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A better understanding of pharmacological activities and uses of phytochemicals of *Lycopodium clavatum*: A review

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ABSTRACT
Lycopodium clavatum commonly known as club moss belongs to family Lycopodiaceae is a plant of great interest and is used in combating a wide variety of diseases in European and Asian countries. Crude extracts from various parts of the plant contain quinolizidine alkaloids. Several workers have reported various alkaloids derived from this plant and their therapeutic potentials, however, some reports are available on toxicity studies of alkaloids of this plant. In such a scenario, there is a need for better understanding of its ameliorating potential and its toxic actions. This review summarizes scientific findings and suggests areas where further research is needed.

Keywords: *Lycopodium clavatum*, alkaloids, pharmacology, antioxidant.

1. Introduction
Lycopodium clavatum, commonly known as Club moss, Clubfoot Moss, Foxtail, Ground Pine, Sulfer, Wolf's Claw is one of the most widespread species belonging to family Lycopodiaceae. It is a pteridophyte which is abundantly found in tropical, subtropical and in many European countries. This spore bearing vascular plant is used in various traditional system of medicines viz. stomach pain, against rheumatic disease, muscle pain, Alzheimers disease etc.[1]. These plants grow best in various areas such as high altitude, in the highlands, in grassy areas. The leaves are small (3-5 mm long) and about 1mm broad; the spore cones are green to yellow in colour. Though many diseased conditions, even today are being treated with *Lycopodium* both in traditional and homeopathic medical systems, but it has some self-limitations therefore, its therapeutic ability needs critical evaluation. The current review summarizes scientific findings of other investigators on *Lycopodium clavatum* and suggests areas where further investigations / research are desired.

2. Uses in traditional medicine systems
Clubmoss is used in homeopathy for treatments of aneurisms, constipation, chronic lung and bronchial disorders, fevers. It also reduces gastric inflammations, simplifies digestion and helps in treatments of chronic kidney disorders [2]. Further, if the powdered spores are applied on the skin it reduces skin irritation and itching. *Lycopodium* is used to treat various mental conditions like anxiety, forgetfulness, and also relieves from tiredness and chronic fatigue [3-4]. It is also used by several tribes for imperfect erections and also reduces prostrate in aged persons. Traditionally Club moss is used in treatments of kidney stones, urinary tract infections and digestive aliments by various tribes.

3. Phytoconstituents.
The *Lycopodium* alkaloids are of great importance due to their biological activity and unique chemical structures, however, many compounds have not been well studied. There are several reports that this plant contains vanillic, coumaric, ferulic acids and syringic acids. In addition, it contains huperzine A, lycopodine, lycoflexine, Alpha-onocerin and sporopollenin etc [5]. A flavonoid polyphenol called apigenin isolated from *Lycopodium clavatum* which has a potent antioxidant property [6]. Further study yielded alkaloids lycopodine as major alkaloid, clavatine and clavatoxine; polyphenolic acids, including dihydrocaffeic and triterpenes (see figure 1) model [11].

4. Mechanisms of action

4.1 Anticancer properties

Mandal *et al.* (2010) [7] investigated that lycopodine from *Lycopodium clavatum* extract inhibits proliferation of HeLa cells through induction of apoptosis via caspase-3 activation. They showed that lycopodine induced chromatin condensation, internucleosomal DNA fragmentation and enhanced cell population in sub-G1 region along with an increase in reactive oxygen species generation and mitochondrial membrane potential depolarization, release of cytochrome c and activation of caspase-3 which are the events closely involved in apoptosis [7]. Studies conducted by other investigators revealed that apigenin exhibits anticancer potential in A375 and A549 cells that may be mediated through DNA interaction, damage and mitochondrial dysfunction either by direct or indirect action on mitochondria [8]. Further studies conducted by Samadder *et al*. 2013 showed that the highly-diluted, dynamized homeopathic remedies of *Lycopodium clavatum* 5C and 15C demonstrated their capabilities to induce apoptosis in HeLa cells, signifying their possible use as supportive medicines in cancer therapy [9]. Bishayee *et al.* 2010 showed that lycopodine is a promising candidate suitable for therapeutic use as an anti-cancer drug, they tested lycopodine against hormone sensitive (LnCaP) and refractory (PC3) prostate cancer cells in vitro. They found that administration of lycopodine down-regulated the expression of 5-lipoxygenase and EGF receptor, and caused up-regulation of cytochrome c with depolarization of mitochondrial inner membrane potential, without palpable change in p53 activity, resulting in apoptosis and ultimately reduced proliferation of cancer cells; concomitantly, there was an externalization of phosphotidyl serine residues. CD spectroscopic analysis revealed intercalating property of lycopodine with the DNA molecule, implicating its ability to block cellular DNA synthesis. Thus lycopodine may be an important candidate in cancer research [10].

4.2 Hepatoprotective activity

We have also investigated protective ability of *Lycopodium clavatum* extract against p-dimethylaminoazobenzene induced hepatocarcinogenesis [11]. Chronic feeding of carcinogens p-dimethylaminoazobenzene (initiator) and phenobarbital (promoter) for 90 and 120 days elevated activities of biomarkers of toxicity viz. acid and alkaline phosphatase lipid peroxidation, levels of blood glucose and cortisol and decreased the activities of glutathione reductase, succinate dehydrogenase, and blood cholesterol and hemoglobin contents, and levels of serum estradiol and testosterone in mice. Levels of these biomarkers in both liver and spleen tissues were positively altered along with a significant reduction of tumor incidence in liver of carcinogen intoxicated mice treated with spore extract of *Lycopodium clavatum*.

4.3 Effects on enzymes

Prolyl endopeptidase is an enzyme which plays a role in metabolism of proline-containing neuropeptides, such as vasopressin, substance P and thyrotropin-releasing hormone (TRH), which were suggested to be involved with learning and memory processes. It has been investigated by Tezuka *et al.* 1998 that the methanolic extract of *Lycopodium clavatum* has strong prolyl endopeptidase inhibitory activity [12] and it reduces lipid peroxidation, acid and alkaline phosphatase, transaminases which increased during the p-DAB induced carcinogenesis in mouse model [11].

4.4 Antioxidant activity

Ferulic acid, a phenolic acid has wide distribution in the plant Kingdom and is more bioavailable than other dietary flavonoid and monophenolics studied**.** It has been reported by other investigators to be a potent antioxidant, anti-inflammatory, and is reported to terminate the free radical chain reactions [13-14]. Another study by Das *et al*. 2010 revealed that Apigenin, a bioactive flavonoid from *Lycopodium clavatum*, stimulates nucleotide excision repair genes to protect skin keratinocytes from ultraviolet B-induced reactive oxygen species and DNA damage. His group postulated that apigenin accelerated reversal of UV-B-induced DNA damage through up-regulation of NER genes, removal of cyclobutane rings, inhibition of ROS generation, and down-regulation of NF-κB and MAPK further apigenin-induced apoptosis in A375 and A549 cells through selective action and dysfunction of mitochondria [8].

4.5 Antiprotozoal and antiviral activity

Petroleum ether and chloroform fractions of *Lycopodium clavatum* have been reported to inhibit growth of P. *falciparum* [15]. Further, it has been reported by the same group that extracts of *Lycopodium clavatum* viz petroleum ether fractions, chloroform fractions displayed leishmanicidal effect which might be due to the presence of high amount of terpenes, alkaloids and flavonoids.

4.6 Analgesic and behavioral activity

Sundaram *et al*. (2013) [16] investigated the analgesic activity of *Lycopodium clavatum* in experimental rats. They evaluated the analgesic effect by using hot plate, ice plate and Randall-Selitto tests and behavioural effect by using rota rod and open field tests and came to conclusion that there was an increase in the latency time to thermal as well as cold stimulus. Different potencies of *Lycopodium clavatum* made as per homeopathic procedures was evaluated for motor coordination activity in Wister rats using grip strength tests, it was found that, there was a decrease in the grip strength of the rats when measured 30 minutes after the administration of the different potencies of *Lycopodium clavatum* for 30 days. Further, there was a decrease in the locomotor activity of the rats when measured on the 10th day of the experiment 30 minutes after administration of different potencies [16].

4.7 Anti-inflammatory

A study conducted by Namsa *et al*. 2009 showed that extracts of *Lycopodium* have anti-inflammatory activity probably due to the alkaloid compounds and supports its folkloric use by the Lohit community of Arunachal Pradesh, India [17].

4.8 Effects on reproductive systems

Lycopodium is most effective homeopathic remedy in treating impotency of young men. Further, it reduces enlarged prostrate in old people. In females in the case of dryness of vagina with burning sensation during and after coition, is reduced by administration of *Lycopodium*.

4.9 Central Nervous system

Konrath *et al*. (2012) investigated *in vitro* and *ex vivo* acetylcholinesterase and antioxidant activities of traditionally used *Lycopodium* species. The effects of *Lycopodium clavatum* were tested *in vitro* and *ex vivo* on the inhibitory action on acetylcholinesterase (AChE) using rat brain homogenates after a single administration of the alkaloid extracts in mice [18]. Alpha-onocerin: an acetylcholinesterase inhibitor from *Lycopodium clavatum* was first reported by Orhan *et al*, 2003 [19]. Bioassay-guided fractionation of the chloroform extract of *L. clavatum* led to the isolation of alpha-onocerin which is a well-known triterpenoid.

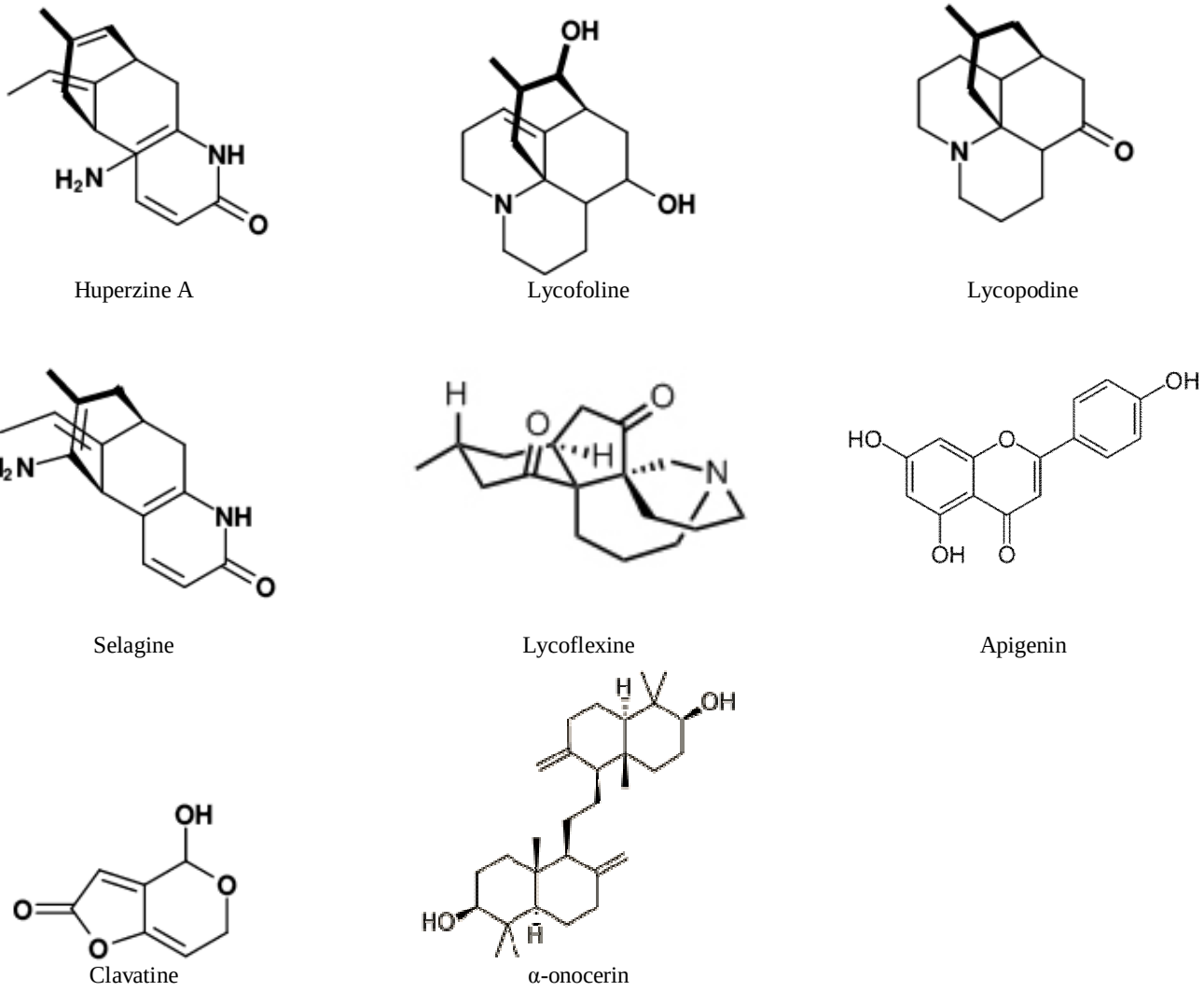

Fig 1: Depicts some of the chemical structures of the compounds commonly found in *Lycopodium clavatum*

4.10 Immunomodulatory properties

In tests on mice, a formulation of *Lycopodium clavatum* (as the vaccine delivery vehicle) and ovalbumin (as the vaccine antigen), consistently produced a very strong immune response. The immune response achieved with the *Lycopodium clavatum* spores was far stronger than that achieved with a cholera toxin or ovalbumin formulation. In medical science cholera toxin is used to test a vaccine's effectiveness, but, due to its toxicity, it is not a suitable option for vaccine administration. These tests show that pollen grains (including plant, moss, fern, algae, and bacterial spores) are superior to vaccine testing mechanisms currently in use and present a viable option for oral vaccination [20-21].

4.11 Other effects

Andersen *et al,* (1993) investigated *Lycopodium* spores in transrectal ultrasound-guided core biopsies of the prostate [22]. Sporopollenin from *Lycopodium clavatum* has been found to be capable of acting as a solid support for peptide synthesis because it is stable against chloromethylation and routinely used deblocking procedures [23]. Zimudzi 2007 investigated that lycopodine stimulates the peristaltic movements of the intestine and causes contraction of the uterus in animals. It removes state of tiredness and a chronic fatigue. Specifically huperzine A was reported to have in vivo antioxidant activity in several studies [24-26]. Another *Lycopodium* species of New Zealand i.e., *L. varium* was shown to possess insecticidal activity towards the flies *Anthrenocerus australis, Lucilia cuprina,* and *Tienda bisselliella,* which led to the isolation of huperzine A as the active component [27]. *L. clavatum,* the most common species has been reported to have a healing effect on wounds and dermatological diseases, including rash in babies and, therefore, called "belly powder" [1]. The spores of *Lycopodium clavatum* are routinely used by native Americans in treating nose bleeding and in wound healing. *Lycopodium clavatum* ethanolic extracts have been found to inhibit markedly CYP3A4 thereby has an additional potential to be used as antidiabetic [28].

5. Conclusion

We have reported hepatoprotective ability of *L. clavatum* in induced hepatocarcinogenesis. It would be prudent to investigate its constituents singly and in combination, how they modulate pathological changes and which form is more potent or effective. Time of collection of plant materials, place of collection, extraction procedures, and its storage might affect its active compounds both quantitatively and qualitatively. The information summarizes here

concerning *L. clavatum* is intended to serve as a reference to researchers involved in ethno-pharmacological research. Positive results obtained in animal models (rats/mice) could be extrapolated in a meaningful and convincing manner for their possible human use.

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