Goldenseal
(*Hydrastis canadensis* L.)

and Two of Its Constituent Alkaloids

Berberine
[2086-83-1]
and
Hydrastine
[118-08-1]

Review of Toxicological Literature

Prepared for

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EXECUTIVE SUMMARY

The nomination of goldenseal and two of its constituent alkaloids, berberine and hydrastine, for testing is based on the potential for human exposure and the lack of chronic or carcinogenicity data.

Goldenseal products are produced from the dried rhizome and root of the plant. Berberine can be produced from cultures of *Coptis japonica* and *Thalictrum rugosum* cells. Hydrastine can be produced from berberine.

Production and import volumes were not located for these compounds.

Goldenseal is a member of the plant family Ranunculaceae. The major constituent alkaloids are hydrastine [as (-)-hydrastine], berberine, and berberastine. Canadine and several other minor alkaloids are also present.

Goldenseal tea is commercially available in health food stores. Goldenseal is also an ingredient in some over-the-counter (OTC) herbal dietary supplements, eardrops, feminine cleansing products, cold/flu remedies, allergy relief products, laxative products, and digestive support products. Berberine chloride and berberine sulfate are ingredients in some commercial eyewash products. Hydrastine is an ingredient in some decongestant nose sprays and feminine cleansing products.

Goldenseal has been used to treat digestive and hemorrhagic disorders, disorders of the genitourinary tract, upper respiratory inflammation and congestion, mucous membrane inflammation, eczema, pruritus, otorrhea, tinnitus, congestion/inflammation of the ear, and conjunctivitis. It is claimed to be effective in treating cancers, particularly of the ovary, uterus, and stomach. Goldenseal is stated also to possess antiseptic, astringent, and hemostatic qualities when applied topically. It has been used as a tonic, antiperiodic, diuretic, and as a vaginal douche. OTC products containing goldenseal are sold under the claim that they are effective in treating menstrual disorders, minor sciatica, rheumatic and muscular pain, motion sickness and nausea, and chronic diarrhea from protozoal, fungal, and bacterial infections. Goldenseal is sometimes used to treat AIDS symptoms and claims have been made that it is able to prevent the detection of illicit drugs in urine.

Berberine has been used as a bitter tonic, diaphoretic, and antipyretic, and for the treatment of skin diseases, eye infections, liver diseases, and diarrhea. Studies have identified antiplatelet, anticerebral ischemic, vasodilatory, and antirrhythmic pharmacologic properties. It is believed to be the active ingredient in *Coptis rhizoma* (used to treat amnesia). Berberine has been used to treat bacterial and parasitical infections and may be effective in improving cardiac performance in patients with heart failure. It is also used as a fluorescent stain in medical research.

Hydrastine is claimed to be an abortifacient, antibiotic, antitussive, antiuterotic, antivaginitic, bactericide, central nervous system depressant, choleretic, convulsant, hemostat, hypertensive, hypotensive, pesticide, sedative, uterotonic, and vasoconstrictor. In the treatment of diarrhea, it has been found to have anti-microbial, antimotility, and antisecretory properties.

Exposure to goldenseal occurs orally as a tea or capsule, or it can be applied dermally as a skin lotion or to the eye as an eyewash. It is also applied as a vaginal douche and as eardrops. Berberine and hydrastine are also applied to the eyes as an eyewash. Hydrastine exposure occurs also from the use of hydrastine-containing decongestant nose sprays and feminine hygiene products.

In humans, goldenseal may cause convulsions and irritation of the mouth, throat, and stomach when taken orally in toxic doses. Paresthesia, paralysis,

respiratory failure, and death may follow. Chronic use may inhibit vitamin B absorption, and sublethal doses may induce labor if taken during pregnancy. While markedly improving cardiac performance in patients with heart conditions, berberine also induces ventricular tachycardia in some subjects. Berberine had an anesthetic effect when injected subcutaneously (s.c.). Both berberine and hydrastine produced parasympatholytic and anesthetic effects when applied to the eyes. Hydrastine induced labor when taken orally by pregnant women.

No chemical disposition, metabolism, or toxicokinetics data were found for goldenseal or hydrastine. In humans, berberine sulfate is absorbed through the skin. Following oral administration (species not provided), berberine was absorbed slowly, taking 4 hours to reach peak concentrations in plasma and another 4 hours to clear. In rats orally administered tritiated berberine chloride, blood levels of the compound leveled off at 4 - 24 hours; peak levels in liver and muscles occurred at 12 hours, while urinary excretion peaked at 12-24 hours. At 48 hours, the majority of the administered dose had been excreted in feces. Following intravenous (i.v.) administration to rats, the highest concentrations of berberine were found in the kidneys, with lower concentrations in the liver, lung, and brain. In rabbits, 24 hours after administration by gavage, small amounts of berberine were found in the heart, liver, and kidneys. Uptake into cells and across epithelia may involve a cation exchange mechanism.

No acute exposure data for goldenseal were located. In mice, the oral LD_{50} dose for berberine is 329 mg/kg (0.98 mmol/kg), and the s.c. LD_{50} dose is 18 mg/kg (0.054 mmol/kg). The i.p. LD_{50} for berberine sulfate in mice is 24.3 mg/kg (0.056 mmol/kg). In rats, the i.p. LD dose for berberine is greater than 500 mg/kg (>1.49 mmol/kg), while the i.p. LD_{50} for berberine sulfate is 88.5 or 205 mg/kg (0.20 or 0.47 mmol/kg). Also, for rats, the LD_{50} doses for berberine sulfate are 14.5 mg/kg (0.033 mmol/kg) when administered intramuscularly, and greater than 1000 mg/kg (>2.31 mmol/kg) when administered orally. In rabbits, the s.c. LD_{Lo} dose for berberine is 100 mg/kg (0.30 mmol/kg). In rats, the i.p. LD_{50} dose for hydrastine is 104 mg/kg (0.271 mmol/kg).

Berberine chloride and berberine sulfate, when injected i.p., did not inhibit amphetamine toxicity in mice. Following i.p. administration, berberine sulfate induced lachrymation, pilomotor erection, and ptosis in mice, with maximal depression occurring two hours after drug administration. In rats, berberine sulfate, administered i.p., reduced rectal temperature. A single intraintestinal injection of berberine sulfate into the duodenum of rats had no effect on the volume or acidity of gastric fluid, nor did it effect the severity of gastric ulcers. Berberine sulfate, administered i.v. reduced blood pressure in rats, dogs, and cats and significantly increased the number of apomorphine-induced vomits in dogs. Cats administered berberine chloride i.v. were sedated, inactive, showed no interest in surroundings, and did not consume food. Ocular application of berberine did not have an anesthetic effect on rabbits or dogs.

Conflicting information regarding the toxicity of hydrastine were found. In one study, hydrastine hydrochloride caused convulsions within one minute in 50% of the mice administered one i.v. injection. In another study, hydrastine did not cause convulsions in mice, even when administered at lethal doses (dose range not provided).

No subchronic information was found for goldenseal or hydrastine. Subchronic exposure of rats to berberine chloride by gavage for 7 or 14 days significantly reduced scopolamine-induced amnesia effects but did not significantly alter motor activity. Subchronic exposure of rats to berberine

sulfate orally for 6 weeks did not induce histopathological changes in tissues or organs.

No chronic exposure data were found for any of the compounds.

No teratogenicity or embryotoxicity studies were found for any of the compounds.

No data were found on carcinogenicity, although some studies tested the potential anticarcinogenicity of berberine. Berberine injected i.p. into mice was effective in preventing the growth of P388 leukemia cells (administered i.v. or intracerebrally). Berberine sulfate also significantly inhibited the tumor yield and the incidence of mice bearing tumors which were initiated with DMBA and promoted with teleocidin. However, in two mouse experiments, berberine did not inhibit the growth of mouse sarcoma-180 tumor cells. In rats implanted in the brain with 9L-2 rat gliosarcoma cells, treatment with berberine effectively killed 81% of the gliosarcoma cells.

No genotoxicity data were found for goldenseal or hydrastine. Berberine binds to DNA by intercalation with an AT base pair preference. As a DNA intercalating agent, berberine caused a general reduction in cellular RNA and protein synthesis and overproduction of the β and β1 subunits of RNA polymerase in *Escherichia coli*. In sonicated and superhelical calf thymus DNA, berberine chloride induced DNA unwinding. Berberine chloride induced *his* gene mutations in *Salmonella typhimurium* strain TA98 in the absence but not in the presence of metabolic activation. It was not mutagenic in strain TA100, with or without metabolic activation. When *Saccharomyces cerevisiae* were cultured in growth medium in the absence of metabolic activation, berberine chloride induced *hom*3-10 frameshift mutations and cytoplasmic 'petite' mutations in a dose dependent manner; it also induced *cyh* crossing over. However, mutations at the *hom*3-10 or 'petite' mutations were not induced when *S. cerevisiae* were treated while incubated in saline, nor were *leu* gene conversions or *lys*1-1 and *his*1-7 point mutations induced when cells were treated while in growth medium or saline. In a DNA damage assay, berberine chloride did not induce β-galactosidase activity in *E. coli* strain PQ37 in the presence or absence of S9. Treatment with berberine induced a dose dependent increase in sister chromatid exchanges in 9L rat intracerebral gliosarcoma cells.

No immunotoxicity data were found for goldenseal or hydrastine. Berberine inhibited DNA synthesis in mitogen-stimulated human lymphocytes, but it did not inhibit polymorphonuclear leukocyte activation, nor was it cytotoxic to target cells, in a C3H/He mouse MM2 tumor cell assay.

Berberine inhibited the growth of cultured mouse sarcoma-180 tumor cells, an effect that was ameliorated by cotreatment with glucose. It also inhibited the growth of cultured human brain tumor cells and HepG2 human hepatoma cells, and was cytotoxic to Molt-4 and L1210 human leukemia cells and P-388 murine leukemia cells. However, berberine was not cytotoxic towards C38 murine colon adenocarcinoma cells or L1210 mouse leukemia cells. Berberine and berberine chloride induced apoptosis in Balb/c 3T3 fibroblast cells and human HL-60 leukemia cells, respectively. In cultured 9L rat gliosarcoma cells, berberine induced lysis, encystation, and degeneration. Berberine chloride inhibited the growth of cultured HeLa cells, and was toxic to T2/D1 human teratocarcinoma cells and F9 murine teratocarcinoma cells. Berberine sulfate inhibited the incorporation of $^{32}P_i$ into phospholipids induced by TPA and teleocidin. It also inhibited TPA-enhanced transport of ^{3}H-3-O-methyl-D-glucose into mouse fibroblast 3T3 cells. Berberine reversed the resistance of BEL-7402 human liver cancer cells to the cytotoxic effects of vincristine, but not of MCF-7/Adr human

breast cancer cells to adriamycin. Down-regulation of the Ki-ras2 protooncogene was induced by berberine chloride in treated T2/D1 human teratocarcinoma cells.

Berberine did not induce phototoxicity in *E. coli* strains RT7h, RT8h, RT9h, or RT10h.

Berberine acted as an anticholinergic in isolated guinea pig ileum, isolated tracheal muscles of dogs, and isolated rectus muscle of frogs. Berberine sulfate exhibited an antiadrenergic response in isolated rabbit aortic strips, guinea pig seminal vesicle, and rat aortas. At low concentrations, berberine sulfate elicited a spasmogenic response in isolated guinea pig ileum, while at higher concentrations, a spasmolytic effect was observed. Berberine sulfate had no effect on tracheal muscle preparations from dogs or guinea pigs, isolated rabbit aortic strips, depolarized guinea pig ileum, or guinea pig seminal vesicle, but showed an antihistaminic response in isolated guinea pig ileum. It also potentiated calcium chloride-induced contractions and PGE_1-, $PGF_{1\alpha}$-, and $PGF_{2\alpha}$-induced contractions in isolated guinea pig ileum. Berberine sulfate was reported to both inhibit and potentiate $PGF_{2\alpha}$-induced contractions in isolated guinea pig ileum. It had a negative inotropic effect on isolated heart preparations and rabbit intestine; it had a positive inotropic and positive chronotropic effect on spontaneously beating atria of rats, guinea pigs, and rabbits. In isolated guinea pig ventricular papillary muscle, berberine inhibited the hypoxic-condition- or cromakalim-induced shortening of action potential and effective refractory period.

Hydrastine had no effect on adrenaline-induced contractions in rabbit aortic strips, nor on unstimulated guinea pig ileum or mouse vas deferens preparations. It had a negative inotropic but positive chronotropic effect on spontaneously beating rat atrium, and inhibited electrically evoked contractions in guinea pig ileum. Hydrastine induced a positive inotropic effect on electrically evoked contractions of isolated mouse vas deferens.

Berberine sulfate reduced bull sperm motility in a dose dependent manner.

Berberine and protoberberine alkaloids have varying DNA binding affinities, but generally binding is very weak; substitution in bulky groups of protoberberine alkaloids inhibited DNA binding. Another study found that only quaternary salts bind with DNA. Berberine inhibited topoisomerase II, but not topoisomerase I. The increased planarity of berberine is thought to account for its enhanced activity in topoisomerase II inhibition. Cleavage of both methylenedioxy and methoxyl groups from berberine forms a compound which is a potent topoisomerase I poison; minor variations in the protoberberines may substantially alter their pharmacological properties.

With regard to induction of convulsion following i.v. injection into mice, (-)-hydrastine was much less potent than (+)-hydrastine. In addition, (-)-β-hydrastine and (±)-β-hydrastine were less potent than (+)-α-hydrastine and (±)-α-hydrastine in inducing binding to the $GABA_A$ receptor in rat brain synaptic membranes.

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1.0 BASIS FOR NOMINATION

The nomination of goldenseal and two of its constituent alkaloids, berberine and hydrastine, for testing is based on the potential for human exposure and the lack of chronic or carcinogenicity study data.

2.0 PROPERTIES

Goldenseal (*Hydrastis canadensis* L.)

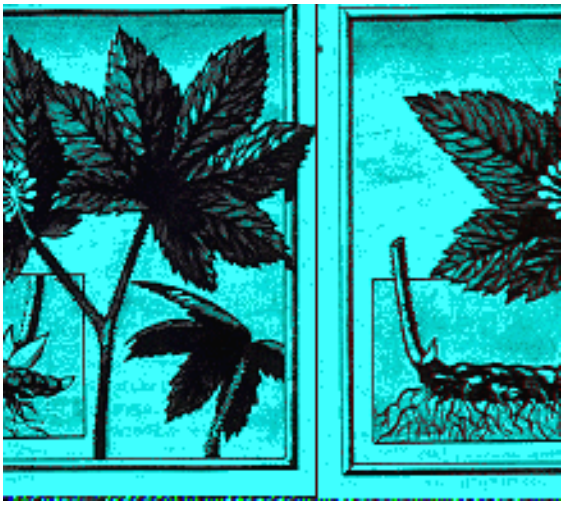
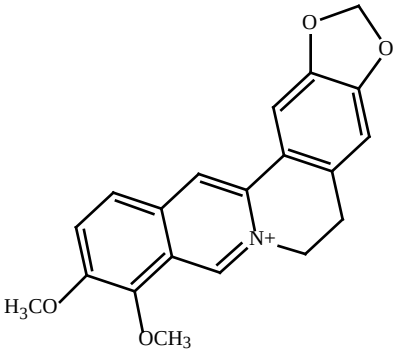
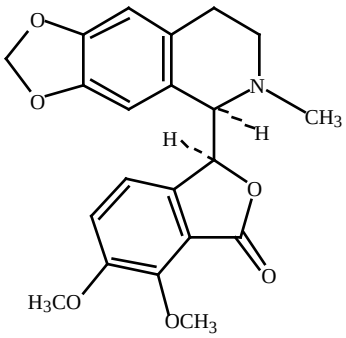


Illustration from Hamon (1990)

Berberine
[2086-83-1]



Hydrastine
[118-08-1]



2.1 Identification

Goldenseal (*Hydrastis canadensis* L.) is also called:

Eye balm	Ohio curcuma	Yellow paint root
Eye root	Orange root	Yellow puccoon
Ground raspberry	Tumeric root	Yellow root
Indian turmeric	Yellow eye	Yellow seal
Jaundice root	Yellow indian plant	Yellow wort

Berberine ($[C_{20}H_{18}NO_4]^+$, mol. wt. = 336.37) is also called:

Benzo[*g*]-1,3-benzodioxolo[5,6-*a*]quinolizinium, 5, 6 dihydro-9,10-dimethoxy- (9CI)
Berbinium, 7,8,13,13*a*-tetradehydro-9,10-dimethoxy-2,3-(methlyenedioxy)- (8CI)
Berbinium, 7,8,13,13*a*-tetrahydro-9,10-dimethoxy-2,3-(methylenedioxy)-
Berbericine
Berberin
5,6-Dihydro-9,10-dimethoxybenzo[*g*]-1,3-benzodioxolo[5,6-*a*]quinolizinium
9,10-Dimethoxy-2,3-(methylenedioxy)-7,8,13,13*a*-tetrahydroberbinium
7,8,13,13*a*-Tetradehydro-9,10-dimethoxy-2,3-(methylenedioxy)berbinium
Umbellatin
Umbellatine

Hydrastine ($C_{21}H_{21}NO_6$, mol. wt. = 383.40) is also called:

[*S*-(*R**,*S**)]-6,7-Dimethoxy-3-(5,6,7,8-tetrahydro-6-methyl-1,3-dioxolo[4,5-*g*]isoquinolin-5-yl)-1(3*H*)-isobenzofuranone
1-Hydrastine
β-Hydrastine
1-β-Hydrastine
(-)-Hydrastine
1(3*H*)-Isobenzofuranone, 6,7-dimethoxy-3-(5,6,7,8-tetrahydro-6-methyl-1,3-dioxolo[4,5-g]isoquinolin-5-yl-, [S-(R*,S*)] - (9CI)
Phthalide, 6,7-dimethoxy-3-(5,6,7,8-tetrahydro-6-methyl-1,3-dioxolo[4,5-*g*]isoquinolin-5-yl)-

Berberine and hydrastine salts mentioned in this report have the following molecular weights:

Berberine chloride ($C_{20}H_{18}NO_4 \cdot Cl$; sometimes called berberine hydrochloride) = 371.83

Berberine sulfate ($C_{20}H_{18}NO_4 \cdot HSO_4$; sometimes called berberine bisulfate) = 433.44

Hydrastine hydrochloride ($C_{21}H_{21}NO_6 \cdot HCl$; sometimes called hydrastine chloride) = 419.86

2.2 Physical-Chemical Properties

2.2.1 Goldenseal

Goldenseal is a member of the plant family Ranunculaceae (Budavari, 1996). It is a perennial, hairy plant with one long-stalked basal leaf, a single stem, and two smaller leaves on the

flowering stem. Usually, there are 2 rounded, lobed, and double-toothed leaves on a forked branch, with one being larger than the other. The plant has a knotted yellow rhizome and a solitary terminal flower with three white sepals and many greenish-white stamens in clusters. It has a small, red raspberry-like fruit (Anonymous, 1997b). The plant grows to a height of about one foot (30.5 cm) (Palmer, 1975).

The major alkaloid constituents of goldenseal are (-)-hydrastine (1.5-4%), berberine (0.5-6%), and berberastine (2-3%) (Hamon, 1990). Canadine and several other minor alkaloids are also present (concentrations not given).

2.2.2 Berberine

Property	Information	Reference
Color	yellow	Budavari (1996)
Physical State	needles from ether	Budavari (1996)
Melting Point, °C	145	Budavari (1996)
Boiling Point, °C	no information available	
Specific Gravity at 25°C	no information available	
Dissociation Constant (pK)	2.47	Budavari (1996)
Odor	no information available	
Odor Threshold, ppm; v/v	no information available	
Solubility:		
Water	dissolves slowly	Budavari (1996)
Organic Solvents	insoluble in ether	Martin and Cook (1961)

Berberine behaves as a quaternary base.

2.2.3 Hydrastine

Property	Information	Reference
Color	creamy white to white	Martin and Cook (1961)
Physical State	orthorhombic prisms from alcohol	Budavari (1996)
Melting Point, °C	132	Budavari (1996); HODOC (1997)
Boiling Point, °C	no information available	
Specific Gravity at 25°C	no information available	
Dissociation Constant (pK)	7.8	Budavari (1996)
Odor	no information available	
Odor Threshold, ppm; v/v	no information available	
Solubility:		
Water	insoluble	Budavari (1996); HODOC (1997)
Organic Solvents	freely soluble in acetone, benzene, and in alcohol, chloroform, and ether	Budavari (1996); HODOC 1997); Martin and Cook (1961)

2.3 Commercial Availability

Goldenseal tea is available in health food stores (Mikkelsen and Ash, 1988). Goldenseal is also an ingredient in some over-the-counter (OTC) herbal supplements (Anonymous, 1997f), eardrops (Anonymous, 1994b), feminine cleansing products (Anonymous, 1994e), cold/flu remedies (Anonymous, 1997e), allergy relief products (Anonymous, 1997d), laxative products (Anonymous, 1991), and digestive support products (Anonymous, 1997c).

Berberine hydrochloride and berberine bisulfate are ingredients in some commercial eyewash products (CTCP, 1985).

Hydrastine is available commercially in the form of (-)-hydrastine (Huang and Johnston, 1990). Hydrastine is an ingredient in some decongestant nose sprays and feminine hygiene products (CTCP, 1985).

3.0 PRODUCTION PROCESSES

Goldenseal products, when produced from the dried rhizome and root of the plant, contain at least 2.5% of the alkaloids, including berberine and hydrastine (Martin and Cook, 1961).

Berberine can be produced from cultures of *Coptis japonica* cells (Fujita and Tabata, 1987) and *Thalictrum rugosum* cells (Kim et al., 1990). Adding gibberellic acid to *C. japonica* cell cultures (Fujita, 1988), or cupric sulfate to *T. rugosum* (Kim et al., 1991) cell cultures, increases the yield. Also, producing *C. japonica* and *T. rugosum* at high cell density is essential for maximizing production yields (Kim et al., 1990; Piehl et al., 1988).

Hydrastine can be produced from berberine (Moniot and Shamma, 1976). The first step in the synthesis involves a ferricyanide oxidation of berberine to yield oxybisberberine. Treatment with methanolic hydrogen chloride yields 8-methoxyberberinephenolbetaine, which after hydration yields the hydrochloride salt of dehydronorhydrastine methyl ester. *N*-Alkylation then gives dehydrohydrastine methyl ester, and direct sodium borohydride reduction gives a 90% yield of a 1:2 mixture of (±)-α-hydrastine and (±)-ß-hydrastine.

4.0 PRODUCTION AND IMPORT VOLUMES

No data were found.

5.0 USES

Goldenseal was first used by Native Americans to treat wounds, ulcers, digestive disorders, and skin and eye ailments (Hamon, 1990). Over the years, goldenseal has been used to treat a variety of digestive and hemorrhagic disorders. It is thought to possess slight antiseptic,

astringent, and hemostatic qualities when applied topically. It is claimed that goldenseal is effective in the treatment of hemorrhoids, disorders of the genito-urinary tract, upper respiratory inflammation and congestion, mucous membrane inflammation, eczema, pruritus, otorrhea, tinnitus and congestion/inflammation of the ear, and conjunctivitis, as well as for cancers, particularly of the ovary, uterus and stomach. Goldenseal has been used as a tonic, antiperiodic, diuretic (Hamon, 1990), and as a vaginal douche (Anonymous, 1994e). It is commonly consumed as an herbal tea (Hamon, 1990). For external use, it may be prepared by adding a teaspoon of root to 0.5 pint of water and used as a skin lotion. To prepare an eyewash, one teaspoon of ground goldenseal root and one teaspoon of boric acid are dissolved in one pint boiling water. The mixture is stirred, cooled, and the liquid is collected. For use as an eyewash, one teaspoon of the liquid is added to 0.5 cup of water (Anonymous, 1997b).

OTC preparations containing goldenseal are currently sold under the claim that they are effective in treating menstrual disorders, minor sciatica, and rheumatic and muscular pain (Hamon, 1990). OTC labels also assert goldenseal-containing products to be effective in treating allergy symptoms (Anonymous, 1997d), cold and flu symptoms (Anonymous, 1997e), motion sickness and nausea (Anonymous, 1996b), chronic diarrhea from protozoal, fungal, and bacterial infections (Anonymous, 1996a), and earaches and ear infections (Anonymous, 1994b). Goldenseal is included in dietary vitamin and mineral supplement tablets (Anonymous, 1997f) and is sometimes used to treat AIDS symptoms (Anonymous, 1996a). It is claimed to have the ability to cleanse the body of mucus, toxins, and waste (Anonymous, 1994c). Goldenseal is available in OTC preparations as an easy to swallow gel cap (Anonymous, 1996c); doses vary from 100 and 200 mg (Anonymous, 1994d) to 470 mg (Anonymous, 1994a).

Claims have been made that goldenseal is able to prevent the detection of illicit drugs in urine by inducing the rapid elimination of these compounds (Hamon, 1990). In one study (Mikkelsen and Ash, 1988), 15 g/L goldenseal tea produced false-negative results for tetrahydrocannibus (THC), but not for amphetamines, barbiturates, benzodiazepines, cocaine, or opiates. In another study (Schwarzhoff and Cody, 1993), adulteration of human urine with goldenseal root caused an apparent decrease in the concentrations of THC and barbiturate.

Berberine has been used as a bitter tonic (to improve stomach function), diaphoretic (sweat inducer), and antipyretic (Kulkarni et al., 1972), and for the treatment of skin diseases (including psoriasis) (Müller et al., 1995), liver diseases (Chi et al., 1994), eye infections, and diarrhea (Sabir et al., 1978), although it may not be effective for non-cholera diarrhea (Khin-Maung et al., 1985). In the treatment of psoriasis, the effectiveness of berberine appears to be related to its

ability to inhibit hyperproliferation (Müller et al., 1995). Findings of one study did not support the usefulness of berberine in treating peptic ulcers and hyperacidity (Sabir et al., 1978).

Studies of berberine identified antiplatelet (Chu et al., 1994; cited by Peng et al., 1997), anticerebral ischemic (Wu and Liu, 1995; cited by Peng et al., 1997), vasodilatory (Chiou et al., 1991; cited by Peng et al., 1997), and antirrythmic (Wang and Tan, 1994; cited by Peng et al., 1997) pharmacologic properties. It is thought to increase ileal contractility and acetylcholine retention (Shin et al., 1993; cited by Peng et al., 1997) and is believed to be the active ingredient in *Coptis rhizoma*, which is used to treat amnesia (Lee, 1986; cited by Peng et al., 1997).

Berberine has also been used to treat a number of bacterial and parasitical infections, including cholera (Kulkarni et al., 1972), giardiasis (Choudry et al., 1972; Sabir et al., 1978), amoebiasis (Sabir et al., 1978), and dermal leishmaniasis (Sabir et al., 1978; Martin et al., 1978; Vennerstrom et al., 1990). There is conflicting evidence of the efficacy of berberine in the treatment of malaria (Vennerstrom and Klayman, 1988).

Results from a clinical trial also indicated that berberine is effective in improving cardiac performance in patients with heart failure (Marin-Neto et al., 1988). It appears to exert a direct depressive action on myocardial, vascular, and smooth musculature (Hahn et al., 1966; Herman and Chadwick, 1974; both cited by Creasey, 1977) and may have anticholesterolase activity (Sabir and Bhide, 1971).

In addition to its therapeutic uses, berberine is also used as a fluorescent stain in medical research (Kim et al., 1990). It is used to stain cells (Borodina et al., 1979), chromosomes (Ridler and Jennings, 1983), and energized mitochondria (Mikes and Dadák, 1983; Mikes and Yaguzhinskij, 1985).

Hydrastine is claimed to be an abortifacient, antibiotic, antiuterotic, antivaginitic, bactericide, central nervous system depressant, choleretic, convulsant, hemostat, hypertensive, hypotensive, pesticide, sedative, uterotonic, and vasoconstrictor (Beckstrom-Sternberg and Duke, 1997a).

6.0 ENVIRONMENTAL OCCURRENCE

Goldenseal is a small perennial plant indigenous to the hardwood forests of the eastern U.S. and Canada (Anonymous, 1997a). Currently, it is rarely found in the wild (Hamon, 1990).

Berberine is usually present in plants as a sulfate (HSDB, 1997). It is found in the rhizomes of Chinese Goldthread (*Coptis chinensis* FRANCH., 40,000-90,000 ppm), Generic Goldthread (*Coptis* spp., 40,000-90,000 ppm), and Huang-Lia (*Coptis japonica,* 40,000-70,000 ppm); in the roots of goldenseal (5000-60,000 ppm); in the bark of Huang Po (*Phellodendron*

amurense RUPR., 8300-10,000 ppm); and in the plant parts of Barberry (*Berberis vulgaris* L., 10,000-30,000 ppm) and Prickly Poppy (*Argemone mexicana* L., 410 ppm) (Beckstrom-Sternberg and Duke, 1997b). Berberine is also a constituent (concentrations not given) of *B. aristata, B. lamberti, B. asiatica, B. heterobotrus, B. crataegina, B. cretica, B. thunbergii, B. kawakamii, B. mingetsensis, B. morrisonensis, B. francesciferdinandi, B. koreana, B. iliensis, B. guimpeli, B. lycium, B. peteolaris,* and *B. amurensis* var (Ikram, 1975).

Hydrastine is a constituent of goldenseal (15,000-40,000 ppm) (Beckstrom-Sternberg and Duke, 1997a) and *B. laurina* (concentration not given) (Ikram, 1975).

7.0 HUMAN EXPOSURE

Exposure to goldenseal occurs orally as a tea (Hamon, 1990) or capsule (Anonymous, 1996c), or it can be applied dermally as a skin lotion (Anonymous, 1997b), to the eye as an eyewash (Anonymous, 1997b), or as a vaginal douche (Anonymous, 1994e). It is also applied to the ear as eardrops (Anonymous, 1994b). Berberine and hydrastine are also applied to the eyes as an eyewash (CTCP, 1985). Hydrastine exposure occurs from use of decongestant nose sprays and feminine hygiene products containing hydrastine (CTCP, 1985).

8.0 REGULATORY STATUS

Under 21 CFR part 310 (Federal Register, 1993), the Food and Drug Administration (FDA) issued a final rule on the Federal Food, Drug, and Cosmetic Act (the Act), effective November 10, 1993, that certain active ingredients in OTC products are not generally recognized as safe and effective or are misbranded. Among these, *H. canadensis* (goldenseal) is not generally recognized as safe and effective and is misbranded when labeled as an OTC drug for use as a digestive aid or an orally administered menstrual drug. Any product that is labeled as such and is initially introduced or delivered after the effective date is in violation of sections 502 and 505 of 21 U.S.C. part 201.

Previous versions of the Act included the following regulations: Goldenseal may not be sold and labeled in products as an acne remedy (FDA, 1995), as a blood purifier with antiseptic and antibacterial effects (FDA, 1993a), as an herbal antibiotic (FDA, 1993b), as a cold symptom reliever (FDA, 1987) or as an aphrodisiac (FDA, 1992). Berberine may not be labeled or sold as an antibiotic (FDA, 1993b). Hydrastine hydrochloride may not be labeled or sold as an eye irritation reliever (FDA, 1989).

Since the demand for goldenseal has grown at a rate similar to the 30% growth rate experienced by the herbal curative market, the U.S. Forest Service has stated that “current

conditions suggest [that goldenseal] cannot be traded in perpetuity unless conservation of the species improves" (Anonymous, 1997a).

9.0 TOXICOLOGICAL DATA

Summary: In humans, goldenseal may cause convulsions and irritation of the mouth, throat, and stomach when taken orally in toxic doses (doses not specified). Paresthesia, paralysis, respiratory failure, and death may follow. Chronic use may inhibit vitamin B absorption, and sublethal doses (doses not specified) may induce labor if taken during pregnancy. Berberine, on the other hand, was found to markedly improve cardiac performance in patients with heart conditions when taken at 0.02 mg/kg/min (59 nmol/kg/min) for 30 minutes, followed by 0.2 mg/kg/min (0.59 μmol/kg/min) for an additional 30 minutes. However, further elucidation of effects from berberine administration to heart failure patients is necessary due to the finding of ventricular tachycardia in some subjects. Berberine had an anesthetic effect when injected s.c (dose not specified). Both berberine and hydrastine produced parasympatholytic and anesthetic effects when applied to the eyes. Hydrastine induced labor when taken orally by pregnant women (0.5 g; 1.30 mmol).

No chemical disposition, metabolism, or toxicokinetics data were found for goldenseal or hydrastine. In humans, berberine sulfate is absorbed through the skin. Following oral administration (species not provided), berberine was absorbed slowly, taking 4 hours to reach peak concentrations in plasma and another 4 hours to clear. In rats orally administered tritiated berberine chloride, blood levels of the compound leveled off at 4 - 24 hours; peak levels in liver and muscles occurred at 12 hours, while urinary excretion peaked at 12-24 hours. At 48 hours, the majority of the administered dose had been excreted in feces. Following i.v. administration to rats, the highest concentrations of berberine were found in the kidneys, with lower concentrations in the liver, lung, and brain. In rabbits, 24 hours after administration by gavage, small amounts of berberine were found in the heart, liver, and kidneys. Uptake into cells and across epithelia may involve a cation exchange mechanism.

Berberine sulfate injection reduced urine volume and urinary concentrations of sodium and chloride in dogs and rats. Additionally, dogs had lower urinary creatinine concentrations and rats had lower urinary potassium levels.

No acute exposure data for goldenseal were located. In mice, the oral LD_{50} dose for berberine is 329 mg/kg (0.98 mmol/kg), the s.c. LD_{50} dose is 18 mg/kg (0.054 mmol/kg), and the i.p. LD_{50} for berberine sulfate is 24.3 mg/kg (0.056 mmol/kg). In rats, the i.p. LD dose for berberine is greater than 500 mg/kg (>1.49 mmol/kg); while the LD_{50} doses for berberine sulfate are 14.5 mg/kg (0.033 mmol/kg) intramuscularly, 88.5 or 205 mg/kg (0.20 or 0.47 mmol/kg) i.p., and greater than 1000 mg/kg (>2.31 mmol/kg) orally. In rabbits, the s.c. LD_{Lo} dose is 100 mg/kg (0.30 mmol/kg). In rats, the i.p. LD_{50} dose for hydrastine is 104 mg/kg (0.271 mmol/kg).

When tested as protection from amphetamine toxicity, berberine chloride (5 mg/kg; 0.013 mmol/kg) and berberine sulfate (15 mg/kg; 0.035 mmol/kg) injected i.p. had no significant effect in mice. Berberine sulfate reduced rectal temperature in albino rats injected i.p. with 50 mg/kg (0.12 mmol/kg) and a single intraintestinal injection (10 mg/kg; 0.023 mmol/kg) into the duodenum of rats had no effect on the volume or acidity of gastric fluid, nor did it effect the

severity of gastric ulcers. When injected i.v. at 6 mg/kg (0.014 mmol/kg), it significantly increased the number of apomorphine-induced vomits in dogs. Berberine sulfate also reduced the blood pressure in rats, dogs, and cats following i.v. administration of 0.1-6.0 mg/kg (0.00023-0.014 mmol/kg).

Following i.p. administration at 10 and 15 mg/kg (0.023 and 0.035 mmol/kg), berberine sulfate induced lachrymation, pilomotor erection, and ptosis in mice, with maximal depression occurring two hours after drug administration. Cats administered 100 μg/0.2 mL or 1 mg/0.2 mL (0.0013 or 0.013 mM) berberine chloride i.v. were sedated, inactive, showed no interest in surroundings, and did not consume food; these responses were more pronounced at the higher dose. In contrast, oral administration of berberine chloride to mice (dose not provided) did not depress spontaneous movement or coordinative motor activity and i.p. injection of 5 mg/kg (0.013 mmol/kg) berberine chloride also had no tranquilizing or anticonvulsant properties. Ocular application of berberine (0.1% solution) did not have an anesthetic effect on rabbits or dogs.

Conflicting information regarding hydrastine toxicity was found. In one study, hydrastine hydrochloride (29.8 mg/kg; 71.0 mmol/kg) caused convulsions within one minute in 50% of the mice administered one i.v. injection, while in another study, hydrastine did not cause convulsions in mice, even when administered at lethal doses (dose range not provided).

No subchronic information was found for goldenseal or hydrastine. Subchronic exposure of rats given berberine chloride by gavage, 100 or 500 mg/kg (0.27 or 1.34 mmol/kg) for 7 or 14 days, significantly reduced scopolamine-induced amnesia effects but 100 mg/kg (0.27 mmol/kg) administered for 14 days did not significantly alter motor activity. Subchronic exposure of rats to 500 mg/kg/day (1.15 mmol/kg/day) berberine sulfate orally for 6 weeks did not induce histopathological changes in tissues or organs.

No chronic exposure data or teratogenicity or embryotoxicity studies were found for any of the compounds.

No data were found on carcinogenicity, although some studies tested the potential anticarcinogenic effect of berberine. In two experiments using mice, berberine did not inhibit the growth of mouse sarcoma S-180 tumor cells in ascites form. Berberine injected i.p. into mice was effective in preventing the growth of P388 leukemia cells (administered i.v. or intracerebrally). Berberine sulfate also significantly inhibited the tumor yield and incidence of mice bearing tumors which were initiated with DMBA and promoted with teleocidin. In rats implanted in the brain with 9L-2 rat gliosarcoma cells, berberine administration effectively killed 81% of the gliosarcoma cells.

No genotoxicity data were found for goldenseal or hydrastine. Berberine binds to DNA by intercalation with an AT base pair preference. As a DNA intercalating agent, berberine caused a general reduction in cellular RNA and protein synthesis and overproduction of the β and β1 subunits of RNA polymerase in *E. coli*. In sonicated and superhelical calf thymus DNA, berberine chloride induced DNA unwinding. Berberine chloride induced *his* gene mutations in *S. typhimurium* strain TA98 in the absence but not in the presence of metabolic activation. It was not mutagenic in strain TA100, with or without metabolic activation. When *S. cerevisiae* cells were cultured in growth medium in the absence of metabolic activation, berberine chloride induced *hom*3-10 frameshift mutations and cytoplasmic 'petite' mutations in a dose dependent manner; it also induced *cyh* crossing over. However, berberine chloride did not induce mutations at the *hom*3-10 or 'petite' mutations when added to cells incubated in

saline, nor did it induce *leu* gene conversions or *lys*1-1 and *his*1-7 point mutations when cells were cultured either in growth medium or saline. In a DNA damage assay, berberine chloride did not induce β-galactosidase activity in *E. coli* strain PQ37 in the presence or absence of S9. Treatment with berberine induced a dose dependent increase in sister chromatid exchanges (SCE) in 9L rat intracerebral gliosarcoma cells.

No immunotoxicity data were found for goldenseal or hydrastine. Berberine inhibited DNA synthesis in mitogen-stimulated human lymphocytes, but did not inhibit polymorphonuclear leukocyte activation, nor was it cytotoxic to target cells in a C3H/He mouse MM2 tumor cell assay.

Berberine inhibited the growth of cultured mouse sarcoma-180 tumor cells, an effect that was ameliorated by cotreatment with glucose. It also inhibited the growth of cultured human brain tumor cells and HepG2 human hepatoma cells, and was cytotoxic to Molt-4 and L1210 human leukemia cells and P-388 murine leukemia cells. However, berberine was not cytotoxic towards C38 murine colon adenocarcinoma cells or L1210 mouse leukemia cells. Berberine and berberine chloride induced apoptosis in Balb/c 3T3 fibroblast cells and human HL-60 leukemia cells, respectively. In cultured 9L rat gliosarcoma cells, berberine induced lysis, encystation, and degeneration. Berberine chloride inhibited the growth of cultured HeLa cells, and was toxic to T2/D1 human teratocarcinoma cells and F9 murine teratocarcinoma cells. Berberine sulfate inhibited the incorporation of $^{32}P_i$ into phospholipids induced by TPA and teleocidin. It also inhibited TPA-enhanced transport of ^{3}H-3-O-methyl-D-glucose into mouse fibroblast 3T3 cells. Berberine reversed the resistance of BEL-7402 human liver cancer cells to the cytotoxic effects of vincristine, but not of MCF-7/Adr human breast cancer cells to adriamycin. Down-regulation of the Ki-ras2 protooncogene was induced by berberine chloride in treated T2/D1 human teratocarcinoma cells.

Berberine did not induce phototoxicity in *E. coli* strains RT7h, RT8h, RT9h, or RT10h.

Berberine acted as an anticholinergic in isolated guinea pig ileum, isolated tracheal muscles of dogs, and isolated rectus muscle of frogs. Berberine sulfate exhibited an antiadrenergic response in isolated rabbit aortic strips, guinea pig seminal vesicle, and rat aortas. At low concentrations, it elicited a spasmogenic response in isolated guinea pig ileum, while at higher concentrations, a spasmolytic effect was observed. Berberine sulfate had no effect on tracheal muscle preparations from dogs or guinea pigs, isolated rabbit aortic strips, depolarized guinea pig ileum, or guinea pig seminal vesicle, but showed an antihistaminic response in isolated guinea pig ileum. It also potentiated calcium chloride-induced contractions and PGE_1-, $PGF_{1\alpha}$-, and $PGF_{2\alpha}$-induced contractions in isolated guinea pig ileum. Berberine sulfate was reported to both inhibit and potentiate $PGF_{2\alpha}$-induced contractions in isolated guinea pig ileum. It had a negative inotropic effect on isolated heart preparations and rabbit intestine; it had a positive inotropic and positive chronotropic effect on spontaneously beating atria of rats, guinea pigs, and rabbits. In isolated guinea pig ventricular papillary muscle, berberine inhibited the hypoxic-condition- or cromakalim-induced shortening of action potential and effective refractory period.

Hydrastine had no effect on adrenaline-induced contractions in rabbit aortic strips, nor on unstimulated guinea pig ileum or mouse vas deferens preparations, but it had a negative inotropic but positive chronotropic effect on spontaneously beating rat atrium, and inhibited electrically evoked contractions in guinea pig

ileum. Hydrastine induced a positive inotropic effect on electrically evoked contractions of isolated mouse vas deferens.

Berberine sulfate reduced bull sperm motility in a dose dependent manner.

Berberine and protoberberine alkaloids have varying DNA binding affinities, but generally binding is very weak; substitution in bulky groups of protoberberine alkaloids inhibited DNA binding. Another study found that only quaternary salts bind with DNA. Berberine inhibited topoisomerase II, but not topoisomerase I. The increased planarity of berberine is thought to account for its enhanced activity in topoisomerase II inhibition. Cleavage of both methylenedioxy and methoxyl groups from berberine forms a compound which is a potent topoisomerase I poison; minor variations in the protoberberines may substantially alter their pharmacological properties.

With regard to induction of convulsion following i.v. injection into mice, (-)-hydrastine was much less potent than (+)-hydrastine. Further, (-)-β-hydrastine and (±)-β-hydrastine were less potent than (+)-α-hydrastine and (±)-α-hydrastine in inducing binding to the $GABA_A$ receptor in rat brain synaptic membranes.

9.1 General Toxicology

9.1.1 Human Data

Goldenseal can cause convulsions, and irritation of the mouth, throat, and stomach when taken orally in toxic doses (doses not specified) (Hamon, 1990). Paresthesia, paralysis, respiratory failure, and death may follow. Chronic use of goldenseal may inhibit absorption of vitamin B. If taken in sublethal doses (doses not specified) during pregnancy, goldenseal may induce labor.

In a group of 12 patients with heart conditions refractory to conventional medical treatment, berberine markedly improved cardiac performance (Marin-Neto et al., 1988). Berberine was administered for 30 minutes by intravenous (i.v.) infusion at a rate of 0.02 mg/kg/min (0.000059 mmol/kg/min) followed by infusion at a rate of 0.2 mg/kg/min (0.00059 mmol/kg/min) for an additional 30 minutes. However, 4 of the 12 patients experienced “torsades de pointes” morphology (ventricular tachycardia characterized by a marked QT prolongation on the electrocardiogram) within 20 hours after berberine infusion, suggesting further studies need to be undertaken before berberine administration is extended to other heart failure patients.

Berberine had an anesthetic effect when injected (amount not specified) s.c. in a volunteer (Seery and Raymond, 1940). Additionally, berberine (0.5%) had parasympatholytic and anesthetic activity when applied to the eyes of one human volunteer (Medow and Greco, 1975). In another study conducted by Medow and Greco (1976), dilation of the pupils, temporary paralysis of pupil ciliary muscles, and corneal anesthesia were observed in a human volunteer treated ocularly with 3 drops of 0.1% and 0.5% berberine in methyl cellulose. Eye treatment with hydrastine (0.5%) exhibited the same parasympatholytic and anesthetic effects as berberine in a study of one human volunteer (Medow and Greco, 1975).

As occurs with goldenseal, oral administration of 0.5 g (1.3 mmol) hydrastine to pregnant women may induce labor (Grismondi et al., 1979).

9.1.2 Chemical Disposition, Metabolism, and Toxicokinetics

Chemical disposition, metabolism, and toxicokinetics data were not found for goldenseal or hydrastine. The following summarizes information found for berberine and berberine salts.

In humans, berberine sulfate is absorbed through the skin (HSDB, 1997). In another study, berberine was slowly absorbed following oral administration (dose and species not provided) (Rennick, 1981; cited by Baird et al., 1997). It took 4 hours to reach a peak concentration in plasma and another 4 hours for berberine to clear. As an organic cation, berberine uptake into cells and across epithelia may involve a cation exchanger mechanism.

When 35 mg/kg (0.10 mmol/kg) berberine was administered i.v. to Sprague-Dawley rats, the highest concentrations were found in the kidney, with lower concentrations found in the liver, lung, and brain, respectively (time since administration not provided) (Bodor and Brewster, 1983). Berberine was rapidly lost from the tissues, except for the brain.

In rats (strain not provided) orally administered tritiated berberine chloride (dose not provided), blood levels of the compound leveled off at 4-24 hours (Sakurai et al., 1976). Peak levels in the liver and muscles occurred at 12 hours, while urinary excretion peaked at 12-24 hours. At 48 hours, 2.7% of the administered dose had been excreted in the urine and 86% of the dose had been excreted in feces.

In another study (Yamahara et al., 1972), 72 hours after oral administration of 10 mg (0.027 mmol) berberine chloride to rats (strain not provided), 60-91% of the dose was recovered in feces.

Twenty-four hours after administration of berberine (dose not provided) by gavage to rabbits, only small amounts (not specified) of the compound were detected in the heart, liver, and kidneys (Wang et al., 1995).

Berberine sulfate (10 or 30 mg/kg; 0.023 or 0.069 mmol/kg) administered i.p. to adult male albino rats preloaded orally with normal saline caused a reduction in urinary volume over the following 24-hour period accompanied by a decline in urinary sodium and an increase in urinary potassium and chloride concentrations (Sabir et al., 1978). There was no change in urinary pH.

In dogs (breed not provided) i.v. administered sodium chloride solution, subsequent treatment with berberine sulfate (0.1-10 mg/kg; 0.00023-0.023 mmol/kg) by i.v. injection consistently reduced the urine volume and urinary concentrations of sodium, chloride, and

creatinine (Sabir et al., 1978). Urinary potassium concentration and urinary pH remained unchanged. The duration of the observation period was not specified.

9.1.3 Acute Exposure

Acute toxicity values for berberine, berberine sulfate, and hydrastine are presented in **Tables 1**, **2**, and **3**, respectively; acute toxicity values for goldenseal were not available. Other *in vivo* acute exposure data presented in this section are outlined in **Table 4**.

9.1.3.1 Oral Administration

No data were found for hydrastine.

There was no depression of spontaneous movement or coordinative motor activity in mice (strain not provided) treated orally with berberine chloride (dose and duration of exposure not provided) (Yamahara, 1976). There was also no inhibition of chemical- and electro-shock-induced convulsion, morphine-induced Straub's tail reaction, apomorphine-induced masticating motion, or aggressive behavior induced by electrical stimulation. Berberine chloride also did not potentiate a loss of righting reflex induced by hypnotics (compounds not specified).

9.1.3.2 Ocular Administration

No data were found for hydrastine.

Ocular application of a 0.1% solution of berberine (solvent not specified) to rabbits and dogs had no anesthetic effect (Seery and Raymond, 1940).

9.1.3.3 Intravenous Injection

In two studies, berberine sulfate at 0.1-6.0 mg/kg (0.00023-0.014 mmol/kg) (Sabir and Bhide, 1971) and 0.15-0.30 mg/kg (0.00035-0.00069 mmol/kg) (Sabir et al., 1978) reduced the blood pressure of rats (strain not provided). There was a reduction in blood pressure, left ventricular systolic pressure, and left ventricular end diastolic pressure in rats (strain not provided) injected i.v. with berberine (dose and duration of exposure not provided) (Fang et al.,

1987). Heart rate was initially increased, but then gradually decreased, and cardiac contractility values were unchanged to increased.

Berberine sulfate reduced the blood pressure in dogs and cats (0.1-6.0 mg/kg [0.00023-0.014 mmol/kg]) (strains not provided) (Sabir and Bhide, 1971).

There was a significant increase in tone and amplitude of intestinal movements in dogs administered 1 or 5 mg/kg (0.0023 or 0.012 mmol/kg) berberine sulfate i.v. (Kulkarni et al., 1972).

Table 1. Acute Toxicity Values for Berberine

Route	Species (sex and strain)	LD_{50}	Reference
oral	mouse (sex and strain n.p.)	329 mg/kg (0.98 mmol/kg)	Journal of Pharmacy 82:726 (1962; cited by RTECS, 1996)
s.c.	mouse (sex and strain n.p.)	18 mg/kg (0.054 mmol/kg)	Russian Pharmacology and Toxicology (English translation of Farmakologiya I Toksikologiva 31:129 (1968; cited by RTECS, 1996)
		LD_{Lo}	
i.p.	rat (sex and strain n.p.)	>500 mg/kg (>1.49 mmol/kg)	Natl. Acad. Sci.5:17 (1953; cited by RTECS, 1996)
s.c.	rabbit (sex and strain n.p.)	100 mg/kg (0.30 mmol/kg)	Abdernalden's Handbuch der Biologischen Arbeitsmethoden 4:1289 (1935; cited by RTECS, 1996)

Abbreviations: i.p. = intraperitoneal; n.p. = not provided; s.c. = subcutaneous

Table 2. Acute Toxicity Values for Berberine sulfate

Route	Species (sex and strain)	LD_{50}	Reference
i.m.	rat (sex and strain n.p.)	14.5 mg/kg (0.033 mmol/kg)	Kowalewski et al. (1975)
i.p.	rat (albino, sex n.p.)	205 mg/kg (0.47 mmol/kg)	Kulkarni et al. (1972)
	rat (sex and strain n.p.)	88.5 mg/kg (0.20 mmol/kg)	Kowalewski et al. (1975)
	mouse (male and female albino)	24.3 mg/kg (0.056 mmol/kg)	Sabir and Bhide (1971)
oral	rat (sex and strain n.p.)	>1000 mg/kg (>2.31 mmol/kg)	Kowalewski et al. (1975)

Abbreviations: i.m. = intramuscular; i.p. = intraperitoneal; n.p. = not provided

Table 3. Acute Toxicity Values for Hydrastine

Route	Species (strain)	LD_{50}	Reference
i.p.	rat (sex and strain n.p.)	104 mg/kg (0.248 mmol/kg)	MacDougal (year n.p.; cited by RTECS, 1996)

Abbreviations: i.p. = intraperitoneal; n.p. = not provided

Table 4. Acute Exposure to Berberine and Hydrastine

Species, Strain, Age	Number and sex of Animals	Chemical Form, Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
9.1.3.1 Oral Administration						
mouse (strain and age n.p.)	n.p.	berberine hydrochloride, purity n.p.	n.p.	n.p.	There was no depression of spontaneous movement or coordinative motor activity. There was also no inhibition of chemical- and electro-shock-induced convulsion, morphine-induced Straub's tail reaction, apomorphine-induced masticating motion, or aggressive behavior induced by electrical stimulation. Berberine hydrochloride did not potentiate a loss of righting reflex induced by hypnotics (compounds not specified).	Yamahara (1976)
9.1.3.2 Ocular Administration						
rabbit (strain and age n.p.)	n.p.	berberine, purity n.p.	0.1% solution (solvent n.p.)	n.p.	There was no anesthetic effect. No other experimental details were provided.	Seery and Raymond (1940)
dog (strain and age n.p.)	n.p.	berberine, purity n.p.	0.1% solution (solvent n.p.)	n.p.	There was no anesthetic effect. No other experimental details were provided.	Seery and Raymond (1940)
9.1.3.3 Intravenous Injection						
rat (strain and age n.p.)	n.p.	berberine sulfate, purity n.p.	0.15-0.30 mg/kg (0.00035-0.00069 mmol/kg) or 0.1-6.0 mg/kg (0.00023-0.014 mmol/kg)	n.p.	There was a reduction in blood pressure. Rats were anesthetized with urethane.	Sabir et al. (1978); Sabir and Bhide (1971)
rat (strain and age n.p.)	n.p.	berberine, purity n.p.	n.p.	n.p.	There was a reduction in blood pressure, left ventricular systolic pressure, and left ventricular end diastolic pressure. Heart rate was initially increased, but then gradually decreased. Cardiac contractility values were unchanged to increased. The rats were conscious during testing.	Fang et al. (1987)
dog (strain and age n.p.)	n.p.	berberine sulfate, purity n.p.	0.1-6.0 mg/kg (0.00023-0.014 mmol/kg)	n.p.	There was a reduction in blood pressure. Dogs were anesthetized with sodium pentobarbitone (35 mg/kg i.p.).	Sabir and Bhide (1971)

Abbreviations: M = male; n.p. = not provided; s.c. = subcutaneous

Table 4. Acute Exposure to Berberine and Hydrastine

Species, Strain, Age	Number and sex of Animals	Chemical Form, Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
cat (strain and age n.p.)					There was a reduction in blood pressure. Cats were anesthetized with sodium pentobarbitone (35 mg/kg i.p.).	

Table 4. Acute Exposure to Berberine and Hydrastine (continued)

Species, Strain, Age	Number and sex of Animals	Chemical Form, Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
dog (strain and age n.p.)	n.p.	berberine sulfate, purity n.p.	1 or 5 mg/kg (0.23 or 0.012 mmol/kg)	n.p.	There was a significant increase in tone and amplitude of intestinal movements. The dogs were anesthetized. No other experimental details were given.	Kulkarni et al. (1972)
dog (strain and age n.p.)	exposed: 6 controls: 6 male and female, not broken down by sex	berberine sulfate, purity n.p.	1 or 6 mg/kg (0.23 or 0.014 mmol/kg)	single dose; observation period n.p.	Apomorphine hydrochloride (0.1 mg/kg) was administered intramuscularly 1 h after injection of berberine sulfate. Berberine sulfate at 6 mg/kg (0.014 mmol/kg) significantly increased the number of vomits in dogs, as compared to dogs injected only with apomorphine hydrochloride.	Sabir et al. (1978)
cat (strain and age n.p.)	3, sex n.p.	berberine hydrochloride	100 µg/ 0.2 mL (0.0013 mM) in 1 cat and 1 mg/ 0.2 mL (0.013 mM) in 2 cats	single dose; observed continuously for 3 h an then unspecified intervals until the cats returned to normal	Cats were sedated, inactive, disinterested, and did not consume food. Effects were dose-dependent. The cat administered 100 µg/0.2 mL (0.0013 mM) recovered after 20 h. At the 1 mg/0.2mL (0.013 mM) dosage, the recovery time was 36-40 h.	Shanbhag et al. (1970)
mouse (strain and age n.p.)	n.p.	hydrastine hydrochloride, purity n.p.	n.p.	single injection; 1-min observation period	The CD_{50} (dose producing convulsions in 50% of animals within 1 minute of injection) for (+)-hydrastine hydrochloride was 0.16mg/kg (0.00038 mmol/kg) and for (-)-hydrastine hydrochloride was 29.8 mg/kg (71.0 mmol/kg).	Huang and Johnston (1990)
mice (strain and age n.p.)	n.p.	hydrastine, purity n.p.	n.p.	single dose; observed for the 1st 30 m and then 12 and 24 h later	β-Hydrastine did not induce convulsions, even at lethal doses. No other experimental details were given.	Bartolini et al. (1990)
9.1.3.4 Intraperitoneal Injection						

Abbreviations: M = male; n.p. = not provided; s.c. = subcutaneous

Table 4. Acute Exposure to Berberine and Hydrastine (continued)

Species, Strain, Age	Number and sex of Animals	Chemical Form, Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
albino mice (age n.p.)	M, number n.p.	berberine hydrochloride (purity n.p.)	5 mg/kg (0.013 mmol/kg) after 4 mg/kg amphetamine	single dose; observation period n.p.	Amphetamine administration markedly increased motor activity. Berberine administration at peak activity of amphetamine sedated the mice in 5 min., reducing motor activity below the control (no treatment).	Shanbhag et al. (1970)
	40 (10 at each dose level), sex n.p.		5 mg/kg (0.013 mmol/kg) berberine followed in _ h by 25, 30, 35, or 40 mg/kg pentobarbitone		Berberine significantly increased sleeping time at the 35 and 40 mg/kg pentobarbitone dose levels.	
albino mice (age n.p.)	10M	berberine hydrochloride (purity n.p.)	5 mg/kg (0.013 mmol/kg) berberine followed in 0.5 h by 15 mg/ kg amphetamine	single dose; observed for 20 h	No significant modification in aggregate or segregate amphetamine toxicity.	Shanbhag et al. (1970) (cont.)
	30 (10 at each dose level), sex n.p.	berberine hydrochloride (purity n.p.)	5 mg/kg (0.013 mmol/kg) berberine followed in 0.5 h by 20, 50, or 75 mg/ kg leptazol	single dose; observation period n.p.	Berberine had no significant action on convulsions caused by leptazol.	
	20M (10 treatment and 10 control)		5 mg/kg (0.013 mmol/kg) berberine followed a shock of 120 mA for 0.2 sec.		Berberine did not protect mice from supramaximal electroshock convulsions.	
freshly weaned albino mice	32 (16 controls, and 8 at each dose level), sex n.p.	berberine sulfate (purity n.p.)	10 and 15 mg/kg (0.023 and 0.035 mmol/kg)	single dose; observed after 5, 15, and 30 min. and after 1, 2, and 4 h and then daily for 4 days.	Maximal overall depression by berberine exposure observed after 2 h. Stimulation of respiration observed more with the higher dose group. Complete recovery noted after 24 h.	Mardikar et al. (1973)

Abbreviations: M = male; n.p. = not provided; s.c. = subcutaneous

Table 4. Acute Exposure to Berberine and Hydrastine (continued)

Species, Strain, Age	Number and sex of Animals	Chemical Form, Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
	n.p.		15 mg/kg (0.035 mmol/kg)	single dose; observation period n.p.	The rotating rod test was used with previously-trained mice. Berberine-treated animals lost balance quickly and fell down in less than 300 sec.	
	10 for each test, sex n.p.			single dose; 2 and 4 h., respectively for the two tests.	Previously screened mice were used in both the chimney and the traction tests. Mice which could reach the 20 cm mark within 30 sec. without treatment (chimney test) were not able to perform the test for up to 4 h after berberine treatment. In the traction test, the animal must touch a wire within 5 sec. to be sucessul. 50% of the animals could not perform the test in the 2 h immediately after drug administration.	
freshly weaned albino mice	20, sex n.p.	berberine sulfate (purity n.p.)	15 mg/kg (0.035 mmol/kg)	single dose; 15 minute observation period	In the evasion test, mice were placed in a small open box within a large trough to test residual curiosity. Two inclined planes allowed escape from the small box. Berberine treatment significantly lowered residual curiosity levels.	Mardikar et al. (1973) (cont.)
	20 (10 treated and 10 control), sex n.p.			single dose; half hourly for 4 _ h	Berberine treatment lowered rectal temperature, with a peak drop after 2 h.	
	20 (10 treated and 10 control)		treatment:15 mg/kg (0.035 mmol/kg) berberine followed in 2 h by 40 mg/kg pentobarbitone control: 40 mg/kg pentobarbitone	n.p.	Berberine pretreatment dramatically increased pentobarbitol-induced sleeping time.	
	40 (20 treated and 20 control)		treatment: 15 mg/kg (0.035 mmol/kg) berberine followed by 14 mg/kg amphetamine control: 14 mg/kg amphetamine	24 h	Amphetamine-induced mortality rates were not changed by berberine pretreatment. However, immediately after amphetamine administration, animals pretreated with berberine did not show hyperactivity as compared to controls.	

Abbreviations: M = male; n.p. = not provided; s.c. = subcutaneous

Table 4. Acute Exposure to Berberine and Hydrastine (continued)

Species, Strain, Age	Number and sex of Animals	Chemical Form, Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
rats (strain and age n.p.)	30 (10 in each treatment group), sex n.p.	berberine hydrochloride (purity n.p.)	5 mg/kg (0.013 mmol/kg) berberine in untreated rats, rats treated with 5 mg/kg morphine, or 15 mg/kg pentobarbitone (berberine was injected _ h prior to treatment)	single dose; observation period n.p.	Hot wire test was used to test pain threshold. Berberine did not produce any effect.	Shanbhag et al. (1970)
rat (albino adult)	M, number n.p.	berberine sulfate, purity n.p.	10, 30, or 50 mg/kg (0.023, 0.069, or 0.12 mmol/kg)	single dose; 5-h observation period	The highest dose significantly lowered rectal temperature; hypothermia persisted over the entire observation period. The 2 lower doses had no effect on rectal temperature. Rectal temperature was the only parameter evaluated.	Sabir et al. (1978)
rat (albino, age n.p.)	10 M in each group (exposed and controls)	berberine sulfate, purity n.p.	10 or 30 mg/kg (0.023 or 0.069 mmol/kg)	single dose; 5-h observation period	All rats were previously injected with Brewer's yeast suspension s.c. Only rats that exhibited an increase in temperature of at least 1.5°C after 18 h were included in this study. Berberine sulfate (10 or 30 mg/kg; 0.023 or 0.069 mmol/kg) significantly lowered the rectal temperature of pyretic rats.	Sabir et al. (1978) (cont.)
cat (strain and age n.p.)	9 (3 at each dose level), sex n.p.	berberine hydrochloride (purity n.p.)	5, 20, or 40 mg/kg (0.013, 0.054, or 0.11 mmol/kg) in a volume of 5 mL	single dose; observed continuously for 3 h and then hourly for the next 12 h	The following effects were noted: sedation, retching, urination, and defecation with straining in 8 cats (effects lasted less than 2 h); rage reaction in 1 cat (returned to normal after 1 h).	Shanbhag et al. (1970)
9.1.3.5 Intraintestinal Injection						
rat (Shay, age n.p.)	12 in both exposed and control groups, sex, n.p.	berberine sulfate, purity n.p.	10 mg/kg (0.023 mmol/kg)	single dose; 18-h observation	Dose was injected into the duodenum. Controls injected with distilled water. Berberine sulfate had no effect on the volume or acidity of gastric fluid, or on the severity of gastric ulcers.	Sabir et al. (1978)

Abbreviations: M = male; n.p. = not provided; s.c. = subcutaneous

Berberine sulfate significantly increased the number of apomorphine-induced vomits in dogs (strain not provided) injected i.v. with 6 mg/kg (0.014 mmol/kg) (Sabir et al., 1978). A lower dose of berberine sulfate (1 mg/kg; 0.0023 mmol/kg) had no effect on the number of vomits.

Cats showed a dose-dependant response to berberine chloride when given in doses of 100 µg/0.2 mL (0.0013 mM) and 1 mg/0.2 mL (0.013 mM), respectively (Shanbhag et al., 1970). With berberine chloride administration, cats were sedated, inactive, showed no interest in surroundings, and did not consume food.

Hydrastine hydrochloride caused convulsions in male mice (strain not provided) when treated once by i.v. injection (Huang and Johnston, 1990). The CD_{50} (dose producing convulsions in 50% of animals within 1 minute of injection) was 29.8 mg/kg (71.0 mmol/kg). However, in another study, hydrastine did not induce convulsions even when administered at lethal doses (dose range not provided) to mice (strain not provided) (Bartolini et al., 1990).

9.1.3.4 Intraperitoneal Injection

No data were found for hydrastine.

Berberine administered i.p. appears to affect the central nervous system, producing sedation in mice (Shanbhag et al., 1970). However, it was found to have no tranquilizing or anticonvulsant properties in white mice administered a 5 mg/kg (0.013 mmol/kg) i.p. dose of berberine chloride. Berberine also had no analgesic properties when administered i.p. (5 mg/kg, 0.013mmol/kg) as treatment for aggregate or segregate amphetamine toxicity in mice.

Berberine sulfate doses of 10 and 15 mg/kg (0.023 and 0.035 mmol/kg) administered i.p. to male and female albino mice produced maximal depression of the autonomic system two hours after drug administration (Mardikar et al., 1973). It caused lachrymation, pilomotor erection, and ptosis. Treatment with 15 mg/kg (0.035 mmol/kg) lowered rectal temperature significantly, with

a peak drop occurring after two hours. No significant effect was identified when berberine sulfate (15 mg/kg; 0.035 mmol/kg i.p.) was tested as protection against amphetamine toxicity.

Berberine had no analgesic properties when administered i.p. (5 mg/kg, 0.013 mmol/kg) as a pain threshold reducer in rats (Shanbhag et al., 1970). Berberine sulfate (50 mg/kg; 0.12 mmol/kg) significantly lowered the rectal temperature in adult male albino rats injected once i.p. and observed for 5 hours (Sabir et al., 1978). Administration of lower doses (10 or 30 mg/kg; 0.023 or 0.069 mmol/kg) had no effect on the normal rectal temperatures. However, when the lower doses were administered to male albino rats that had been previously injected s.c. with Brewer's yeast suspension, rectal temperature was significantly decreased.

Berberine produced sedation in cats (Shanbhag et al., 1970). Nine cats were dosed with 5, 20, or 40 mg/kg (0.013, 0.054, 0.11 mmol/kg) berberine hydrochloride (3 at each dose level). Eight cats experienced sedation, retching, urination, and defecation with straining, with effects lasting less than 2 hours; one cat experienced a rage reaction, but returned to normal after 1 hour.

9.1.3.5 Intraintestinal Injection

No data were found for hydrastine.

A single injection of berberine sulfate (10 mg/kg; 0.023 mmol/kg) into the duodenum of Shay rats had no effect on the volume or acidity of gastric fluid, or on the severity of gastric ulcers (Sabir et al., 1978).

9.1.4 Short-Term and Subchronic Exposure

No information relating to short-term and subchronic exposure was found for goldenseal or hydrastine. The studies described in this section are presented in **Table 5**.

Berberine chloride (150 mg/kg; 0.00040 mmol/kg) did not stimulate liver regeneration in partially hepatectomized Charles River and Holtzman male rats when injected s.c. daily for 7 days (Gershbein and Pedroso, 1985). The rats were killed 3 days after the last injection.

Administration by gavage of berberine chloride (100 or 500 mg/kg; 0.27 or 1.34 mmol/kg in 0.9% saline) for 7 or 14 days significantly reduced scopolamine-induced amnesia effects in male Sprague-Dawley rats (Peng et al., 1997) when amnesia was measured using performance of a passive avoidance response task. The antiamnesic effect of berberine chloride was significantly increased by administration of physostigmine or neostigmine, and was completely reversed by administration of scopolamine *N*-methyl bromide. There was not a significant change in motor activity in male Sprague-Dawley rats treated by gavage with berberine chloride (100 mg/kg; 0.27 mmol/kg in 0.9% saline) for 14 days (Peng et al., 1997). Combined administration of scopolamine and berberine chloride (500 mg/kg; 1.34 mmol/kg) also did not affect motor activity of rats, as compared to the motor activity of rats treated only with scopolamine.

There were no histopathological changes in tissues or organs of rats (strain not given) treated orally with berberine sulfate (500 mg/kg/day; 1.15 mmol/kg/day) for 6 weeks (Kowalewski et al., 1975). It was not specified which tissues and organs were examined.

9.1.5 Chronic Exposure

No data were found.

Table 5. Short-Term and Subchronic Exposure to Berberine

Species, Strain, Age	Number and sex of Animals	Chemical Form, Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
Charles River and Holtzman rats (age n.p.)	13-18 M	berberine chloride	150 mg/kg (0.00040 mmol/kg), s.c.	berberine chloride administered for 7 d	Rats were partially hepatectomized before berberine hydrochloride administration. Berberine chloride did not stimulate liver regeneration.	Gershbein and Pedroso (1985)
rat (Sprague-Dawley, age n.p.)	exposed: 12-18 M per group controls: 12-18 M	berberine hydrochloride, purity n.p.	100 or 500 mg/kg (0.27 or 1.34 mmol/kg) by gavage in 0.9% saline	7 or 14 days; training trial carried out 1 h after administration of last dose	Rats were previously treated i.p. with 1.0 mg/kg scopolamine and tested for compound-induced amnesia using performance of a passive avoidance response task as an indicator. Administration of berberine hydrochloride (100 or 500 mg/kg; 0.27 or 1.34 mmol/kg) for 7 or 14 days significantly improved scopolamine-induced amnesia. The antiamnesic effect of berberine hydrochloride was significantly increased by administration of physostigmine (0.02 mg/kg i.p.) or neostigmine (0.02 mg/kg i.p.), and was completely reversed by administration of scopolamine *N*-methylbromide (0.05 mg/kg i.p.).	Peng et al. (1997)
	exposed: 6 M per group controls: 6 M			14 days; motor activity measurement carried out 1 h after administration of last dose	There was no change in motor activity of berberine hydrochloride-treated rats, as compared to vehicle-treated controls. Combined administration of scopolamine (1.0 mg/kg i.p.) and berberine hydrochloride (500 mg/kg; 1.34 mmol/kg) also did not affect motor activity of rats, as compared to the motor activity of rats treated only with scopolamine.	
rat (strain and age n.p.)	n.p.	berberine sulfate, purity n.p.	500 mg/kg/d orally (1.15 mmol/kg/d)	6 wk; observation period n.p.	There were no histopathological changes in tissues or organs (not specified).	Kowalewski et al. (1975)

Abbreviations: i.p. = intraperitoneally; M = male; n.p. = not provided

9.2 Teratogenicity and Embryotoxicity

No data were found.

9.3 Carcinogenicity

No data on carcinogenicity were found, although some studies were conducted to test the anticarcinogenicity of berberine. These studies are described in **Section 9.4**.

9.4 Anticarcinogenicity

The studies described in this section are presented in **Table 6**; no information was available for goldenseal or hydrastine.

Berberine injected i.p. into dd mice inoculated with mouse sarcoma-180 ascites cells did not exhibit antitumor activity (measured as the total packed cell volume [TPCV] of sarcoma-180 ascites cells) (Hoshi et al., 1976). Mice were administered 3, 10, or 30 mg/kg (0.0089, 0.030, or 0.089 mmol/kg) berberine i.p. daily for 5 days, starting 24 hours after i.p. transplant of the ascites cells.

Creasey (1979) performed a comparative study of the biochemical interactions of berberine on sarcoma-180 tumor cells in the ascites form using *in vivo* and *in vitro* analyses. The first *in vivo* analysis investigated the effect of berberine on the lifespan of Swiss white mice inoculated i.p. with sarcoma-180 ascites cells and then administered 2.5, 5, 10, 15, or 20 mg/kg/day berberine chloride (0.0067, 0.013, 0.027, 0.040, or 0.054 mmol/kg/day) i.p. for 5 days. The berberine chloride doses were initially given 24 hours after transplantation of tumor cells. Survival of tumor-inoculated mice was not increased by treatment with berberine chloride; in fact a dose-dependent decrease in life span compared to mice inoculated with tumor cells was noted. The second *in vivo* analysis assessed Swiss white mice administered 10 mg/kg (0.027 mmol/kg) berberine chloride i.p. 30 minutes before injection of [1-^{14}C]glycine or [methyl-^{3}H]thymidine. Berberine chloride had only minimal effects on the incorporation of the precursors, inhibiting incorporation of glycine into protein by 17% and thymidine into DNA by 14%. In a third

analysis, cells were harvested 30 minutes after treating Swiss white mice *in vivo* with 10 mg/kg (0.027 mmol/kg) berberine chloride i.p. The harvested cells were washed and then incubated with labeled glycine *in vitro*. Treatment with berberine chloride resulted in an 85% inhibition of glycine into protein. In a fourth analysis using an *in vitro* approach, 5 µg/mL (0.013 µM) berberine chloride and glucose (varying doses) were incubated in medium with the sarcoma-180 cell suspension for 30 minutes before the addition of [^{14}C]glycine or [^{3}H]thymidine. This treatment partially or completely blocked the berberine chloride-induced inhibition of glycine and

Table 6. Anticarcinogenic Activity of Berberine

Species, Strain, Age	Biological Endpoint	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
sarcoma-180 ascites transplanted i.p. into dd mice	total packed cell volume (TPCV)	berberine, purity n.p.	3, 10, or 30 mg/kg/d (0.0089, 0.030, or 0.089 mmol/kg/d) i.p. for 5 d, starting 24 h after transplant of tumor cells	Berberine had no antitumor activity.	TPCV was measured 7 days after transplant of ascites.	Hoshi et al. (1976)
sarcoma-180 ascites transplanted i.p. into Swiss white mice	survival	berberine hydrochloride, purity n.p.	2.5, 5, 10, 15, or 20 mg/kg/d (0.0067, 0.013, 0.027, 0.040, or 0.054 mmol/kg/d) i.p. for 5 d, starting 24 h after transplant of tumor cells	There was no prolongation of survival. Life span decreased with increasing berberine hydrochloride dose.	Only 3 injections of the 15 and 20 mg/kg doses were tolerated by the mice.	Creasey (1979)
	incorporation of thymidine into DNA and glycine into protein		10 mg/kg (0.027 mmol/kg) i.p. 30 min before injection of [1-^{14}C]glycine or [methyl-^{3}H]thymidine	Glycine and thymidine uptake were inhibited by 17% and 14%, respectively, *in vivo*.	To test effects of glucose, berberine hydrochloride and glucose were incubated *in vitro* 30 min before the addition of [1-^{14}C]glycine or [methyl-^{3}H]thymidine. Addition of glucose to the incubation medium partially or completely blocked the berberine hydrochloride-induced inhibition of thymidine into DNA and glycine into protein, respectively. The author noted that the failure of berberine to inhibit growth *in vivo* may be related to the effects of glucose.	
	incorporation of glycine into protein		10 mg/kg (0.027 mmol/kg) i.p. 30 min. before cells were harvested, washed, and then incubated with [1-^{14}C]glycine *in vitro.*	Incorporation of glycine into protein was inhibited by 85%.		
BDF mice (male, age n.p.)	24 (6 at each treatment level and 6 in control group)	berberine, purity n.p.	P388 lymphocytic leukemia cells administered i.p. or intracerebrally. Berberine administered i.p. 3 times a day on day 2, 6, and 10 at treatment levels of 5, 10, or 20 mg/kg (0.015, 0.030, or 0.059 mmol/kg).	Prevented growth of lymphocytic leukemia cells.		Bodor and Brewster (1983)

Abbreviations: DMBA = 7,12-dimethylbenz[*a*]anthracene; i.p. = intraperitoneally; n.p. = not provided

Table 6. Anticarcinogenic Activity of Berberine (continued)

Species, Strain, Age	Biological Endpoint	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
mouse (8-wk-old female ICR)	tumor induction	berberine sulfate, purity n.p.	initiation: Single application of 100 µg DMBA onto dorsal skin promotion: Teleocidin (2.5 µg) applied dermally twice/wk for 18 wk starting 1 wk after initiation. Berberine sulfate (0.5 mg; 0.0012 mmol) in ethanol/dimethyl sulfoxide applied dermally 40 min before each teleocidin application	Tumor yield and incidence of tumor-bearing mice was significantly inhibited by berberine sulfate. At wk 18, approximately 85% of controls had tumors versus approximately 12% of berberine-sulfate-treated mice. The location of the tumors was not specified.	Controls received DMBA and teleocidin only. Berberine sulfate was not administered alone to any animals.	Nishino et al. (1986)
9L or BCNU-resistant 9L-2 rat gliosarcoma cells implanted in brain of adult male Fischer 344 rats	*in vitro* colony forming efficiency of implanted cells	berberine, purity n.p.	*9L cells*: berberine alone (10 mg/kg; 0.030 mmol/kg i.p.); BCNU alone (4.43 mg/kg i.p.); or berberine (10 mg/kg; 0.030 mmol/kg i.p.) and BCNU (4.43 mg/kg i.p.) *9L-2 cells*: berberine alone (10 mg/kg; 0.030 mmol/kg i.p.); BCNU alone (6.66 mg/kg i.p.); or berberine (10 mg/kg; 0.030 mmol/kg i.p.) and BCNU (6.66 mg/kg i.p.) (dose was administered 14-15 d after tumor cell implantation)	In 9-L tumor-bearing rats, berberine alone and BCNU alone achieved 81% and 76% cell death, respectively. Treatment with both compounds produced 95.2% cell death. In 9L-2 tumor-bearing rats, berberine alone was inactive.	Rats were killed 24 h after berberine and/or BCNU treatment. Brain tumors were removed and converted into single-cell suspensions which were incubated for 12-14 days.	Zhang et al. (1990)

Abbreviations: DMBA = 7,12-dimethylbenz[*a*]anthracene; i.p. = intraperitoneally; n.p. = not provided

thymidine incorporation. The author concluded that the failure of berberine to inhibit growth *in vivo* may be related to the effects of glucose.

Berberine (purity not given) at 5, 10, and 20 mg/kg (0.015, 0.030, and 0.059 mmol/kg) i.p. was effective in preventing the growth of P388 leukemia cells administered i.v. or intracerebrally to male BDF mice (Bodor and Brewster, 1983). Berberine was administered on days 2, 6, and 10.

In a 2-stage mouse skin carcinogenicity study, berberine sulfate significantly inhibited the tumor yield and the incidence of tumor-bearing animals initiated with 7,12-dimethylbenz[*a*]anthracene (DMBA) and promoted with teleocidin (Nishino et al., 1986). Female ICR mice were initiated with a single application of DMBA onto dorsal skin. One week later, teleocidin was applied dermally twice/week for 18 weeks; berberine sulfate (0.5 mg; 0.0012 mmol) dissolved in ethanol/dimethyl sulfoxide was applied dermally 40 minutes before each teleocidin application. At week 18, approximately 85% of controls had tumors, whereas only approximately 12% of berberine-sulfate-treated mice did. Berberine sulfate was not administered alone to any animal.

Zhang et al. (1990) evaluated the anticarcinogenic activity of berberine and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) in adult male Fischer 344 rats implanted in the brain with 9L or BCNU-resistant 9L-2 rat gliosarcoma cells. Rats bearing 9L tumor cells were treated i.p., 14-15 days after implantation, with berberine alone (10 mg/kg; 0.030 mmol/kg), BCNU alone (4.43 mg/kg), or a combination of berberine (10 mg/kg; 0.030 mmol/kg) and BCNU (4.43 mg/kg). Rats bearing 9L-2 tumor cells were similarly treated, but received a higher dose of BCNU (6.66 mg/kg). Rats were killed 24 hours after treatment. Brain tumors were removed and converted into single-cell suspensions which were incubated for 12-14 days. In 9-L tumor-bearing rats, berberine alone and BCNU alone achieved 81% and 76% cell death, respectively. Treatment with both compounds produced 95.2% cell death. In 9L-2 tumor-bearing rats, berberine alone was inactive.

9.5 Genotoxicity

No information was found for goldenseal or hydrastine. The studies on berberine that are described in this section are also presented in **Table 7**.

9.5.1 Acellular Assays

Berberine chloride induced DNA unwinding in sonicated and superhelical calf thymus DNA (Davidson et al., 1977). It binds to DNA by intercalation (Rungsitiyakorn et al., 1981; Smekal and Kubova, 1982), with an AT base pair preference (Kumar et al., 1993).

Table 7. Genotoxicity of Berberine

Test System	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
9.5.1 Acellular Assays							
sonicated and superhelical calf thymus DNA	induction of DNA unwinding	n.p.	berberine chloride	0.002-0.1 mol	positive for both sonicated and superhelical DNA		Davidson et al. (1977)
sonicated calf thymus DNA	DNA binding	n.p.	berberine, purity n.p.	0.000034 mol	positive (probably by intercalation)		Rungsitiyakorn et al. (1981)
calf thymus DNA	DNA binding	n.p.	berberine chloride, chemically pure	1 and 48.44 µM	positive (binds by intercalation)		Smekal and Kubova (1982)
calf thymus DNA	DNA binding	n.p.	berberine chloride, purity n.p.	n.p.	positive (showing an AT base pair preference)		Kumar et al. (1993)
9.5.2 Prokaryotic Systems							
Salmonella typhimurium strains TA98 and TA100	*his* gene mutations	+/-	berberine hydrochloride, purity n.p.	n.p.	positive (TA98 without S9) negative (TA98 with S9; TA100 with or without S9)	Berberine hydrochloride was weakly mutagenic (2 revertants/µg/plate) in TA98 in the absence of S9.	Nozaka et al. (1990)
Escherichia coli strain PQ37	induction of β-galactosidase activity	+/-	berberine chloride, purity n.p.	1, 5, 10, 50, 100, 500, 1000, 5000, or 10000 ng/plate (0.0027, 0.013, 0.027, 0.13, 0.27, 1.34, 2.68, 13.4, or 26.8 nM) for 2 h	negative at all doses, with and without S9	SOS chromotest was used.	Pasqual et al. (1993)
9.5.3 Lower Eukaryotic Systems							
Saccharomyces cerevisiae haploid strain XV-185-14c	*hom*3-10 frameshift mutation	-	berberine chloride, purity n.p.	in saline, 75, 150, 225, or 300 µg/mL (0.20, 0.40, 0.60, or 0.80 µM) in growth medium, 10, 20, 30, 40, or 50 µg/mL (26.8, 53.6, 80.5, 107, or 134 nM)	positive (20-50 µg/mL; 53.6-134 nM in growth medium) negative at 10 µg/mL (26.8 nM) in growth medium and 75-300 µg/mL (0.20-0.80 µM) in saline	Cells were maintained either in saline or in growth medium.	Pasqual et al. (1993)
	cytoplasmic 'petite' mutation						

Abbreviations: n.p. = not provided

Table 7. Genotoxicity of Berberine (continued)

Test System	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
S. cerevisiae haploid strain XV-185-14c	*cyh* crossing-over	-	berberine chloride, purity n.p.	in saline, 75, 150, 225, or 300 µg/mL (0.20, 0.40, 0.60, or 0.80 µM) in growth medium, 25, 50, 75, or 100 µg/mL (67.1, 134, 201, or 268 µM)	positive at 25-100 µg/mL (67.1-268 µM) in growth medium negative at 75-300 µg/mL (0.20-0.80 µM) in saline	cells were maintained either in saline or in growth medium	Pasqual et al. (1993) (cont.)
	leu gene conversion				negative		
	*lys*1-1 point mutation			in saline, 75, 150, 225, or 300 µg/mL (0.20, 0.40, 0.60, or 0.80 µM) in growth medium, 10, 20, 30, 40, or 50 µg/mL (26.8, 53.6, 80.5, 107, or 134 nM)	negative		
	*his*1-7 point mutation						
9.5.4 *In Vitro* Mammalian Systems							
9L rat intracerebral gliosarcoma cells	sister chromatid exchange (SCE)	n.p.	berberine, purity n.p.	25-150 µg/mL (0.07-0.45 µM) for 2 h	There was a dose-dependent increase in SCE, up to 2.7-fold at the highest dose, as compared to untreated cells.	Cells were washed after treatment and incubated for 2 replication cycles (approximately 30 h)	Zhang et al. (1990)

Abbreviations: n.p. = not provided

9.5.2 Prokaryotic Systems

Berberine chloride (dose not provided) induced *his* gene mutations in *S. typhimurium* strain TA98 in the absence but not the presence of metabolic activation, and was nonmutagenic in strain TA100 in the presence or absence of metabolic activation (Nozaka et al., 1990).

Using the SOS chromotest, berberine chloride (1-10,000 ng, 0.0000027-0.027 µM for 2 hours) did not induce β-galactosidase activity in *E. coli* strain PQ37 in either the presence or absence of S9 (Pasqual et al., 1993).

9.5.3 Lower Eukaryotic Systems

Berberine chloride was tested for induction of mutation and recombination in *S. cerevisiae* which were either cultured and maintained in growth medium or were incubated in saline (Pasqual et al., 1993). All experiments were conducted in the absence of metabolic activation. In cells maintained in growth medium, berberine chloride (10-50 µg/mL; 0.027-0.13 µM) induced *hom*3-10 frameshift mutations and cytoplasmic 'petite' mutations in a dose-dependent manner; at doses of 25-100 µg/mL (0.07-0.27 µM), it induced *cyh* crossing-over. However, berberine chloride (75-300 µg/mL; 0.20-0.80 µM) did not induce *hom* 3-10 or 'petite' mutations when added to cells incubated in saline, nor did it induce *leu* gene conversions when added to cells maintained in growth medium (25-100 µg/mL;0.07-0.27 µM) or in saline (75-300 µg/mL; 0.20-0.80 µM). Berberine chloride did not induce *lys*1-1 or *his*1-7 point mutations at any dose tested in either medium.

9.5.4 *In Vitro* Mammalian Systems

There was a dose-dependent increase in sister chromatid exchange (SCE) in 9L rat intracerebral gliosarcoma cells treated with 25-150 µg/mL (0.07-0.45 µM) berberine for 2 hours (Zhang et al., 1990).

9.6 Immunotoxicity

No data were found for goldenseal or hydrastine. The immunotoxicity studies described in this section are also presented in **Table 8**.

In an *in vitro* cellular proliferation study using mitogen-stimulated human peripheral blood lymphocytes, the authors concluded that the effects of berberine, particularly the anti-inflammatory

Table 8. Immunotoxicity of Berberine

Test System	Biological Endpoint	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
Human peripheral blood lymphocytes, *in vitro*	inhibition of [^{3}H]thymidine incorporation	berberine, purity n.p.	2.5-20 µg/mL (7.43-59.4 nM)	85% inhibition with 20 µg/mL (59.4 nM) berberine for phytohaemagglutinin and concanavalin A-stimulated lymphocytes and with 10 µg/mL (29.7 nM) for pokeweed mitogen-treated lymphocytes. Berberine induced a dose-dependent inhibition of DNA synthesis.	Cells were stimulated to divide by the addition of phytohaemagglutinin, concanavalin A, or pokeweed mitogen.	Ckless et al. (1995)
C3H/He mouse MM2 transplantable ascites tumor	inhibition of PMN activation and cytotoxicity	berberine, purity n.p.	n.p.	Berberine showed neither toxicity to the target tumor cells nor inhibition of PMN activation.		Kinoshita et al. (1992)

Abbreviations: n.p. = not provided; PMN = polymorphonuclear leucocytes

action, may occur from the inhibition of DNA-synthesis (Ckless et al., 1995). In lymphocyte cultures stimulated with phytohemagglutinin and concavalin A, berberine at 20 µg/mL (0.059 µM) inhibited DNA synthesis by 85%. Inhibition of 85% was achieved in lymphocytes stimulated with pokeweed at a dose of 10 µg/mL.

Kinoshita et al. (1992) investigated the effects of berberine on polymorphonuclear leukocyte (PMN) activation using a PMN cytotoxicity assay system. Berberine (dose not specified) showed neither toxicity to the target MM2 tumor cells from a spontaneous mammary carcinoma in a C3H/He mouse nor inhibition of PMN activation.

9.7 Other Studies

9.7.1 Cytotoxicity and Apoptosis *In Vitro*

Berberine chloride (1 or 5 µg/mL; 0.0027 or 0.013 µM) caused a marked, but temporary, inhibition of growth of cultured mouse sarcoma-180 ascites cells (Creasey, 1979). Berberine chloride (4 or 40 µg/mL; 0.011 or 0.11 µM) also interfered with the *in vitro* production of nucleic acids, residual proteins, and lipids, and the oxidation of glucose in mouse sarcoma-180 ascites cells. The synthesis of nucleic acids, residual proteins, and lipids was measured by incorporation of thymidine[methyl-^{3}H], glycine[1-^{14}C], and sodium acetate[2-^{14}C], respectively.

In HeLa cells, berberine sulfate (25 or 50 µM for 4 hours) inhibited the incorporation of $^{32}P_i$ into phospholipids induced by the tumor promoters 12-*O*-tetradecanoylphorbol-13-acetate (TPA; 50 nM) and teleocidin (23 nM) (Nishino et al., 1986). The cells were not incubated with berberine sulfate alone. Berberine sulfate (25, 50, or 100 µM for 4 hours) inhibited TPA (50 nM)-enhanced transport of ^{3}H-3-*O*-methyl-*D*-glucose into mouse fibroblast Swiss 3T3 cells.

Berberine (25-150 µg/mL; 0.07-0.45 µM) was marginally effective in inhibiting the *in vitro* growth of human brain tumor cell lines; dose-dependent colony inhibition occurred in only 2/6 cells lines tested (Zhang et al., 1990). The cells were treated for 2 hours, washed, and then incubated in berberine-free medium for 12-14 days. Incubation of the cells for 2 hours with

berberine (25-150 µg/mL; 0.07-0.45 µM), followed by BCNU (23 µM) had an additive effect; dose-dependent colony inhibition was observed in 6/6 cell lines treated with both compounds and then washed and incubated for 12-14 days.

In NT2/D1 human teratocarcinoma cells, berberine chloride at doses of 0.5 mg/mL (1.34 µM) and higher (higher doses not specified) was toxic; cell degeneration and death occurred 1 day after treatment (Chang et al., 1990; Chang, 1991). Lower doses (0.01-0.2 mg/mL; 0.027-0.54 µM) of berberine chloride were not toxic, but induced morphologic differentiation. In another portion of the experiment conducted by Chang et al. (1990; Chang, 1991), the latency period for induction of morphologic differentiation by berberine chloride in T2/D1 cells was investigated. Cells were incubated with 0.1 mg/mL (0.27 µM) berberine chloride for 1, 2, 3, 4, or 5 days. Morphologic differentiation was observed even with only 1 day of treatment. Differentiation started to occur the following day and rapidly progressed during subsequent days.

In F9 murine teratocarcinoma cells, however, neither cytotoxicity nor morphologic differentiation was observed following treatment of the cells with 0.1-0.2 mg/mL (0.27-0.54 µM) berberine chloride (Chang et al., 1990; Chang, 1991). Higher doses (not provided) were cytotoxic, but did not induce morphologic differentiation. The duration of the incubation period was not specified.

When the effect of berberine on the expression of glucocorticoid receptors (GR) and its relation to cell cycle progression of HepG2 human hepatoma cells was examined, berberine (1-50 µM for 1-3 days) caused a dose-dependent inhibition of growth in HepG2 cells (Chi et al., 1994). However, the inhibition was not cytotoxic because cells continue to grow after removal of berberine from the culture media; viability of berberine-treated cells was greater than 90% in all treatment groups. Berberine also significantly reduced the S-phase fraction and increased GR levels. Berberine inhibited cell secretion of α-fetoprotein.

Berberine (dose not provided), either alone or in combination with argon laser treatment, induced lysis, encystation, and degeneration in 9L rat glioma cells (Chen et al., 1994).

In Molt-4 and L1210 human leukemia cells, berberine-induced cytotoxicity was enhanced by arabinose-cytidine monophosphate (ara-CMP) and isoguanosine monophosphate (IGMP) (Lee et al., 1995).

Berberine chloride treatment of human HL-60 leukemia cells induced a dose-dependent decrease in cell viability and an increase in apoptosis when cells were treated with 5 to 50 µg/mL (0.013.4-0.134 µM) berberine chloride and observed over a period of 48 hours (Kuo et al., 1995). When the cells were treated with 25 µg/mL (0.037 µM) and observed over the same time period, changes in the cell cycle distribution were observed after 6 hours; the number of S-phase cells and the amount of DNA content decreased, but no changes were observed in the total number of G_1 and G_2-M cells. The authors suggested that cells in S-phase may be the cell subpopulation which is undergoing rapid apoptosis following berberine chloride exposure.

Berberine (0.032-500 mg/mL; 0.10-1486 µM) exhibited cytotoxicity against P-388 murine leukemia cells (Lee et al., 1995). Incubation of the cells with a mixture of berberine and arabinose cytidine monophosphate (0.0.32-500 mg/mL, 1:1 molar ratio) or of berberine and isoguanosine monophosphate (0.0.32-500 mg/mL, 1:1 molar ratio) enhanced the berberine-induced cytotoxicity by 2 to 20 times.

Berberine reversed the resistance of BEL-7402 human liver cancer cells to vincristine (VCR)-induced cytotoxicity by 8.3-fold (Pan and Tian, 1996). Cells were incubated with VCR (dose not provided) and 0 or 27.0 µM berberine for 72 hours. The cells exhibited innate resistance to VCR. However, berberine did not reverse the resistance of MCF-7/Adr human breast cancer cells to adriamycin (ADR)-induced cytotoxicity. Cells were incubated with 10 µM ADR and 0 or 10 µM berberine for 3 hours. MCF-7/Adr cells were selected by stepwise exposure of MCF-7 parental cells to ADR.

Berberine at 200 µg/mL (0.59 µM) but not 100 µg/mL (0.30 µM) induced apoptosis in over 90% of Balb/c 3T3 fibroblast cells (Wen Yang et al., 1996).

Berberine (16 µg/disk) was not cytotoxic towards C38 murine colon adenocarcinoma cells or L1210 mouse leukemia cells (Valeriote et al., 1996).

9.7.2 Oncogene Expression

Treatment with berberine chloride (0.1 to 0.02 mg/mL; 0.27-0.05 µM, purity not specified) induced down-regulation of the Ki-ras2 protooncogene when measured as the reduction in the amount of poly $(A)^+$ RNA (Chang et al. 1990; Chang, 1991). Treated T2/D1 human teratocarcinoma cells were compared to untreated T2/D1 cells and untreated MRC-5 embryonic human lung fibroblast cells. Actin-mRNA production was not impaired even after berberine-induced differentiation. It was not clear whether the down-regulation of Ki-ras2 expression was causally related to neuronal differentiation.

9.7.3 Topoisomerase Inhibition

Berberine inhibited topoisomerase II but not topoisomerase I *in vitro* (Makhey et al., 1995).

9.7.4 Phototoxicity

Berberine did not induce phototoxicity in *E. coli* strains RT7h, RT8h, RT9h, or RT10h (Tuveson et al., 1986). Approximately 10^5 cells/mL were placed onto thin-layer chromatographic plates spotted with psoralen and α-T. The cells were treated with berberine (dose not provided) in agar for 2 minutes and were then exposed to 43.7 kJ of broad-spectrum near UV.

9.7.5 Anticholinergic Activity

In isolated guinea pig ileum, pretreatment with 20 µg/mL (0.046 µM) berberine sulfate blocked the response to 0.1 µg/mL acetylcholine (Kulkarni et al., 1972). Pretreatment of isolated

tracheal muscle of dogs with 20-30 µg/mL (0.046-0.069 µM) berberine sulfate blocked the response to 0.1 µg/mL acetylcholine by more than 80%, and 60 µg/mL (0.14 µM) berberine sulfate completely inhibited the response of rectus muscle of frogs to 0.1 µg/mL acetylcholine.

In depolarized guinea pig ileum at doses of 0.001-0.01 mg/mL (0.0023-0.023 µM), berberine sulfate inhibited carbachol-induced contractions by 27-60%; the inhibition was quickly reversible (Sabir et al., 1978).

9.7.6 Antiadrenergic Activity

In isolated rabbit aortic strips, berberine sulfate (0.003- 0.03 mg/mL; 0.0069-0.069 µM) inhibited adrenaline and noradrenaline-induced contractions in a dose-dependent manner (Sabir et al, 1978). Berberine chloride (0.01-0.5 mg/mL; 0.023-1.15 µM) inhibited adrenaline-induced contractions in isolated guinea pig seminal vesicle by approximately 10, 30, 50, and 70% at doses of 0.01, 0.03, 0.06, and 0.10 mg/mL (0.023, 0.069, 0.14, and 0.23 µM), respectively. Noradrenaline-induced contractions were also inhibited by approximately 14, 17, 34, 44, and 80% by 0.01, 0.03, 0.06, 0.10, and 0.30 mg/mL (0.023, 0.069, 0.14, 0.23, and 0.69 µM) berberine sulfate, respectively.

Palmery et al. (1993) found that berberine (4.33 mg/mL; 12.9 µM) inhibited adrenaline-induced contractions in rabbit aortic strips, but did not produce vasoconstrictive effects.

In a study investigating the mediators of induced contractions, berberine relaxed potassium chloride- and phenylephrine-induced contractions in isolated aortas of Sprague-Dawley rats (Lee and Chang, 1996). Berberine exhibited a greater inhibition of the contractile response induced by phenylephrine than by potassium chloride, which implied that berberine exhibits α-adrenoceptor blocking activity. The aortas were incubated with 1.0-10 µM berberine and phenylephrine for 10 minutes or with 1.0-10 µM berberine and potassium chloride for 30 minutes.

Hydrastine (4.05 mg/mL; 10 µM) had no effect on adrenaline-induced contractions in rabbit aortic strips and did not produce vasoconstrictive effects (Palmery et al., 1993).

9.7.7 Antihistiminic Activity

In isolated guinea pig ileum, 60 µg/mL (0.14 µM) berberine sulfate completely inhibited the response to 0.1 µg/mL histamine (Kulkarni et al., 1972).

9.7.8 Spasmolytic/Spasmogenic Effects

In isolated guinea pig ileum, berberine sulfate elicited a spasmogenic response at concentrations of 0.001-0.01 mg/mL (0.0023-0.023 µM) but had a spasmolytic effect at concentrations of 0.020-0.10 mg/mL; 0.046-0.230 µM (Kulkarni et al., 1972). It did not inhibit contractions in tracheal muscle preparations from dogs and guinea pigs (dose not provided).

Berberine sulfate had no effect on the uninduced contraction rate of isolated rabbit aortic strips, depolarized guinea pig ileum, or guinea pig seminal vesicle at concentrations of 0.003- 0.03 mg/mL (0.0069-0.069 µM), 0.001-0.30 mg/mL (0.0023-0.69 µM), and 0.01-0.5 mg/mL (0.023-1.15 µM), respectively (Sabir et al., 1978).

Hydrastine was inactive on unstimulated guinea pig ileum and unstimulated mouse vas deferens preparations at doses of 30-500 µM and 10-300 µM, respectively (Bartolini et al., 1990).

9.7.9 Inotropic/Chronotropic Effects

In isolated rabbit heart preparations, berberine sulfate (dose not provided) had a negative inotropic effect (decreased the amplitude of contraction) (Kulkarni et al., 1972). At concentrations of 0.010-0.050 mg/mL (0.023-0.115 µM), berberine sulfate also had a negative inotropic effect and caused changes in tone (not described) in isolated rabbit intestine.

Berberine sulfate (0.003-0.10 mg/mL; 0.0069-0.23 µM) had positive inotropic (increased amplitude of contraction) and positive chronotropic (increased rate of contraction) effects on

spontaneously beating isolated atria of rats, guinea pigs, and rabbits (Sabir et al., 1978). On rabbit atria, the amplitude of contraction was increased by 8, 15, 30, and 70% and the rate of contraction was increased by 10, 14, 20, and 30% with 0.003, 0.01, 0.03, and 0.10 mg/mL (0.0069, 0.023, 0.069, and 0.23 µM) berberine sulfate, respectively (Sabir et al., 1978). Percentages of increase were not given for rats or guinea pigs.

Hydrastine produced dose-related positive inotropic but negative chronotropic effects in spontaneously beating rat atrium (Bartolini et al., 1990). It also inhibited electrically evoked contractions in isolated longitudinal muscle of guinea pig ileum at high doses (ED_{50} = 620, CI = 386-993 µM). In the range of 10-500 µM, hydrastine induced a dose-related positive inotropic effect on electrically evoked contractions of isolated mouse vas deferens (Bartolini et al., 1990).

9.7.10 Inhibitory Effects on Potassium Channels

In isolated guinea pig ventricular papillary muscle, incubation with 3 to 100 µM berberine for 30 minutes partially inhibited (low dose) or completely abolished (high dose) the shortening of action potential duration and effective refractory period induced by hypoxic conditions or by cromakalim (Wang et al., 1996).

In guinea pig ventricular myocytes under whole-cell voltage clamp conditions, berberine (3 to 100 µM) partially or completely inhibited cromakalim-induced outward K^+ currents (Wang et al., 1996). In inside-out membrane patches exposed to 0.1 mM ATP, berberine inhibited K_{ATP} channel activity in a dose-dependent manner.

9.7.11 Effects on Calcium Chloride-Induced Contractions

In depolarized guinea pig ileum, berberine sulfate (0.001-0.003 mg/mL; 0.0023-0.0069 µM) potentiated calcium chloride-induced contractions by 7-23% and did not produce an inhibitory effect even when administered at doses as high as 0.30 mg/mL (0.69 µM) (Sabir et al.,

1978). Berberine sulfate reversed the inhibitory effect of polysorbate 80 and papaverine on calcium chloride-induced contractions by 50-60%.

9.7.12 Effects on Prostaglandin-Induced Contractions

In 6/9 experiments conducted by Sabir et al. (1978) using isolated guinea pig ileum, berberine sulfate (0.001-0.006 mg/mL; 0.0023-0.014 µM) inhibited PGE_2-induced contractions by 10-64%; in 7 other experiments, berberine sulfate (0.0001-0.03 mg/mL; 0.00023-0.069 µM) inhibited $PGF_2\alpha$-induced contractions by 8-67%. At 0.001-0.01 mg/mL (0.0023-0.023 µM), berberine sulfate potentiated prostaglandin (PG)E_1-induced contractions by 18-280% and $PGF_1\alpha$-induced contractions by 20-560% in isolated guinea pig ileum. In 3/9 experiments investigating PGE_2-induced contractions, berberine sulfate potentiated the induced contractions by 15-165%. In 5 other experiments, berberine sulfate (0.001-0.01 mg/mL; 0.0023-0.023 µM) increased $PGF_2\alpha$-induced contractions by 84-300%.

9.7.13 Inhibition of Sperm Motility

British Friesian bull sperm samples treated *in vitro* with berberine sulfate became immotile in 217 minutes at 3 mg/mL (6.92 µM); in 153 minutes at 10 mg/mL (23.1 µM); in 20 minutes at 50 mg/mL (120 µM); and immediately after treatment with 100 mg/mL (231 µM) berberine sulfate (Sabir et al., 1978).

10.0 STRUCTURE-ACTIVITY RELATIONSHIPS

Berberine and protoberberine alkaloids have varying DNA-binding affinities (Smekal et al., 1980; Silikas et al., 1996). Compared to that of berberine, the relative fluorescence intensity of the complexes with DNA decreases in the following order: pseudoberberines, berberines, oxyberberines, tetrahydroberberines, planar derivatives, and protoberberine. Binding properties are influenced by the position and type of substituent, and the presence of charge on the protoberberine skeleton (Smekal et al., 1980). From an analysis of berberine chloride and 12 protoberberine analogues, Silikas et al. (1996) stated that substitution in bulky groups of

protoberberine alkaloids inhibited binding to DNA and binding activity of the alkaloids was generally fairly weak. Cushman et al. (1979) found that only the quaternary salts bind with DNA.

In an experiment testing berberine and 12 of its analogues as topoisomerase poisons, it was suggested that increased planarity of berberine may correlate with its enhanced activity as a topoisomerase II poison (Makhey et al., 1995). With cleavage of both methylenedioxy and methoxyl groups from berberine, the compound acts as a potent topoisomerase I poison. Thus, minor variations in the protoberberines may substantially alter their pharmacological properties.

In a study that compared the convulsant potencies of (-)-hydrastine hydrochloride and (+)-hydrastine hydrochloride injected i.v. into male mice, (-)-hydrastine hydrochloride had 180 times less convulsant potency than (+)-hydrastine (Huang and Johnston, 1990).

In male Wistar rat brain synaptic membranes, the concentrations of hydrastine isomers which induced binding to the γ-aminobutyric acid (GABA) A receptor (measured as the concentrations which displaced 50% of GABA bound to receptor sites, IC_{50}) in a TRIS assay varied: (-)-β-hydrastine and (±)-β-hydrastine, IC_{50} = approx. 1000 µM each versus (+)-α-hydrastine and (±)-α-hydrastine, IC_{50} = greater than 1000 µM each (Kardos et al., 1984).

11.0 ONLINE DATABASES AND SECONDARY REFERENCES

11.1 Online Databases

Chemical Information System Files

CTCP (Clinical Toxicology of Commercial Products)
SANSS
TSCATS (Toxic Substances Control Act Test Submissions)

DIALOG Files

Chemical Econ. Handbook
DIOGENES
Kirk-Othmer Encyclopedia of Chem. Technol.
Federal Register
NIOSHTIC

National Library of Medicine

EMIC AND EMICBACK (Environmental Mutagen Information Center)

STN International Files

AGRICOLA (Agricultural Online Access)
BIOBUSINESS
BIOSIS (Biological Abstracts)
CABA
CANCERLIT
CAPLUS (Chemical Abstracts)
CBNB
CEN (Chemical and Engineering News)
CHEMLIST
CROPB
CROPU
CSNB (Chemical Safety News Base)
DDFB
DDFU
DRUGLAUNCH
EMBASE (Excerpta Medica)
FSTA
HODOC
IPA
LIFESCI
MEDLINE (Index Medicus)
NAPRALERT
PHIN
PROMPT
RTECS (Registry of Toxic Effects of Chemical Substances)
TOXLINE
TOXLIT

TOXLINE includes the following subfiles:

Toxicity Bibliography	TOXBIB
International Labor Office	CIS
Hazardous Materials Technical Center	HMTC
Environmental Mutagen Information Center File	EMIC
Environmental Teratology Information Center File (continued after 1989 by DART)	ETIC
Toxicology Document and Data Depository	NTIS
Toxicology Research Projects	CRISP
NIOSHTIC7	NIOSH
Pesticides Abstracts	PESTAB
Poisonous Plants Bibliography	PPBIB
Aneuploidy	ANEUPL
Epidemiology Information System	EPIDEM
Toxic Substances Control Act Test Submissions	TSCATS
Toxicological Aspects of Environmental Health	BIOSIS
International Pharmaceutical Abstracts	IPA
Federal Research in Progress	FEDRIP
Developmental and Reproductive Toxicology	DART

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