3 h after hesperidin administration. Carrageenan induced pleurisy was also prevented by hesperidin. There was a reduction in the volume of exudates and migrating leucocyte count was decreased.Similarly, there was a decrement in an acetic acid induced abdominal constriction in animals, but hesperidin demonstrated no effect on tail flick test. A moderate degree of antipyretic effect was also observed. Fever was lowered after 4 h. Hesperidin demonstrated the cytoprotective effect on gastric cells as there were no gross lesions on gastric mucosa in animals pretreated with hesperidin[33].

**Anti-gout and anti-arthritic effects**

Inhibition of xanthine oxidoreductase enzyme may serve to decrease uric acid levels and thus leading to anti-hyperuricemic effect. Hesperidin derivatives and

metabolites were studied for possible xanthine oxidase inhibitory effect. Kinetics study revealed hesperidine to be the competitive inhibitor of xanthine oxidoreductase[34]. Hesperetin demonstrated strong inhibition of xanthine oxidase with $IC_{50}$ =53 mM. Similarly, atherogenic index of hesperetin treated group was also significantly low. Another study confirmed the a synergistic effect of orange juice and hesperetin supplementation in the treatment of gout in experimental animals. Allopurinol (standard drug) was unable to increase serum antioxidant and decrease lipid peroxidation butorange juice (rich in hesperidin) mediated such effects[35]. Similarly, hesperidin administration in complete Freund's adjuvant-induced arthritis in rats resulted in the restoration of serum immunoglobulin G, cartilage oligomeric matrix protein, serum myeloperoxidase, and glutathione. Histopathological analysis of hesperidin treated animals revealed restoration of 'joint architecture'[36]. The antiarthritic effect was seendue to suppression of TNF-α and IL-1β in synoviocyte proliferation rat adjuvant arthritis model [37-39]. Similar protective effects were observed with α-glucosylhesperidin[40]. Docking studies of hesperidin with TNF-α, IL-1β and IL-6 revealed some crucial interactions with these immunomodulatory targets. The binding energy of hesperidin for TNF-α was −6.96 kcal/mol, there were some important interaction which was observed at Serine 69, leucine 120, and tyrosine 151 ; with IL-1β the binding energy was −6.64 kcal/mol and binding at glutamic acid 37 and lysine 65 (1.55 Å) from A chain were observed. In the case of IL-6, the binding energy was found to be −7.07 kcal/mol with interaction at methionine 67, glutamic acid 172 and arginine 179 (B chain)[41]. Similar interactions were observed with rutin[31]. Hesperidin prevented bone loss in ovariectomized-female rats and increase bone mineral density[42].

**Cardiovascular effects**

***Antihypertensive effects***

Antihypertensive effects of hesperidin and its analogues have been extensively studied in animal models. Short-term administration of G-hesperidin and hesperitin to spontaneously hypertensive rats resulted in a dose-dependent showed reduction in systolic blood pressure. The combination of enalapril or prazosin with hesperidin decreased blood pressure significantly which was due to nitric oxide-mediated vasodilation[43]. In spontaneously hypertensive rats, long-term hesperidin and glucosyl hesperidin supplementation in the diet showed regulation of blood pressure and heart rate to normal[44]. In stroke-prone spontaneously hypertensive rats, hesperidin treatment promoted inactivation of NO and shielded endothelial function[45].

**Organ protective effects**

***Hepatoprotective effects***

Hesperidin demonstrated protective effects on histo-architecture and cellular integrity of hepatocytes. Hesperidin administration in male Sprague-Dawley rats exposed to γ-irradiation (1 Gy, 3 Gy, and 5 Gy) resulted in restoration of ALP (alkaline phosphatase), AST (aspartate transaminase), ALT (alanine transaminase), (GGT) γ-glutamyl transpeptidase, serum ceruloplasmin and LDH (lactate dehydrogenase) levels. Histopathological studies were in affirmation with this results. Hesperidin (200 mg/kg) helped to overcome cellular deterioration, as there was no change in 'lobular structure'. In acetaminophen intoxicated Wistar rats, hesperidin administration caused restoration of AST and ALT levels which were supported by histological studies[46]. Hesperidin demonstrated antifibrotic effects against dimethylnitrosamine-induced liver fibrosis in rats[47]. Due to enzymatic modification, hesperidin demonstrated inhibition of lipid peroxidation and restoration of activity of antioxidant enzymes providing beneficial effects on 'alcohol-induced liver disease'[48]. In another study, hesperidin (80 mg/kg) restored biochemical and histochemical changes in iron-induced hepatic and renal toxicity in rats[49]. An upregulation of heme oxygenase-1 (HO-1) expression and activation of ERK1/2 in human hepatic L02 cells was observed due to hesperidin revealing its protective effect in hydrogen peroxide-induced damage[50]. Protective effect of hesperidin against hepatic steatosis is also observed[51].

***Neuroprotective effect***

Hesperidin and its aglycone hesperetin demonstrated neuroprotective effects in various experimental models. In a study, administration of 3-nitropropionic acid in rats for five days (20 mg/kg) resulted in decreased locomotor activity, inhibition of prepulse and increase in MDA levels (in cortex, striatum, and hippocampus). Electron microscopy revealed remarkable swelling of mitochondria, perivascular oedema and shrinkage of nerve cells in untreated rats. All such pathogenies were relieved by administration of hesperidin[52]. Similar protective results were seen in the case of excitotoxicity induced

by kainic acid in the hippocampus of rats[53]. In hippocampal whole-cell patch clamp study, hesperidin decreased the rate of 'spontaneous excitatory postsynaptic currents' without changing 'amplitude'[54]. In another study, the integrity of cultured cortical cells (against damage produced by '$H_2O_2$ or xanthine and xanthine oxidase') was protected by hesperetin[55]. Involvement of L-arginine-NO signalling pathway was observed during protective effect of hesperidin against cerebral ischemic reperfusion injury[56].

***Retinoprotective effects***

In streptozotocin-induced diabetic rats, hesperidin administration to animals for four weeks resulted in suppressed 'blood-retina breakdown', the increment in thickness of the retina, decreased aldose reductase activity and reduction in levels of vascular endothelial growth factor. Lipid peroxidation was decreased with increment in SOD levels[57]. In rabbit retinal pigment epithelial cells culture, hesperidin promoted cell growth and prevented NO and iNOS expression[58]. Hesperidin treatment resulted in a decrement in retinal damage caused due to high glucose levels[59].

***Cardioprotective effect***

Cardioprotective effects of hesperidin against various insults have been well documented. In the doxorubicin-induced cardiotoxicity model, hesperidin administration to rat aided in the reduction of oxidative stress, an anomaly in cellular morphology and DNA damage. This effect was mediated by suppression of NF-κB, caspase-3and p38[60]. In streptozotocin-isoproterenol induced cardiotoxicity model, hesperidin aided in an increment of GPX, SOD, and CAT. Levels of creatine kinase-MB isoenzyme, lactate dehydrogenase were also increased. Upregulation of PPAR-γ and Bcl-2 along with downregulation of Bax was seen. Doxorubicin-induced DNA damage and apoptosis were considerably prevented (Agrawal *et al*. 2014a)[61]. Hesperidin also showed protective effects in ischemic reperfusion injury model. Enrichment of the endogenous antioxidant defence system and improved histo-architecture of myocardium was observed (Agrawal *et al*. 2014b)[62].

***Nephroprotective effects***

Hesperidin administration in $CCl_4$ intoxicated animals caused attenuation of thiobarbituric acid reactive substances (TBARS) and increased levels of protective antioxidant enzymes *viz*. CAT, SOD and glutathione[46]. Cisplatin raises total sodium, total potassium, and urinary creatinine levels but administration of hesperidin in cisplatin intoxicated animals aided to restore kidney functions and oxidative stress biomarkers. In this study, there was a significant refurbishment of serum sodium, serum creatinine and blood urea nitrogen in hesperidin treated group[63]. In cisplatin induced oxidative stress over kidneys, hesperidin administration significantly restored the expression of nitric oxide in the kidney. There was a decrease in lipid peroxidation, inflammation due to infiltration of leukocytes and pro-inflammatory cytokine. Necrosis (due to caspase-3 activity) and DNA damage were prevented[64]. In an independent study, gentamicin administration to rats altered endogenous antioxidant enzyme system. Higher body weight and low kidney weight were observed in the same group. Similarly, there was an increase in serum creatinine, urea, uric acid and TBRAS levels. Hesperidin administration served to be fruitful in reverting harmful effects produced by gentamicin in rats and restoration of antioxidant enzyme levels was observed. The histomorphological study revealed normal kidney features with little degenerative changes[65]. Similarly, in trichloroethylene-induced nephrotoxicity model, hesperidin treatment decreased kidney malondialdehyde levels and increased antioxidant enzymes, creatinine and KIM-1 levels[66]. Correspondingly, protective effects of hesperidin on kidney integrity and functioning against acetaminophen-induced toxicity were observed[67]. Nicotine administration in rats for 22 weeks altered levels of AST, ALT, ALP, and LDH. Hesperidin (25 mg/ kg dose) was effective in restoring altered levels of enzymes above in kidneys of nicotine intoxicated rats[68]. In γ-radiation-induced toxicity in rats, hesperidin administration caused little necrotic damage along with significant recovery[69,70].

***Radio modulatory effects***

Radiation therapy is one of the main strategies in the treatment of cancer, but, due to detrimental effects on other healthy tissues, its use is limited. Hesperidin has been screened for radioprotective effects in animal models. In various studies, hesperidin has showed protective effect on 'γ irradiation in mouse bone marrow cells'[71], liver[72], and other tissues[69]. Protective effects on human lymphocytes had been studied, whereby the deteriorative effect of radiation was reverted which was witnessed by the restoration of antioxidant enzymes and decrease in TBARS levels[73,74]. Protective effect of hesperidin was also evaluated against γ-irradiation induced acute renal

damage in rats. The restoration in activity of SOD and GSH (glutathione)and decreased lipid oxidation was seen. The decrease in neurtophils and macrophages was also observed[75].

***Protection against testicular toxicity***

Protective effects of hesperidin on testicular ischemia/reperfusion injury in rats was observed. Levels SOD, CAT and GSHwere restored due to hesperidin administration (Celik *et al*, 2016)[76]. Benzo[α] pyrene-induced testicular toxicity was ameliorated by hesperidin whereby testis weights were increased to normal, there was negligible degree of induced pyknosis, necrobiotic changes and chromatolysis in the nuclei of the spermatocytes in the seminiferous tubules[77]. In another study, harmful effects of cisplatin on the reproductive system were effectively reversed by hesperidin[78]. G-hesperidin demonstrated a protective effect against vanadium, as vanadium exposure to testicles caused decreased availability of androgens. Thus, oxidative damage to testicles was protected due to G-hesperidin administration[79].

**Endocrine effects**

***Antidiabetic and hypoglycemic effects***

In streptozotocin-induced marginal type 1 diabetic rats, hesperidin administration in the diet (10 g/kg diet) caused a decrement in elevated blood glucose levels, with the regulation of adiponectin and lipid levels[80]. Protective effects of hesperidin (50 mg/kg, orally) over CNS activity on streptozotocin-induced diabetic rats were studied where hesperidin treatment caused depletion of neurotoxicity biomarkers, and neuromodulatory effects were observed[81]. Similar, results were observed in high-fat fed/streptozotocin induced type 2 diabetic rats. Hesperidin supplementation (50 mg/kg, orally for 4 weeks) to these animals caused improvement and restoration of antioxidant enzyme defence system. Level of glycosylated haemoglobin was decreased. There was suppression of inflammatory cytokines (TNF-α and IL-6)[82]. Another important mechanism responsible for the hypoglycemic effect of hesperidin could be increased 'hepatic glycolysis' and decreased 'hepatic gluconeogenesis'[83]. In pregnant diabetic mice, attenuation of maternal glycemia, increased number of implantations and an overall number of foetus were observed by hesperidin treatment. Histopathological analysis of foetus showed no sign of embryopathy in hesperidin treated group[84]. There was a significant improvement in diabetic nephropathy which was observed due to modulation TGF-β1 and oxidative DNA damage[85].

Hesperetin caused inhibition of glycolytic enzymes *viz*, amylase and glucosidase. For glucosidase $IC_{50}$ was 150 μM and for amylase, more than 0.50 mM[86].

***Antihyperlipidemic effects***

Hesperidin supplementation in laboratory animals was associated with lipid-lowering effects in many animal models of lipidemia and cholesterolemia. The antihypercholesterolemic effects of hesperidin (0.066 mM/100 g diet) were associated with inhibition of lipidemic enzymes *viz*. HMG-CoA reductase and acyl-CoA:cholesterol acyltransferase. Thus the reduction in cholesterol biosynthesis and esterification was observed[87]. Another study confirmed improvement in fatty liver on hesperidin administration (0.08% hesperidin in diet). Expression of mRNA for retinol binding protein, cutaneous fatty acid-binding protein, and heart fatty acid-binding protein was also regulated (Wang *et al*. 2011)[88]. α-Monoglucosyl hesperidin suppress white fat accumulation[89]. Co-administration of vitamin C and hesperidin was beneficial in reducing the levels of cholesterol[90]. Glucosyl hesperidin (30 mg/kg, orally), a water soluble analogue of hesperidin aided in the improvement of HDL cholesterol synthesis in animals. Hypertrophy in vasculature was also inhibited[90]. In case of *Caenorhabditis elegans*, 100 μM hesperidin decreased the accumulation of fat in high-fat worms. The decrease in the regulation of stearoyl-CoA desaturase was seen. Expression of pod-2, mdt-15, acs-2, and kat-1 genes (lipid metabolism regulating genes) was also decreased[91].

***Antithyroid effect***

Hesperidin administration to L-thyroxine (*l*-T4) induced hyperthyroidism in rats caused suppression of hepatic 5'DI, serum lactate dehydrogenase and serum glutamate pyruvate transaminase. Suppression of hydroxyl radical formation in hepatic tissue was observed by electro paramagnetic resonance (EPR) spectra[92].

**Antiulcer effects**

Hesperidin as well as in combination with other drugs/phytochemicals has been evaluated for antiulcer effects. In indomethacin-induced peptic ulcers model of rats, hesperidin administration increased GSH content of stomach[93]. In another study, indomethacin and hypothermic restrain stress-induced ulceration models were used to assess the antiulcer effects

of hesperidin. Hesperidin (300 and 450 mg/kg) administration elevated gastric pH with diminution of acidity and ulcer index. There was a significant increase in the levels of mucin, SOD, CAT, and GSH. The protective effect was observed due to cytoprotective, muco-protective and antioxidant activity[94].

**Antiallergic effects**

In chronic airway inflammation and airway remodelling (ovalbumin-induced airway inflammation) situation, hesperidin supplementation (5, 10, and 30 mg/kg) decreased 'infiltrating inflammatory cells' count, and 'Th2 cytokines' in alveolar space, serum levels of OVA-specific IgE were also decreased. There was reduced resistance to inhaled 'methacholine' airway hyperresponsiveness in animals. Histopathological evidence also supported above mentioned protective effects[95]. Ovalbumin (OVA) obtained from the chicken egg is an often used asallergen to induce allergic pulmonary inflammation in laboratory animals. Sensitivity in airways is seen soon after inducing allergens to animals. Alum may also be combined with OVA to achieve a significant degree of inflammation in animals[96]. Antiasthmatic effects of hesperidin were due to' suppression of production of IL-5, IL-17, and OVA-specific IgE' especially 'Th2 cytokines (IL-5)'[97].

**Chemotherapeutic effects**

***Antibacterial effects***

Lethal effect of hesperidin against *Aeromonas hydrophila* hasbeen studied in detail. Minimum bactericidal concentration was determined and *in vivo* studies on mouse infection model was performed. Zone of inhibition was 8 mM for 12.5 mg/mL and 13 mM for 100 mg/mL concentration. Minimum inhibitory concentration and minimum bactericidal concentration of hesperidin were found to be 3125 and 12500, respectively. In mouse infection model, bacterial load in untreated group of infected animals was 7.842 ± 0.128 Log10 CFU/mL where as it was (3.2875 ± 0.085 Log10 CFU/mL in hesperidin treated group[98]. Antimicrobial effect of hesperidin on *Proteus mirabilis* and *Staphylococcus aureus* revealed that it's MIC90 was 12 times lower when compared to chloramphenicol[99].

Sulphonated hesperidin demonstrated inhibitory effects against *Chlamydia trachomatis* and *Neisseria gonorrhea*[100]. Hesperitin, a hesperidin aglycone demonstrated antibacterial effects against *E. coli*, *Pseudomonas putida*, *Salmonella enterica*, Gram-positive bacteria *Listeria inocula*, *Bacillus subtilis*, *Staphylococcus aureus*, *Lactococcus lactis* above 1000 µg/ mL[101]. Shock due to Salmonella typhimurium was suppressed by hesperidin. TNF-α and high mobility group box chromosomal protein 1 (HMGB-1) expression was suppressed[102].

***Antifungal effects***

Against *Saccharomyces cerevisiae*, inhibitory concentrations of hesperetin above 1000 µg/mL were observed[101]. Antifungal effects of hesperidin against *Aspergillus parasiticus*, *Aspergillus flavus*, *Fusarium semitectum* and *Penicillium expansum*are well documented. Patulin (a mycotoxin) accumulation was also prevented[103].

***Antiviral effects***

Inhibition of HIV, and Herpes Simplex virus type 2 (HSV-2) was observed due to hesperidin [100]. Inhibitory effects over sindbis virus [$IC_{50}$= 20.50 µg/mL][104] and rotavirus [$IC_{50}$=10 µM][105] has also been studied. The Inhibition against canine distemper virus was observed[106]. Glucosyl hesperidin, a water-soluble derivative of hesperidin, by inhibiting sialidase (neuraminidases), prevented Influenza A virus replication as evidenced by studies on Madin-Darby canine kidney cells[107].

***Antiparasitic effects***

Survival of *Brugia malawi* was prevented by hesperetin. The death of the test organism was witnessed. More than 50% MTT reduction was seen at 31.22 µg/mL concentration (Lakshmi, *et al*. 2010)[108].

***Anthelmintic Effects***

Protective effect of hesperidin against *Schistosoma mansoni* infestation in mice model was studied. Hesperidin administration demonstrated a significant reduction in 'worm burden' in infected animals. '*Schistosoma mansoni* specific IgG level' in infected animals were considerably augmented[109].

***Anticancer effects***

Anticancer study of hesperidin and its analogs have been well documented[110,111]. Hesperetin administration in a rat model of colon carcinogenes is causes a decrease in 'lipid peroxidation' levels in colon tissue. Restoration of SOD, CAT, GPX and GR was observed[112]. In an independent study, the anticarcinogenic effect of hesperidin over benzo (α) pyrene induced lung carcinogenesis in mice was studied. Benzo (α) pyrene (a carcinogen) administration

resulted in an increment in lung specific tumour marker carcinoembryonic antigen specifically along with increased levels of aryl hydrocarbon hydroxylase, lactate dehydrogenase, 5'nucleotidase, and glutamyl-trans peptidase. Depletion of defensive antioxidant enzyme system was observed. Treatment of animals with hesperidin revealed 'potent anticancer effect'.The histopathological analysis also confirmed the same[113]. Anticancer effects of hesperidin on Ehrlich solid carcinoma were determined on mice model. The increment in serum and tissue glutathione and decrement in tumour weight and volume was observed. Modulation of 'mdr1a gene expression' along with the decreased expression of 'p53 and VEGF' were possible mechanisms behind it[114]. In a study, hesperidin suppressed of cell proliferation in the colonic crypts[115]. Co-treatment of doxorubicin and hesperidin MCF-7/Dox cells demonstrated synergistic effect possibly due to inhibition of P-glycoprotein (Pgp) expression[116]. In gall bladder cancer cells, hesperidin treatment resulted in cell cycle arrest at $G_2$/M phase[117]. Anticancer effect of hesperidin is possibly due to suppression of migration and invasion of non-small cell lung cancer cells by inhibiting of SDF-1/CXCR-4 pathway[118]. Hesperidin treatment resulted in programmed cell death which was due to down-regulation of non-genomic estrogen receptor signalling pathway in endometrial cancer cells[119]. Outcomes of various studies about the anticancer effects of hesperidin are listed in (Table 1 and Fig. 1).

**Immunomodulatory effects**

In irradiated mice, hesperidin supplementation resulted in an increase in percentages of 'CD4(+) and CD8(+) lymphocytes'; a decrease in the levels of serum cytokines *viz*. tumour necrosis factor-α, interleukin-6, and interleukin-1β and increase in total protein after 30 days of irradiation was observed[120]. Effect of hesperidin administration on 'macrophage'

Table 1 — Anticancer effects of hesperidin and putative mechanisms

| Cell line | Type | $IC_{50}$ | Mechanism | Reference |
|---|---|---|---|---|
| MCF-7 | Breast | 1.67 µg/mL | Cytotoxic effect | 111 |
| HEp-2 | Larynx | 3.33 µg/mL | Cytotoxic effect | 111 |
| HeLa | Cervix | 4.17 µg/mL | Cytotoxic effect | 111 |
| HepG-2 | Liver | 4.58 µg/mL | Cytotoxic effect | 111 |
| SNU-C4 | Colon | 65% cell viablilty reduction | Apoptotic effect | 112 |
| COS7 | Kidney | 29 µg/mL | Cytotoxic effect | 113 |
| Ramos | Lymphoblast | 50 µM | Apoptotic effect | 114 |
| MSTO-211H | Mesothelioma | 152.3 µM | Cytotoxic effect | 115 |

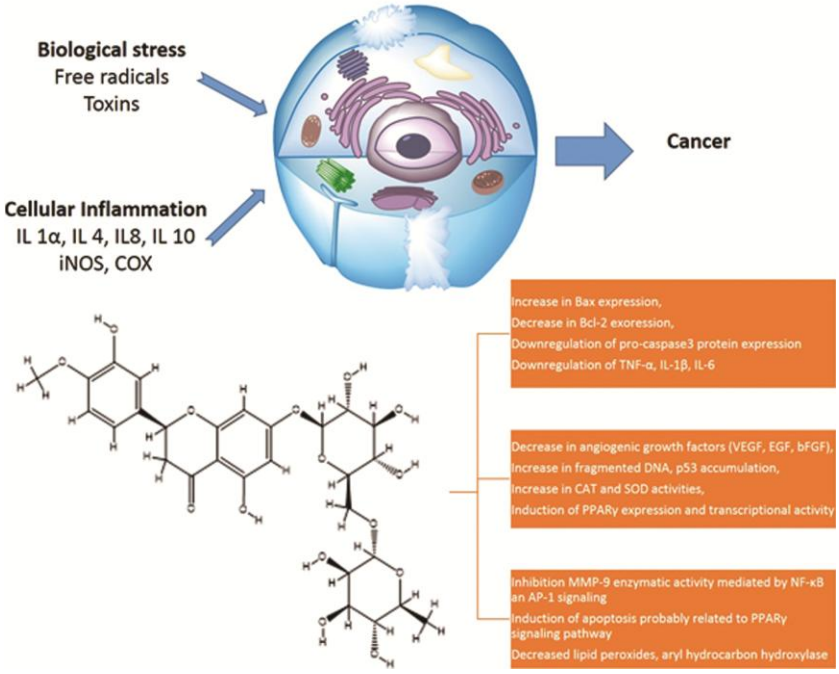


Fig. 1 — Anticancer effects of Hesperidin

integrity was observed whereby, decrement in NO, IL-10, IL-12, and TNF-α were seen. Suppression of inflammatory response was observed[121]. In another study, improvement in 'immunity and the morphometry of small intestine' in lipopolysaccharide challenged broiler chickens was observed (Kamboh & Zhu 2014)[122]. Immunomodulatory effects and anti-infective effects of hesperidin due to *Aeromonas hydrophilia* were observed in the murine model. Levels of anti-LPS and anti-ECP IgA levels hesperidin treated group were reduced[98].

**Pharmacokinetics**

Hesperidin is well absorbed from the large intestine. It has poor bioavailability as compared to hesperetin. Hesperidin ((1.51 ± 0.78 × $10^{-6}$ cm/s) has less retina to scleral permeability when compared to hesperetin (2.52 ± 0.51 × $10^{-6}$ cm/s). Hepato-biliary elimination of hesperidin takes place against a concentration gradient. Glucuronidation and sulphate conjugation are important pathways for their metabolism (Fig. 2). Hesperetin is one of the important active metabolites. Details of various pharmacokinetic parameters are summarised in (Table 2). Hesperidin (Fig. 3) is stable in acidic media. Some novel formulations of hesperidin like nanosuspensions[123], organogel-emulsion[124], microemulsion[125] and hydroge[126], gastro-resistant microparticles[127], dermal smartCrystals®[128], have been formulated for achieving better bioavailability.

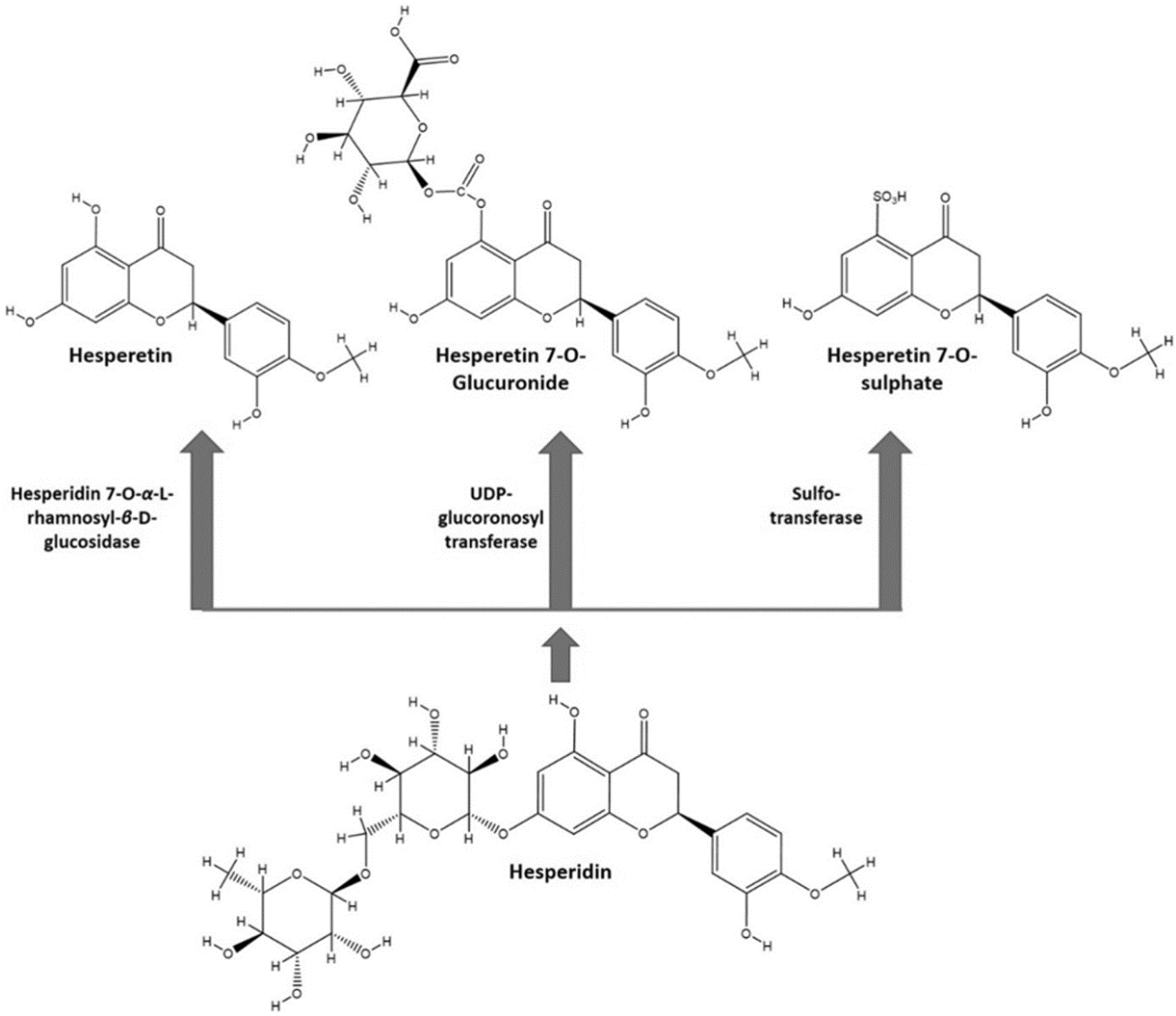


Fig. 2 — Metabolism of hesperidin

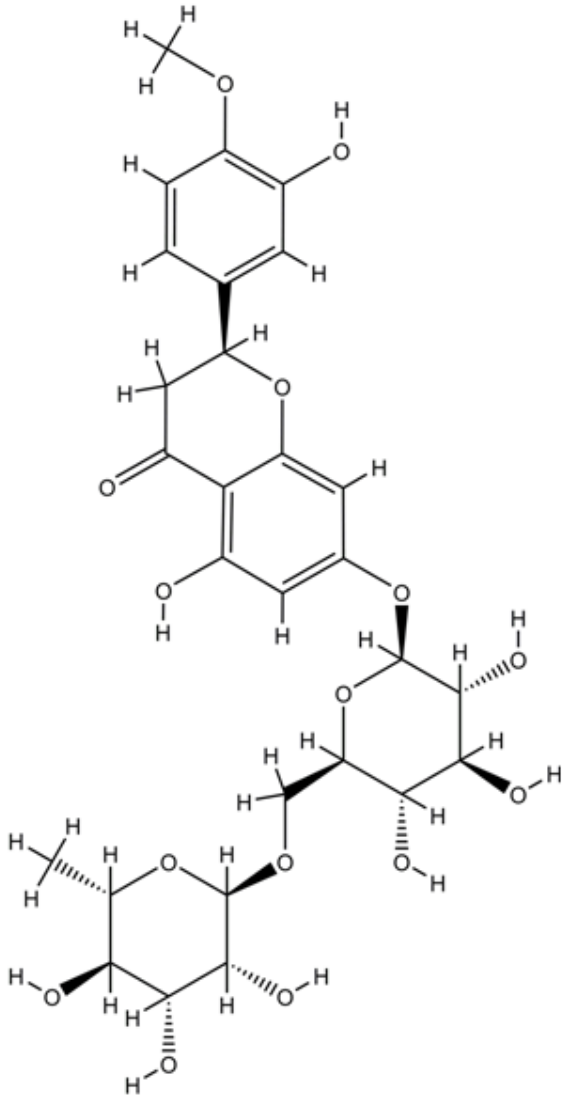

Fig. 3 — Structure of hesperidin

Table 2 ─ Pharmacokinetics of Hesperidin

| | | |
|---|---|---|
| Absorption | Oral | 116 |
| Distribution | Widely distributed | 117 |
| Metabolism | Deglycosylation | 118 |
| Excretion | Faecal | 119 |
| Active metabolite | Hesperetin | 118 |
| Half life | 6.989±0.258 h | 120 |
| AUC | 30.1 nM . h/mL | 120 |

## Conclusion

Hesperidin and its aglycone hesperetin on the animal as well as human have demonstrated various beneficial, protective and therapeutic effects on various biological systems Hesperidin has shown antioxidant and radical scavenging effects which could be a possible reason behind therapeutic effects. Hesperidin aided in the restoration of an antioxidant enzyme defence system. Anticancer effects of hesperidin and its analogues are due to apoptosis and cytotoxic effects. A wide variety of inflammatory mediators and their expression has been suppressed by hesperidin. The activity of some enzymes is also inhibited by hesperidin. Antidiabetic and antihypertensive effects of hesperidin are well studied. Apart from animal studies, numerous human studies have revealed anti-obesity, anti-hyperlipidemic, and anti-hemorrhagic effects.

In the future, hesperidin/hesperetin analogues with adequate lipid solubility and unique receptor/enzyme binding affinity could be developed that will have targeted therapeutic effect on cells and organs. Hence, hesperidin can be considered as an ‘essential phytochemical’ which is obligatory to be studied comprehensively to establish effective safety profile in human to get therapeutic benefits.

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