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An overview on the chemical and biological aspects of lycorine alkaloid

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Abstract

Amaryllidaceae plants are currently appreciated as a plentiful source of unique bioactive alkaloids. Among them, lycorine is one of the major pyrrolophenanthridine alkaloids commonly isolated from different species of this family. Over the past years, lycorine has attracted great research interest owing to its superlative biological potential and pharmacological actions, including antitumor, antiangiogenic, antiviral, antibacterial, antimalarial, anti-parasite, antioxidant, hepatoprotective, analgesic, anti-inflammatory activities, and inhibition of ascorbic acid synthesis, among many others. Therefore, this review was undertaken to highlight the chemical, chromatographic, spectral, and biological aspects of this promising Amaryllidaceae alkaloid, together with a future perspective for its potential applications in the pharmaceutical field.

Key words
Amaryllidaceae,alkaloids, lycorine, chemical properties, biological activities

1. Introduction

Alkaloids constitute a diverse class of plant secondary metabolites with varied chemical structures and biological activities. In the context of their taxonomic distribution, family Amaryllidaceae, commonly known as the Amaryllis or Daffodil family, is one of the twenty most important alkaloid-containing families in the plant kingdom [1]. Amaryllidaceae belongs to the monocotorder Asparagales and comprises more than 75 genera and 1600 species that are mainly categorized into 15 tribes and 4 subfamilies [2]. Plants of this family are perennial, bulbous, and flowering herbs with a wide distribution in the tropical and subtropical regions of the world [1]. Amaryllidaceae plants have been used for centuries as folk herbal remedies against several disorders, especially for inflammatory, circulatory, and neurological conditions [3].In southern Africa, different Amaryllidaceae species are also used to treat swelling of the body, urinary tract problems, and itchy rashes [4]. To date, most reports have predominantly attributed the medicinal value of Amaryllidaceae plants to their tyrosine-derived alkaloids, which are produced exclusively within this family [5]. In this point of view, a total of about 357 Amaryllidaceae alkaloids with diverse skeletons have been isolated and identified so far, and were also reported to possess a wide range of biological properties, including cytotoxic, antinociceptive, anti-inflammatory, antimicrobial, antidepressant, and cholinesterase inhibitory activities [6]. As a result, family Amaryllidaceae is undoubtedly regarded as a great treasure house for novel drug discovery [7].

From a chemical point of view, Amaryllidaceae alkaloids are classified into nine main structural classes, comprising belladine, lycorine, homolycorine, crinine, haemanthamine, tazettine, galanthamine, montanine, and narciclasine types, in addition to other minor groups, such as cherylline, ismine, plicamine, phenanthridine, phenanthridone, and mesembrine alkaloids. Lycorine-type alkaloids, which exhibit a unique pyrrolophenanthridine skeleton, represent the most common alkaloidal group, of which lycorine is the major alkaloid commonly found in the leaves and bulbs of all Amaryllidaceae plants [8]. This alkaloid was firstly isolated in 1877 from *Narcissus pseudonarcissus* L. and its structure was elucidated by *Nagakawa et al.* in 1956 [9]. Over the past decades, lycorine has attracted a special interest owing to its outstanding biological properties, including among others, analgesic, anti-inflammatory, antiviral, antibacterial, antifungal, antiprotozoal, as well aswide-ranging cytotoxic effects against numerous tumor cell lines, e.g., leukemia, cervical cancer, and prostate cancer [7]. Accordingly, this review aims to recapitulate the chemical, spectral, and biological features of this important Amaryllidaceae alkaloid, along with a future perspective for its possible application in the pharmaceutical field.

Biosynthesis of lycorine

Lycorine-type alkaloids are formed biogenetically by intramolecular ortho-paraoxidative coupling of the key intermediate O-methylnorbelladine, derived from the amino acids L-phenylalanine and L-tyrosine (**Figure 1**) [10]. Lycorine is considered as a prototypical member of over 70 related pyrrolophenanthridine alkaloids that have been described from Amaryllidaceae till now [11].

Plant sources of lycorine

Lycorine was firstly isolated fromthe Amaryllidaceous plant *Narcissus pseudonarcissusin* 1877, then its structure was elucidated in 1956 by *Nagakawa et al* [9]. Being the most

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commonly encountered alkaloid within Amaryllidaceae species, it has been further identified from different genera of family Amaryllidaceae, such as Ammocharis, Boophane, Brunsvigia, Crinum, Galanthus, Haemanthus, Hippeastrum, Hymenocallis, Leucojum, Lycoris, Narcisuss, Sternbergia, and, Zephyranthes [12].It is worth mentioning that lycorine can be obtained in considerable amounts up to grams during acid-base fractionation of most Amaryllidaceae plants [13]. Moreover, an in vitro biosynthetic system was designed to increase the amount of lycorine in the tissue cultures of Leucojum aestivumL. Plant, which reached to 20 mg/100 g plant (0.02%) [4].

Chemical, physical, and chromatographic properties

Lycorine, also named as galanthidine, amarylline, or narcissine [13] ,is 2, 4, 5, 7, 12b, 12c-hexahydro-lH- (1,3) dioxolo (4,5-j)-pyrrolo (3,2,1-de) phenanthridine-1-diol (**Figure 1**), has the chemical formula C16H17NO4 and the molecular weight 287.315 [14] .It is usually isolated in the form of colorless prismatic crystals, which melt at 260–262 °C, whereas its hydrochloride salt is needle-shaped crystals with a melting point of 217 °C [4]. Owing to its remarkable stability, lycorine can be preserved at room temperature for about three years [4]. Lycorine is completely insoluble in petroleum ether, diethyl ether, and chloroform but displays moderate solubility in ethyl acetate, acetone, and methanol [13]. Lycorine shows green fluorescence under UV light (254 nm) during thin layer chromatographic (TLC) analysis and gives an orange color after spraying with modified Dragendorff's reagent, in addition to a yellow color with 10% sulphuric acid [13].A wide range of solvent systems can be used for TLC analysis or purification of lycorine on silica gel, e.g., petroleum ether-ethyl acetate(1:1) [Rf= 0.36] and chloroform-methanol (95:5) [Rf= 0.43] [13].

Analysis and quantification of lycorine

So far, a variety of analytical techniques have been described for the qualitative and quantitative determination of lycorine in various parts of Amaryllidaceae plants, including capillary gas chromatography-mass spectrometry (CGC-MS), reversed-phase high performance liquid chromatography (RP-HPLC),as well as spectrophotometric and fluorimetric techniques. Evidente *et al.* have validated a convenient and reliable method for determination of lycorine using RP-HPLC. The analysis was performed on a Perkin-Elmer C18/10 stainless-steel column (25 x 4.6 mm I.D.) using acetonitrile-0.01M ammonium carbonate (47:53 v/v) as the mobile phase at a flow rate of 2 ml/min. The standard solution was prepared by dissolving 10 mg of recrystallized lycorine in 100 ml of 1% sulphuric acid. Lycorine was detected by monitoring the chromatographic effluent at 290 nm with a detection limit of 5 ng. The retention time of lycorine under these chromatographic conditions was 2.55 min. [15].

In another study, an accurate, specific, repeatable, and robust high performance thin layer chromatographic (HPTLC) method was developed for the determination of lycorine in plant extracts. The silica gel HPTLC plates, developed with chloroform-methanol (9:1) as the mobile phase, showed compact, flat, and fluorescent bands of lycorine (R_F= 0.3) when viewed under UV light at 368 nm. The peak areas and the amount of the applied alkaloid showed a linear relationship over the range 0.2-1.21 g spot. The three-point calibration curve exhibited also a linear regression correlation coefficient of 0.99972 [16].

In light of the wide pharmacological potential of lycorine, a liquid chromatography-mass spectrometry method (LC-MS) has been reported for the quantitative bio analysis of lycorine in mice plasma to uncover its pharmacokinetics. However, this method suffered from long analytical time, large volume of plasma needed, as well as complicated liquid-liquid extraction procedure [17]. In contrast, another simple, rapid, and sensitive HPLC-MS/MS method was developed and validated for determining lycorine in rat plasma. Plasma samples of rats, intraperitoneally injected with lycorine, were prepared by a simple protein precipitation with methanol containing dextrorphan as the internal standard. The chromatographic separation was carried out on Kromasil 60-5CN column (3 µm, 2.1 mm x 150 mm) with methanol-water (containing 0.1% formic acid) (40:60, v/v) at a flow rate of 0.2 ml/min. The total analytical run time was 5 min, whereas the detection was performed on a triple quadrupole tandem mass spectrometer equipped with electronic spray ion by selected reaction monitoring (SRM) of the transitions at *m/z* 288.1 to 147.1 for lycorine and at *m/z* 258.1 to 157.2 for dextrorphan. Interestingly, lycorine was found to be sufficiently stable under all the applied analytical conditions [18].

Spectral analysis of lycorine

UVspectral analysis of lycorine in methanol displaysthree absorption bands at λ_{max} 292 nm (strong peak), 235 nm (strong peak), and 217 nm (shoulder), which is characteristic for lycorine-type alkaloids with methylenedioxy aryl chromophore [19]. The EI-MS spectrum of lycorine shows a molecular ion peak at *m/z* 287 (100%) in agreement with its molecular formula $C_{16}H_{17}NO_4$. It also exhibits two diagnostic fragment ion peaks of lycorine-type alkaloids at *m/z* 227 (73%) and 226 (53%) corresponding to $C_{14}H_{13}NO_2$ and $C_{14}H_{12}NO_2$ due to the loss of $C_2H_4O_2$ and $C_2H_5O_2$, respectively. The formation of these intense fragment ions is attributed to the loss of carbon atoms C-1 and C-2 and their substituent (**Figure 2**) [20]. Some other characteristic fragments appear also at *m/z* 270 (10%), 269 (36%), and 250.0 (19%) corresponding to $[M^+–OH]$, $[M^+–H_2O]$, and $[M^+–2H_2O]$, respectively [2].

^{1}H-NMR spectrum of lycorine (500 MHz, DMSO-d_6)shows the following signals (ppm): 4.24 (1H,br.s, H-1), 3.94 (1H, br.s, H-2), 5.33 (1H, br.s, H-3), 2.38 (2H, m, H-4), 2.15 (1H, ddd, *J*= 9.0, 8.4, 8.4 Hz , H-5α), 3.15 (1H, t-like, *J*= 9.0, 7.65 Hz,H-5β), 3.26 (1H, d, *J*= 13.8 Hz, H-7α), 3.96 (1H, d, *J*= 13.8 Hz, H-7β), 6.64 (1H, s, H-8), 6.77 (1H, s, H-11), 2.46 (1H, br.d, *J*= 9.9 Hz, H-11b), 2.55 (1H, br.d, *J*= 9.9 Hz, H-11c), 4.79 (OH-1, br.d, *J*= 3.8 Hz), 4.90 (OH-2, br.d, *J*= 6.1 Hz), 5.91-5.92 (-OCH_2O-, 2s). The ^{13}C-NMR spectrum (175 MHz, DMSO-d_6) shows the following signals (ppm): 70.65 (C-1), 72.16 (C-2), 118.93 (C-3),

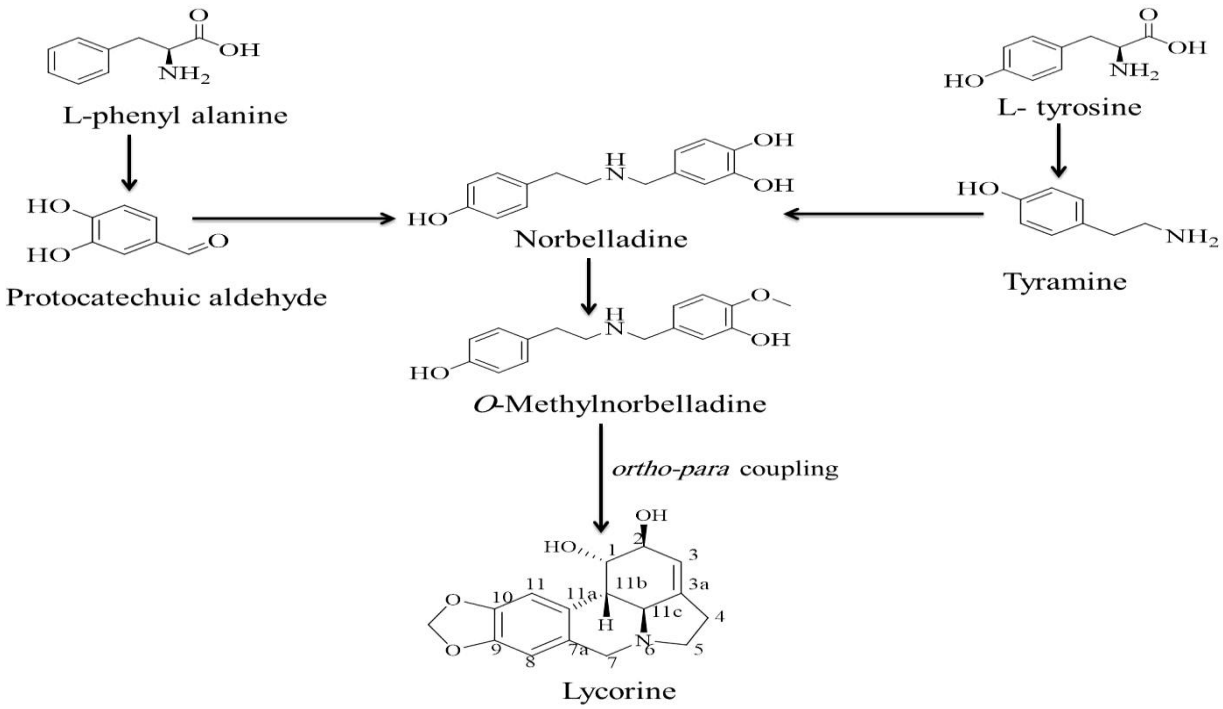

Figure 1: The biosynthetic pathway of lycorine

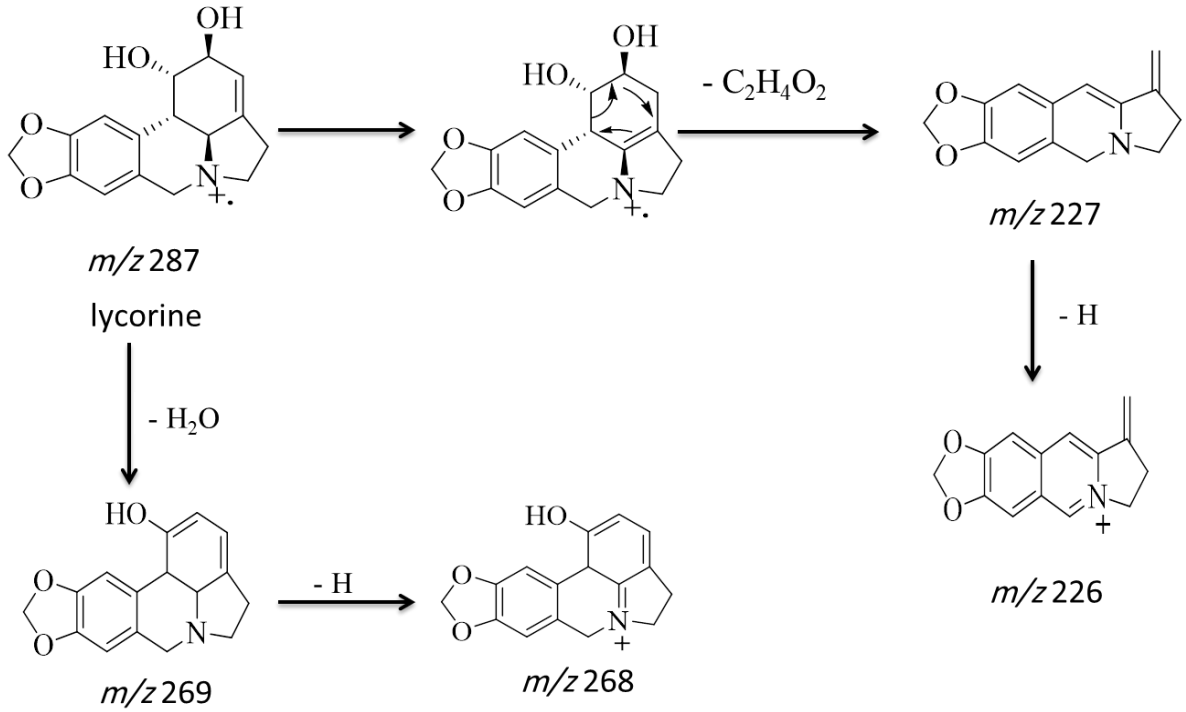

Figure 2: Mass fragmentation pattern of lycorine.

142.14 (C-3a), 28.57 (C-4), 53.76 (C-5),57.18 (C-7), 130.2(C-7a), 105.52 (C-8), 146.10 (C-9), 145.66 (C-10), 107.49 (C-11), 130.02 (C-11a), 40.61 (C-11b), 61.29 (C-11c), 101.03(-OCH_2O-) [13].

Biological activities of lycorine

Cytotoxic and antitumor activities

Lycorine was the first identified cytostatic compound from Amaryllidaceae [21]. After that, has been intensively investigated in various preclinical models of human cancers both *in vitro* and *in vivo*. In general, lycorine is recognized as a low micromolar antiproliferative agent against a wide range of multidrugresistant and apoptosis-resistant tumor cells [22, 23], showing a selective cytotoxicity via mitochondrial pathways and induction of apoptosis. Lycorine was found to down-regulateMcl-1 in human leukemia cells [24]. The apoptotic effect of lycorine involves cell cycle regulation in HL60 and KM3 cell lines, cytochrome-c release, and caspase activation [24]. It was also reported that lycorine can decrease HDACenzymatic activities in K562 cells and up-regulate the expression of p53 and its target gene product p21, thus inhibits the proliferation of K562 cells [25] Additionally, lycorine hydrochloride was also shown to effectively suppress metastatic melanoma C8161cell-dominant formation of capillary-like tubes *in vitro* as well as the generation of tumor blood vessels *in vivo* with a low toxicity. Such effects of lycorine hydrochlorideare mediated by inhibition of melanoma C8161 cell-dominant vasculogenicmimicry by reducing VE-cadherin gene expression and diminishing cell surface exposure of the protein [22]. Recently, the lycorinederivative 4-ethyl-5-methyl-5,6-dihydro-[1,3] dioxolo [4,5-j] phenanthridine (HLY78) has been identified as an activator of the Wnt/β-catenin signaling pathway in a Wnt (Wingless and INT-1) ligand-dependent manner. HLY78 was also found to promote LRP6 phosphorylation and Wnt signaling transduction, which is of value against breast and prostate cancers and glioblastoma [26].

It has been reported that lycorine exhibits cytostatic effects rather than cytotoxic actions, mostly through impairing the actin cytoskeleton organization in a large panel of apoptosis-resistant cancer cell lines. Furthermore, lycorine provides significant therapeutic benefits in mice bearing brain grafts of the B16F10 melanoma model at non-toxic doses, with a beneficial *in vitro* therapeutic potential that isover 15 times more active against cancerthan normal cells [27]. Such therapeutic potential has been also demonstrated in a number of mouse and human cancer models, including Hey1B ovarian cancer, LLC lungcarcinoma, and HL-60 leukemia [28, 29]. The *in vitro* inhibitory actions of lycorine against a number of tumor cell lines are summarized in (**Table 1**).

It was also indicated that lycorine can inhibit the proliferation of five human leukemia cell lines in a dose-dependent manner with IC_{50} values ranging from 1.5 to 5.5 μM, while the viability of the normal peripheral blood mononuclear cells from five healthy subjects was not significantly affected by the same treatment at lycorine concentrations of up to 50 μM. Therefore, the sensitivity of cancer cells to lycorine was described as 15-fold higher than that of the corresponding normal cells [22, 24, 28].

Table 1: In vitro growth inhibitory activities of lycorine on various tumor cell lines.

Tumor type	Cell line	IC50 (μM)	References
Leukemia	HL-60	1 ± 0.0	(28, 30)
	K562	3.6 ± 1.2	(5, 31)
	U937	2.42 ± 0.0	(30)
	CEM	1.6 ± 0.0	(5, 22)
Ovarian cancer	Hey1B	1.2 ± 0.0	(32)
Multiple myeloma	KM3	1.25 ± 0.0	(4, 33)
Breast adenocarcinoma	MCF-7	13.0 ± 2.9	(5, 22)
Colon adenocarcinoma	HT-29	3.2 ± 0.0	(28)
Hepatoma	HepG2	3.7 ± 0.0	(28)
	G-361	5.0 ± 0.3	(22, 31)
	C8161	1.2 ± 0.0	(32)
Melanoma	SKMEL-28	8.5 ± 0.3	(5, 23)
	B16F10	6.3 ± 0.2	(24, 34)
Cervical adenocarcinoma	Hela	10.6 ± 0.9	(5, 22)
Lung cancer	A549	4.2 ±0.4	(24, 34)
	LLC	0.5 ± 0.0	(4, 35)
Glioblastoma	Hs683	6.9 ± 0.5	
	U373	7.6 ± 0.4	(24, 34)
Eophageal cancer	OE21	4.5 ± 0.7	
	BJ	1.9 ± 0.1	(31)

Antiangiogenic activity

In 1971, Folkman raised a theory of tumor angiogenesis; suggesting that endothelial propagation angiogenesis is a necessary event for malignant tumor growth and metastasis [36]. Tumor cell-mediated neovascularization significantly promotes tumor growth, progression, and cancer metastasis, and is closely associated with poor prognosis of cancer patients [37]. In this context, lycorine was shown to effectively stop metastatic melanoma cell-dominant vasculogenic simulation, reduce melanoma cell-mediated development of capillary-like tubes *in vitro*, and prevent generation of tumor blood vessels *in vivo* [22]. These effects were attributed to inhibition of the activity of VE-cadherin promoter and reduction of VE-cadherin expression [22]. Likewise, lycorine was also demonstrated to suppress ovarian cancer cell neovascularization through destroying the formation of capillary-like tubes by Hey1B cells *in vitro*, in addition to the ovarian cancer cell-dominant vascularity *in vivo* when administered to Hey1B-xenotransplanted mice. Such antiangiogenic action of lycorine is related to diminishing the expression of several key angiogenic and vasculogenic genes, including VE-cadherin, vascular endothelial growth factor (VEGF), and semaphorin 4D (Sema4D). the ability of lycorine to reduce Akt phosphorylation and block Akt signaling pathway in Hey1B cells was also reported [28].

Inhibition of topoisomerase I

Lycorine displayed significant inhibitory effects on DNA topoisomerase I activity that is required for cancer cell growth [38]. In a comparative study on the topoisomerase I inhibitory potential of a number of pyrrolophenanthridine alkaloids using genetically engineered mutants of the yeast *Saccharomyces cerevisiae* (strains RAD+, RAD52Y, and RS321), lycorine was found to be highly effective. Accordingly, lycorine as well as its related derivatives might represent a promising starting point for novel anti-cancer drug discovery with regard to topoisomerase inhibition [28].

Anticholinesterase activity

Amaryllidaceae alkaloidsare widely known for their acetyl cholinesterase (AChE) and butyryl cholinesterase (BuChE) inhibitorypotential; however, lycorine exhibit no or very weak AChE inhibitory actionswith an IC_{50} of 213±1μM [5, 39, 40]. Therefore, a variety of structurally modified bioactive derivatives of lycorine have been developed, such as 2-O-*ter*-butyldimethylsilyl-1-*O*-(methylthio) methyllycorine, which is a dual inhibitor of human AChE and BuChE with IC_{50} values of 11.40±0.66 and 4.17±0.29 μM, respectively [41], whereas the acylated/etherified derivatives of lycorine as well as lycorin-2-one are more potent molecules against BuChE than AChE [42]. In the same way, 1-*O*-acetyllycorineis a powerful inhibitor of AChE (IC_{50}= 0.96±0.04 μM) and is two-fold more potent than galanthamine [5, 40]; however, 1,2-*O*-diacetyllycorine was found to exhibit no or very weak inhibitory effects (IC_{50}= 211±10.0μM) [5, 39].

Antibacterial activity

In a study by Evidente *et al*., lycorine displayed no significant inhibitory actions against *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeroginosa*. using the agar disc diffusion technique [43]. In another work, the antibacterial activities of lycorine and its analogues were evaluated against two isolates (ALM-00-173 and BioMed) of the Gram-negative bacterium *Flavobacterium columnare*, which occurs in channel catfish and causes columnaris disease. Results indicated that the substitution at C-1 or C-2 is pivotal to the antibacterial efficacy of these compounds with the maximum activity were recorded by the disubstituted lycorine *O*-analogues. Among them, the carbamate analogue was the most active against both *F. columnare* isolates with an IC_{50} (24 h) value of 3.0 mg/l [5]. Likewise, 1-*O*-acetyllycorine was more effective than lycorine against both isolates [44].

Antiviral activity

Preliminary evaluation of the antiviral effects of lycorine and its derivatives indicated theirpossible*in vitro*/*in vivo* activities against *Flaviviridae*, *Bunyaviridae*, and *Japanese encephalitis* [45]. It is reported that lycorine inhibited *flaviviruses* mainly through suppression of viral RNA synthesis. Furthermore, lycorine strongly hinders RNA synthesis in *flaviviruses* and weakly inhibits viral protein translation [5, 46]. Lycorine also exhibits strong activities against influenza A virus N5H1 *in vitro*. Mechanistic studies showed its ability to delay the export of nucleoprotein from the nucleus to the cytoplasm in single and multiple replications [5, 47]. It has been also demonstrated that lycorine can inhibit the cytopathic effect induced by severe acute respiratory syndrome associated coronavirus (SARS-CoV), with an EC_{50} value of15.7±1.2 nM [48]. In another work, the antiviral activity of lycorine against poliovirus using a cellular fluorescence resonance energy transfer assay has been reported. It was found that lycorine can reduce 1 log 10 unit of virus titer at 2.5 mM without cytotoxicity [49]. Moreover, Zhang and his co-workers have reported that lycorine can inhibit human enterovirus 71 (EV71) infectionin rhabdomyosarcoma cells [30]. Similarly, lycorine exerts powerful antiviral effects on several RNA and DNA viruses, such as Coxsackie B_2, Poliomyelitis, and Herpes type I [50], but is inactive against some other viruses, including alphavirus (Western equine encephalitis virus) and rhabdovirus (vesicular stomatitis virus) [4, 46].

Anti-parasitic activity

The cytotoxicityof lycorine in *Trichomonas vaginalis* was found to be different in comparison with its activities against a variety of tumor cell lines mentioned before. For instance, lycorine arrests the parasite cell cycle but fails to fulfill the criteria for apoptosis or apoptosis-like death. However, some similarities to paraptotic cell death described for multicellular organisms were observed [51]. Lycorine was found to strongly inhibit the activities of nucleoside triphosphate diphosphohydrolase (NTPDase) and ecto-50-nucleotidase in the 24 h-treated *Trichomonas* parasites, while the transcript levels of NTPDase A or B were notaltered by lycorine [51]. A preliminary study of the structure-activity relationship has indicated that the cell death evoked by lycorine in *T. vaginalis* is independent of the presence of hydroxyl groups at C-1 and C-2, whereas the greatestanti-parasitic activity was obtained after esterification of lycorine at C-2 with a lauroyl group [52]. On the other hand,lycorine was found to be active against *Entamoeba hystolitica* with an IC_{50} of 0.23 μg/ml [53]. It also displays a significant aphicidal activity to other parasites, such as *Tribolium castaneum* and *Aphis gossypii* [54].

Antimalarial activity

Lycorine exhibited a dose-dependent antimalarial activity when tested against *Plasmodium falciparum* (T9.96) and *P. falciparum* (K1) at four doses (0.04, 0.2, 1.0, and 5.0 μg/ml) with IC_{50} values of 1.026 and 0.379 μg/ml, respectively [55]. In another work, the antimalarial activity of new synthetic lycorine derivatives against the drug-resistant K1 strain and the drug-sensitive FCR3 strain of *P. falciparum* was evaluated. All the tested compounds, including 1,2-di-*O*-butanoyllycorine, 1-*O*-propanoyl lycorine, 1-*O*-(3'*R*)-hydroxy butanoyl lycorine, and 1-*O*-(3'*S*)-hydroxy butanoyl lycorine showed potent antimalarial activities with IC_{50} values of 0.67, 0.37, 0.60, and 0.62 μg/ml for

the K1 strain and of 0.53, 0.30, 0.45, and 0.49 µg/ml for the FCR3 strain, respectively [56]. The results suggested that the best antimalarial activities are achieved with lycorine derivatives having free hydroxyl groups at C-1 and C-2 or esterified as acetates or isobutyrates. The presence of a double bond between C-2 and C-3 was also shown to be necessary for their antimalarial potential [5, 57].

Anti-inflammatory activity

Lycorine exerts good anti-arthriticpotential when tested in various animal models [12, 58-60]. It successfully blocks rat paw edema induced by carrageen an with an ED_{50} of 0.514 mg/kg [12]. It also efficiently reduces the cytotoxicity of calprotectin, a pro-inflammatory factor contributing to the development of inflammation leading to tissue destruction in severe inflammatory diseases [61]. Moreover, lycorine blocks lipopolysaccharide (LPS)-induced production of pro-inflammatory mediators and also decreases LPS-induced mortality in mice [60]. The involved mechanisms include controlling of the LPS-induced up-regulation of inducible nitric oxidesynthase and cyclooxygenase-2 protein levels in RAW264.7 cells. Furthermore, lycorine reduces the release of nitric oxide, PGE2, TNF-alpha, and IL-6 from LPS-treated RAW264.7 cells, and inhibits LPS-induced activation of P38 MAPK and Jak-STAT signaling pathways. Such appreciable anti-inflammatory activity of lycorine provides new insight into the development of anti-arthritis and anti-inflammatory therapies.

Immunological activity

Lycorine has been patented as an immunosuppressor and can be useful in suppression of the immune systems of mammals for the treatment of autoimmune diseases, complex immune syndromes, allergic and rheumatic conditions, as well as for prophylaxis against transplant rejections [62, 63].

Antioxidant, hepatoprotective, and metabolic effects

Lycorine showed significant DPPH scavenging effects [64]. It also exerted protective effects on human erythrocytes against oxidative damage induced by 2-amidinopropane due to its antioxidant nature [65]. Lately, lycorine has been shown to exhibit significant hepatoprotective effects against CCl_4-induced oxidative stress in Swiss albino mice at 5 mg/kg that were also comparable to Silymarin. It effectively normalized the increased generation of lipid peroxidation products and reduced the elevated levels of malondialdhyde, glucose, urea, bilirubin, and hepatic marker enzymes. It also restoredboth the levels of glutathione and vitamin C and the activities of superoxide dismutase, catalase, glutathione-*S*-transferase, and glutathione reductase. Moreover, the histological and ultra-structural observations have evidenced the protective effects of lycorine on hepatocytes against CCl_4-induced oxidative damage without disturbing their cellular metabolic functions [8, 66-68].

Effects on sexual functions

Application of lycorinethe testes and ovaries of immature rats inhibited cell division in the spermatogonia or primary spermatocytes. No spermatid cells were also found in the tested animals, whereas follicles were found to be smaller and less in number in rat's ovaries [63, 69].

Inhibition of ascorbic acid biosynthesis

Ascorbic acid is synthesized in plants from glucose and acts as a free-radical scavenger in a variety of cell partitions [70]. It is also a common antioxidant component in the apoplast and is known to be a significant *in vitro* inhibitor of peroxidases [4]. Lycorine has been proved to be an inhibitor of ascorbic acid biosynthesis both *in vitro* and *in vivo* [70]. It powerfully inhibits the *in vivo* conversion of galactono-gamma-lactone to ascorbic acid [71]. At a dose of 50 µM, lycorine was found to significantly inhibit ascorbic acid biosynthesis, and the effect continued even when the alkaloid was removed from the incubation medium. Further studies showed that lycorine selectively inhibit the activity of L-galactono-gamma-lactone dehydrogenase, but does not affect the activities of ascorbate peroxidase, ascorbate free radical reductase, and dehydroascorbate reductase [4, 71, 72]. In this regard, lycorine may be applied in multiple fields, such as plant breeding and tissue cultures [73, 74].

Other biological activities

It was reported that lycorinehasa greater analgesic activity than aspirin and indomethacin [68, 75]. It also displayed anticonvulsant effects when evaluated by the electrical stimulation test in rats [64]. A number of Amaryllidaceae alkaloids, including lycorine, have been shown to have vasorelaxing effects due to their smooth muscle relaxing actions that are mostly attributed to their resemblance to isoquinoline alkaloids, e.g., papverine [76].

In a study to investigate the effects of twenty Amaryllidaceae alkaloid on the blood pressure of normotensive rats at 1.5 mg/kg, lycorine did not affect the normal blood pressure of rats [77]. Lycorine was also shown to inhibit both the production of nitric oxide and the induction of inducible nitric oxide synthase [60, 78]. Lycorine displayed a marked choleretic effect at 1 mg/kg in rats anaesthetized with urethane [79].

Lycorine was shown to possess growth inhibitory actions on several *Saccharomyces cerevisiae* strains, e.g., rho^+, rho^-, and mit^-; while those lacking mitochondrial DNA are resistant to lycorine at concentrationsexceeding 200 µg/ml; suggesting that such mitochondrialgenes are convoluted in the effects of lycorine [80, 81].The resistance of rho^o stains to lycorine was also reported to be associated with the removal of RTGgene, a key mitochondrial functional gene [82]. Besides, lycorinewas found to efficiently inhibit the growth of isogenic rho^+ RTG and rho^oDrtg strains, but does not affect the growth of isogenic rho^ostrain with RTG nuclear genotype.

On the other hand, lycorine is a powerful inhibitor of growth and cell division in higher plants, algae, and yeasts [83], while lycorine 1-*O*-glucoside is a potent promoter of root growth and

seed germination in higher plants, e.g., *Allium cepa* [84, 85].Additionally, lycorine can control circadian period length [86]. When the cells were pretreated with lycorine and then the active principle was removed, the period length was returned as compared with that of the control cells; demonstrating that the elongation of the circadian period induced by lycorine is reversible. In this regard, lycorine was found to modulate the transcription of the key clock gene, namely brain and muscle Arnt-like protein- 1 (Bmal1), resulting in the circadian period changes.

Toxicological studies

Lycorine showsvery minor toxicity to normal cells. Toxicological experiments on animals revealed that the LD_{50} values of lycorine in mice were 112.2±0.024 and 344 mg/kg mg/kg via intraperitoneal injection and gastric lavage injection; suggesting its very low toxicity after gastrointestinal administration [87]. Subcutaneous injection of 3mg/kg lycorine into beagle dogs only induced a short salivation increase, meanwhile, the dogs with the same dosage were found to have mild continuous vomiting and diarrhea [87]. In an open, randomized, and controlled trial, subcutaneously administered lycorine induced nausea and emesis at 0.5 mg/kg body weight that were statistically significant at 1.0 mg/kg, whereas the maximum emetic dose was 2 mg/kg bodyweight [88]. The induced nausea and emesis were short-lasting and extended not later than 2.5 h post dose. The tested dogs did not show any other noticeable adverse effects; proposing that lycorine is very low toxic. Results suggested that neurokinin-1 and to a lesser extent 5-hydroxytryptamine receptors are predominantly involved in the lycorine-induced emesis. Besides, both the biochemical and hematological parameters indicated no pathological signs[89].

Conclusion and perspectives

Amaryllidaceae is a prolific and exclusive source of unique bioactive alkaloids with divergent chemical structures, of which lycorine-type alkaloids are predominantly biosynthesized by all Amaryllidaceous species. As the most important alkaloid, the physical, chemical, spectral, and biological properties of lycorine have long been extensively studied, and it was reported to exhibit a wide range of significant biological activities, including antitumor, antiviral, anti-parasitic, antimalarial, and anti-inflammatory effects. Despite of the comprehensive progress in investigating the biological potential of lycorine and its related derivatives, *some important points* have to be considered in future research studies. Firstly, comparing the bioactivities and structure-activity relationship of lycorine as well as its natural and synthetic analogues should be necessarily accompanied with clarifying the probable mechanisms of action, particularly with regard to their promising antitumor, anti-parasitic, antimalarial, and antiviral potential. Exploring the cellular and molecular features of such effects will be of a great value in designing new effective compounds based on lycorine. Besides, the development of additional synthetic derivatives of lycorine should also focus on improving the efficacy and safety, together with widening the scope of their biological testing. Unfortunately, in spite of the interesting pharmacological and therapeutic potential of lycorine and its available derivatives, none of them has been translated into existing market products till now; therefore, future research should consider some detailed preclinical and clinical analyses of such potential drug candidates. Finally, for enhancing the production of lycorine and its biosynthetically-related alkaloids in substantial quantities, more attention should be paid to tissue culturing of Amaryllidaceae plants as an alternative and more convenient approach to replace the routine phytochemical isolation and purification of lycorine from these plants.

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