REVIEW: HEPATOPROTECTIVE AND MICROBIOLOGICAL STUDIES OF THREE GENERA: *Equisetum*, *Lycopodium*, AND *Gentiana*

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Abstract. Liver injury treatments are among the most important of today's research domains, because with every passing year there is a more acute need for liver transplants. Many of the everyday drugs that people use, and all the toxic influences and unhealthy food lead to some form of liver disorder. That is why today's attention is drawn to the potentials of a few miracle plants that have the ability to reduce or cure liver damage. *Equisetum*, *Lycopodium* and *Gentiana* genera species are well known homeopathic plants in the Northern Hemisphere. Their properties are used in many disorders, and in the recent studies they are tested for their microbiological and hepatic curative actions.

Keywords: hepatoprotective, *Gentiana, Equisetum, Lycopodium*, microbiological activity

INTRODUCTION

The hepatic organ plays some of the most important roles in the bodies of all mammals. It is implicated in almost all the vital functions among which: metabolism, secretion of bile and storage of glycogen, vitamins, iron and body fats [4, 7, 22, 26, 52]. The liver's P450 cytochrome is the detoxifying complex of foreign substances, and the hepatocyte's role is to metabolize sugars, peptides and lipids. The detoxifying action is vital to the body but it can also lead to the death of hepatic cells. Large doses of drugs, xenobiotics, alcohol, poisons, toxic foods or bacteria infections are the main ways to affect the liver. But the chemical process which leads hepatic cells to enter apoptosis is the production of oxidative stress, no matter the factor [1, 7, 16, 22, 27, 33, 42, 59]. There are thousands of known plants and almost as many known substances with a certain level of protective or healing activity for the liver, or antibacterial effects, few of them being studied and officially recognized, and even fewer used for clinical administration on patients [1, 5, 12, 15, 20, 43, 58]. But there is today, a continuous growing tendency to use natural products instead of synthetic drugs [1, 36, 60]. The aim of this review is to summarize some of the recent work on the topic of hepatoprotective and antibacterial activities of a few species of the *Equisetum*, *Lycopodium* and *Gentiana* genera.

MATERIALS AND METHODS

In the present review, the literature search was made using the following keywords: "hepatoprotective", "acetaminophen-induced liver injury", "drug-induced liver injury", "hepatic failure", "hepatoprotective activity of *Equisetum* (*Lycopodium*, *Gentiana*)", and the needed information was found in on-line articles or books on Pubmed, Wiley Publications, official site of NIH and WHO, and Google Scholar libraries.

RESULTS

In the scientific literature, diverse plant activity on the liver has been reported, using animal models (mice and rats), genetically modified or not, or hepatic cell lines, and for some of the most studied plants scientists have conducted microbiological tests and chemical structure analysis. For the beneficial effects of the natural products to be observed, the plant products were extracted in a range of solvents: water, ethanol, methanol, dichlormethane, petroleum ether, chloroform, ethyl acetate, n-butanol. The animal models were sickened with substances like acetaminophen, carbon tetrachloride, alcohol, chloroform or methylen chloride [16]. Although there is a large variety of liver damage-inducing substances (like the many drugs retrieved from the market: iproniazid, benoxaprofen, bromfenac, trovafloxacin, ibufenac, dilevalol, and others), these are not recommended for analyzing the liver's response to a certain treatment, because most of them have no quantifiable physiological action, as for those mentioned above which present concentration or dose-related injury response of the liver [24, 42, 46].

One of the widely used liver injury inducing substance is paracetamol (acetaminophen) which is normally used for mild pain and for reducing high body temperature, but if ingested in large doses per body weight it can cause hepatic cell death, systemic toxicity [9, 18, 19, 47] and if taken for long term in high dosage it can cause general organ failure and death. Paracetamol is reduced and eliminated trough the glucuronide pathway or trough the glutathione complexation. High dosage drastically reduces glutathione and the toxic metabolite produces oxidative stress. *N*-acetyl-*p*-benzoquinone imine seems to be the metabolite that, even though normally produced in small amounts, in the metabolizing process of the acetaminophen, induces the toxic oxidative stress, which in turn always determines the lipid peroxidation in paracetamol-induced liver injury [13, 21, 35, 55].

Carbon tetrachloride intoxication is another model for hepatic toxicity. The metabolic residue which causes the oxidative stress is the trichloromethyl

radical, which destroys the phospholipids and induces membrane integrity loss [38]. At the tissue level it destroys the hepatocyte arrangement, determines necrosis, stasis in the central-lobular veins, infiltration of lymphocytes and multiplication of Kupffer cells [2]. This is why plants need to contain high concentrations of antioxidants in order to have the healing or protective strength to reduce the inner stress of the cells [38].

Some of the papers reported the use of *Silybum marianum* or *Cynara scolymus* extracts for positive control [2]. It has also been reported that liver failure is accentuated by the starving of animals, due to the deacetylation of mitochondrial proteins [25] therefore some of the recent papers presented that mice or rats were starved prior to the administered treatments. Depending on the authors desire to demonstrate the plants effects, the phytocompounds were administered in one of three ways: prior to induced-liver damage, starting with the moment of the induction of liver damage, or after a certain period from the toxic administration. Some of the papers' authors watched for the immediate reactions of the liver, sacrificing the animals at certain determined hours after the administration of the damaging factor, or after days or longer periods of herbal extract intake.

In order to establish the liver's state a few analyses are required. There are four major types of liver injury: hepatocellular, autoimmune, cholestatic, and infiltrative. Hepatocellular damage is identifiable trough elevations of serum transaminases. ALT and AST are released from an injured hepatocyte, but the AST is found in large quantities in other organs as well (eg. striate muscle), therefore its specificity is low for liver conditions whereas the ALT is found in high concentration only in the liver [7, 13]. An infiltrative injury manifests an elevation in serum alkaline phosphatase (5′-nucleotidase, γ-glutamyltransferase, leucine aminopeptidase, fractionated alkaline phosphatase, which are zinc metalloenzymes localized at the level of microvilli of the bile canaliculus), and a nonremarcable elevation in serum transaminases. Cholestatic injury presents the same symptoms as the infiltrative injury, plus a high concentration of bilirubin in the blood. Serum albumin and prothrombin time tests are also relevant for the hepatic function, but only to ensure a certain diagnostic [13, 30].

Studies on plant extract activity were conducted on almost every continent, the most well known ones being the Chinese, Indian, African, Turkish and Brazilian herb studies, mostly because of the popularity of the plant utility among the local people.In this review the attention was concentrated on articles referring to results of some studies of the hepatoprotective and antibacterial effects of *Lycopodium*, *Equisetum* or *Gentiana* genera species.

The horsetail is a common pteridophyte in all the Northern Hemisphere and it has well known popular utilizations due to its highly studied phenolic, alkaloid and phytosterolic components, but mostly it is known for its remineralisation properties due to its high content of minerals like silica, calcium, magnesium, selenium, iron, potassium and zinc. This is why it is a widely used natural product even today [11]. *Equisetum* genus contains 32 species spread along almost all Northern hemisphere continents, from lowlands up to the mountains, mostly near waters [3, 49].

Oh *et al* (2004) [34] isolated two phenolic petrosins and four flavonoids from methanol extracts of *Equisetum arvense,* which were proven to have hepatoprotective activity [1, 34]. Milovanovic *et al*. (2007) [28] analyzed five Serbian species of *Equisetum*: *E. arvense, E. sylvaticum, E. fluviatile, E. palustre, E. telmateia*, and found that the major constituents in the hydroalcoholic extracts were 3-O-glucoside and aglycone quercetin (*E. arvense*), and kaempferol 3-O-glucoside-7-O-rhamnoside (for the other four), all containing caffeic acid derivatives. In Table 1 we synthesized all the compounds found in various species of *Equisetum* genus. The same authors underwent a micronucleus test for these species, and obtained a concentration dependent degree of micronucleus formation [6].

Several analyses have shown that different doses of *Equisetum* do not produce any liver damage on mice [10] and in 2010, a few Serbian authors presented the antiproliferative activity of different extracts of *E. arvense* on cervix epidermoid carcinoma cell line, colon adenocarcinoma, and breast adenocarcinoma human tumor cell lines, for the first time. The HeLa human cervix epidermioid tumor cells were the most responsive to all extracts [11]. Pattewar *et al* (2011) [41] states that *E. arvense* manifests a certain cognitive enhancement, due to its high antioxidant content, which was identified *in situ* by confocal laser scanning microscopy on a few plant organs, by Hutzler *et al*. (1998) [17]. It was also demonstrated that it has a soothing effect in rats, acting as a mild anesthetic and being a better anti-anxiety drug than diazepam [45, 48].

When Milanovic *et al*. (2007) [28] analyzed the antioxidant capacity of *Equisetum* species, they found that *E. telmateia* and *E. arvense* are powerful antioxidants, comparing to *E. palustre* and *E. fluviatile* that have a lower capacity to fight free radicals. These results are sustained by the high level of phenolic content detected [28, 53, 54]. They seem to have an antibacterial activity also, inhibiting *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa,Streptococcus enteritidis* or producing sensibility to *Aspergillus niger* and *Candida albicans,* when compared to antibiotics [28].

Gentiana genus species are known hepatoprotective plants, found in mountain areas of Europe, Asia, North America, North-Western Africa, Australia and New Zeeland. Most of them are scientifically recognized of having anti-inflammatory properties. This is why in France, Germany and other countries, some species are cultivated and their roots used for many medical purposes [12]. *Gentiana* species are very appreciated due to their high content of natural antioxidants and alkaloids, almost all species containing them (Table 2).

A few studied species of *Gentiana: macrophylla, manshurica* and *olivieri* are known to contain gentianine, iso-orientin, swertiamarin, gentiopicroside, sweroside and other antioxidant, anti-inflammatory and bitter components which protect and stimulate the liver [60]. The most studied *Gentiana* species is *G. olivieri*. It is traditionally used as a bitter tonic or as a blood pressure controller [51]. *Gentiana olivieri* aerial parts extracts have proven to contain an anti-lipid peroxidase and compared to positive controls, the standard liver analysis (ALT-AST and malondialdehyde) presented the same or improved results [2].

Histopathology studies and biochemical assays made on rats showed some tissue improvement of the liver with a dose of 15 mg/kg of isoorientin, extracted from *Gentiana olivieri* [36]. Satnam *et al*. (2011) [51] also proved an immunomodulatory property of this plant at 200 mg/kg body weigh concentration of ethanol extract. Another species of the Gentianaceae family, *Centauraea americana*, was studied for its hepatoprotective activity on carbon tetrachloride intoxicated hepatic cell lines, and even though its capacity to reduce lipid peroxidation was noticed, the authors sustain the fact that the subject needs further studying [57].

In alternative medicine *G. punctata, G. chirayita* and *G. scabra* are used for treating the flu, the lack of appetite, by stimulating the function of the liver and pancreas and the production of gastric juices and saliva. It is also used for treating liver problems and fungal infections [3, 56]. *G. scabra, G. asclepiadea* and *G. olivieri* were demonstrated to have the potential to stimulate the immune response, when administered as an ethanolic or buthanolic extract, and the oil extract of *G. lutea* has a calming effect, proved on rats and guinea pigs [49, 62].

The antibacterial activity of *G. lutea* was seen against *Staphylococcus aureus* in an ethanol extract, and against *Aspergillus fumigatus, A. niger, Botrytis cinerea, Fusarium oxysporum* and *Penicillium digitatum* in a water based extract [62].

Table 1. Chemical compounds of Equisetum species

Chemical compound group	Chemical compound	Species	References
Floavonoids	izoquercetin (quercetin-3-o-glucose)	*E. sylvaticum, E. palustre, E. arvense*	Milovanovic *et al*. (2007) [28]
	kaempferol-3-o-glucose-7-o-ramnose	*E. sylvaticum, E. telmateia*	
	caffeic acid	*E. sylvaticum, E. fluviatile, E. telmateia*	
	kempferol-3,7-o-diglucose and derivatives	*E. fluviatile*	
	kempferol-3-o-rutinose-7-o-soforose	*E. palustre*	
	kempferol-3-o-rutinose-7-o-glucose		
Phenolic acids	silicic acid	*E. arvense*	Ardelean and Mohan (2008) [3], Robu and Milică (2004) [49]
	malic acid		Ardelean and Mohan (2008) [3]
Alkaloids	equisetonin		
	metoxipiridin		
	nicotin		
	palustrin		
	palustridin		
Fatty acids	ω-3 and ω-6 essential fatty acids		
Vitamins	C vitamin		
Minerals	Si, Mg		
Glucosides	3-metoxipiridin		Mocanu and Răducanu (1983) [29]
	saponins		Robu and Milică (2004) [49]
	articulatidin		
	izoarticulatidin		
	gluteolin		
	aconitic acid		
	oxalic acid		
	silicic oxid		
Minerals	Si, Ca, K, Fe, Mg, Na		
Alkaloids	onitin		Oh *et al*. (2004) [34]
	luteolin		
	thymol		Radulovic *et al*. (2006) [44]
	1,8-cineol		
	linalool		
	hexahidrofarrnesil		
	cis-geranil acetone		
	trans-ionone		
	aglicon-quercetin		Milovanovic *et al*. (2007) [28]

Table 2. Phytochemical compounds of *Gentiana* species

Chemical compound group	Chemical compound	Species	References
Monoterpenes	gentiopicroside	*G. lutea, G. scabra, G. asclepiadea*	WHO (2007) [62], Ardelean and Mohan (2008) [3], Franz *et al.* (2005) [12]
	zwertiamarin	*G. lutea, G. scabra*	WHO (2007) [62]
	zweroside		
	amarogentin	*G. lutea*	WHO (2007) [62], Franz *et al.* (2005) [12]
	gentiamarin	*G. scabra, G. asclepiadea*	WHO (2007) [62], Ardelean and Mohan (2008) [3], Franz *et al.* (2005) [12]
	ophelic acid	*G. chirayita*	Sampath Kumar *et al.* (2010) [50]
	chiratin		
Xanthones	gentisin	*G. lutea, G punctata*	WHO (2007), Ardelean and Mohan (2008) [3]
	gentianose		
	gentianin		
	izogentisin	*G. lutea*	WHO (2007) [62]
	gentiosid		
	gentiobinose	*G. punctata*	Ardelean and Mohan (2008) [3]

Lycopodiales family species are pteridophytes spread only in the Northern hemisphere and *Lycopodium* genus is comprised of more than a hundred species characterized by the formation of sporophils at the end of a stem. The aerial parts reach maturation and produce spores only after a period of at least four to six years of vegetation. Due to their long evolutionary history many of its members are extinct or on the verge of extinction [32]. Nevertheless the remaining popular species are used by local people for their anti-inflammatory properties, and in recent years for many other applications, which were discovered and tested. The main physiologically active components seem to be the alkaloids, lycopodine being the most studied one them [38, 39]. *Lycopodium serratum* (China) and *L. varium* (New Zealand) were proven to contain huperzine A and B, with great antioxidative processing activity and neuroprotective effects for Alzheimer patients. But *L. clavatum* and *L. complanatum* subsp. *chamaecyparissus* species from Turkey presented no such alkaloid [36]. *L. selago* was documented to contain lycopodine, sellagine and nicotine and likewise, *L. annotinum* contain the alkaloids annotidine, annapodine, isolycopodine, clavatine and nicotine [3]. Studying the same two species, Halldorsdottir *et al*. (2010) [14] found lycodoline, lycoposeramine, lycopholine, lannotidine, acripholine and fitostigmine (Table 3.).

Complementary medicine recommend the use of *Swertia chirata* [50], *L. selago, L. annotinum, L. japonicum* and *L. cernum* to treat kidney and bone disorders, alcohol and cigarette dependence or skin and lung problems, and *L serratum* for enhancing the processes of learning and memory and in Alzheimer [3, 23, 58]. An ethanolic extract of *L. serratum* increased the healing capacity of the epithelium [31] and *L. complanatum* ether and chloroform based extract had an antioxidant effect [39].

Microbiological tests have shown a good antimicrobial activity of *L. clavatum* and *L. complanatum* against both bacteria and fungi like *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Acinetobacter baumannii, Staphylococcus aureus, Enterococcus faecalis, Candida albicans* and *C. parapsilosis* due to their phenolic content: dihidrocaffeic acid, vanillic acid, p-hidroxibenzoic acid , syringic acid, p-coumaric acid and feroulic acids known for their bactericidal activity [38]. Orhan *et al*. (2007, 2009) [38, 39] and Pathak *et al*. (2006) [40] also found a protective activity against induced tumor *in vivo* and micronuclei formation after treatment with a *Lycopodium* extract.

WHO estimated in a report made in 1997 that up to 80% of the population of developing countries use plant based treatments to heal different diseases, and their request is getting bigger by the day [20]. For many of these plants the pharmaceutical industry has developed so much that they have become extinct by uncontrolled exploitation but there are still many more to be discovered and put in use [5]. In the twentieth century, due to a demographic explosion and an increase of population movement, an exchange of phytoculture between regions became possible. This way spices and exotic treatments from Asia, Africa and Latin America became known and utilized by the Europeans and North Americans and after the monopoly of synthetic drugs for more than a century, plants began to win terrain in medical treatments.

This happened mostly at the end of the 20th century when diet pills and natural supplements became very popular amongst the developed countries people [8]. This together with the fact that synthetic drugs are very expensive and inaccessible, and many of them have high risk second effects pushed the general attention towards the benefits of using natural treatments [20]. Counting more than 250000 species of considered superior plants on Earth, it is thought that more than a quarter of them could be used for various treatments in medicine [20, 43]. Studying only a few of them it was found that there are many natural chemical compounds that have o protective or antimicrobial activity, such as: phenols, quinones, flavones, tanins, coumarins, therpenes or isocyanate [12].

The studied species of the three genera presented hepatoprotective effects on laboratory animal models, and cell lines, and no risk of bacterial infection. In fact, they seem to be able to protect against the most common strains of pathogens. On the subject of liver protection activity there is a clear need for further analysis for both the composition of the plants, and

Table 3. Phytochemical compounds of *Lycopodium* species

Chemical compound group	Chemical compound	Species	References
Alkaloids	lycopodine	*L. clavatum*	Orhan *et al*. (2007) [37, 38], Katakawa *et al*. (2009)
	fawcettimane		
	fawcettidane		Orhan *et al.* (2007) [37,38]
	alopecurane		
	serratinine		
	maggelanine		
	flabellidan		
	phlegmarane		
	cernuan		
	lycoposeramin–R	*L. serratum*	Katakawa *et al* (2009)
	lycoposeramin–T		
	N–dimetil–betaobscurin		
	A, B and C hupersins (selagine)	*L. serratum, L. selago*	Orhan *et al*. (2007) [37,38], Ardelean and Mohan (2008) [3]
	nicotine	*L. selago, L. annotinum, L. clavatum*	Ardelean and Mohan (2008) [3]
	annotidin	*L. annotinum*	
	annapodine		
	isolicopodine		
	licopamin		Halldorsdottir *et al*. (2010) [14]
	licopaseramine		
	licofoline		
	lannotinidine		
	acrifoline		
	fisostigmin		
	annotin	*L. clavatum*	Ardelean and Mohan (2008) [3]
Lactone	clavatine	*L. annotinum, L. clavatum*	
Terpens	triterpens	*L. clavatum*	
Flavonoids	apigenin		
Minerals	Mg, S		
Phenolic acids	dihidrocaffeic acid		Orhan *et al*. (2007) [37,38]
	vanillic acid		
	p-hidroxibenzoic acid		
	syringic acid		
	p-coumaric acid		
	ferulic acid		

more importantly for the establishment of the medicinal or medical implementation of plant extract containing treatments [20, 61].

DISCUSSIONS

When testing the medical capabilities of a plant, one should have some knowledge about the plant's chemical content, active principles, and how to extract and concentrate them, while keeping the important ingredient in active form. This is one of the reasons why for the final product to be a good medical supplement the plants need to be tested from different point of views and for different therapies or malignancies, often coming across the problem of not having consistent results. One has to take into consideration the fact that plants are very much influenced by the environmental factors and a strict standardization of plant product is difficult to obtain even in a controlled environment. But new discoveries of plant proprieties offer a good future prospect for a natural treatment of liver affections [20].

There are considerably more recent studies made on the *Lycopodium* and *Equisetum* species than on the *Gentiana* genus. This is probably because the first two genera have a wider spreading in the world, and the local communities used them for their healing purposes for centuries and nowadays there is a struggle to produce commercial products out of local vegetal healers. Some *Gentiana* species were discovered to have important and medical relevant effects on treating gastrointestinal disturbances, especially liver problems, and the scientific community concentrated its attention on them. After the world recognition of yellow gentian healing powers, the efforts were directed to using that knowledge into creating pharmaceuticals from it, and not to explore the other species of the genus [61].

In this review we concentrated the recent studies and results concerning three widespread genera, *Lycopodium*, *Equisetum* and *Gentiana*, focusing on their medical importance. Almost all studied species belonging to these genera contain several compounds with a certain degree of medical relevance, as antibiotics, relaxing, antitumor or hepatoprotective agents.

Medicinal plants are the most resourceful source of medicinal substances, nutrients, natural supplements and pharmaceutical compounds. Many plants are used for their compounds in perfume and food industry, cosmetics and complementary therapy [15].

Plant based treatments need to be further studied because there is clear evidence that they have the capacity to ameliorate and even heal different metabolic disturbances. Centuries old oral transmitted

popular medicines and today's state of the art studies all show the benefits of using natural plant derived extracts to treat or to protect against malignances or to help and enhance various physiological functions.

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