Biochanin A: A Novel Bioactive Multifunctional Compound from Nature

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Abstract

Natural products (NPs) will continue to serve humans as matchless source of novel drug leads and inspiration for the synthesis of non-natural drugs. As our scientific understanding of 'nature' is rapidly expanding, it would be worthwhile to illuminate the pharmacological distinctions of NPs to the scientific community and public. Flavonoids have long fascinated scientists with their remarkable structural diversity as well as biological functions. Consequently, this review aims to shed light on the sources and pharmacological significance of a dietary isoflavone, biochanin A, which has been recently emerged as multitargeted and multifunctional guardian of human health. Biochanin A possesses anti-inflammatory, anticancer, neuroprotective, antioxidant, anti-microbial, and hepatoprotective properties. This anticancer isoflavone combat cancer development by inducing apoptosis, inhibition of metastasis and arresting cell cycle via targeting several deregulated signaling pathways of cancer. It fights inflammation by blocking the expression and activity of pro-inflammatory cytokines via modulation of NF-κB and MAPKs. Biochanin A acts as a neuroprotective agent by inhibiting microglial activation and apoptosis of neurons. As biochanin A has potential to modulate several biological networks, thus, we anticipate that this therapeutically potent compound might serve as novel lead for drug development in the near future.

Key words: Biochanin A, isoflavone, anticancer, anti-inflammatory, antioxidant, neuroprotective, anti-microbial

1. Introduction

It is no more mystic that over the centuries, human pharmaceutic armamentarium is remarkably indebted to natural products (NPs) [1]. NPs predominate as an endless source of novel scaffolds for developing effective drugs to cure numerous pathological conditions. In spite of the emerging synthetic chemistry-based approaches in pharmaceutics, the contribution of NPs for the prevention as well as cure of diseases is still enormous [2]. NPs have been reported as the richest source of novel mediators of biomolecular functions [3] which have improved human health by serving as useful drugs of choice [4]. To date, NPs and their derived compounds account for approximately 50% of anti-infective and 50% of anticancer drugs [5] such as paclitaxel, silibinin, and lovastatin which are all nature-inspired drugs [6]. Eighty percent of the human populations still depend on plant-derived drugs to meet their healthcare demands [7]. NPs exhibit various pharmacological activities such as anticancer [8], anti-inflammatory [9], antioxidant [10], and anti-microbial [11].

NPs are categorized on the basis of their structural similarities into following major classes: polyphenols (flavonoids, tannins, lignins) alkaloids, terpenoids, and saponins [12]. Isoflavones, a subclass of flavonoids, are phytoestrogens that have similarity to 17-β-estradiol in chemical composition. Isoflavones are mainly found in the plants of legume family such as soybeans, peanuts, chickpeas and green beans. Isoflavones-rich diets have attracted attention of scientific community because of their health advantages such as chemoprevention, anti-osteoporosis and maintenance of cognitive functions [13].

Biochanin A (4′-methoxy-5, 7-dihydroxy isoflavone) is a naturally occurring isoflavone isolated from red clover, soy, chickpea, and many other plants [14, 15]. Pharmacological and biological activities of biochanin A are well-documented as anticancer [16], anti-inflammatory [17], antioxidant [18], neuroprotective [19], anti-microbial [20], and hepatoprotective [21].

This review article aims to highlight the researches related to biological and pharmacological efficacy of biochanin A which will hopefully pave a way for scientific community for further research on biochanin A. The literature was assessed via various e-sites such as PubMed, Scopus, Science Direct, and Elsevier. Key words utilized for searching data are “biochanin A”, “natural products”, “antioxidant”, “neuroprotective”, “anti-inflammatory”, and “anticancer”.

2. *Natural sources of biochanin A*

Biochanin A ($C_{16}H_{12}O_5$) was firstly isolated from the leaves and stems of *Trifolium pretense* L., of family Leguminosae. *T. pretense* is utilized on large scale to relieve postmenopausal problems in women and to cure asthma, eczema, cough, and eye disorders [22, 23]. Further, biochanin A was also isolated from *Astragalus membranaceus*, which is a traditionally used medicine in China [24]. Some other natural sources of biochanin A include soy (*Glycine max*) [25, 26], peanuts (*Arachis hypogaea*) [27], chickpea (*Cicer arietinum*) [28], Indian rosewood (*Dalbergia sissoo*), [29], golden tree (*Cassia fistula*) [20], and alfalfa sprouts (*Medicago sativa*) [30, 31] (Figure 1). Yield of biochanin A was recorded as 1.79–3.32 mg g^{-1} from dry leave matter of *T. pretense* [32]. 5mg of biochanin A was isolated from 20 mg chloroform fraction of *D. sissoo* leave extract [33]. Comparison of yield of isoflavones from chickpea shows that biochanin A is most abundant isoflavone with highest yield of 15.7 mg/150 mg among other isoflavones. As chickpea is a common dietary food, thus, biochanin A is an easily available and cost-effective pharmacologically active compound [34]. Biochanin A was isolated at the concentration of 0.133 mg/ 100 g of hexane-defatted dry matter of *A. hypogaea* [35] The major sources of biochanin A are summarized in Table 1.

Table 1. Natural sources of biochanin A

Plant Name	Common Name	Part Used	Fraction	Yield of Biochanin	Functions	References
Cicer arietinum	Chickpea	Seeds and sprout	n-hexane, ethyl acetate	15.7 mg/150mg	Anticancer, anti-obesity	[34]
Trifolium pratense	Red clover	Above ground parts, flower heads	---	0.07–0.33% Above ground parts, 0.070–0.14% flower heads	Anti-proliferative	[36]
Casia fistula	Amaltas	Fruit	Dichloro-methane	---	Anticancer, antiviral, Anti-inflammatory	[20]
Dalbergia paniculata	Phansi	Stem, bark and leaves	Chloroform	---	Antimicrobial, antioxidant, anti-inflammatory, anti-diarrheal	[37]
Arachis hypogaea	Peanut	Peanut skin	n-hexane	0.964mg/100g, 0.133mg/100g	Antiviral, anti-inflammatory, Anti-carcinogenic	[27, 35]
Medicago sativa	Alfalfa	Leaves	---	---	Antioxidant, anti-ulcer, neuroprotective	[38, 39]
Astragalus membranaceus	Goat's horn	Roots	Acetonitrile,	0.006 mg/g	Anti-tumor, antioxidant, antiviral	[40]

3. *Pharmacological activities of biochanin A*

A natural bioactive compound “biochanin A” has been well-known for its wide range of pharmacological traits (Figure 2). Various *in vitro* as well as *in vivo* studies on biochanin A have reported its bioactivities and associated mechanisms of actions.

3.1 *Anticancer activity*

Cancer is a hyperproliferative disorder which is second leading cause of mortalities around the globe. Existing chemotherapeutic drugs for cancer have delimited clinical applications due to their non-selectivity and cytotoxic effects towards normal cells. These shortcomings provoke the need for the development of agents with improved pharmacological profiles against cancer [41]. Natural compounds such as flavonoids, phenolic compounds and glycosides are reported to suppress proliferation and halt metastatic capabilities of cancer cells [42]. NPs proved to be efficacious lead structure for the discovery of anti-cancer agents because of low toxicity profiles, adequacy of activity, biosafety and common availability [43].

Biochanin A, an isoflavone, has been affirmed to mediate its anti-tumorigenic effects by prohibiting cellular growth, activating cancer cell apoptosis, blocking angiogenesis, halting metastasis, and arresting cell cycle (Figure 3) [24]. Biochanin A possesses anti-cancer properties against bladder, breast, pancreatic, prostate, osteosarcoma and liver cancers (Table 2) [44].

3.1.1 *Biochanin A and cell cycle arrest*

Cell cycle progression is directed by the activation of cyclin-dependent kinases (CDKs) and accumulation of cyclins [45]. Thus, targeting cell cycle regulatory proteins particularly CDKs and cyclins to halt cellular growth presents innovative approaches for drug discovery against cancer [46]. Isoflavones have been utilized as promising chemopreventive agents from several years [47], because they have potent ability to mediate the functioning of CDKs and cyclins along with various regulatory proteins of cell cycle [4].

Biochanin A has potential to arrest cell cycle at G1, G2/M and G0/G1 phases in various cancer cells. In LNCaP and PC-3 cancer cells, it induced arrest at G2/M and G0/G1 phase via enhancing p21 and reducing cyclin B expression [48, 49]. Combinatorial treatment of biochanin A and

sorafenib to HepG2 cells induced G0/G1 phase arrest and downregulated the gene expression of cyclin D, Ki-67 as well as survivin [50]. Furthermore, biochanin A mediated cell cycle arrest at G2/M phase in SW-480 colon cancer cells, S phase in A549 cells and G1 phase in U87 glioma cells via up-regulating p53 and its downstream target p21 while reducing the expression of cyclin A and CDK2 (Figure 3) [47, 51, 52].

Table 2. Molecular targets of biochanin A in different cancer types

Cancer type	**Cell lines**	**Treatment condition**		**EC_{50}**	**Molecular targets**	**Cell cycle arrest**	**References**
		No. of cells/well	**Treatment time**				
Breast	T47D, MCF-7	4×10^3	24 h, 48 h	50 μM	ERα↑, miR-375↑, Bcl-2↓, Bax↑, HER-2↑, MMP-9, DPC4↓, p300↓, p53↑, TGFβR2↓, gadd45↓, IκBα↑, CYP19↓, iNOS↓	G2/M	[24, 53-61]
	MCF-7	5×10^5, 2×10^4	1 h, 48 h, 72 h	5 μM, 2-6 mg/kg, 25 μg/mL			
	MDA-MB-231	2×10^4	72 h	10 μM			
	SK-BR-3, MCF-7	2×10^5, 3×10^6	24 h, 72 h	2.5, 8, 12.5, 50 μM			
Glioma	C6, bEnd.3	5×10^5, 1×10^6, 2500 cells	72 h, 48 h	35 μM/L	VEGFR 2⊥, HIF-1α↓, eIF4E↓, p-p53↑, p-ERK↓, EGFR↓, p-Akt↓, c-myc↓, MMP-2⊥, MT1-MMP protein expression↓	G1	[15, 52, 62-64]
	U87	--	72 h	50 μM			
	U87-MG, T98 G	0.5-1×10^6, 2500, 10×10^3	72 h	50 μM, 70 μM			
	U251, U87, F98, GL261	1000-3500 cells	4 days	50 μM/L			
Lung	A549, 95D	--	24 h, 48 h	40 μM, 200 and 240 nmol/L	Akt⊥, ERK1/2⊥, NF-κB⊥, p21↑, cyclin A↓, CDK2↓, cleaved capase-3↑, Bcl-2↓, Bax↑	S phase	[51, 65-67]
	A427	3×10^3	24 h	40 μM			
	NCI-H460	5×10^3	24 h	1 μM			
Leukemia	AML-193	3×10^3	24 h	40 μM	--	--	[66, 68, 69]
	WEHI-3B	4×10^3	48 h	50 μM			
Prostate	LNCaP	4×10^4, 1×10^5, 5×10^4	24 h, 48 h, 6 days	50 ng/ml, 8, 13 μg/ml, 5 μM	NF-κB↓, p21↑, PLK-1⊥, EGFR⊥, caspase-3^{Act}, PCNA↓, Bax↑, Bcl-2↓, cyclin B↓, PI3K/Akt⊥, p-JNK↑, p-ERK1/2↓	G2/M, G1, G0/G1	[48-50, 70-73]
	LNCaP, PC3	2×10^3	72 h	50-100 μM			
	PC3	2×10^4	24 h	8.33 μM			
Liver	Huh-7, HepG2, SNU-449	3,000 cells	72 h	40 μM (Huh-7), 22 μM (HepG2)	Bax↑, Bcl-2↓, Bcl-xL↓, caspase-9↑, caspase-3↑, Ki-67↓, cyclin D↓,	G0/G1	[50, 74]

	HepG2, Hep3B, Huh-7, HA22T	3000 cells/well, 1×10^4	3 days	0.1-1000 µM, 26±2 µg/ml (HepG2), 20±2 µg/ml (Hep3B), 24±5 µg/ml (Huh-7), 15±1 µg/ml (PLC), 19±3 µg/ml (HA22T)	survivin↓		
Colon	HCT-116, SW-480	5000 cells/well	24, 48, 72 h	~50 µM, 100 µM	p53/21↑, P-gp expression↑	G2/M	[47, 75]
	LS-180	--	1/5 days	20 µM			
Gastric	HSC-41E6, HSC-45M2, SH101-P4	2×10^4	48 h	7.6 µg/ml (HSC-41E6), 9.8 µg/ml (HSC-45M), 10.2 µg/ml (SH101-P4)	--	---	[76]
Pharynx	FaDu	0.2×10^5 cells/ml	24, 48 h	25, 50 µM	Cleaved caspase-8↑, FasL↑, PARP↑, Bcl-2↓, Bcl-xl↓, Bad↑, caspase-9↑, MMP-2/-9↓, p38MAPK↓, NF-κB↓	--	[77]
Melanoma	SK-Mel-28	2×10^6 cells/ml	24, 48, 72 h	0, 10, 25, 50, 75 and 100 µM	p-MAPKs↑	--	[78, 79]
	B16	--	48 h	22 µM			
Pancreatic	Panc1, AsPC-1	2500 cells	72 h	70 µM	Activation of EGFR↓, activation of Akt and MAPK↓, MMP-2↓	--	[80]
Osteosarcoma	MG-63	2×10^3, 5×10^3 cells/ml	6, 12, 24, and 48 h	20±0.3 µg/mL, 4 and 8 µM	PARP↑, Bcl-2↓, Bcl-xL↓, Bax↑, caspase-3/-9↑, BGLAP↓, ATF3↑, TP53↓	G0/G1	[44, 81-83]
	U2OS	--	--	10-20 µg/mL			

Down-Regulation ↓, Up-Regulation ↑, Inhibition ⊥, B-Cell Lymphoma 2 (Bcl-2), Bcl-2-Associated X Protein (Bax), B-Cell Lymphoma-Xl (Bcl-xL), c-Jun N-Terminal Kinase (JNK), Mitogen-Activated Protein Kinase (MAPK), Nuclear Factor Kappa B (NF-κB), Tumor Necrosis Factor-α (TNF- α), Mammalian Target of Rapamycin (mTOR), Extracellular Signal-Regulated Kinase (ERK), Cytochrome c (Cyt c), Poly (ADP-Ribose) Polymerase (PARP), Matrix Metalloproteinase (MMP), Epidermal Growth Factor Receptor (EGFR), Tumor Protein (p53), Proliferating Cell Nuclear Antigen (PCNA), Interleukin (IL), Vascular Endothelial Growth Factor Receptor 2 (VEGFR 2), Inducible Nitric Oxide Synthase (iNOS), Transforming Growth Factor Beta Receptor 2 (TGFβR2), Bone Gamma Carboxyglutamate Protein (BGLAP), Activating Transcription Factor 3 (ATF3).

3.1.2 *Biochanin A and apoptosis*

Carcinogenesis emerges from the impairment of genes that control cellular growth and division. Thus, blocking of tumorigenesis by stimulating apoptosis in immortalized cancerous cells is an effective approach to combat cancer [84]. Apoptosis can be triggered by extrinsic or intrinsic pathways. These signaling pathways lead to caspase cascade activation [85]. Upon stimulation, initiator caspases via proteolytic cleavage trigger the activation of downstream effector caspases to induce apoptosis [86]. Thus, small bioactive molecules that trigger apoptosis are promising candidates to combat cancer [87].

Collected information by the researchers recommends that natural bioactive compounds have a potential to induce apoptosis via regulation of variety of signaling molecules [88-91]. Anticancer activity of biochanin A has been reported to be associated with inducing apoptosis via mediating the Bcl-2 family proteins, pro-inflammatory cytokines [24, 44, 50, 66, 77, 81, 82], MAPK (mitogen activated protein kinase) signaling network [p38 MAPK, JNK (c-Jun amino-terminal kinase), and ERK (extracellular signal regulated kinase)] [15, 44, 58, 73, 92], NF-κB (Nuclear factor kappa B) [70, 77], discharge of cytochrome c [44], and activation of caspase family members including caspase-3/-8/-9 [50, 73, 77, 81] (Figure 3).

3.1.3 *Biochanin A and NF-κB signaling pathway*

NF-κB performs an imperative role in various biological processes such as survival, proliferation, inflammation and immune response [93]. Aberrant activation of NF-κB leads towards the development and progression of cancer. Hence, targeting NF-κB has potential to cure cancer [94].

Recently, NF-*κ*B has been reported as a target for several phytochemicals including isoflavones [95]. Biochanin A halted proliferation of head and neck cancers via blocking the activation of NF-κB [77]. Moreover, treatment of prostate [48-50, 70-73] and lung [55, 84, 85] cancerous cells with biochanin A led to stimulation of apoptosis via dose-dependent inhibition of NF-κB pathway (Table 2). It can be concluded that biochanin A triggers apoptosis via inhibiting NF-κB but whether it directly blocks NF-κB pathway or by its upstream signaling pathways (STAT3, JAK) yet need to be investigated by the researchers.

Biochanin A inhibited the degradation as well as phosphorylation of IκBα, thus, blocking the activation of NF-κB which reduced the proliferation of HT-29 cells [16]. However, surprisingly, one study also reported the up-regulation of NF-κB and MAPK signaling pathway after treatment with biochanin A in SK-Mel-28 cancer cells [78]. Thus, this contradictory issue of modulation of NF-κB pathway by biochanin A needs to be resolved by further investigations in future.

3.1.4 *Biochanin A and MAPK and PI3K/Akt pathway*

The MAPK family is characterized by three members as p38MAPK, JNK, and ERK. MAPK/ERK & Akt signaling cascades have a critical role in cellular growth, migration, differentiation, and apoptosis [96, 97]. Therefore, these pathways are reported as promising therapeutic targets for cancer therapy [98].

Biochanin A blocked the activation of pro-angiogenic proteins (ERK/Akt) as well as blocked VEGF and HIF-1α (Hypoxia-inducible factor 1 alpha) in glioma C6 cells [15]. It also prohibited growth and migration of FaDu cancerous cells via down-regulating MMP-2/-9 (matrix metalloproteinase-2/-9) that is mediated by reduction in p38MAPK and Akt pathways [77]. Treatment with biochanin A has inhibitory effects on the activation of MAPK/Akt which in turn reduce EGFR (endothelial growth factor receptor) expression (Figure 3) [80]. Moreover, combinatorial treatment of biochanin A and temozolomide reduced the levels of EGFR, p-ERK, p-Akt, and c-Myc in glioma (T98, U87) cells [52]

3.2 *Anti-inflammatory activity*

Inflammation is regarded as the usual host response to a tissue injury or a protective attempt by the organism against infections or any other stimuli. In case of chronic inflammation, a microenvironment arises in which various immune cells, stromal cells and cancer cells coexist. All of these cellular species collaborate for the production of inflammatory mediators such as growth factors and cytokines [99].

Biochanin A shows anti-inflammatory effects via inhibition of NF-κB and MAPK as well as the release of inflammatory mediators as TNF-α (tumor-necrosis factor-alpha), IL-1β (interleukin 1 beta), iNOS (inducible nitric oxide synthase), NO, and PGE2 (prostaglandin E2) in LPS-induced BV2 microglia cells [100]. In another study, biochanin A showed anti-inflammatory response via

up-regulating PPAR-γ (peroxisome proliferator-activated receptor gamma), thus, reducing the activation of NF-κB and blocking the expression of TNF-α, IL-8, VCAM-1 (vascular cell adhesion protein 1) and ICAM-1 (intercellular adhesion molecule 1) in HUVEC cells [30]. Biochanin A treatment also reduced the LPS-activated expression of NO and iNOS via blocking NF-κB and inflammatory pathways in macrophages [16]. Biochanin A enhanced the expression of HO-1 (heme oxygenase-1) and Nrf2 (nuclear factor erythroid-2-related factor 2) in dose-mediated mode in LPS-induced acute liver injury. Moreover, biochanin A also inhibited inflammasome activation via reducing the interaction among NLRP3 and thioredoxin-interacting protein (TXNIP) (Figure 4) [101].

Biochanin A protected rat chondrocytes against IL-induced inflammation via reduction of NO synthase 2, COX-2, inflammatory cytokines, and PGE2. Besides this, biochanin A also repressed the phosphorylation of NF-κB, thus, protecting rat chondrocytes from inflammation (Figure 4) [102]. Biochanin A was found effective to suppress TNF-α and IL-6 in RAW264.7 macrophages [103]. Biochanin A blocked the expression of Solar UV (sUV)-mediated COX-2 enzyme in mouse JB6 P+ cells and human HaCaT keratinocytes [104]. Biochanin A along with phloretin attenuates the production of allergic cytokine via suppression of intracellularly produced ROS, suggesting that they may have potent anti-allergic property *in vitro* in RBL-2H3 cells [68].

3.3 *Antioxidant activities*

In living systems, imbalance between ROS generation and antioxidant defensive systems can cause deterioration of biologically important targets, thus, causing various pathologies such as inflammation, allergies, and cardiovascular ailments. Oxidative stress is also a key contributor to neurodegeneration, aging, cancer, and diabetes [105]. Therefore, eradicating free radicals and activating the cytoprotective defense system has beneficial health effects [106].

Biochanin A has the capability to protect HepG2 liver cells against t-BHP-mediated oxidative injury by the activation of Nrf2, thereby, stimulating the expression of downstream cyto-protective enzymes such as HO-1 and NADPH quinone oxidoreductase 1 [107]. Biochanin A has a promising antioxidant potential to mitigate arsenic induced hepatotoxicity via up-regulating the antioxidant enzymes like glutathione, superoxide dismutase and catalase in rat livers [108]. Furthermore, biochanin A displayed DPPH free radical scavenging activity (IC_{50}=129.11±2.6

μg/ml), NO radical scavenging activity (IC_{50}=60.76±5.3 μg/ml), metal chelating activity (IC_{50}=112.62±6.12 μg/ml) and LPO inhibitory activity (IC_{50}=58.66±3.4 μg/ml) [109].

3.4 *Neuroprotective activities*

Biochanin A mitigated the Aβ25-35-mediated cytotoxicity in PC12 cells via preventing caspase activation and mitochondrial dysfunction [19]. The anti-apoptotic properties of biochanin A have also been reported against L-glutamate-stimulated cytotoxicity in PC12 cells (Figure 5) [110].

Protective mechanism of biochanin A was also examined on LPS-induced damage in dopaminergic neurons both *in vitro* and *in vivo*. The result shows that biochanin A blocked the activation of microglia and loss of neurons while inhibited MDA (malondialdehyde) production and increased SOD (superoxide dismutase), NADPH oxidase and glutathione peroxidase (GPx) activities. Biochanin A also down-regulated NO, TNF-α, IL-1β levels while inhibited ROS production *in vitro* (Figure 5) [18, 111, 112]. Biochanin A displayed neuroprotective activities in cerebral ischemia or reperfusion models of male rats by reducing brain edema and infarct volume [113].

Interestingly, biochanin A considerably attenuated the depletion of striatal dopamine, elevated the glutathione content and reduced the MDA content in substantia nigra of neurochemical deficit model of female and male rats [114]. Biochanin A also subsequently reduced the phosphorylation of p38MAPK and superoxide formation in neurochemical deficit mice models [115]. Biochanin A was recognized as the most promising mediator of glutamate oxaloacetate transaminase (GOT) gene expression in neural cells. Biochanin A enhanced the mRNA and protein expression of GOT and protected against the cell death caused by glutamate *in vitro* [116].

3.5 *Anti-microbial activities*

Microbial infections persisted to be major threat to human health worldwide in spite of increasing efforts in developing modern pharmacology [117]. Overreliance on anti-microbial medications to treat some infections caused by pathogens has led to increase resistance in various strains of microorganisms [118, 119]. Treatment of microbial infections with natural bioactive compounds is a traditionally employed practice [120]. Biochanin A inhibited the replication of the H5N1 virus, blocked the H5N1-induced activation of ERK1/2, Akt and NF-κB and virus-

induced production of IL-6/-8/-10 [17]. Biochanin A has been reported to possess anti-leishmanial property against promastigotes of *Leishmania chagasi* (EC_{50} = 18.96 µg/ml) [20] and anti-bacterial activity against *C. pneumoniae* with IC_{50} value of 12 µM [121]. Biochanin A has been reported to possess synergistic effect with ciprofloxacin on the efflux system of resistant strain of *Staphylococcus aureus*. At concentration of 40 pg/mL, biochanin A reduced the MIC value of ciprofloxacin from 64 µg/mL to 8 µg/mL [122]. Biochanin A also displayed anti-microbial potential against eight *Clostridia spp.* [123]. Synergistic antimicrobial activity of biochanin A along with fluoroquinolones was also noticed against antibiotic-resistant *Ureaplasmas* spp. [124]. Biochanin A was investigated as mycobacterial efflux pump inhibitor (EPI) in *Mycobacterium smegmatis*. It exhibited better efflux pump blocking activity than other flavonoids such as luteolin and resveratrol [125].

3.6 *Hepatoprotective activities*

Liver injuries or dysfunctions pose a serious threat to human health since times [126]. Approximately 2,50,000 new cases and 20,000 demises due to liver ailments have been reported yearly [127]. ROS-mediated stress is the root cause for the progression of various types of diseases in the liver. Hepatic dysfunctions are originated by several lethal agents including alcohol, viruses, chemically reactive metabolites or their bio-activated products. Although modern system of therapeutics has been established, still there is urgent need of bio safe hepatoprotective medications. So, NPs from medicinal herbs are considered to be effectual and safe for the cure of liver ailments [128].

Biochanin A has been reported for hepatoprotective effect in the carbon tetrachloride-mediated liver toxicity in rats that could be attributed to antioxidant, anti-inflammatory as well as immunomodulatory activities of biochanin A [21]. The hepatoprotective properties of biochanin A against arsenic-mediated hepatic injury were evaluated in rat models. Administration of biochanin A (20 mg/kg) decreased the arsenic-induced liver toxicity by activating GSH, SOD and catalases [108].

3.7 *Miscellaneous biological activities*

Biochanin A provides effective photo-protection to skin and is a good ingredient for safety against the ultraviolet (UV) photo-damage [129]. It is also responsible for its physiological role in preventing the postmenopausal osteoporosis [130]. Biochanin A exerts anti-hypertensive

effects in ovariectomized rats [131]. The gastroprotective activity of this compound could be credited to improve cellular metabolic pathways e.g., increase in the actions of NO and SOD as well as increase in MDA levels and expression of Hsp70 [132]. Biochanin A has been reported for having potential anti-hyperglycemic action in streptozotocin mediated diabetic rats and its beneficial anti-diabetic effect has also been investigated in the male Wistar rats [26, 133]. In high-fat diet-stimulated hyperlipidemic mice, biochanin A decreased total cholesterol levels which gave new insights into the role of biochanin A as hypolipidemic agent [134]. Biochanin A has also been proved as an interesting agent for halting TCDD-stimulated wasting syndrome. Mechanism of action involves up-regulation in adipogenesis-linked factors like PPAR-γ and adiponectin (which are reduced by TCDD) as well as prohibition of TCDD-mediated decrease in the formation of glucose transporter-4 and insulin receptor substrate 1, respectively [135]. Another study was conducted to unveil the biomimetic and lipophilic property of phytoestrogens including biochanin A. Results concluded that biochanin A along with other isoflavones possess high lipophilicity, AGP-binding and Caco-2 permeability [136]. Biochanin A might be beneficial as an alternative for estrogen therapy to improve cutaneous and renal changes in postmenopausal women [137].

4. Bioavailability of biochanin A

Bioavailability refers to the rate of absorption of drug at the site of drug action in systematic circulation. Several investigations have reported the bioavailability of flavones as <1% μmol/L after administration in the humans [138]. Drugs which have lower bioavailability are required to be administrated orally at a high dosage as only a limited portion of the drug is absorbed in systematic circulation [139, 140].

Bioavailability of biochanin A was recorded as 2.6% after oral administration of 5 mg/kg dose while 1.2% after oral administration of 50 mg/kg dose in rats. The free fraction of biochanin A in rat plasma was found to be 1.5%. The absorption of biochanin A was reported to be rapid because of high permeability of biochanin A, however, biliary elimination and extensive metabolism might be the major factors for its poor bioavailability [141]. While in case of simultaneous administration of five flavonoids to rats, bioavailability of biochanin A was found to be 21.3% which suggests that combination of flavonoids might have improved the bioavailability of biochanin A [142].

5. Structure –activity relationship of biochanin A

Isoflavonoids in comparison to isoflavone and isoflavanone exhibit good biological activities due to the structural modifications. Presence of 2, 3-double bond in ring C plays a vital role in biological activities of isoflavonoids. Biochanin A has 2, 3-double bond and 7-OH in ring A that increase the activity [143]. Intra isoflavonoid comparison showed that the DPPH radical scavenging activity of biochanin A (>100 %) is stronger than ascorbic acid (ascorbic acid 92.22% while biochanin A >100 %) [144]. The potent antioxidant potential of biochanin A is due to the presence of 7-OH in ring A and 4′-OCH3 in ring B that greatly increase the antioxidant activity of biochanin A [145]. Different derivatives of biochanin A showed that the position-7 in ring A, position-4' in ring B and 2, 3-double bond in ring C are important in reactivity behavior. Free hydroxyl group at position-7 increases the antitumor activity while hydroxyl at position-5 decreases the antioxidant activity because it prevents the production of active radicals [146]. For example, esterification at 7-position in ring A enhanced the anti-proliferative activity against MCF-7 cells [57] (Figure 6).

6. Concluding remarks

At present, nature-derived bioactive compounds are gaining interest due to their pharmacological potential. In this article, we have highlighted the anticancer, antioxidant, anti-inflammatory, anti-microbial, and neuroprotective properties of biochanin A. Collected data from various researches have affirmed potent role of biochanin A in the treatment of several cancer types. Critical evaluation of reported researches has unveiled the fact that this isoflavone displays best potency towards breast cancerous cells with IC_{50} 0.32 μM. Thus, it would be worthwhile to examine about mechanism of action of biochanin A against breast cancer because this cancer type is most prevalent and lethal among the women. It would also be useful to explore the structural activity relationship of biochanin A by organic chemists to synthesize more bioavailable derivatives of biochanin A. Moreover, toxicological profiling of biochanin A such as hepatotoxicity and nephrotoxicity also needs to be explored. In the light of forerunner investigations, it could be hypothesized that biochanin A might serve as a novel and potential lead isoflavone for drug development but further research and preclinical studies are required to explore full range of its pharmacological properties.

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Conflicts of interest

None.

Contribution of authors

Ayesha Sarfraz and Maria Javeed made significant contribution in writing manuscript and designing figures of the manuscript. Azhar Rasul has made substantial contribution in designing and conceptualization the manuscript. Dr. Muhammad Ajmal Shah has proof read the article. Ghulam Hussain has contributed in acquisition of data. All authors have read and approved final manuscript.

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