Journal Pre-proof

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PII: S1043-6618(20)31393-1

DOI: https://doi.org/10.1016/j.phrs.2020.105085

Reference: YPHRS 105085

To appear in: *Pharmacological Research*

Received Date: 21 June 2020

Revised Date: 12 July 2020

Accepted Date: 14 July 2020

Please cite this article as: Mandal SK, Maji AK, Mishra SK, Ishfaq PM, Devkota HP, Silva AS, Das N, Goldenseal (*Hydrastis canadensis* L.) and its active constituents: a critical review of their efficacy and toxicological issues, *Pharmacological Research* (2020), doi: https://doi.org/10.1016/j.phrs.2020.105085

Goldenseal (*Hydrastis canadensis* L.) and its active constituents: a critical review of their efficacy and toxicological issues

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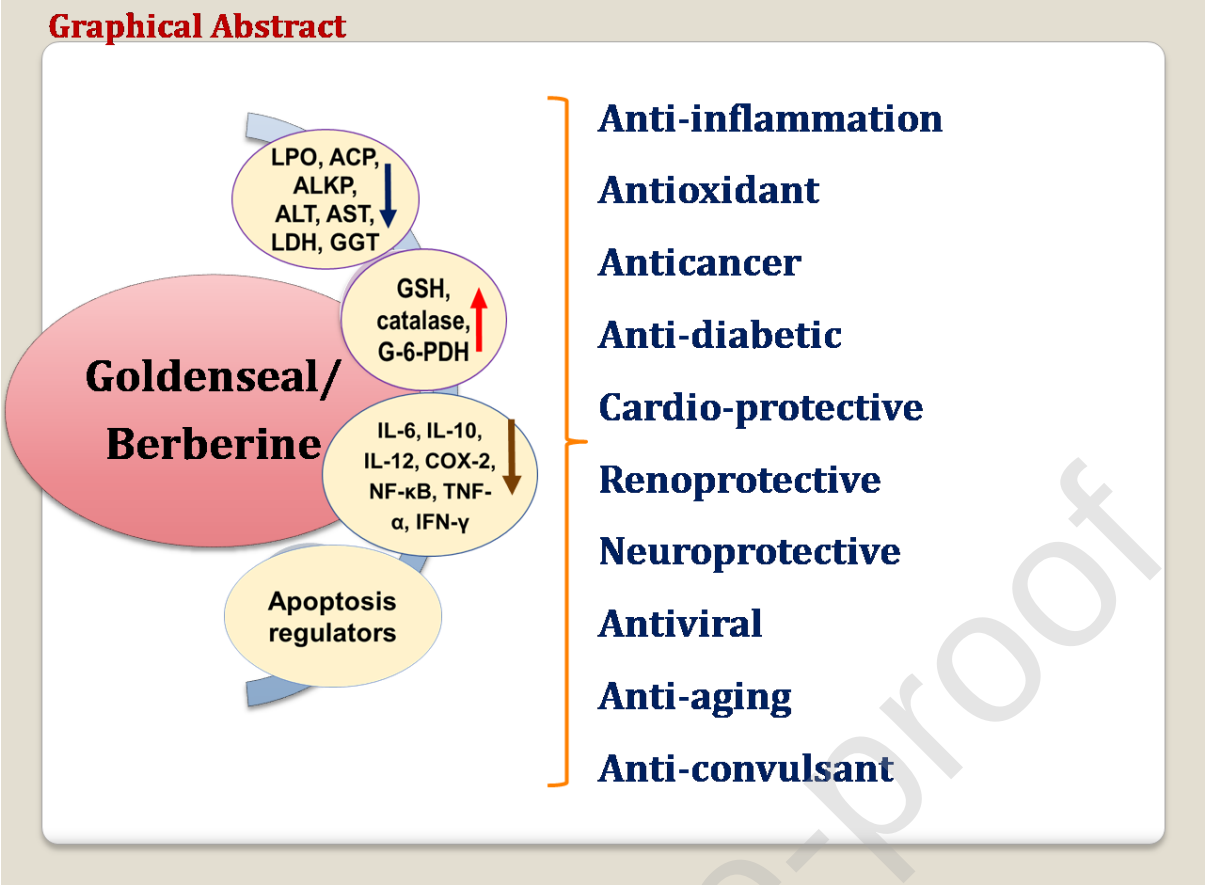
Graphical Abstract.



Highlights

- Goldenseal is widely used as a traditional medicinal herb and food supplement
- Goldenseal alkaloids berberine showed numerous of therapeutic effects
- Critically evaluated the pharmacological and toxicological effects of goldenseal and berberine
- Also evaluated the pharmacokinetic profile and food safety efficacy of goldenseal
- Further detailed research is necessary to provide scientific evidences for unelucidated mechanisms

ABSTRACT

Goldenseal (*Hydrastis canadensis* L.) is a medicinal plant widely used in various traditional systems of medicine and as a food supplement. It has been traditionally used by Native Americans as a coloring agent and as medicinal remedy for common diseases and conditions like wounds, digestive disorders, ulcers, skin and eye ailments, and cancer. Over the years, goldenseal has become a food supplement in the USA and other regions. The rhizome of this plant has been used for the treatment of a variety of diseases including, gastrointestinal disorders, ulcers, muscular debility, nervous prostration, constipation, skin and eye infections,

cancer, among others. Berberine is one of the most bioactive alkaloids that has been identified in different parts of goldenseal. The goldenseal extract containing berberine showed numerous therapeutic effects such as antimicrobial, anti-inflammatory, hypolipidemic, hypoglycemic, antioxidant, neuroprotective (anti-Alzheimer's), cardioprotective, and gastrointestinal protective. Various research finding suggest the health promoting effects of goldenseal components and their extracts. However, few studies have also suggested the possible neurotoxic, hepatotoxic and phototoxic activities of goldenseal extract and its alkaloids. Thus, large randomized, double-blind clinical studies need to be conducted on goldenseal supplements and their main alkaloids to provide more evidence on the mechanisms responsible for the pharmaceutical activity, clinical efficacy and safety of these products. Thus, it is very important to review the scientific information about goldenseal to understand about the current scenario.

List of abbreviations

ACP, acid phosphatase;

ALKP, alkaline phosphatase;

ALT, alanine amino transferase;

AST, aspartate amino transferase;

COX-2, cyclooxygenase-2;

CT-DNA, circulating tumor-DNA;

CYP P450, cytochrome P450;

G-6-PDH, glucose-6-phosphate dehydrogenase;

GGT, gamma glutamyl-transferase;

GSH, glutathione;

iNOS, inducible nitric oxide synthase;

LC-MS/MS, liquid chromatography-mass spectrometry;

LDH, lactate dehydrogenase;

LPO, lipid peroxidation;

MIC, minimum inhibitory concentration;

mRNA, messenger ribonucleic acid;

NF-κB, nuclear factor-kappa B;

NO, nitric oxide;

p-DAB, p-dimethylaminoazobenzene;

TNF-α, tumor necrosis factor-α.

Keywords: Goldenseal, *Hydrastis canadensis*, pharmacological activities, alkaloids, toxicity, herb-drug interactions, pharmacokinetics, food safety.

1. Introduction

In recent years, there is a growing interest on the plant extract or phytochemical based for supplements and functions foods for health promoting activities [1-3]. However, proper understanding of their chemical composition and mechanism of action are yet to be explored for various products to support the health claim. On the other hand, many such popular products are often adulterated with extracts/phytochemicals of other plant origin [4]. Thus, proper understanding of the original of extracts, their bioactivie chemical constituents and pharmacological/functional properties is very essential. On the other hand, it is important to assess their safety profile, pharmacokinetics and possible herb-drug interactions [5]. Goldenseal (*Hydrastis canadensis* L.) is one of the most commonly used food supplement in USA [6]. It is a perennial, herbaceous plant of the Ranunculaceae family. It is found in the rich hardwood forests of the northeastern United States and Canada. Native Americans have long been used goldenseal to treat several diseases and health conditions such as wounds healing, gastric and digestive disorders, peptic ulcers and colitis, skin and eye ailments, cancers [6-8]. With the time goldenseal has become a traditional remedial food supplement for treating digestive and haemorrhagic disorders, as well as for treatment of upper respiratory tract infections, stuffy nose and hay fever, gastritis, diarrhoea and hemorrhoids [9]. Interestingly, various preparations of rhizome extract of goldenseal have been used for centuries in traditional medicine to treat variety of conditions including gastritis, ulcers, muscular debility, intestinal catarrh, urinary disorders, nervous prostration, constipation, hepatic congestion, dysmenorrhea, blood stasis, skin, mouth, and eye infections, upper respiratory disorders, diarrhea, cancer, and so on [6-8, 10, 11]. In addition, different, various parts of this plant especially rhizome contains numerous alkaloids including berberine, hydrastine, palmatine, canadine, hydrastinine and lesser amount of flavonoids (e.g. sideroxylin, 8-desmethyl-sideroxylen, 6-desmethyl-sideroxylin etc), organic acids (e.g. neochlorogenic acid, chlorogenic acid etc) [12]. Among these constituents, berberine is the pharmacologically most active component and is considered in modern clinical applications against the variety of diseases including infections, diabetes, dyslipidemia and cardiovascular diseases. Berberine is a quaternary ammonium salt from the protoberberine group of

isoquinoline alkaloids which is found in a variety of plants including *H. canadensis* [13, 14]. Berberine containing medicinal plants have been reported for beneficial properties including health tonic, antimicrobial, antipruritic, antipyretic, antiemetic, antioxidant, anti-inflammatory, anti-hypertensive, sedative, antinociceptive, anticholinergic and cholagogue, hepatoprotective, preventive in leishmaniasis, malaria and gall stones. Berberine has shown significant anti-inflammatory and antioxidant activities, neuroprotective and cardiovascular protective effects, and lipid-lowering and insulin-resistance improving actions [13, 15]. Berberine has shown antitumoral and antimetastatic activity and regulated the epithelial to mesenchymal transition (EMT) activity in several cancer cells [16-19]. Berberine undergoes extensive metabolic process after oral administration and thus shows an extremely low concentration in plasma exposure. Thus, the metabolites of berberine are believed to contribute to multiple pharmacological effects, which raises the interest in analyzing the metabolic pathways and metabolic organs covering the pharmacological activities of *H. canadensis* and berberine [20]. These reports on berberine suggest it possible applications in the management of chronic disease through various mechanisms and thus the importance of goldenseal is further established as it contains berberine in abundance.

The main aim of this review is to collectively and critically evaluate accessible and up-to-date literature of bioactive chemical constituents as well as pharmacological effects of goldenseal (*H. Canadensis*) against various diseases with underlying the mechanisms of action. In addition, it provides the toxicological and herb-drug interactions associated with goldenseal extract and its particular alkaloid berberine. A Scopus search (www.scopus.com, May 11, 2020) with the key words "goldenseal" or "*Hydrastis canadensis"* resulted in total 302 publications [**Figure 1** (**a**)] and United States was leading country in number of publications [**Figure 1** (**b**)] which shows close resemblance with the market. This review may be useful to identifying the gaps in the above parameters for future research studies to develop suitable therapeutic agents from goldenseal or its alkaloids against several chronic diseases.

2. Methodology

An extensive literature search was conducted in various scientific databases such as PubMed, Scopus, SciFinder, Sciencedirect, Medline and Google Scholar. Queries in literature search were concerning with ethnomedicinal use, phytochemical constituents, pharmacological activity, drug interactions, CYP450 enzymes and toxicity of Goldenseal. All databases were searched using the keywords 'Goldenseal' or '*Hydrastis canadensis'* or '*H.*

canadensis' or 'Berberine' in combination with 'constituents', 'traditional uses', 'drug interactions', 'pharmacological activity' biological activity', 'clinical efficacy', 'CYP450 enzymes' and 'toxicity. For this review, we have followed the preferred reporting items for systematic review and meta-analysis (PRISMA) criteria [21] and high-quality publications were collected for the period of 1990 to May, 2020.

3. Bioactive chemical constituents

The plant goldenseal is the rich sources of alkaloid phytochemicals. Various studies have been performed on the chemical constituents of different plant parts of the plant. The roots and rhizomes of goldenseal contain three major alkaloids including berberine, hydrastine and canadine [22]. Apart from these, various flavonoids, fungal metabolites and phenolic acid derivatives are also reported [8, 11, 23-25]. Rhizomes are the most chemically explored parts and contain numerous alkaloids including berberine, hydrastine, palmatine, canadine, hydrastinine (Table 1, **Figure 2**) and lesser amounts of flavonoids (e.g. sideroxylin, 8-desmethyl-sideroxylen, 6-desmethyl-sideroxylin etc) (**Figure 3**), phenolic acids (e.g. neochlorogenic acid, chlorogenic acid etc.), sterol and other compounds (**Figure 4**) [6, 8, 11, 12]. The detailed list of reported compounds (**1** to **50)** along with plant parts which they were isolated are given in **Table 1**. In addition, the levels of the main alkaloids and phenolic acids in different parts of *H. Canadensis* are illustrated in **Table 2**. Out of the reported 50 compounds, 25 are alkaloids (Sl. No. **1–25**), 10 are flavonoids (Sl. No. **26–35**), 6 are phenolic acid derivatives (Sl. No. **36–41**), one sterol (Sl. No. **42**), and 8 are other compounds (Sl. No. **43–50**).

4. Pharmacological activities

4.1. Activities related to metabolic disorders

4.1.1. Hypolipidemic

Several studies have demonstrated that the goldenseal rhizome extract and berberine exhibited strong lipid lowering activity. Oral administration of rhizome extract and berberine effectively reduced total cholesterol (TC), triglycerides (TG) and low-density lipoprotein (LDL)-cholesterol levels in the serum of hyperlipidemic hamsters as well as humans. It acted through up-regulation of the expression of hepatic LDL-receptor (LDLR) mRNA and LDLR protein and down-regulation of the expression and secretion of the LDLR modulator,

proprotein convertase subtilisin/kexin type 9 in the liver [26-28]. Interestingly, the rhizome extract was more effective than berberine as such, because of the presence of canadine, a strong up-regulator of LDLR expression. Besides, berberine was also able to significantly ameliorated dyslipidemia by reducing serum levels of phosphatidate phosphohydrolase, TG, TC, VLDL and MDA in rats and mice [29, 30]. Moreover, another study demonstrated that the lipid-lowering effect of berberine was mediated by inhibiting the salt hydrolase activity and elevating the levels of tauro-conjugated bile acids, especially tauro-cholic acid in the intestine. This could suppress suppressing CD36 expression by activating intestinal farnesoid X receptor signaling pathway that led to reduce absorption of long chain fatty acids in the liver [31]. Berberine could regulate cholesterol homeostasis in a condition of excessive cholesterol induced cardiovascular diseases model by ameliorating the hepatic autophagic flux blockade. It showed hypolipidemic activity by lowering hepatic cholesterol level and modulating the cholesterol distribution via reduction in the sterol carrier protein 2 and inhibitoi of COX-2 [32]. Non-alcoholic fatty liver disease (NAFLD) is rasing metabolic syndrome associated with high-fed diet (HFD) and that leads to liver carcinoma. Berberine showed beneficial effects in obesity-associated NAFLD by improving hepatic steatosis and decreasing hepatic lipid accumulation. It enhanced autophagy in lipotoxicity induced in hepatocytes through inhibition of the ERK/mTOR pathway [33]. These reports showing autophagic response of berberine and regulating ERK/Akt/mTOR pathways in liver under cholesterol overload are suggestive of the potentials of berberine as a therapeutic agent hyperlidemic conditions.

4.1.2. Anti-obesity activity

The lipid metabolism modulating activities of compounds is sometimes also associated with the glucose metabolism and the conditions of diabetes. An *in vivo* study showed the anti-obesity effect of berberine mediated by inhibition of adipogenesis as evidenced by the reductions of epididymal adipose mass and the serum levels of triglyceride, insulin, and leptin. It also inhibited fat diet-induced elevated levels of proteins CD36 and CCAAT/enhancer-binding protein α (C/EBPα) in epididymal adipose tissues of mice [34]. Besides it improved the lipid dysregulation and fatty liver in obese mice through activation of AMPK in peripheral tissues including liver and muscle [35]. In cells treated with berberine inhibited the differentiation of adipocyte and reduced the lipid accumulation in adipocytes by regulating the expressions of various lipogenic genes including the up-regulation of GATA-2 and 3 genes and down-regulation of peroxisome proliferator-activated receptor γ2 (PPARγ2),

EBPα, adiponectin, leptin, sterol regulatory element-binding protein 1 (SREBP-1c), fatty acid synthase, acetyl coenzyme A carboxylase, acyl-CoA synthase, lipoprotein lipase, and CD36 [36-38]. It also reduced the diversity of gut microbiota of rats by increasing the population of short-chain fatty acids (SCFA)-producing bacteria, including *Allobaculum, Bacteriodes, Blautia, Butyricoccus, and Phascolarctobacterium* which could also contribute to the control of obesity [39].

4.1.3. Hypoglycemic activity

The insulin dependent response of goldenseal may also associate with the reverse state of diabetes, the hypoglycemia, with variable mechanism and conditions. Berberine was found to have significant hypoglycemic effect, which was evidenced by the increased of insulin sensitivity and secretion, glucose absorption and metabolism [28, 40]. Administration of berberine significantly enhanced the plasma glucagon level by increasing proglucagon mRNA expression, population of L-cells in intestine and β-cells in the pancreas as well as increasing the insulin levels in plasma and pancreas in diabetic rats [41]. It has been shown that berberine increased glucose uptake by adipocytes and myocytes by inhibiting protein tyrosine phosphatase 1B activity and increasing phosphorylation of insulin receptor, insulin receptor substrate 1 and AMP-activated protein kinase in adipocytes and myocytes [42, 43]. Detailed study also showed that berberine increased insulin sensitivity and InsR mRNA expression as well as protein kinase C (PKC) activity in the liver [44, 45]. Berberine also stimulated insulin-independent glucose transport system in adipocytes and skeletal muscle [46, 47]. It also improved glucose metabolism by stimulating glycolysis and inhibiting insulin-independent gluconeogenesis pathways [48]. Besides, berberine significantly reduced hyperglycemia associated oxidative stress and inflammation through enhancing antioxidant defenses and up-regulation of PPARγ mRNA expression in streptozotocin-induced diabetic rats [49]. Thus, the extracts of goldenseal and berberine need a conditional use in management of hyper or hypoglycemia, whichever the case maybe.

4.2. Antimicrobial activity

4.2.1. Antibacterial activity

Goldenseal rhizome extract has traditionally been used against variety skin infections due to its strong antimicrobial activity. The antibacterial activity was studied through evaluation of the minimal inhibitory concentration (MIC) in several studies. Rhizome extract of the plant showed antibacterial activity against *Staphylococcus aureus*, *Streptococcus*

sanguis, S. mutans, S. pyogenes, Pseudomonas aeruginosa, *Escherichia coli* and the activity were attributed by the alkaloids berberine, canadine, and canadaline [50, 51]. The extract showed bactericidal activity as measured by the "killing time" on a low-density bacterial inoculum, as well as it showed bacteriostatic activity in liquid bacterial culture medium. Berberine and other compounds from extract contributed to antibacterial activity from 3 mg/ml to 1.5 mg/ml against all the tested strains. While it showed bacteriostatic activity at an average of 0.12 mg/ml against Gram-positive strains [50]. Another study with goldenseal rhizome ethanol extracts showed the highest recovery of alkaloids compared to the mixed solvent (ethanol/glycerin/water) and glycerin extracts [51]. Two alkaloids (berberine and hydrastine) were quantified from the extract, and the ethanolic extracts showed greater antibacterial activity than the mixed solvent extracts. Berberine showed antibacterial activity against *E. coli, P. aeruginosa, S. aureus, S. mutans,* and *S. pyogenes* with MIC_{50} values 4, 2, 0.5, 1 and 0.125 mg/mL, respectively [51]. In addition to these alkaloids, two flavonoids 6, 8-di-*C*-methylluteolin 7-methyl ether and 6-*C*-methylluteolin 7-methyl ether were isolated from goldenseal rhizome which showed strong antimicrobial activity against oral pathogens *S. mutans* and *Fusobacterium nucleatum*. Interestingly, the combination of compounds, 6, 8-di-*C*-methylluteolin 7-methyl ether and berberine, showed synergistically higher antimicrobial activity against *S. mutans* [8]. Another study indicated that the methanol extract of goldenseal rhizome also had stronger antimicrobial activity (MIC_{50} 12.5 µg/ml) against *Helicobacter pylori* and the active constituents in the extract were identified as berberine and β-hydrastine with MIC_{50} values of 12.5 and 100 µg/ml, respectively [52]. Berberine is the major active component of goldenseal that was reported to a potent antimicrobial agent against *Mycobacterium tuberculosis* [53], *M. smegmatis*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *S. aureus* [54], *Salmonella typhi* [55], and *S. agalactiae* [56]. The antimicrobial activity of goldenseal leaves extract was also reported and remarkably the activity of leaves extract was stronger than berberine alone (MIC values 75 µg/mL and 150 µg/mL, respectively) against multi-drug resistant *S. aureus* [57]. The major flavonoids of leaves including sideroxylin, 8-desmethyl-sideroxylin, and 6-desmethyl-sideroxylin in association with berberine may synergistically increase the antimicrobial activity of the leaves against *S. aureus*; however, these flavonoids did not show inherent antimicrobial activity [24]. Berberine also showed strong synergistic antibacterial activity in combination with penicillin, lincomycin, amoxicillin, clindamycin, linezolid, cefoxitin and erythromycin against various species of *Staphylococccus* including *S. haemolyticus, S. epidermidis, S. capitis, S. galinarium, S.*

hominis, *S. intermedius*, and *S. lugdunensis* [58, 59]. It was also found to be active against some species of *Streptococcus* including *S. mutans*, *S. sanguinis*, and *S. oralis* [60].

4.2.2. Antifungal activity

Goldenseal is one of the most important antifungal plants used in various skin infections due to the presence of active antimicrobial alkaloid berberine. *In vitro* study of berberine showed significant antifungal activity against various fungal pathogens such as *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Trichophyton mentagrophytes*, *Microsporum canis*, *Trichophyton rubrum*, *Epidermophyton floccosum*, and *Microsporum gypseum* [61, 62]. Antifungal activity of berberine was attributed through the alterations of plasma and mitochondrial membranes integrity and DNA damage which led to cell death, possibly by apoptosis [61]. Moreover, several studies have shown that berberine showed a strong synergistic antifungal potential in combination with other antifungal drugs such as fluconazole, amphotericin and terbinafine [63-65]. Such an *in vitro* study showed that berberine inhibited the growth of various *Candida* species (MIC range 0.98-31.25 mg/L) including *C. krusei*, *C. kefyr*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis* and *C. albicans*. However, berberine alone was not significantly inhibited the biofilm formation by *C. albicans*, but in combination with miconazole reduced biofilm formation by more than 91% after 24 h [66].

4.2.3. Antiviral activity

Number of research reports suggest the antiviral activity of goldenseal alkaloid berberine against different viruses especially influenza, herpes simplex, cytomegalo, chikungunya and Enterovirus [67-69]. Berberine showed a strong growth inhibitory potential against H1N1 influenza A in the *in vitro* study on human lung epithelial cells and murine bone marrow macrophages [67]. Another study showed that berberine decreased the mortality of influenza infected mice as well as reduced the production of various cytopathogenic factors like tumor necrosis factor-α (TNF-α), nitric oxide (NO) and inducible nitric oxide synthase (iNOS) [68]. Varghese et al. [69] reported the potential anti-chikungunya effect of berberine which was attributed through the inhibition of virus-induced activation of cellular mitogen-activated protein kinase signaling pathway [69]. Several reports have specifically showed the dose-dependent antiviral activity of berberine as anti-herpes simplex, HIV-1, anti-enterovirus 71 and anti-cytomegaloviral effects via interfering viral replication, RNA transcription and protein synthesis [70-73]. Berberine showed potent activity against HIV-1

NL4.3 virus in CEM-GFP cell lines with EC_{50} 0.13 μM [71]. However, berberrubine and two other compounds shwoed lesser activity as compared to berberine in the same study. Berberine chloride when assessed for the anti-human cytomegalovirus (HCMV) activity showed potent anti-HCMV activity with IC_{50} 0.68 μM [70]. The plausible mechanism for the anti-HCMV activity was inhibition of the HCMV replication and interference with intracellular events after virus penetration into the host cells and before viral DNA synthesis [70]. Berberine tested for the antiviral effect against herpes simplex virus (HSV) infection showed that it potently inhibited the early stage HSV replication at concentration 6.25 μM [72]. It inhibited the viral attachment/entry in to host cell and suppressed genomic DNA replication. It further significantly reduced HSV-induced NF-κB activation and IκB-α degradation and p65 nuclear translocation, followed by suppression of HSV-induced c-Jun N-terminal kinase (JNK) phosphorylation [72]. Likwesie, another study showed that berberine does-dependently reduced EV71 RNA and protein synthesis in a concentration range of 6.25 to 200 μM [73]. It inhibited the activation of MEK/ERK signaling pathway and suppressed the EV71-induced autophagy by activating AKT protein and inhibiting the phosphorylation of JNK and PI3KIII [73]. **Figure 5** suggest the collectively mechanisms for the effect of goldenseal and berberine with potent antiviral activity agains several viral infectons through discreet molecular mechansism.

4.3. Anti-protozoal activity

In addition to antibacterial, antifungal, and antiviral activities, berberine showed significant anti-protozoan activity against various protozoan parasites including *Blastocystis hominis*, *Entamoeba histolytica*, *Giardia lamblia*, *Trichomonas vaginalis*, and *Leishmania donovani* [54]. A number of medicinal plants were used to treat endoparasites and stomach problems in dogs, cats and pigs and the anthelmintic activity of plant extracts was assessed. Amongst various plants including *H. canadensis* showed anthelmintic efficacies with proposal for their ethnoveterinary uses [74].

4.4. Antitumor activity

A number of natural products have been reported to exhibit significant anti-cancer actions. Developing prospects of using phytochemicals have shown a recent therapeutic concept for the utilization of phytochemicals as pharmacological alternatives against human malignancies in a drug repositioning approach [75]. In particular phytochemicals that have shown multiple properties like antidiabetic, antimicrobial, anti-inflammatory,

antiproliferative, and growth receptor antagonistic activities have been utilized in cancer therapy [75]. Several studies have been performed to evaluate the anticancer potential of goldenseal rhizome extract. Karmakar et al. [76] demonstrated that the ethanol extract of goldenseal rhizome exhibited strong anticancer potential against p-dimethylaminoazobenzene (p-DAB)-induced hepatocarcinogenesis in mice. Results of the study revealed that the ethanol extract decreased lipid peroxidation (LPO), and the activities of acid phosphatase (ACP), alkaline phosphatase (ALP), alanine amino transferase (ALT), aspartate amino transferase (AST), lactate dehydrogenase (LDH), gamma glutamyl-transferase (GGT) activities as well as increased the level of reduced glutathione (GSH) content and the activities of catalase, glucose-6-phosphate dehydrogenase (G-6-PDH) in p-DAB treated mice [76]. This indicates that goldenseal rhizome extract could exert antioxidant and anti-inflammatory effects which, led to tumor regression in the hepatocarcinogenesis model. Saha et al. [77] tested chemotherapeutic potential of goldenseal extract against HeLa cells with emphasis on its drug-DNA interaction and apoptosis induction ability. The extract showed potent anti-cancer activity through induction of apoptosis and interaction with circulating tumor-DNA (CT-DNA). Goldenseal extract showed activation of cytochrome C activity and Bax translocation in mitochondrial and cytosolic fractions and Caspase-3 activity. The plausible mechanism of action was mediated by p53-dependent regulation of NF-κB and TNF-α [77]. Ethanol extract of goldenseal rhizome has been used in homeopathic medicine for the treatment different types of cancers. A research report demonstrated that homeopathic goldenseal preparation (200C) significantly increased the lifespan of Ehrlich Ascites Carcinoma (EAC) and Dalton's Lymphoma Ascites (DLA) induced tumor-bearing mice by 69.4% and also reduced tumor volume by 95.8% after 30 days of treatment [78]. Recently, it was found that ultra-high diluted ethanol extract of goldenseal altered DNA methylation and the expression of certain genes linked to carcinogenesis [79]. Das et al. [80] reported the anticancer potential of silver nanoparticle of ethanolic goldenseal extract against skin melanoma A375 cells. Their results indicated that silver nanoparticle of goldenseal extract induced apoptosis of the skin melanoma cells through DNA fragmentation, cell cycle arrest at G2/M phase and activation of caspase 3 [80].

A large number of reports have been published on the anticancer potential against various cancer cells with mechanism of actions involving berberine as major compound from *H. canadensis*. The anticancer mechanisms of berberine were involved in the regulation of oncogene and carcinogenesis-related gene expressions as well as interaction with DNA and transcriptional regulation. Besides, it showed a strong enzyme inhibitory property that affects

N-acetyltransferase, cyclooxygenase-2 (COX-2), and topoisomerase activities and gene/protein expression. This adds to the reports that berberine may interact at transcriptional and translational level in the chronic conditions. All along with the regulation of reactive oxygen species production, mitochondrial transmembrane potential, and nuclear factor-kappa B (NF-κB) activation contributed to its anti-proliferative and proapoptotic effects [54]. NF-κB acts as transcriptional regulator for apoptosis related caspases as well as regulator for inflammation mediators especially COX-2 and iNOS [81, 82, 83]

In connection with NF-κB, *H. canadensis* extract shwoed the anti-cancer potential through pro-apoptotic activity mediated by p53-dependent regulation of NF-κB and TNF-α [77]. Berberine was found to synergistically suppress tumor growth and metastasis in combination with other anticancer agents. Such as, berberine potentiated the inhibitory effects of arsenic trioxide on migration and invasiveness of glioma tumor cell possibly by disturbing the PKC signaling that led to reduce the level of matrix metalloproteinase-2 (MMP-2) involved in cancer cell migration during metastasis [84]. Balakrishna and Kumar [85] had shown the synergetic anticancer activity (> 77%) of berberine (< 45%) with curcumin (< 54%) in a ratio of 1:1 on different human cancer cell lines. A similar synergetic anticancer effect of berberine and doxorubicin (DOX) combination was observed against human lung carcinoma, cervix carcinoma and hepatoma cell lines [86]. However, other reports indicated that berberine significantly attenuated the anticancer potential of other chemotherapeutic agents, including fluorouracil, camptothecin, and paclitaxel [87]. Besides, β-hydrastine is another important active constituent of goldenseal that induced early apoptosis, and suppressed proliferation and invasion of lung adenocarcinoma cells through inhibition of the kinase activity of p21-activated kinase 4 (PAK4) [88]. Berberine activated stress-induced autophagic death in cancer cells by enhancing glucose regulated protein 78 (GRP78) and binding to the autophagy-related vacuolar protein sorting 34 (VPS34) [89]. These observations suggest the chemotherapeutic potential of goldenseal extracts and berberine and as a promising candidate for chemoprevention. The antitumoral activities of goldenseal and berberine are summarized in **Figure 6** which, comprehends the four major molecular regulations associated with antitumor activity, namely oxidative balance, inflammation, apoptosis, and growth and proliferation.

4.5. Antiangiogenic and antimetastatic activity

Certain reports demonstrated the antiangiogenic potential of berberine. *In vitro* studies showed that berberine inhibited mRNA level expression of various proangiogenic factors,

such as vascular endothelial growth factor (VEGF), COX-2, iNOS, and hypoxia-inducible factor-1α (HIF-1α) and secretion of proinflammatory mediators, such as interleukin (IL)-1β, IL-6, TNF-α, and granulocyte macrophage colony-stimulating factor (GM-CSF) [90]. In addition, berberine inhibited various transcription factors implicated in tumor development and angiogenesis such as NF-κB, c-Fos, cAMP response element-binding protein (CREB), and activation transcription factor-2 (ATF-2) [90]. Apparently, berberine inhibited endothelial cell's motility, proliferation, migration, vessel sprouting and endothelial tube formation [90, 91]. VEGF induced angiogenesis confers efficient tumorigenicity through escalation of epithelial–mesenchymal transition (EMT) and stemness of cancer cells. The EMT ability of cancer cells contributes to their metastatic potential involving modulation of the expression of several genes and proteins such as recent intervention showing transglutaminase-2 facilitated extracellular vesicle-mediated metastasis in breast cancer [92]. Invasion of the non-small cell lung cancer to brain resulted in a shift of the blood-brain barrier (BBB) and brain metastasis [93]. Transforming growth factor-beta (TGF-β) is another key signaling molecule which triggered EMT and induced the formation of ribonuclear protein processing (P)-bodies and metastasis in mammary epithelial cells [94]. Also the requirement of tyrosine kinase-mediated autophagy [95], fibronectin accumulation [96, 97], and pyruvate carboxylase expression [98, 99] in EMT plasticity and metastasis in breast cancer is notable signaling events which can be targeted as a therapeutic option to limit metastatic tumors. A correlation NF-κB and VEGF along with apoptosis regulatory proteins Bcl-2 was drawn in the case of multiple myeloma with a therapeutic approach using bortezomib [82]. Berberine exerted potent inhibitory effects on the invasion and migration of highly metastatic prostate cancer cells by repressing a panel of mesenchymal genes that regulate the developmental EMT. Berberine specifically downregulated the expression levels of high BMP7, NODAL and Snail genes of metastatic prostate cancer tissues [100]. Berberine suppressed EMT in mouse melanoma B16 cells through regulation of the cross-talk between the PI3K/Akt pathway and RARα/RARβ expression which suggest that berberine can be a useful adjuvant therapeutic agent in treating metastatic tumors [16]. Berberine inhibited EMT, metastasis and tumor-induced angiogenesis in human cervical cancer cells through regulation of epithelial marker E-cadherin and inhibition of mesenchymal markers such as N-cadherin and snail-1 [17]. Berberine inhibited TGF-β-induced invasive tumor growth and EMT through regulation of increased E-cadherin and decreased vimentin proteins nasopharyngeal carcinoma. Berberine showed the antiproliferative ability in nasopharyngeal carcinoma cell and induced cell cycle arrest and apoptosis [18]. Berberine

was reported to suppresses cell motility through downregulation of TGF-β1 in triple negative breast cancer cells (most critical form of breast cancers) and its lung metastasis [19]. PPARγ dependent modulation of apoptosis and invasion and migration has also been approached in cancer therapy [101], while berbeine has shown to modulate PPARγ signaling in diabetic condition [38, 39, 49] but not explored yet in cancer. Collectively these reports indicate that berberine could reduce EMT, metastasis and angiogenesis in different types of cancers and thereby constituting as an adjuvant treatment for limiting metastasis.

4.6. Immunomodulatory activity

In the United States, China and other Asian countries, the goldenseal has been used in herbal products as an immune stimulant. Phytochemcials with glycan domains have shown interactions with antigens complexed to act as nanocarriers for the immune cells and immunomodulation through cytotoxic T lymphocyte immune response mediated by CD8+ T cells [102]. It has been found that goldenseal extract has the ability to increase primary IgM response to an antigen in mice [103]. Bactericidal effect of berberine was well documented and it was found to enhance the bacterial killing abilities by augmenting inflammasome activation evidenced by the augmentation of caspase-1p10 and IL-1β production in macrophages [104]. Such effects of berberine were suppressed by AMP-activated protein kinase (AMPK) inhibitor that indicated the inflammasome activation was mediated through the activation of AMPK signaling pathway. Administration of higher dose (10 mg/kg) of berberine showed immunosuppressive effects in mice which were evidenced by reduction of splenocyte proliferation, production/differentiation of T- and B-cells followed by suppression DTH and HA responses. Besides, berberine was also found to be decrease total number of blood leukocytes, lymphocytes, neutrophils, and the absolute numbers of splenic $CD4^+$, $CD8^+$ T-cells and $CD19^+$ B-cells [105]. In an experimental rat model, berberine was found to ameliorate autoimmune neuritis by suppressing P0 peptide 180-199-induced lymphocyte ($CD4^+$ T cell) proliferation, Th1 (TNF-α) and Th2 (IL-10) cytokines production and anti-P0 peptide 180-199 IgG1 and IgG2a production [106]. The immunomodulatory effects in terms of allergic response also need to address with reference to goldenseal extract. Berberine was reported to provide significant protection against peanut-induced anaphylaxis and inhibited the synthesis of IgE in murine model. Results showed that berberine inhibited IκBα phosphorylation, IgE production, expression of ε-germline transcription with simultaneous increased in the mRNA expressions of transcription factor T-box TBX21, signal transducer and activator of transcription-3 in peripheral blood mononuclear cells [107].

4.7. Anti-inflammatory activity

It was found that goldenseal extract was able to modify lipopolysaccharide-stimulated macrophages responses such as reduction of TNF-α, IL-6, IL-10, and IL-12 production in a dose-dependent manner [108]. Similarly, berberine significantly inhibited TNF-α-induced expression of IL-6, IL-8, and monocyte chemo-attractant protein (MCP)-1 at both protein and mRNA levels in ARPE-19 cells [109]. These inhibitory effects were mediated by downregulating the phosphorylation of p38, extracellular signal-regulated kinase (ERK1/2), and c-Jun N-terminal kinase (JNK), which are the cell growth regulatory signaling pathways. Further, *in vivo* and *in vitro* studies showed that berberine dose-dependently reduced prostaglandin E2 (PGE2) production followed by the reduction of COX-2 protein [110]. Berberine may also have a beneficial effect on inflammatory bowel disease (IBD) which is a chronic state of intestinal inflammation. Berberine was found to ameliorate the dextran sulfate sodium (DSS)-induced weight loss, myeloperoxidase activity, and colon shortening, injury, and inflammation scores in mice [111]. Berberine inhibited the production of proinflammatory cytokines especially TNF-α, IFN-γ, KC, and IL-17 through the inhibition of MAPK and NF-κB signaling pathways in colonic macrophages and epithelial cells of DSS-treated mice [111]. These results strongly corroborate the anti-inflammatory effects of *H. canadensis* and berberine in chronic inflammatory pathophysiological conditions including cancer.

4.8. Anti-arthritis activity

Inflammation and immunomodulation correlate with the inflammatory arthritis conditions. Berberine was found to ameliorate the collagen and Freund's complete adjuvant-induced rheumatoid arthritis by regulating specific immune responses. In animal studies, berberine significantly increased dendritic cell apoptosis as well as decreased the expressions of TNF-α, IL-1β, IL-6, IL-17, CD34, VEGF and increased the expressions of IL-10 and transforming growth factor-β (TGF-β) [112-114]. Apparently, the histopathological study indicated that berberine diminished synovial hyperplasia and infiltration of inflammatory cells in joint tissues [114]. In gouty arthritis, urate crystal-induced inflammation is a critical factor. Recent study showed that berberine relieved monosodium urate crystal-induced inflammation in gouty arthritis, by down regulating NLRP3 inflammasome and IL-1β expressions [115]. *In vitro* and *in vivo* studies, berberine showed protective role against the development of osteoarthritis by increasing the expression of metalloproteinase inhibitor and

inhibiting NO production, IL-1β-induced glycosaminoglycan release, MMP-1, -3 and -13 expressions [116].

4.9. Anticonvulsant activity

The neuronal and physiological convulsions are the disease state in which epileptic seizures and neuronal dysfunctions are observed. A seizure is the clinical condition showing an abnormal, excessive, hypersynchronous discharge of cortical neurons. While epilepsy is a neuronal disorder characterized by recurrent seizures unprovoked by an acute systemic or neurologic insult. Terminologically, the drugs that induce neuronal convulsions are named as convulsant while the drugs, which inhibit them are named as anticonvulsant [117]. Thus, anticonvulsant drugs do find the space in managing epileptic seizures by various mechanism especially involving antioxidant impacts. Berberine containing plants such as barberry and goldenseal has traditionally been used as an anticonvulsant. Studies showed that the berberine (10 and 20 mg/kg, i.p.) was significantly decreased kainic acid and maximal electroshock-induced convulsion and mortality rate of mice [118]. Another recent study showed that administration of berberine (25, 50 and 100 mg/kg, i.p.) significantly decreased the rate of seizure incidence in kainate-induced temporal lobe epilepsy in rats [119]. Sadeghnia et al. [120] found that berberine significantly delayed the initiation of 4-aminopyridine-induced convulsion by reducing the release of excitatory amino acids, glutamate and aspartate, in rat hippocampus [120]. Therefore, these studies indicated that berberine showed significant anticonvulsant activity by modulating the neurotransmitter systems.

4.10. Antioxidant activity

Oxidative stress generation through excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) has strongly been associated with chronic inflammatory pathophysiological conditions like diabetes, obesity, cardiovascular diseases, neurodegenerative diseases and cancer. Overproduction of ROS and RNS can cause oxidative damage to the cellular macromolecules including lipids, proteins, and DNA. Furthermore, ROS and RNS are associated with the activation of several inflammation, antioxidative and cellular defense pathways especially involving NF-κB, nuclear factor-erythroid 2-related factor 2 (Nrf2), and antioxidant-responsive elements (ARE). Berberine was reported to inhibit oxidative stress in a variety of tissues including liver, adipose tissue, kidney and pancreas [121, 122].

The antioxidant potential of berberine was studied in different *in vivo* and *in vitro* models. Luo and Fan [123] reported the antioxidant potential of berberine in various radical scavenging models including 2,2-diphenyl 1-picrylhydrazyl (DPPH) radical, superoxide anion, hydroxyl radical, and 2,2-azino bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) radical scavenging assays [123]. Berberine was reported to prevent H_2O_2-induced penile erectile dysfunction by increasing cell viability and SOD activity and decreasing lactate dehydrogenase release and malondialdehyde (MDA) production in corpus cavenosum smooth muscle cells of rabbit [124]. Berberine improved intestinal antioxidant status which was evidenced by the increase of reduced glutathione levels, activities of catalase and glutathione peroxidase (GSH-Px) enzymes and concurrent decrease in oxidative damage biomarkers including H_2O_2, malondialdehyde, nitrite/nitrate, iNOS and protein carbonyl content [125].

Recently, berberine was also reported to prevent stress-induced liver damage by restoring the hepatic levels of thiol groups, total antioxidant capacity, the activities of superoxide dismutase, catalase, glutathione peroxidase, and glutathione-S-transferase, and reduced *versus* oxidized glutathione ratio in rats [126]. Besides, canadine was found to exhibit significant antioxidant potential [127]. A popular preparation of Traditional Chinese Medicine (TCM) named Huang-Lian-Jie-Du-Decoction (HLJDD) showed potent preventive effects against conditions of oxidative stress especially ischemic stroke. The major compound reported in HLJDD are berberine, baicalin, and jasminoidin, which ameliorated the abnormal metabolism and reduced oxidative stress, neuronal death, and inflammation [128]. Thus, berberine containing medicinal plants like goldenseal has been advocated for supplementation in the pathophysiological conditions of oxidative stress.

4.11. Relaxant activity

The Goldenseal extract and berberine were reported to exhibit relaxing activity. *In vitro* studies demonstrated that goldenseal extract reduced 5HT-, oxytocin- and acetylcholine-induced smooth muscle contraction of rat uterine and carbachol-induced contraction of guinea-pig trachea [129]. Further studies demonstrated that this activity was due to its major alkaloids berberine, β-hydrastine, canadine and canadaline [130]. However, the relaxant effect of berberine was studied more extensively. *In vitro* study showed that berberine effectively reduced tonic contraction of gastric fundus muscle isolated from rats by preventing the entry of calcium from extracellular fluid [131]. It also induced relaxation of vascular endothelial and vascular smooth muscle [132]. In addition, the antagonistic effect of

berberine on muscarinic acetylcholine receptors also led to reduce the contraction of tracheal muscle [133].

4.12. Cardioprotective activity

As stated above, berberine has antioxidant and anti-inflammatory activities that led to provide protection against myocardial ischemia/reperfusion injury. In post-ischemia, administration of berberine improved cardiac function, decreased infarct size, apoptotic index, serum creatine kinase and lactate dehydrogenase levels and up-regulated silent information regulator 1 (SIRT1), Bcl-2 expressions as well as down-regulated Bax and caspase-3 expressions in rats [134, 135]. Additionally, it increased myocardial levels of SOD with subsequent reduction in superoxide generation, MDA concentration and inflammatory markers (i.e. TNF-α, IL-6 and myeloperoxidase activity) [135]. Berberine pretreatment was also significantly reduced plasma endothelin-1, von Willebrand factor levels, myocardial necrosis, inflammatory cell infiltration, microthrombosis as well as decreased the levels of TNFα, IL-1β, intracellular adhesion molecule-1 (ICAM-1) and regulated on activation, normal T cell-expressed and secreted (RANTES) in serum and heart tissues in a rat model of coronary microembolization [136].

Chang et al. [137] reported that pretreatment with berberine provided significant cardioprotection by increasing AMPK activity, protein kinase B (AKT) phosphorylation and reducing glycogen synthase kinase 3β (GSK3β) activity in non-ischemic areas in diabetic heart. It also prevented ischemia/reperfusion-induced myocardial injury by activating the signal transducer and activator of transcription 3/janus-activated kinase 2 (STAT3/JAK2) signaling pathway and attenuating endoplasmic reticulum stress [138]. Modulation of STAT3/JAK2 signaling pathways for apoptosis induction has been reported with potentials in cancer therapy [139, 140]. Cardioprotective role of berberine also arbitrated through its vasorelaxant effect by blocking the release of intracellular Ca^{2+}, stimulating endothelium-derived relaxing factor release and activation of large-conductance Ca^{2+}-activated K^+ channel [141, 142]. Besides, it suppressed atrial fibrillation induced by acetylcholine through increasing the effective atrial refractory period and prolonging action potential duration of atrial myocytes in rabbits [143]. It also prevented certain drug-induced cardiotoxicity by inhibiting their cytoplasmic metabolism, as well as by reducing the accumulation of cardiotoxic metabolites in the heart [144]. The TCM preparation HLJDD containing berberine was proven effective in ischemic stroke condition through its antioxidant properties

[128]. These observations suggest that goldenseal extracts and berberine could potentially prevent the cardiovascular dysfunctions and cardiac stress.

4.13. Anti-platelet aggregations

Platelet aggregation is defined as clumping of platelets together in the blood and it a part of the sequence of events leading to the formation of a thrombus clotting. These complex hemostatic mechanisms are involved in the pathophysiological conditions especially cardiovascular diseases. Various medicinal plants and traditional medicine systems has showed natural supplementation could reduce platelet aggregation [145]. Studies showed that berberine inhibited collagen-induced platelet aggregation. Result of radioimmunoassay, showed that berberine significantly inhibited synthesis of thromboxane A2 in rabbit platelets that could be mediated by the inhibition of arachidonic acid metabolism in platelets and endothelial cells [146]. Berberine was also reported to a direct inhibitor of thrombin, and showed an inhibitory potential (IC_{50} 2.92 μM) against thrombin-induced platelet aggregation [147]. Berberine was recently found to inhibit platelet aggregation and reduction in the ROS and superoxide formation via modulation of AR, NOX, and glutathione reductase activities in diabetes model. Berberine inhibited calcium release, ERK phosphorylation, granules release and platelet adhesive properties through inhibition of p38-p53 mediated BAX activation [148]. Yet the effects of goldenseal on coagulation need elucidation; some studies suggest that berberine inhibited platelet-activating factor and aggregation of platelets with a reported 50% inhibition at a concentration of 38 μg/mL. It inhibited the binding of platelet-activating factor in rabbit platelets by 50% inhibition at a concentration of 480 μg/mL [149]. Some other studies have also reported the inhibitory effects berberine on platelet aggregation through involvement of ADP, arachidonic acid, and collagen, and reductions in the thromboxane-B2 in ischemic cerebral rat model [150].

4.14. Anti-hypopigmentation activity

The human skin is often exposed to the ultraviolet radiation which can influence the function and survival of several types of skin cells and may also induce skin cancer. Skin pigmentation is traditionally believed to have important photoprotective factor because of melanin, which can not only absorb the UV broadband but also mediates the antioxidant and radical scavenging mechanisms [151]. Reduction in melanin production by the decrease or absence of melanocytes leads to the development of various hypopigmentation disorders. Recent studies showed that berberine dose dependently induced darkening of the skin and

increased the number of melanophores with melanin-loaded dendrites in skin melanophores of *Bufo melanostictus* [152, 153]. Since, the effects of berberine were completely abolished in the presence of propranolol while the effects were highly potentiated by isoprenaline, a β-adrenoceptor agonist. Therefore, it was concluded that the skin darkening effect of berberine was mediated through the stimulation of β2 adrenergic receptors [152, 153]. However, its effects on melanine synthesis were contradictory. An *in vitro* study showed that berberine inhibited melanin synthesis and tyrosinase activity through the down-regulation of the expression of microphthalmia-associated transcription factor (MITF) and tyrosinase in melanoma cells [154].

4.15. Anti-aging activity

Exposure to ultraviolet (UV) irradiation induces IL-6 production, matrix metalloproteinases (MMPs) and reduces the expression of procollagen type I genes which leads to skin aging. *In vitro* study demonstrated that berberine decreased UV-induced MMP-1 expression with the concurrent enhancement of type I procollagen expression in human dermal fibroblast [155]. In another study, berberine was found to inhibit TPA-induced expression and activity of MMP-9 and suppressed IL-6 expression in human keratinocytes [156]. Therefore, they concluded that the anti-aging effect of berberine mediated through the prevention of skin inflammation and the degradation of extracellular matrix proteins, including collagen, by MMPs [156]. The anti-aging effects of goldenseal and berberine are mainly attributed to their antioxidant properties.

4.16. Neuroprotective and Anti-Alzheimer's disease activity

Several studies demonstrated that berberine showed neuroprotective potential in different *in vitro* models of neurotoxicity including glutamate, H_2O_2, oxygen-glucose deprivation (OGD), N-methyl D-aspartate (NMDA) glutamate receptor stimulation and CoCl2-induced hypoxia [157]. *In vivo* and *in vitro* studies showed that berberine ameliorated OGD-induced ischemic neuronal damage that was arbitrated through the inhibition of intracellular ROS generation, cytochrome c release, JNK phosphorylation, caspase 3 activity and the increase of Akt, GSK3β and ERK1/2 phosphorylation [158, 159]. Berberine was reported to attenuate neuronal cell death induced by glucose through the inhibition of ROS generation and increases the expressions of hemeoxygenase-1 (HO-1) and nerve growth factor as well as promoted neurite outgrowth through Nrf2-dependent pathway [160].

In another study, nanomolar concentrations of berberine provided significant neuroprotection against H_2O_2-induced neuronal cells damage It suppressed ROS generation and downregulated cytochrome c release and activation of Bax and caspase 3, and up-regulated Nrf2, HO-1, Bcl-2 expressions and endogenous antioxidants (GSH and SOD) status [161]. It also showed protective role against $CoCl_2$-induced hypoxia-mediated neuronal apoptosis by the down-regulation of HIF-1α, caspase 9 and 3 and Bax, as well as the up-regulation of Bcl-2 [162]. Further, berberine inhibited the release of glutamate from cortical synaptosomes as well as stimulated neuronal autophagy in response to neuronal damages [163, 164]. Further, Kim et al. [165] exerted the neuroprotective effect of berberine against ischemia-induced neuronal apoptosis via activation of phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) signaling pathway in the hippocampus. Berberine also reported to prevent neuronal damages through downregulating various inflammatory mediators (IL-1β, TNF-α, IL-6, MCP-1, and MIP-2), and upregulating anti-inflammatory cytokine (IL-10) as well as preventing endoplasmic reticulum stress-induced neuronal apoptosis [166-168].

Several reports have shown that berberine showed therapeutic potential against various neurodegenerative diseases by alleviating neuroinflammation. Extracellular amyloid plaques and intracellular neurofibrillary tangles (NFTs) as well as synaptic degeneration, hippocampal neuronal loss, neuroinflammation, and oxidative stress are the well-known pathological features of Alzheimer's disease (AD) [169]. Studies showed the beneficial neurobiological effects of berberine against Alzheimer's. Berberine was found to ameliorate beta amyloid (Aβ) pathology, gliosis, cognitive and behavior impairment as well as inhibited hippocampal neurodegeneration and the activity and expression of beta-site amyloid precursor protein (APP) cleaving enzyme, β-secretase in various animal models of AD [170, 171]. *In vitro* studies showed that berberine improved axonal transport impairment by reducing Calyculin A-induced tau hyperphosphorylation and cytotoxicity in neuroblastoma-2a cells [172]. Several studies have demonstrated the neuroprotective role of berberine mediated through its powerful antioxidant effect. In a study, berberine was found to prevent Aβ-induced cell death in rat cortical neurons via decreasing the production of malondialdehyde and reactive oxygen species [173]. Progression of oxidative stress, Aβ accumulation and tau hyperphosphorylation play a key role in neuroinflammation of the brain.

It was suggested that berberine suppressed neuroinflammation in AD by reducing Aβ accumulation, tau hyperphosphorylation and suppressing the progression of oxidative stress in the brain [169]. Besides, berberine was reported to exhibit inhibitory potential against

pathogenic enzymes of AD including acetylcholinesterase, butyrylcholinesterase, monoamine oxidase (MAO)-A and MAO-B [174]. The Alzheimer's disease being a state of neurodegeneration, largely involved in oxidative neuronal damage and impairments of neuronal and cognitive functions. **Figure 7** demonstrate the antioxidant, anti-inflammatory and growth and survival promoting mechanisms of goldenseal and berberine that may be key factor in protective such neurodegenerative signs.

4.17. Gastrointestinal effects

Berberine has been reported to have a significant therapeutic potential in the treatment of gastrointestinal inflammatory disorders. Berberine ameliorated inflammatory responses associated with various gastrointestinal inflammatory diseases by inhibiting the growth of enterobacteria and colonic expression of iNOS, COX-2, IFN-γ, IL-17, IL-6, IL-8, IL-1β and TNF-α with the increase of colonic IL-22 and secretory immunoglobulin A levels [111, 175, 176]. Additionally, it inhibited NF-κB, STAT3 and STAT1 phosphorylation and differentiations of Th17 and Th1 cells, without affecting regulatory T cells [176]. Besides, it also prevented tight-junction injury of colon epithelium and membrane microdomains from intestinal endotoxinemia by down-regulating NF-κB and myosin light chain kinase pathway [177]. Recent study demonstrated that berberine prevented dextran sulfate sodium (DSS)-induced colon damage in addition with the decrease of CD68, tight junction protein-1, occludin and epithelial cadherin expression in colonic tissue [178]. It also prevented intestinal injury and its complications by elevating the activities of catalase, SOD and GSH-Px as well as preventing the activation of toll-like receptor 4 (TLR4) and NF-κB in ileum [179].

In variety of acute or chronic enteropathies, berberine was demonstrated to attenuate IFN-γ and TNF-α-induced the dysfunction of intestine epithelial barrier via inhibiting HIF-1α mediated signaling pathway of myosin light chain kinase (MLCK)-dependent MLC phosphorylation [180]. In a study on rat model of gastroesophageal reflux disease (GERD), berberine was found to reduce esophagic tissue damage and attenuate the severity of GERD by reducing the levels of serum proinflammatory biomarkers such as TNF-α, IL-1β, IL-6 and plasminogen activator inhibitor (PAI) -1 [181]. It was also reduced endoplasmic reticulum (ER) stress in the intestine by inhibiting GRP78 chaperone protein expression and xbp-1 mRNA splicing as well as down regulating c-JNK phosphorylation, and the level of caspase-12 and cleaved caspase-3 [182]. Berberine was found to be preventive the mice model of intestinal inflammation induced by DSS [111]. Berberine protected the case of intestinal

epithelial cells inflammation by suppressing neutrophil influx in intestinal cells as shown my myeloperoxidase activity and reduced the inflammatory scores [111].

4.18. Renoprotective activity

In a clinical study on hypertensive patients with type 2 diabetes mellitus, showed that berberine treatment for 2 years significantly reduced blood and urine biomarkers of renal damage. Results indicated that berberine significantly reduced urine albumin-to-creatinine ratio, osteopontin and kidney injury molecule-1 that are specific biomarkers of early-stage kidney damage [183]. It also reduced hypoxia/re-oxygenation-induced apoptosis of renal proximal tubular cells by suppressing mitochondrial and endoplasmic reticulum stress [184]. Recent study showed that berberine ameliorated kidney damage by increasing the expression of adiponectin receptors (adipoR1 and adipoR2) and serum adiponectin level via AMPK signaling pathway [185]. In streptozotocin (STZ) treated mice, berberine also ameliorated serum creatinine, blood urea nitrogen (BUN) and albuminuria as well as renal fibrosis through the activation of Nrf2 and the inhibition of TGF-β/Smad signaling pathways [186]. The STZ-exposed mice mimic the experimental diabetes which involved dysfunctions of pancreas, liver and kidney as well. Glomerulus of kidneys gets inflammaed and show reduced filtration/absorption ratios. **Figure 5** demonstrates that goldenseal extract and berberine may protect the kidney function by its anti-inflammatory and cell growth promoting mechanisms.

4.19. Anti-osteoporotic activity

The anti-inflammatory potential of goldenseal and berberine can be utilized in other chronic conditions like osteoporosis. In diabetic osteopathy, the bone mineral density is decreased due to the increase of osteoclastogenesis and decrease of osteoblastogenesis. It was hypothesized that the berberine might be helpful in treating osteoporosis, because it decreased osteoclastogenesis and increased osteoblastic differentiation by modulating several factors responsible for decreasing bone mineral density [187]. *In vivo* study showed that administration of berberine diminished glucocorticoid-induced osteoporosis by inhibiting bone resorption and improving bone formation in rats [188]. Studies have demonstrated that berberine enhanced the expression of osteogenic marker genes of osteoblasts including osteopontin and osteocalcin which further promoted the transcriptional activity of key osteogenic transcription factor Runx2. Berberine was also activated p38 mitogen-activated protein kinase and increased COX-2 expression which is the key factors for osteoblast differentiation [189]. Recent *in vitro* study demonstrated that berberine inhibited osteoclasts

formation in addition with the inhibition of the expression of osteoclast marker genes, including cathepsin K (Ctsk), nuclear factor of activated T cells cytoplasmic 1 (NFATc1), tartrate resistant acid phosphatase (TRAcP, Acp5) and vacuolar-type H+-ATPase V0 subunit D2 (V-ATPase d2) in bone marrow macrophages derived osteoclast culture system [190].

4.20. Hepatoprotective activity

The hepatoprotective effect of goldenseal extract and berberine was investigated in different animal models. Goldenseal extract was found to inhibit CYP2E1 activity (IC50: 4.32 µg/ml) that led to prevent acetaminophen metabolism to generate N-acetyl-p-benzoquinone imine, a highly hepatotoxic intermediate of acetaminophen [191]. Different studies showed that both, goldenseal extract (300 mg/kg, p.o.) and berberine (60 mg/kg) significantly attenuated drug-induced elevated serum levels of AST and ALT, while pure berberine attenuated drug induced structural injuries such as vascular congestion, inflammatory cell infiltration, hepatocellular degeneration, necrosis and fibrosis in the liver [191, 192]. Berberine was also found to be inhibited potassium and calcium currents in hepatocytes which also involved in the hepatoprotection [193]. In earlier, it has been described that berberine has strong antioxidant, anti-inflammatory and anti-lipid peroxidation potentials which led to protect the liver from oxidative stresses. Besides, it reduced hepatic MDA and hydroxyproline contents, expressions of TNF-α, COX-2, α-smooth muscle actin, TGF-β and increased reduced glutathione content, glutathione peroxidase, catalase and SOD activities in drug-induced hepatotoxic animals [126, 194-196]. Dkhil et al. [197] reported that berberine treatment restored *Plasmodium chabaudi*-induced histopathological alterations as evidenced by the presence of hepatic lobular inflammatory cellular infiltrations, dilated sinusoids, vacuolated hepatocytes, increased number of Kupffer cells and the malaria pigment, hemozoin. Moreover, studies in rats also confirmed that berberine pretreatment ameliorated DOX-induced hepatorenal toxicity by lowering serum ALT, AST, total cholesterol, BUN, and preventing hemorrhage and focal necrosis of the liver and kidney tissues in addition to its antioxidant capability [198]. Altogether, it has been suggested that the hepatoprotective effect of berberine was mediated through its strong antioxidant and anti-inflammatory mechanism as well as the inhibition of potassium and calcium currents in the liver. The major pharmacological effects of goldenseal and berberine and their molecular pathways are summarized in **Figure 8**.

5. Pharmacokinetics profile of goldenseal extract and their constituents

The pharmacokinetic properties of goldenseal were analyzed from the human urine test following its oral administration which indicated that bioavailability of berberine was quite low as compared to berberine conjugates that showed significantly higher detection [199, 200]. Berberine was found to show low bioavailability for oral administration in dogs [201] and in rats [200]. The bioavailability was <5% bioavailability for berberine hydrochloride.

A validated LC-MS/MS based method was established for determination of *H. canadensis* alkaloids in human serum. This showed that berberine was especially absorbed more rapidly after oral administration of the *H. canadensis* test supplement, while thee was lower absorption of administration of isolated berberine [202]. *H. canadensis* alkaloids including berberine others serve as substrates for the xenobiotic efflux pump pglycoproteins (P-gp). These compounds interact with P-gp inhibitors while P-gp acts to inhibits net absorption of berberine in the gut [203]. Thus, the metabolites of berberine which are biologically active known principals have been detected in the plasma of rats for sustained periods as analyzed precisely by identified using LC/MS [204].

The pharmacokinetic action of *H. canadensis* extract, berberine, hydrastine and canadine was shown on human liver microsomes which indicated that it inhibited the CYP2E1 isoform, with hydrastine as most potent alkaloid amongst tested [205]. It is well reported that *H. canadensis* alkaloids possess methylenedioxyphenyl (MDP) moieties which interact with cytochrome P450 (CYP) enzymes to form complexes [203, 206]. The rhizome extract and isolated alkaloid both inhibited CYP reactions especially the CYP3A4 isoform, in which hydrastine showed high specific inhibition as compared to berberine. The human CYP inhibitory activity for *H. canadensis* extracts was 2.7g and 3.9g (daily administration) for CYP 3A4 and CYP2D6 isoforms, respectively [207-209]. A methanolic extracts of *H. canadensis* significantly inhibited the isoenzymes CYP2B6, CYP2C19, CYP3A4, CYP2D6 and CYP2E1 [210]. This indicated that ingestion of *H. canadensis* could inhibit the metabolism of pharmaceutical drugs when administered concomitantly [203, 206, 207].

The bioavailability of berberine in rats was reported of 0.36% by method of intestinal first-pass elimination [211]. Similar poor degree of absorption of berberine in the gut was reported in hamsters which suggest that berberine ingestion was largely accumulated in the gut and did not proceed for blood circulation; however, it showed much better bioavailability through intraperitoneal route of administration as compared to oral/intragastric route [212]. Berberine oral administration (400 mg) to humans followed by HPLC analysis of plasma showed that it has extremely lower bioavailability with the maximum plasma concentration of about 0.4 ng/ml [213]. The organ specific comparative information suggests that berberine

when administered to animal models, once absorbed it can be distributed into all major organs with the highest amount obtained in the liver and readily excreted via the urine [214]. Amongst the plausible reasons linking the poor bioavailability of berberine and its pharmacology, one of the major links is the gut microbiota which can modulate both the pharmacokinetics profile of berberine and its biological effects. In a beagle dog model oral administration of berberine led to changes in the intestinal bacterial composition and production level of butyrate (a bacterial anerobic oxidation product) in the gut [215]. Berberine administration (7 days) to the dog model caused abundant increase in the level of butyrate- and the nitroreductases-producing bacteria, yet the plasma level of berberine was still very low. This indicated that berberine could induce gut microbiota, which assisted in pharmacological activity yet the maximum detected level of berberine in plasma, remained to 37 ng/ml upon oral administration at 50 mg/kg [215]. These studies highlighting the role of the gut microbiota as a major target for berberine mediated modulation of drug pharmacokinetics was further associated with increased expression of bile acids synthetic enzymes (Cyp7a1 and 8b1) and uptake transporter (Ntcp) in the liver by berberine [216].

Hydrastine, another major alkaloid of *H. canadensis* showed interesting bioavailability. Goldenseal supplement (2.7 g) containing hydrastine (78 mg) was orally administered to healthy subjects and LC-MS/MS based pharmacokinetic parameters for hydrastine were calculated [217]. The maximal serum concentration of hydrastine was about 225 ng/ml at maximum time about 1.5 h, and the elimination half-life time was 4.8 h. Hydrastine was found to metabolize rapidly and extensively by phase I and phase II metabolic biotransformation, and some intermediary metabolites may have pharmacological activity at different levels and conditions [217]. Goldenseal root extract in the form of 570 mg capsules (oral administration twice a day for 14 days) was given to HIV patients (under indinavir therapy at 800 mg single oral dose) and healthy subjects showed no statistically significant pharmacokinetic alterations for the indinavir. The maximal accumulated concentration of goldenseal in plasma ranged from 0.6 to 2.5 mg/L [218]. Despite the plethora of pharmacological activities of berberine and other phytoconstituents of goldenseal to induce protective and preventive effects in several acute and chronic conditions, their bioavailability is a considerable concern in therapeutic applications and drug development.

6. Herb-drug interactions

Herb-drug interaction pays an important role in the metabolism of drug molecules through the modulation of cytochrome P450 enzyme activities. Goldenseal was found to have

some excellent therapeutic potential; however, goldenseal extract and/or its active constituents interacted with various P450 enzymes. Along with berberine, the other alkaloids of *H. canadensis* extracts, especially hydrastine and canadine, showed inhibitor effects on cytochrome P450 2E1 (CYP2E1) and 1A2 (CYP1A2) [205, 219]. These inhibitions may associate with the pharmacological effects of these compounds from the plant and proposes their analysis in terms of drug metabolism and enzymatic systems. *In vivo* and *in vitro* studies reported that goldenseal extract and its constituent; hydrastine, strongly inhibited the enzymatic activity of CYP2C8, CYP2D6 and CYP3A4 [203, 208]. However, the interaction between berberine and CYP450 enzymes was also extensively studied. In a clinical study with 18 healthy human subjects revealed that repeated administration of berberine (900 mg/day) decreased the activities of CYP2D6, CYP2C9, and CYP3A4 enzymes [206].

Further, Chatuphonprasert et al. [220] reported that berberine dose-dependently suppressed the induction of mRNA expression of CYP1A1, CYP1A2, CYP2E1, CYP3A11, CYP4A10 and CYP4A14 enzymes, while increased the expression of CYP2B9 and CYP2B10 enzymes in primary mouse hepatocytes. Interestingly, *in vivo* study demonstrated that berberine itself suppressed the expression of CYP2E1; an enzyme associated with liver adverse event, and restored the up-regulated mRNA expression of CYP3A11, CYP4A10, and CYP4A14 enzymes to normal levels in STZ-induced diabetic mice [220].

7. Studies related to assessment of toxicity of goldenseal extracts and its constituents

Goldenseal extract and its alkaloids, especially berberine showed a large number of therapeutic potentials. However, the safety profile of goldenseal extracts and its alkaloids remains controversial. Some of the reported toxicities from goldenseal alkaloids have been highlighted here.

7.1. Neurotoxicity

Along with several neuroprotective effects, certain neurotoxic effects of goldenseal needed to be mentioned. It was found that the lower concentration of hydratastine possess cytotoxic effect against neuroblastic cells and eosinophilic cells. An *in vitro* study showed that hydrastine (500 μM) reduced the viability of PC12 cells by inducing apoptotic cell death [221]. It was also reported that berberine induced N-mitochondria and NMDA receptor-dependent toxicity in primary neurons which was evidenced by mitochondrial swelling,

increased oxidative stress, decreased mitochondrial membrane potential and depletion of ATP content [222].

7.2. Hepatotoxicity

Two years toxicity studies using the combination of the extracts of goldenseal and milk thistle (*Silybum marianum*) in male and female F344/N rats and B6C3F1 mice increased the incidence of liver tumors because of its berberine-mediated inhibition of the topoisomerase associated DNA repair processes [223]. In similar *in vivo* studies, administration of higher doses of goldenseal root powder significantly increased liver weight and hepatocellular hypertrophy within 15 days. In addition, administration of low to high doses of goldenseal root powdered for 2 years significantly increased the incidence of hepatocyte hypertrophy, eosinophilic focus and hepatocellular adenoma and/or carcinoma in all animals [224]. A recent *in vitro* study has been performed to evaluate the genotoxic potential of goldenseal extract and its five major alkaloids including berberine, palmatine, hydrastine, hydrastinine, and canadine in human hepatoma HepG2 cells [225]. Results of the study showed that berberine and palmatine as well as goldenseal extract were found to the potent inducers of DNA damage in HepG2 cells by suppressing the activities of both topoisomerase I and II [225].

7.3. Phototoxicity

Photochemical and phototoxic response assessment also an important aspect in deriving therapeutic aspect for natural compounds. Goldenseals extract its major alkaloid like berberine and palmatine has been assessed for their photochemical and phototoxic impacts in the medical applications such as in eyewashes and skin lotions by the National Toxicology Program [226]. Berberine was found to produce both $_1O^2$ and radical species in a nonpolar environment. Berberine (50 μM) when applied to the HaCaT keratinocytes irradiated with UVA resulted in an 80% decrease in cell viability and a 3-fold increase in DNA damage [227]. The other alkaloid of goldenseal when applied to the UVA irradiated HaCaT keratinocytes in the presence of palmatine (50 μM) resulted in a 50% decrease in cell viability but no DNA damage. While hydrastine, hydrastinine, or canadine (50 μM) did not cause DNA damage or cell death in keratinocytes after UVA exposure [10]. Further study suggested that berberine phototoxicity could be countered with application of antioxidants like ascorbate (2 mM) and N-acetylcysteine (5 mM). The combination of berberine (10 μM) and palmatine (10 μM) when treated to the UVA irradiated keratinocytes showed mild DNA

damage [228]. In addition, berberine damaged the hRPE cells when irradiated with visible light, and palmatine was not phototoxic to hRPE cells as well. These observations demonstrate that goldenseal containing eyewashes and lotions need careful consideration and exposure to different wavelength lights need to be checked for ocular phototoxicity.

8. Safety efficacy of goldenseal as food supplements

The pharmacokinetic properties of phytochemicals and food supplements and physicochemical properties of their bioactive principles affect the efficacy and toxicity of the medication concomitantly administered with food supplements or food [229]. Adverse reactions and contraindications are an important concern for assessement, and as such no reports of adverse reactions to the administration of goldenseal plant and its extracts or products have been observed exclusively so far. However, some experimental reports of contraindications exist. Goldenseal has been used as a botanical dietary supplement and as a single-entity product or as an ingredient of herbal preparations. Goldenseal remained a record-selling botanical dietary supplement earlier in the United States even without substantially approved clinical or scientific evidences for either safety or efficacy [230]. The Native Americans have traditionally used this plant rhizomes extract as an oxytocic and to induce abortion [230]. Eleven secondary metabolites are also reported from the endophytic fungi associated with goldenseal which include alternariol, alternariol monomethyl ether, 5'*epi*-equisetin, equisetin, 10–11 dehydrocurvu-larin, macrosphelide A, cordipyridone A, sch 52901, sch 52900, verticillin A, and aurofusarin [231].

The Botanical Safety Handbook has classified *H. canadensis* in Class 2(b) which represents that “Not to be used during pregnancy” [232] as well as the fresh plant may cause irritation to gastrointestinal (GI) tract mucosa in patients with inflammatory or infectious GI conditions [233]. The uterine-stimulating effects of goldenseal are suggestive that its use during pregnancy or nursing, and administration to under puberty females shall be avoided [230]. It was also suggested not to administer goldenseal products to neonates as it may potentially disturb the bilirubin level [234]. The ability of berberine to displace bilirubin from albumin may lead to bilirubin deposition in the brain resulting in brain damage, a condition of kernicterus observed in several fatalities [234, 235]. The risk for kernicterus is greater in newborns with jaundice [235] and goldenseal can cause kernicterus in newborns thus preterm neonatal hyperbilirubinemia is a contraindicative complication for mothers using goldenseal during pregnancy or lactation [233]. Thus, the use of goldenseal in newborns needed a check on contraindications and eventually goldenseal could not be recommended for children under

two [233]. Due to these observations, although the safety data is not stipulated, the use of goldenseal products by pregnant women and children shall be taken into careful consideration.

Certain drug agents and formulations with antimicrobial and antioxidant properties could show contraindications on hemolytic anemia in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. Goldenseal, with antimicrobial and antioxidant properties, needs assessement for its impacts on hemolytic anemia in patients with G6PD deficiency [236]. The safety concerns of goldenseal also involve the masking effect of detection of illegal drugs tested in urine [237], as it was reported to mask the detection of morphine and other drugs in racehorses [238]. Although such claims could not be further extended, their verification is required in the assessment of drug abuse as well as toxicity. Hydrastine, from *H. canadensis*, when administered in large doses was reported to produce strychnine-like convulsions and respiratory paralysis, induced hypertension and hypoglycemia, and led to peripheral blood vessels constriction. The contraindications of goldenseal in hypertension may associate with its effects on cardiac muscle leading to increased cardiac output and increased blood pressure [232, 233, 239].

The earlier submission at the British Herbal Pharmacopoeia (2006) suggested the contraindications of *H. canadensis* in hypertension, however, these were later removed in the observations of British Herbal Compendium [240]. Concurrent topical application of goldenseal and tretinoin may cause adverse effects on the skin yet no clear mechanism of the effect was elucidated [233]. In addition, a higher dose of goldenseal products was found to cause severe skin irritation in conditions of inflammatory skin diseases and may irritate the skin and mucus membranes of the mouth, throat, and vagina [10, 226]. These observations suggest that the use of goldenseal in patients with cardiovascular diseases, hypertension, diabetes and skin diseases, and in pregnancy need careful consideration. As many products claiming to be originated from goldenseal are reported to be adultered with other sources, recent studies have also focused on the identification and characterization of such adulterants [4, 5, 241]. Future studies focussing on the safety and quality control of these products is necessitated.

9. Conclusions

This review extensively discusses the traditional uses, phytochemical, pharmacological, toxicological potential, pharmacokinetics, herb-drug interactions, and safety efficacy of goldenseal extract and its main alkaloid berberine. Notably, the majority of

research focused on the biological activities of berberine, of which anti-bacterial, antioxidant, anti-inflammatory, anti-hyperlipidemic, cardioprotective, neuroprotective and anti-hyperglycemic effects have been well characterized. However, others important biological activities of goldenseal extract and berberine include anti-arthritis, anti-obesity, anti-hypopigmentation, anti-aging, anti-Alzheimer's disease, renoprotective, and anti-osteoporotic activities, which require more research studies to be worthy of being exploited. Effects of goldenseal extract and berberine on hepatoprotection and melanine synthesis showed contradictory results that need to be further evaluated scientifically. Goldenseal has a large number of therapeutic applications; however, its alkaloids berberine and hydrastine inhibited the hepatic microsomal enzymes, CYP2D6, CYP2C9 and CYP3A4 that metabolize large numbers of drugs. So, herb-drug interactions should be considered when berberine and hydrastine as well as goldenseal extract are co-administered with the drugs that are metabolized by these enzymes. Goldenseal food supplements have effectively been used for medicinal purposes however they also showed neurotoxic, hepatotoxic and phototoxic concerns associated with specific alkaloids.

The extracts of goldenseal and their alkaloids constituents promote they utilization as a food supplement as well as suggested their utilization in the medicinal systems for therapeutic and preventive purposes. However, in view of their potential toxicity, further studies should be undertaken to determine the effective concentration and the maximum tolerated dose limit in the pharmaceutical formulations and in order to standardize goldenseal food supplements. Large randomized, double-blind clinical studies need to be conducted on goldenseal supplements and their main alkaloids, to provide more evidence on the clinical efficacy and safety of these products. Measures shall also be taken to avoid any safety concerns due to chemical or microbiological contamination (e.g., pesticides, mycotoxins, bacteria, other plants). Finally, recommendations on the use of goldenseal supplements and their main alkaloids shall be given to certain groups of the population (e.g. pregnant and breast-feeding women, infants).

Conflicts of interest

Disclosure statement

There are no conflicts of interest.

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Legends to figures and table:

Figure 1. Trends in goldenseal research. (**a**) Number of publications per year from 1990; this information main subject areas were Pharmacology, Toxicology and Pharmaceutics; Medicine; Biochemistry, Genetics and Molecular Biology; Agricultural and Biological Sciences; Chemistry; Environmental Science and Immunology and Microbiology.
(**b**) Countries with high number of publications (Source: www.scopus.com, accessed on May 11, 2020).

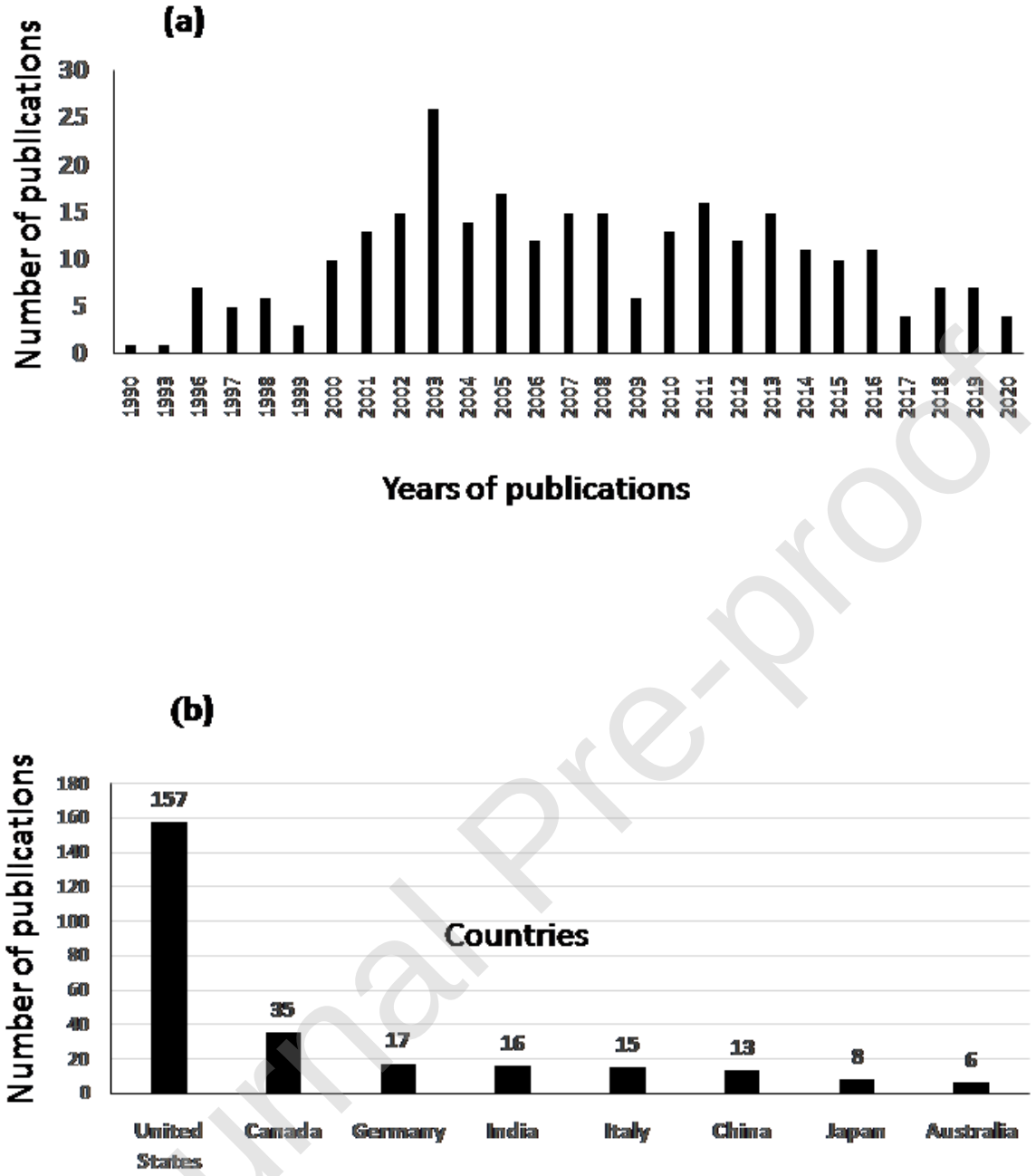

Figure 2. Chemical structures of reported alkaloids from goldenseal (*H. canadensis*). The structures are obtained from pubChem as well as reported citations. The structures are drawn through chemDraw 12.0.2 version.

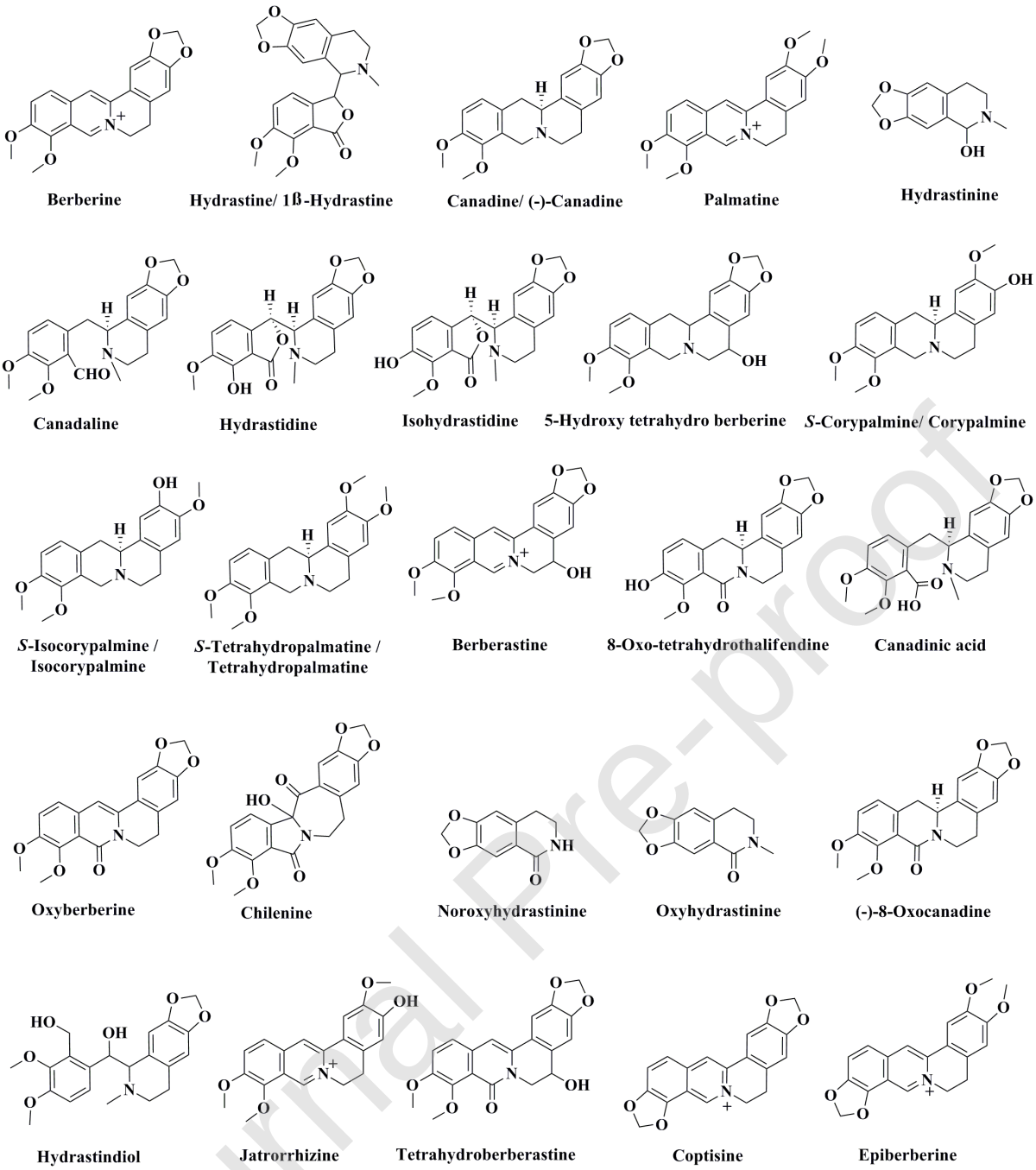

Figure 3. Chemical structures of reported flavonoids from goldenseal (*H. canadensis*). The structures are obtained from pubChem as well as reported citations. The structures are drawn through chemDraw 12.0.2 version.

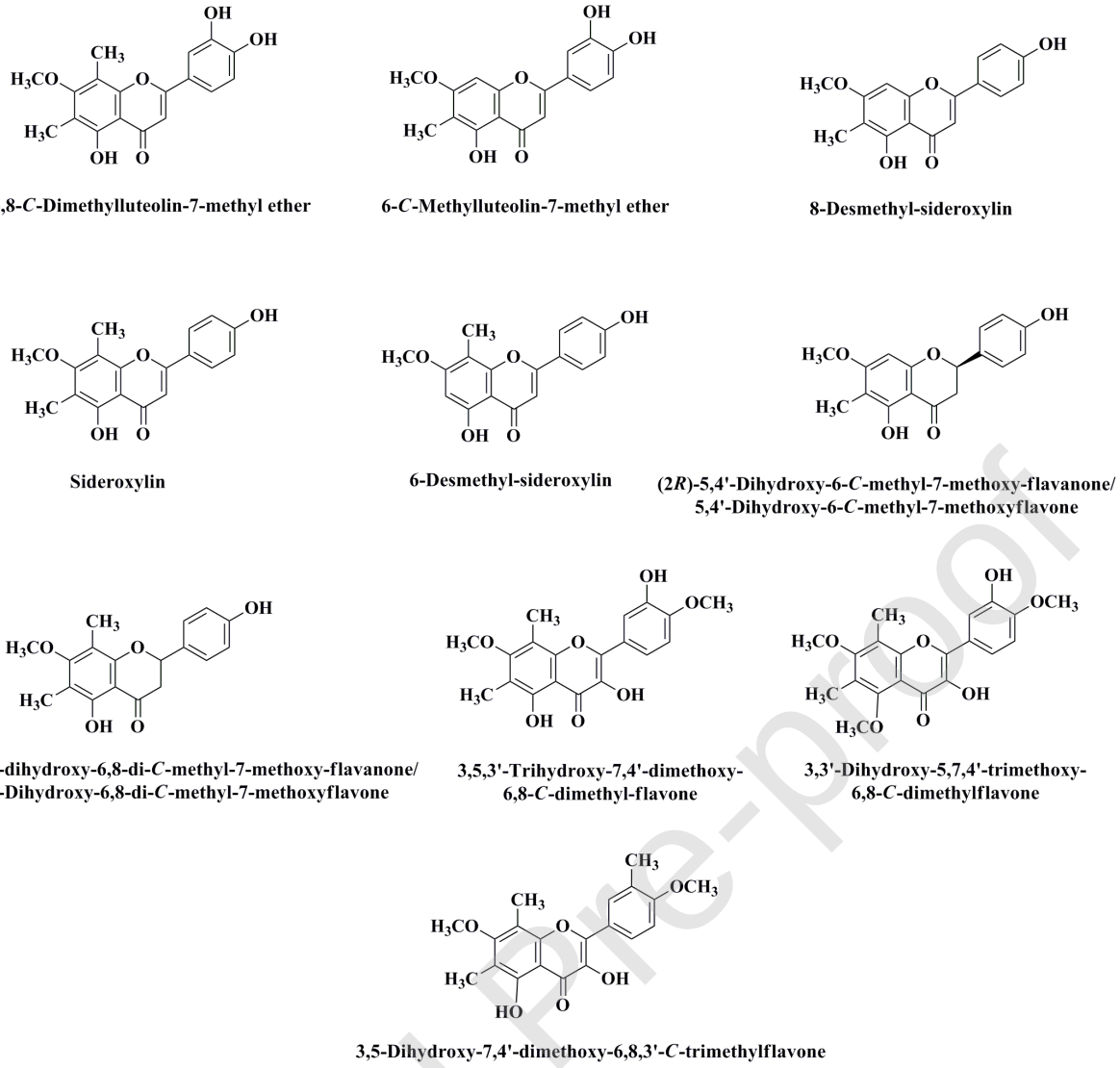

Figure 4. Chemical structures of reported phenolic acid derivatives, sterol and other compounds from goldenseal (*H. Canadensis*). The structures are obtained from pubChem as well as reported citations. The structures are drawn through chemDraw 12.0.2 version.

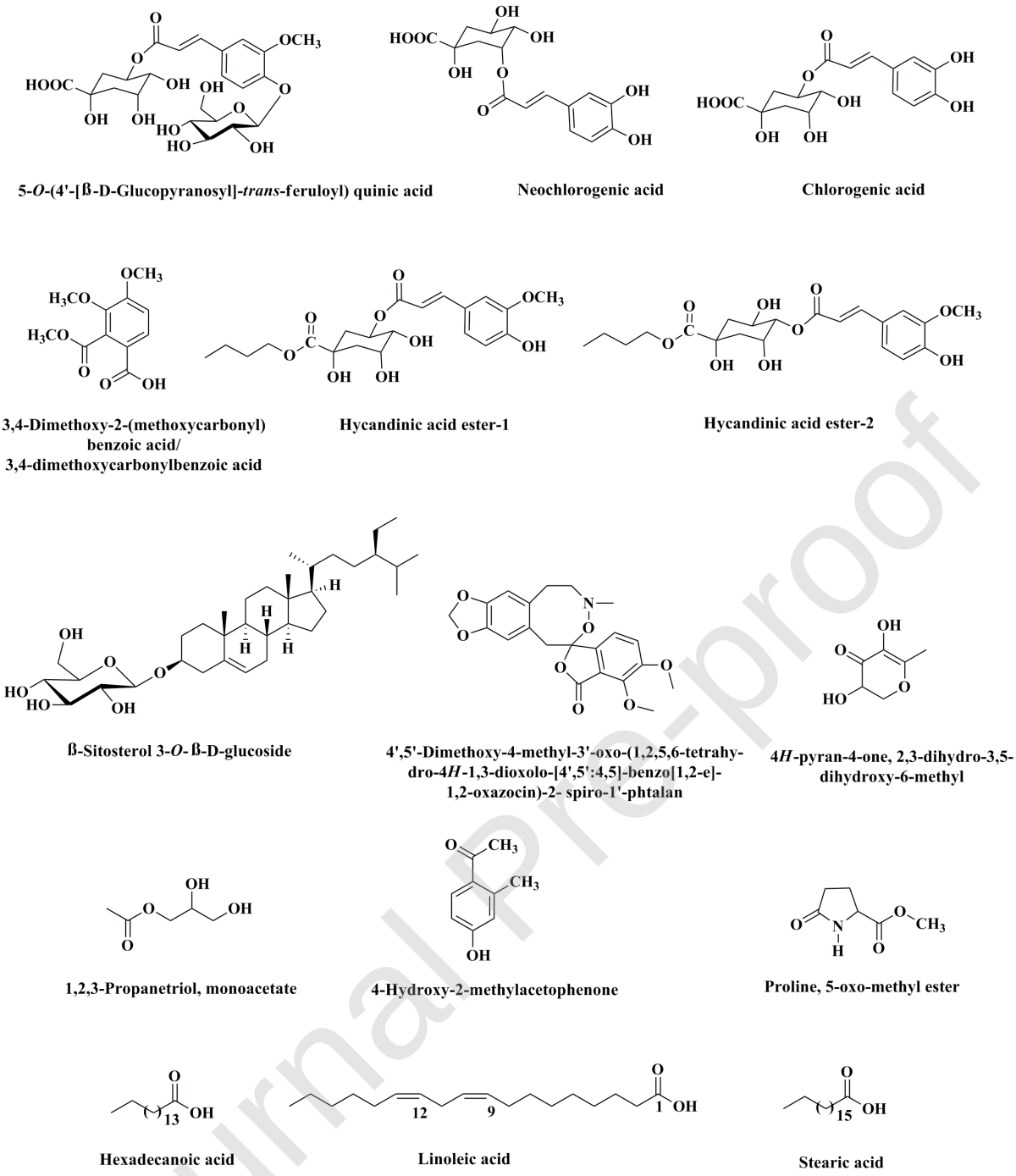

Figure 5. Renoprotective and antiviral effects of goldenseal extract and berberine and its proposed molecular mechanism. Goldenseal/berberine was shown to protect renal dysfunction and kidney damage by improving APMK signalling, and inhibition of Nrf2, TGF-b and Smad. It showed antiviral effects by suppressing the viral attachment to host cells by inhibiting inflammatory signalling pathways and activating the host cell survival mechanisms.

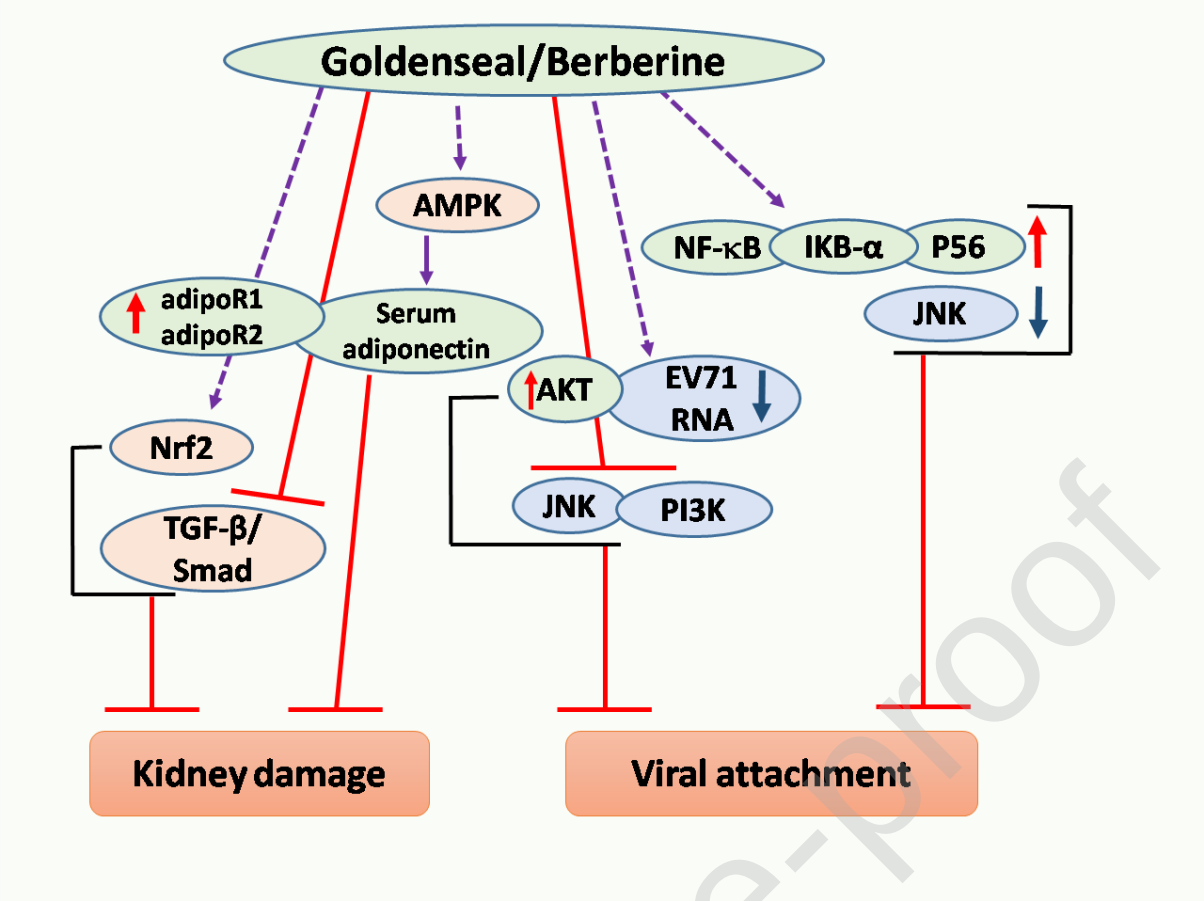

Figure 6. Antitumroal effects of goldenseal extracts and berberine. Goldenseal extract or berberine downregulated the oxidative stress induced growth factors and activated pro-oxidant markers. It induced apoptosis through mitochondrial mechanism. It also modulated the expression of 53 and inhibited MMP-2 levels through PKC as anti-proliferation mechanisms, and inhibited NF-κB and iNOS as anti-inflammation mechanism

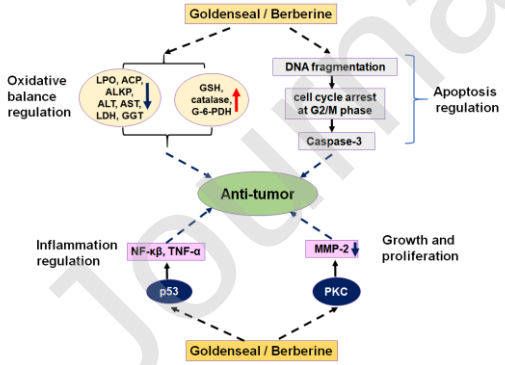

Figure 7. Neuroprotective effects of goldenseal and berberine and its proposed molecular mechanism. Goldenseal/berberine has shown to prevent neuronal cell damage by maintaining the neuronal cell growth and survival through antioxidant and antiinflammation mechanisms. It activates antioxidant enzymes system and suppresses proinflammatory cytokines and

chemokines, and activates anti-inflammatory cytokine. Berberine suppresses apoptosis in neuronal cell and activated Akt/Erk signalling pathways.

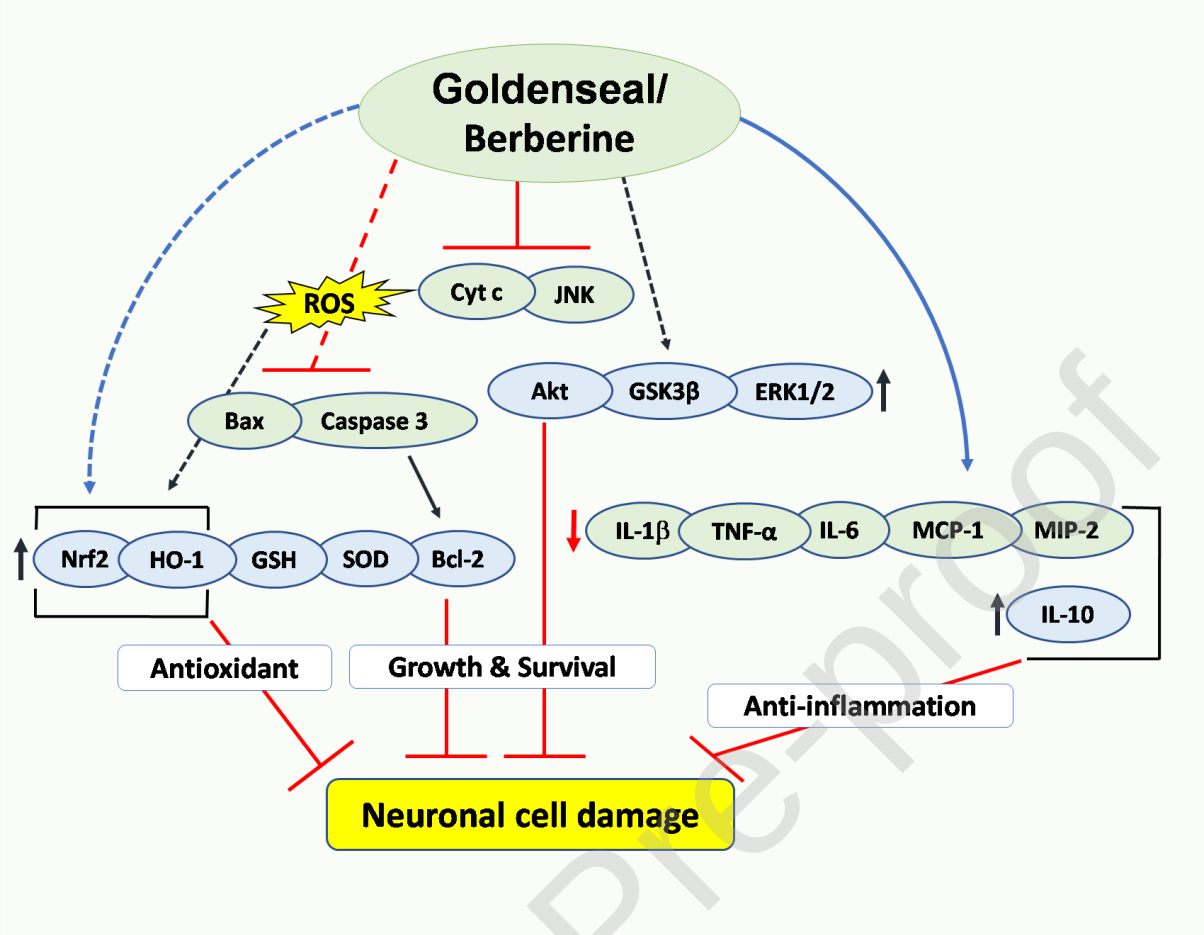

Figure 8. Major effects of goldenseal extracts and berberine in modulation of various biochemical pathways. Goldenseal extract or berberine showed anti-inflammatory effects through downregulation of inflammatory genes and proteins especially NF-κB, TNF-α, IFN-γ and COX-2. It was demonstrated to balance the oxidative stress through activation of anti-oxidant enzymes level. It showed cardioprotective effects through activation of anti-apoptosis genes and inhibition of pro-apoptosis genes. Also, it showed neuroprotective effects mainly *via* downregulation of genes and proteins involved in neurodegenerative processes.

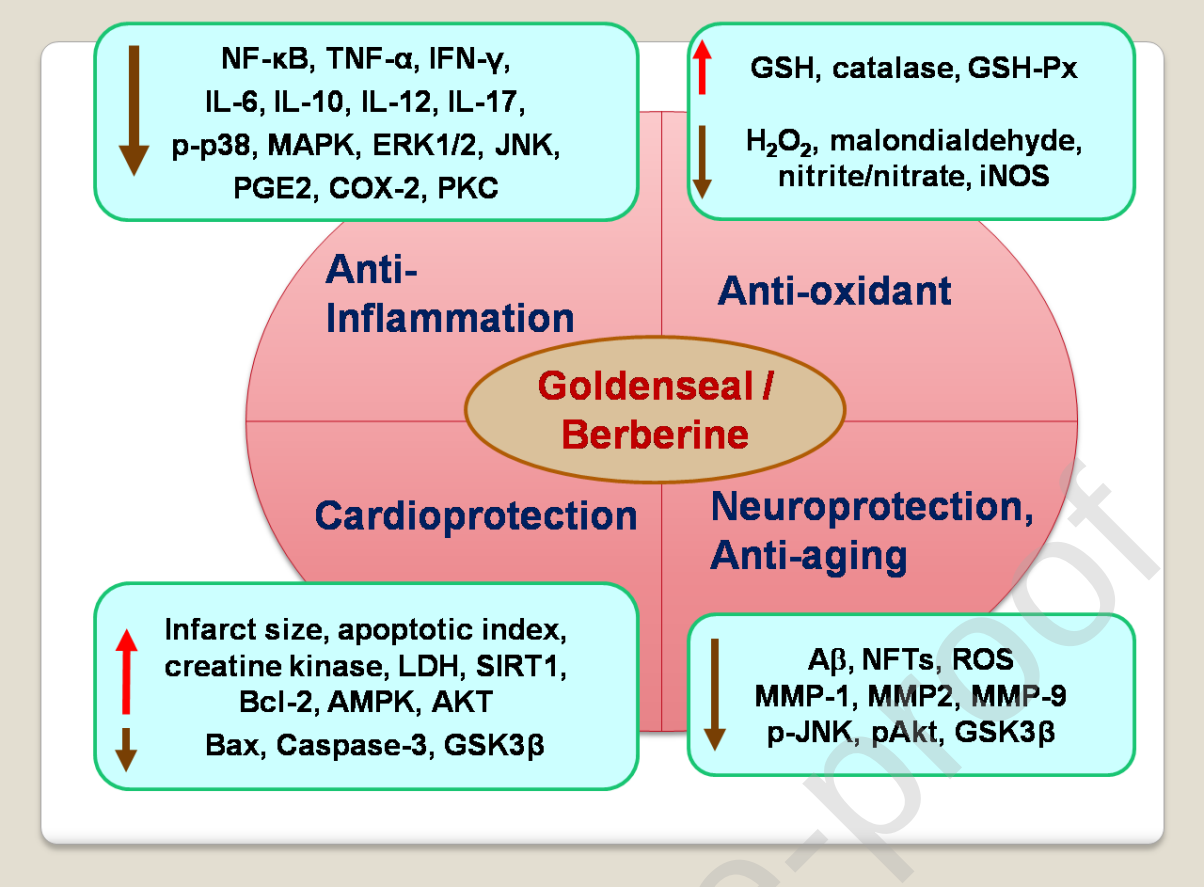

Table 1. List of reported phytochemicals from goldenseal (*H. canadensis).*

Comp. No	Compound name	Plant parts used	Fraction used	Reference(s)
[A]	**Alkaloids**			
1	Berberine	Root, rhizome, stems, and leaves	Methanol extract	[6, 8, 11, 23, 25, 53]
2	Hydrastine/ 1β-Hydrastine	Root, rhizome, stems, and leaves	95% Ethanol extract/ Methanol extract	[6, 8, 11, 22, 23, 25]
3	Canadine/ (-)-Canadine	Root, rhizome, stems, and leaves	Methanol extract	[6, 8, 11, 23, 25]
4	Palmatine	Roots		[6]
5	Hydrastinine	Root, rhizome, stems, and leaves	Methanol extract	[6, 22, 23, 25]
6	Canadaline	Root, rhizome, and leaves	Methanol extract	[6, 8, 22, 25]
7	Hydrastidine	Rhizome and leaves	Methanol extract	[25, 242]
8	Isohydrastidine	Root and rhizome	Methanol extract	[22, 242]
9	5-Hydroxy tetrahydro berberine	Root and rhizome	Methanol extract	[22]
10	*S*-Corypalmine/ Corypalmine	Root, rhizome, and leaves	Methanol extract	[22, 25]
11	*S*-Isocorypalmine / Isocorypalmine	Root, rhizome, and leaves	Methanol extract	[8, 22, 25]
12	*S*-Tetrahydropalmatine / Tetrahydropalmatine	Root, rhizome, and leaves	Methanol extract	[22, 25]
13	Berberastine	Root, rhizome,	Methanol extract	[6, 22, 25]

		and leaves		
14	8-Oxo-tetrahydrothalifendine	Root, rhizome, and leaves	95% Ethanol extract/ Methanol extract	[11, 22, 25, 53]
15	Canadinic acid	Root, rhizome, and leaves	Methanol extract	[8, 22, 25]
16	Oxyberberine	Rhizome and leaves	Methanol extract	[11, 25]
17	Chilenine	Rhizome and leaves	Methanol extract	[25]
18	Noroxyhydrastinine	Rhizome, leaves, and roots	Methanol extract	[11, 25]
19	Oxyhydrastinine	Rhizome and leaves	Methanol extract	[6, 11, 25]
20	(−)-8-Oxocanadine	Leaves	Methanol extract	[11, 25]
21	Hydrastindiol	Roots		[6]
22	Jatrorrhizine	Roots	Methanol / water (80/20 v/v)	[6, 243]
23	Tetrahydroberberastine	Roots and leaves	Methanol extract	[6, 25]
24	Coptisine	Roots	Methanol/water (80/20 v/v)	[6, 243]
25	Epiberberine	Roots	Methanol/water (80/20 v/v)	[243]
[B]	**Flavonoids**			
26	6,8-*C*-Dimethylluteolin-7-methyl ether	Root, rhizome, and leaves	Methanol extract	[8, 22, 25]
27	6-*C*-Methylluteolin-7-methyl ether	Root, rhizome, and leaves	Methanol extract	[8, 22, 25]
28	8-Desmethyl-sideroxylin	Root and leaves	(Ethanol/water, 1:1) extract / Methanol extract	[11, 22, 24, 25]
29	Sideroxylin	Root and leaves	(Ethanol/water, 1:1) extract / Methanol	[11, 22, 24, 25]

			extract	
30	6-Desmethyl-sideroxylin	Root and leaves	(Ethanol/water, 1:1) extract/ Methanol extract	[11, 22, 24, 25]
31	(2*R*)-5,4′-Dihydroxy-6-*C*-methyl-7-methoxy-flavanone/ 5,4′-Dihydroxy-6-*C*-methyl-7-methoxyflavone	Leaves	Methanol extract	[11, 25]
32	5,4′-dihydroxy-6,8-di-*C*-methyl-7-methoxy-flavanone/ 5,4′-Dihydroxy-6,8-di-*C*-methyl-7-methoxyflavone	Leaves	Methanol extract	[11, 25]
33	3,5,3′-Trihydroxy-7,4′-dimethoxy-6,8-*C*-dimethyl-flavone	Leaves	Methanol extract	[11, 25]
34	3,3'-Dihydroxy-5,7,4'-trimethoxy-6,8-*C*-dimethylflavone	Leaves	Methanol extract	[25]
35	3,5-Dihydroxy-7,4'-dimethoxy-6,8,3'-*C*-trimethylflavone	Leaves	Methanol extract	[244]
[C]	**Phenolic acids**			
36	5-*O*-(4′-[β-D-Glucopyranosyl]-*trans*-feruloyl) quinic acid	Root, rhizome, and stems	Methanol extract	[23]
37	Neochlorogenic acid	Root, rhizome, stems, and leaves	Methanol extract	[23]
38	Chlorogenic acid	Root and rhizome	Methanol extract	[23]
39	3,4-Dimethoxy-2-	Leaves	Methanol extract	[11, 25]

	(methoxycarbonyl) benzoic acid/ 3,4-dimethoxycarbonylbenzoic acid			
40	Hycandinic acid ester-1	Roots	95% Ethanol extract	[53]
41	Hycandinic acid ester-2	Roots	95% Ethanol extract	[53]
[D]	**Steroid**			
42	β-Sitosterol 3-*O*-β-D-glucoside	Root and rhizome	Methanol extract	[8, 22, 23]
[E]	**Others**			
43	4′,5′-Dimethoxy-4-methyl-3′-oxo-(1,2,5,6-tetrahydro-4*H*-1,3-dioxolo-[4′,5′:4,5]-benzo[1,2-e]-1,2-oxazocin)-2- spiro-1′-phtalan	Leaves	Methanol extract	[11, 25]
44	4*H*-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	Roots		[6]
45	1,2,3-Propanetriol, monoacetate	Roots		[6]
46	4-Hydroxy-2-methylacetophenone	Roots		[6]
47	Proline, 5-oxo-methyl ester	Roots		[6]
48	Hexadecanoic acid	Roots		[6]
49	Linoleic acid	Roots		[6]
50	Stearic acid	Roots		[6]

Table 2. Levels of the main alkaloids and phenolic acids in different parts of *H. Canadensis*.

Name of the compounds	**Level according to the part of the plant**	**Reference(s)**
Berberine (**1**)	Root/rhizome: 36.4 mM; leaf: 7.78 mM	[24]
	Root powder: 2.52−3.97 %	[6]
	Root: 3.78%; rhizome: 4.62%, lower stem: 1.83%, upper stem 1.25%, leaf: 1.50%	[23]
Hydrastine (**2**)	Root/rhizome: 3.14 mM; leaf: 1.12 mM	[24]
	Root powder: 1.38−2.74 %	[6]
	root: 1.90%; rhizome: 2.77%, lower stem: 0.43%, upper stem: 0.31%, leaf: 1.01%	[23]
Canadine (**3**)	Root/rhizome: 0.0687 mM; leaf: 0.45 mM	[24]
	Root powder: 0.04−0.19 %	[6]
	Root: 0.26%; rhizome: 0.20%, lower stem: 0.26%, upper stem: 0.07%, leaf: 0.43%	[23]
Palmatine (**4**)	Root powder: 0−0.22%	[6]
	Root: n.f.; rhizome: n.f., lower stem: n.f., upper stem: n.f., leaf: n.f.	[23]
Hydrastinine (**5**)	Root: 0.08%; rhizome: n.f., lower stem: 0.018%, upper stem 0.01%, leaf: 0.030%	[23]
5-*O*-(4′-[β-D-Glucopyranosyl]-trans-feruloyl) quinic acid (**36**)	Root: 1.10%; rhizome: 2.26%, lower stem: 0.08%, upper stem: 0.01%, leaf: n.f.	[23]
Neochlorogenic acid (**37**)	Root: 0.19%; rhizome: 0.23%, lower stem: 0.12%, upper stem 0.10%, leaf: 0.90%	[23]
Chlorogenic acid (**38**)	Root: 0.32%; rhizome: 0.17, lower stem: 0.24%, upper stem 0.20%, leaf: 0.51%	[23]

Abbreviation: n.f., not found.