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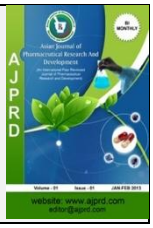

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Review Article

Pharmacognostic Characteristics, Chemistry, Biological Activity and Toxicity of *Lolium* Species

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ABSTRACT

Around seven species of the genous lolium poisonous grasses belonging to the family Poaceae are mutually grown in corps field over the world. In Iraq the prennel ryegrass is locally called "rewatta".The toxicity of these gasses are related to three chemically distinct alkaloids groups; the aminopyrrolizidine; lolines, indole-diterpenes (ergots, loliterms, and paxillines) as well as peramine alkaloids mostly concentrated in their seeds although indole-diterpene alkaloids loliterm B and paxilline biosynthesis requires endophytes symbiosis. The level of loline alkaloids enhances in both late summer-autumn of the year as well as in the infected dry plant materials up to 10 fold. However, paxilline and ergovaline are believed to be the precursor of the most toxic lolium species alkaloids, loliterm B, although, indole-diterpene alkaloids paxillines, loliterms and ergovaline are the actual indicators of Lolium species. In this review we summarize chemical characteristics, biological and toxicological influences as well as their interrelation of the plant of lolium genus. Central as well as peripheral biological/toxicological manifestations are summarized for both loline and indole-diterpene alkaloids. Finally, toxic influences of lolium alkaloids are function of their biological influences mostly exhibited via resembling molecular mechansims centrally as well as peripherallyare concluded.

Key words: Pharmacognostic, lolium, alkaloids, chemistry, biological, phytochemicals, toxicity.

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INTRODUCTION

There are several species of the cool season grass that belongs to the genus *Lolium* which belongs to the family Poaceae or Gramineae subfamily Pooideae [1], including the perennial ryegrass *Lolium perenne* (meadow fescue) [2-5] or also called *Lolium pratense* (=*F. pratensis*, meadow fescue) [6-12], the tall fescue *Loliumarundinaceum* (*Festuca arundinacea*) [7, 13, 14], *Lolium giganteum* (=*F. gigantea*) [6], *Lolium multiflorum*, *Lolium rigidum* [15], *Loliumcuneatum* Nevski [16],and *Lolium temulentum* [15, 17]. In addition, there are hybrids of tall fescue and *Lolium* (tall fescue)or *Lolium-Festuca* [18, 19]. The ryegrasses and broad-leaf fescues of *Lolium* species is distributed Europe and the Mediterranean [1]. In the central parts of Asia the herb Lolium cuneatum Nevski grows as a poisonous weed within the fields wheat, barley, as well as flax [20] while*Lolium temulentum* L. is one of the predominant poisonous plants in Pakistan in the discrete Bannu, Khyber Pakhtunkhwa known as darnel or poisonryegrass besides, its global abundance in the cereal fields of the developing contries where it is considered as the worst weed [21-23]. Remarkably, in Egypt, *Lolium temulentum* (*L. cuneatum*) is reported to be one of five species belongs to the genus *Lolium*[24] contaminating the wheat crop where it is commonly known by the Arabic name "zawan", yet, dernel and invraie in other countries [25]. Nevertheless, thePerennial Ryegrass *Lolium perenne*is detected in Australia and North America, while, the Wimmera ryegrass Lolium rigidum Gaud. is located in Australia, South Africa, and, rarely, North America [26]. The common names of the *Lolium* species are listed in table (1) while their local names are listed in table (2) adapted from (Thomas, et al. 2011) [27]. It has been reported that the seed transmitted fungal clavicipitaceous endophytes including *Neotyphodium* or *Epichloe¨* (Clavicipitaceae) are

associated with plants' production of nitrogen rich loline [1, 2, 6, 7, 9, 11, 15, 28-33], ergot lolitrems, peramine alkaloids [4, 34-38] and Festucine [14] that are chemo-active plant protective mechanism against vertebrate and invertebrate [2, 39]. Besides, various endophytes type fungus symbiosis including *Acremonium coenophialum* for is reported to be crucial for the biosynthesis of the pyrrolizidine as well as pyrrolopyrazine alkaloid in addition to their accumulation in the grasses [9, 35, 36] particularly peramine in *Lolium perenne* in symbiosis with *N. lolii*. [3]. however, *Neotyphodium uncinatum* is involved in the production of loline alkaloids in *Lolium pretense* [11, 12], *Acremonium coenophialum* Morgan-Jones and Gams are involved in the production of the considerable quantities of loline alkaloids in *L. arundinacea Schreb*. [40], yet, *E. uncinata* is involved in these alkaloids production in *L. pratensis* [41]. In this context, (Stegelmeier et al, 2013) have reported that such endophytes symbiosis with different *Lolium* species is associated with their neurotoxicity particularly that of *Lolium perenne* [26]. Remarkably, (Burhan, 1984) have reported the involvement of both endophytes symbiosis as well as plant age are related to the N-acetyl-loline (NAL) and N-formyl-Loline (NFL) production/concentration. In addition, the NAL and NFL levels are the highest after 9-11 weeks of seeding which declines after clipping (8≥weeks after seeding) [42, 43] which is greater in the *Epichloe¨ Lolium-Festuca* hybrids [44] as well as other pyrrolizidine alkaloids [18, 19], however, pyrrolizidine [18, 45] and ergopeptine [44] alkaloids is detected in non-infected(non-Epichloe¨)grasses. Moreover, (Liu, et al., 2001) have reported the direct involvement of L-homoserine in the loline alkaloids biosynthesis pathway in the *Acremonium chrysogenum* fungi [46]. Furthermore, (Craven et al., 2001) have reported that the type/amount of loline alkaloids phytosynthesis is associated with the genotype of fungus as well as its degree of symbiosis with the grass particularly *Lolium pratense*, reporting that the alkaloids level may mount 2% of the grass dry weight [8] which is in accordance to (Justus et al., 1997) who reported the highest level of loline alkaloids in the seeds infected with *N. uncinatum* [10].For example, theperamine mean level have been reported to be 26.21 $\pm$ 2.97 μg/g dry weight, of considerable toxic lolitrem B quantity, hence, exceeding 2 μg/g of dry plant weight (the toxicity level) in E. festucae var. lolii infected L. perenne seeds from New Zealand in late summer, while, their level in fresh grass is 24–37% of the dry plant [2] on one hand.The loline and other related alkaloids producing lolium species-endophytes symbiosis is listed in table (3) adapted from (Schardl, 2007) and other reports [1, 5, 6, 8-10, 13, 15, 17, 30].

Table 1: common names of the *Lolium* species [27].

Species	Common name
Lolium perenne	Perennial ryegrass
Lolium multiform	Italian ryegrass
Lolium rigidum	Annual ryegrass, wimmera ryegrass
Lolium canariense	Canary island ryegrass
Lolium loliaceum	----------
Lolium remotum	Hardy ryegrass
Lolium temulentum	Darnel, poisonous ryegrass
Lolium persicum	Persian darnel

Table: 2 local names of the *Lolium* species [27].

language	Common names of *Lolium* species
English	Bearded darnel, Poison darnel, Annual darnel, Red darnel, Poison ryegrass, Darnel ryegrass, Ray-grass, Tarse, Drake, Drawke, Drunk, Dragge, Study ryle, Cheat, Wonwoer, Chess, Virginina oat, Cokil, Cockle, Evir.
Arabic	Zirwan, Samma, Aqoullab, Zawan, Zuwan, Shaylam, Suwal, Sikra, Danaqa
Basque	Iraka
Breesciano	Fraina, Lergheta, Loi
Breton	Draog, Ivre, Pigal, Pilgere'h
Calabrese	Giogghju
Chinese	Du mai
Colombia	Ballico
Czech	Jelik
Dutch	Dolik, HandsdarivK
Estonian	Uimastav raihein
French	Ivraie annuelle, Ivraie enivrante, Herb a couteau, Herb d'ivrogne, Zizanie
German	Taumellolch, Taumel-Raygras
Hungarian	Konloly
India	Machni, Mochni, Mostaki
Italian	Loglio del Veleno, Loglio ubriacaute, Zizzania
Latin	Lolium temulentum
Morocco	Zwan, Zuwan, Gesmata, I –medhum, Sirkran, Sikra, Saylam, Laichour
Peru	Ballico, Cerisuelo, Sirisuela
Polish	Kakol, Zycicac roczna
portuguese	Joio
Spanish	Borrachuela, Cizana commun, Cizana embriagente, Cominillo, Joyo, Trigollo, Mala hierba, Rabillo
South Africa	Drabok raaigras, Dronkgras, Drabok
Swedidh	Darrepe
Romagnolo	Zizagna, Zizania
Valencian	Brossa
Zulu	Shesi
Welsh	Efrau, Efryn, Yd meddw, Edrau coliog, Pabi'r gwenith, Drewg, Pabi gwenith, Ller, Graban yr hwylydd, Lleren

Table 3: *Lolium* species-endophytes reported symbiosis [1, 5, 6, 8-10, 13, 15, 17, 30, 31].

Specie	Endophyte	Country
Lolium arundinaceum	N. coenophialum	USA
L. arundinaceum	N. coenophialum	Morocco
L. giganteum (L.) S. J. Drabyshire	E. festuca	Europe
L. multiflormLam	N. occultans	South Africa
L. persicum Bioss et Hohen	N. occultans	Iran
L. pretense	N. uncinatum	Europe
L. pretense	N. siegelii craven et al.	Germany
Lolium sp.	Neotyphodium sp. FaTG-3	Tunisia
L. rigidum Gaud.	N. occultans	Egypt
L. temulentum	N. occultans	Greece

However, (Blankenship, 2004) have also reported that the pyrrolizidine alkaloids such as loline class of these alkaloids in *Lolium pretense* is through ornithine-homospermidine pathway [4, 47] where spermidine [48].

LOLINE ALKALOIDS AND THEIR CHEMISTRY:

Globally, pyrrolizidine alkaloids are located in 3% of the flowering plants [49]. The level of these alkaloids varies with season, where in Germany grass lands it inclines to their optimum peak during summer to exceed the toxicity threshold in dry grasses (three folds of the fresh grasses),

while, remains bellow this threshold in fresh ones particularly those of the toxic alkaloids peramine, lolitrem B, and ergovaline [2]. Similarly, in Kentucky, USA it has been reported that the levels of these alkaloids particularly the various loline alkaloids in tall fescue are within the range of low (200-300 μg/g) during winter then inclines gradually during spring to approach their peak level during late summer [50] are reported in other studies [51-54]. In addition, (Bauer et al., 2018) have reported that pyrrolizidine/loline as well as indole-diterpene alkaloids such as paxilline and ergot levels are greater (up to ten folds) in the dry grasses as compared to an equivalent weight of the fresh grasses using immunochemical analysis [55].

(Hartmann, 1999) have classified these alkaloids into five classes these are class I and III which have α,β unsaturated necine core structure along with a macrocyclic, diester bridge between C9 and C7 including senecionine and monocrotaline. Second, Class II which have α,β-unsaturated core structure along with open chaine diesters linked to C9 and C7 comprising triangularine type alkaloid. The third, is class IV and V with single ester side chain at C9 comprising pyrrolizidines [56] as shown in figure (2). Chemically, the core structure of this class pyrrolizidine alkaloids is composed of two fused saturated heterocyclic pentagonal rings with a nitrogen atom at one of the bridgehead with amine group substitution as what is identified in the loline alkaloids causing ring staggering [26]. In addition, these saturated amino-pyrrolizidine alkaloids isolated from *Lolium* species have exocylic oxygen bridge occurs between C2 and C7. These loline alkaloids isolated from endophytes infected *Lolium perenne* L., are reported to be in the greatest level in the seeds while the lowest in rachis, stem, leaf sheath, and leaf blade, nevertheless, the synthesis site is unknown, while, the plant age is also involved in these biologically significant pyrrolizidine alkaloids levels [9]. The seasonal effect on the level of loline alkaloids N-acetylloline (NAL) and N-formylloline (NFL) is also reported to be enhanced from April to be peaked during summer during July up to 1000 μg/g [55, 57] as what is reported for samples from Alabama, USA. However, their level is not affected by the growth conditions as what is observed for perloline [56, 57]. Furthermore, plant clipping 6-7 weeks of seeding has been reported to bring about enhancement of NAL and NFL mean levels [42]. In addition, it is reported that water stress as well as temperature dramatically enhanced the level of NAL and NFL from 2236 to 11063 μg/g within 12 weeks at 21/15 C^0 particularly when tall fescue infected with endophytes which is also inclined as the nitrogen or phosphorus level inclined [45, 49, 58].

In general, loline chemically named hexahydro-N-methyl-2,4-methano-4H-furo[3,2-b]pyrrol-3-amine is a small size molecule a saturated 1-aminopyrrolizidines-type alkaloid originally isolated from *Loliumcuneatum* Nevski with a remarkable rigid simple structure of a characteristic polarity leading to an extraordinary physicochemical properties [4, 16, 30, 59, 60]. Structurally, their two fused saturated pentagonal rings sharing carbon and nitrogen atoms at their fusion ring with an oxygen ether bridge occur at the two carbons C2 and C7 (C2–O–C7 bridge) making this tricyclic ring system a very stained system [4, 30]. Interestingly, the endophyte infecting fungi are responsible for the oxygen ether bridge hence, completing the pyrrolizidine ring system [1]. Nevertheless, its exo-amino group (–NRR') occur at C1 group of different substitutions in various loline alkaloids plant secondary metabolite such as formyl, acetyl, and methyl groups [4] on its unusual tricyclic strained necine ring system (that is of –CH2OR group at C1 position) [33, 60-64]. There are other 1-aminopyrrolizidine alkaloids with α,β-unsaturation besides neither ether bridge at C2 and C7 positions nor amine functionalities at C1 position such as senecionine and retrorsine [65-67].

Loline alkaloids of *exo*-1- aminopyrrolizidine-2,7-ether nucleus structure are first isolated from tall fescue grasses (*Lolium* species) by (Petroski et al., 1989) who have synthesized the other loline alkaloids [40] then are detected in other plants although (Yunusov, Akramov, 1955) have reported the isolation of loline alkaloids from the seeds of *Lolium cuneatum* but with an incorrect elucidation of its chemical structure [16].The first loline alkaloid temuline, later on called norloline, with no N-methyl substitution occur during isolation process as reported by (Dannhardt and Steindl, 1985) [17], is isolated from *Lolium temulentum* for the first time in 1892 by Hofmeister [17, 33, 68, 69] which was reported Longley later on to be the dominant alkaloids in this plant [17], while, (Katz, 1949) has reported no alkaloids existed in *L.temulentum* [70]. In fact, (Yunusov, Akramov, 1955) have reported is reported to be major alkaloid in *L. temulentum* [17]. However, its N-methyl derivatives -$NHCH_3$ substituent are in *exo* position of the pyrrolizidine moiety as proposed by (Yates and Tookey, 1965) [14]. In addition, the term loline is proposed by Yunusov and Akramov who have isolated loline for the first time from rye grass, *L. cuneatum* Nevski at 1955 then its structure is conformed in 1965 and 1972 who conformed the existence of pyrrolizidine core of unique ether linkage at C2 and C7 [16, 69, 71-73]. Besides, the isolation and identification of other loline alkaloids from the same plant including norloline, N-acetylloline (or lolinine) [74], N-acetylnorloline [75] which is available along with other loline alkaloids in *L arundinancea* as a volatile alkaloids [31], N-methylloline, N-formylloline [76], N-formylnorloline [77] are lolines isolated from darnel and tall fescue [31, 78] in addition to N-acetylloline N-oxide [77] and a dimeric chlorine containing alkaloid lolidine is also isolated as [77, 79, 33] where one loline molecule is joined to a saturated pyrrolizidine that exhibit a chlorine at C7 and hydroxyl group at C2 instead of the ether bridge as reported by others [33, 77, 79, 80]. Moreover, (Yunusov and Akramov, 1960) have produced chlorinated and hydroxylated compounds without affecting the oxygen bridge of the pyrrolizidine which are then selectively removed to produce a mixture of *N*-methylpyrrolizidine resulting in *endo*-*N*-methyl-1-aminopyrrolizidine structure and free pyrrolizidine [81, 82] however, festucine without exo-position C1-N-methyl substitution have been isolated from *Lolium arundinaceum* (Schreb.) S.J. Darbyshire [14, 73]. In addition, the extraordinarily chlorinated alkaloid, lolidine is also isolated from the *Lolium* species [79]. Lolidine is structurally hetrogenous dimeric compound composed of loline and N-acetylnorloline fraction which is always isolated from their alkaloids extract ether fraction using chlorine containing solvents in all stages of isolation and purification. The chlorine atom in this halogenated in

the N-acetylnorloline part located at C6 carbon with contact oxygen bridge, yet, its opening in Chlorohydroxyloline and lolidinein [80] that occurs in the seeds of *Lolium* plants in the methanolic fraction [83]. In addition, norloline is obtained from the chlorine containing fraction of lolidine through alkalinization that cause oxygen ether bridge formation [83]. Moreover, (Dannhardt and Steindl, 1985) have reported the isolation of two major alkaloids loline as well as perloline from the caryopses and stem of the aerial parts of *Lolium temulentum* L while no detection of the loline demethylation alkaloid, norloline [17]. Nevertheless, the loline alkaloids isolated from the seeds of *L. temulentum* are loline chemical metabolic intermediate analogues as well as fungal infection final degradation products, norloline, loline, 6-methylloline and lolinine where the final one is supposed to occur due to norloline N-methylation as well as acetylation [84-86].

It is necessary to note that N-formyl loline is a biosynthetic analogue of loline while, N-Senecioyl norloline and acylnorloline, are loline alkaloids metabolites isolated from hours urine which feed on tall fescue grass [1, 87] which are reported to exhibit potent DNA binding potential [65, 66] as well as hepatotoxicity [56]. The *Lolium* species germinated seeds have been reported to exhibit a high level of loline alkaloids. In this context, (Yu et al., 1955) have reported the isolation of total alkaloids of *Lolium cuneatum* Nevski using chloroform and have been found to constitute 0.23% of the dry plant weight and are composed of loline, nortoline, lolinine (N-acetylloline), N-methylloline, and N-acetylnorloline while the aqueous fraction of the extract contains N-formylloline as it is quaternary base alkaloid. Loline constitutes 45% of the chloroform extract while, lolinine constitute 41.7% [79]. Thus, N-formylloline, N-acetylloline, N-methylloline, norloline, N-acetylnofloline and N-formylnofloline are isolated from *Lolium cuneaturn* and *Loliurn temulentum*. Moreover, these alkaloids including the two enantiomers of *N*-formylloline and *N*-acetylloline are reported to be existed in the plant parts of endophyte-infected tall fescue Lolium pretense, yet, the highest concentration is detected in the seeds, while, 1000 µg/ml concentration is reported in the fungal filtrate. Interestingly, (Blankenship, 2004) have reported that the biosynthetic final steps of loline alkaloids follows the following order: norloline → loline → methylloline → *N*-formylloline while ring C1 amine methylation happens before ring cyclization [4]. Nevertherless, the pharmacologically interesting new properties of loline alkaloids [9] has lead to the successful synthesis of (±)-loline by (Tufariello et al, 1986) [88] while, N-formylloline as well as N-acetylloline have been synthesized from loline using ethyl formate at room temperature and acetyl chloride in chloroform respectively [9], however, N-acetylloline structure have been elucidated by (Bates, Morehead, 1972) [72]. Moreover, both of paxilline and ergovaline are reported to be the precursors of the indole diterpene alkaloid loliterm B as end product obtained from the *Neotyphodium lolii* and Epichloë infected grasses including perennial ryegrass, *Lolium perenne* [3, 89-94]. In England immunoassay EIA has demonstrated that the seeds infected with endophyte perennial ryegrass contains (3000 µg/kg) paxilline [95] while, (3000−5200 µg/kg) for that from England and France [96, 97] while, ergovaline level in perennial ryegrass seeds from France is 6200 µg/kg [97]. Others have been reported that ergovaline level in perennial ryegrass dry material from Franse is 2300 µg/kg, yet, 4700 µg/kg from Czech [97, 98]. However, approximately close concentration of paxilline as well as lolitrem B have been reported for perennial ryegrass from New Zealand [99, 100] in addition to lolitrem B in Germany and other European countries [53, 97, 101-103].

Furthermore, (Vikuk, et al., 2020) have reported that the *E. festucae* var. *lolii* seeds of *L. perenne* from New Zealand contains peramine, lolitrem B and ergovaline in a season dependent concentrations that incline during summer while decline during winter. The concentration of peramine is found to be within the range above the toxic range 0.04 and 23.38 μg/g dry weight (2.00 $\pm$ 0.32 μg/g, 6.57 $\pm$ 1.09 μg/g and 3.23 $\pm$ 0.61 μg/g are the mean levels in July, August and September respectively) which is above the toxic one and half of that reported in Germany while its concentration in fresh plant is 13-40% of the dry weight. While, the detected level of lolitrem B is within the range of 0.07 and 23.81 μg/g which is above the toxic one particularly during summer. However, paxilline is not detected while ergovaline is very low 0.3–0.4 μg/g (DW) which is bellow the toxicity threshold however, ergovalline level reaches its peak in July to be 1.33 $\pm$ 0.30 μg/g dry weight while in the fresh plant is 20-40% of its concentration in dry material [2]. Moreover, (Bauer, et al., 2018) have reported that paxilline congers are the dominant lipophilic secondary loline alkaloids metabolites in the ethyl acetate extracts of the seeds and the fresh plant of perennial ryegrass, *L. perenne L*. as well as in the seeds of the Italian ryegrass *L. multiflorum* Fabio obtained from Germany which are 1'-O-acetylpaxilline and 13-desoxypaxilline. Besides, the existence of paxilline-like indole diterpene and ergot alkaloids in the seeds as well as the fresh plant of perennial ryegrass, however, weak concentrations, 7.3 µg/kg, of ergot alkaloids is detected in the seeds of the Italian ryegrass while, no detection to paxilline alkaloid. Remarkably, immunoassay have indicated the availability of high concentration of paxillin alkaloid, (5400 µg/kg), and ergot alkaloids, (260 µg/kg), in the dry matter of perennial ryegrass. Nevertheless, in south Germany the level of paxilline is 110 µg/kg in fresh plant while 270 µg/kg in dry matter which mostly related paxilline-like analogues due to the cross-reactivity to paxilline since according to immunoassay paxilline mean level of 190 190 µg/kg represent > 3% of the total paxilline alkaloids (6800 µg/kg) in perennial ryegrass. In addition, the mean level of ergot alkaloids in the seeds and dry matter of perennial ryegrass is reported to be 1600 and 180 µg/kg ergometrine equivalents, respectively, yet, ergovaline actual levels are of 24000−80000 µg/kg and 3000−9000 µg/kg in the seeds and plant dry mater that make it the major indole-diterpene alkaloids. Thus, paxilline as well as its paxilline-like indole-diterpene analogues levels are good indicators for the plant toxicity [55]. The chemical structures of the summarized loline alkaloids is illustrated in figure (3).

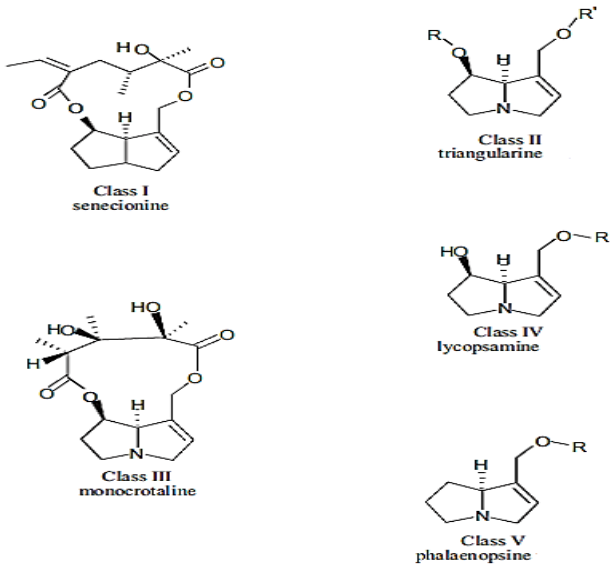

Figure 2: Classes of plants origin pyrrolizidine type alkaloids[56].

Finally, some authors have reported that there is a relationship between the accumulations of peramine and ergovaline through out the year while no correlation between N-acetylloline and N-formylloine accumulation and the accumulation of peramine or ergovaline meaning that both N-acetylloline and N-formylloine are not synthesized by the infecting endophytes [4].

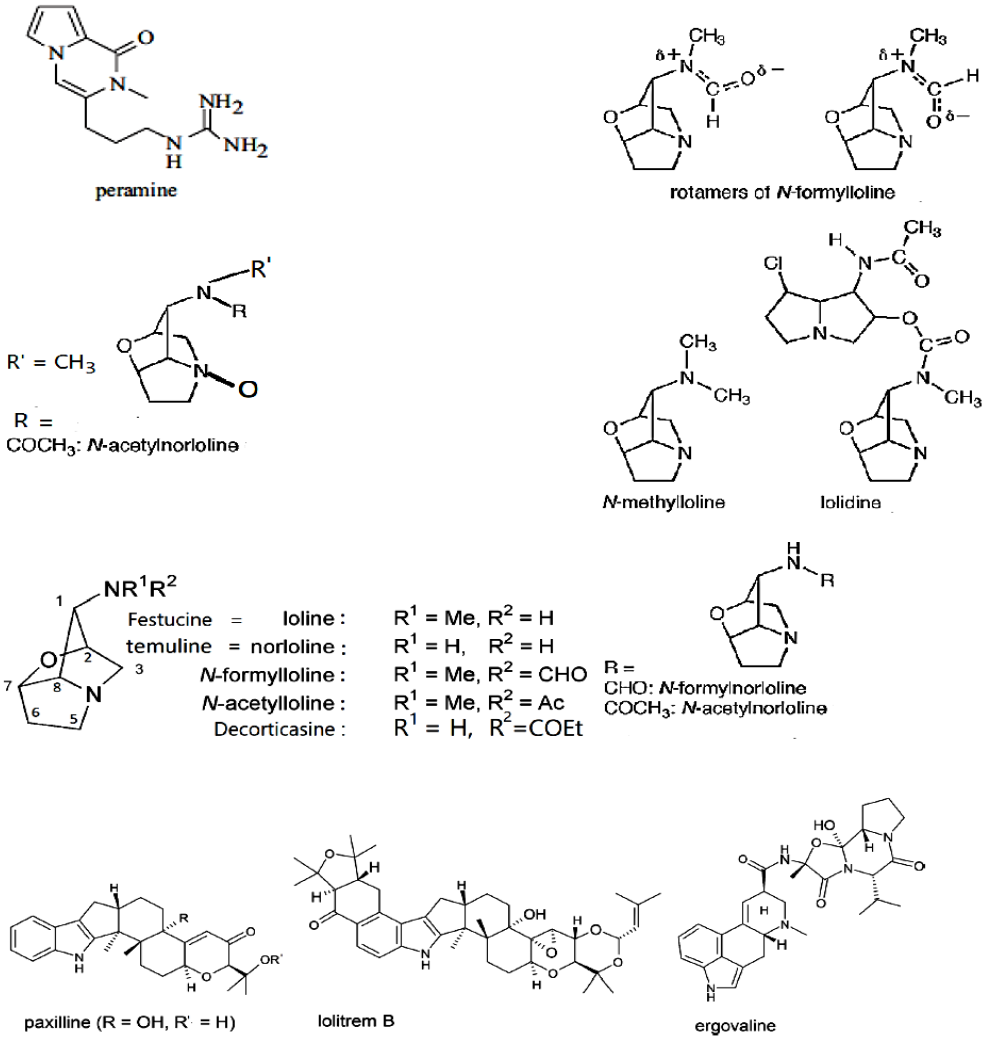

Figure 3: Chemical structure of the summerized lipophilic indole-diterpene and hydrophilic loline alkaloids.

Loline As Well As Paxilline/Paxilline-Like Diterpene Alkaloids Biological Influences and Their Toxicity:

Loline alkaloids particularly temuline from *Lolium temulentum* in darnel beer have been traditionally used as sedative for reliveing pain as well as sedative for nervous conditions [104]. Nevertheless, it is earlier reported that loline alkaloids as well as ergovaline interacts with the α_2-adrenergic, D_2 dopamine, or serotonergic receptors in the blood vasculatures leading to vasoconstriction besides, vasculatures thickening [105, 106], in addition to their immunosuppersive influence in murine model [107]. However, (Dannhardt, Steindl. 1985) have reported that the α1-adrenergic, α2-adrenergic, D_2 dopamine, cholinergic, serotonergic and benzodiazepine receptors bindings in rat and calf brains are not interfered by loline dihydrochloride as it has no affinity to these receptors [17, 108-112]. Whereas, other have suggested various central nervous system receptor antagonizing influences particularly the dopaminergic receptors [17, 113, 114]. Interestingly, unlike ergot alkaloids which are of no influence at concentration of 1.9 ppm, anorexic feed as well as prolactin depressing influences have been reported to a partially purified of tall fescus extract fraction of these pyrrolizidine alkaloids in rat model [115, 116]. In addition, (Strickland, et al. 1994) have reported a dose dependent prolactin suppression influence of loline at elevated concentration of 10^{-4} M via D_2 receptor agonistic effect in rat model [117]. Nevertheless, at similar level other loline derivatives such as N-acetylloline, N-formylloline and N-methylloline have exploited no prolactin inhibition activity in rats [118], particularly, N-acetylloline and N-formylloline that have no D_2 receptor or cAMP counteracting effect even at milimolar level [114]. Tall fescue extract has been reported to exhibit α_2 adrenergic receptor powerful agonistic influence leading to a potent contractility in the cattle's lateral saphenous veins [119] while, N-acetylloline and N-formylloline mixture at 3 x 10^{-5} M have been reported to cause partial inhibition of the norepinephrine mediated vasoconstriction [120]. However, N-acetylloline have been also reported to exploit briefly lower cow lateral saphenous veins and arteries vasoconstrictive effect in hourses [121, 122] indicating serotonergic and α_2 adrenergic receptor antagonistic influences [123]. Nevertheless, N-acetyl loline have been reported to elicit *in vitro* vascular smooth muscle cells at lower concentration while, at higher levels of 10^{-8} and 10^{-9} M have prohibited their growth, yet, at concentration range of 10^{-8}-10^{-11} M stimulate the growth of the dormant cells in bovine models [105]. Meanwhile, it is necessary to note that the in vitro tall fescue lolines vascular as well as endocrinal influences are exploited only at quietly high levels [117, 119, 120]. Moreover, it has been reported that these lolium pyrrolizidine alkaloids in excessive dose may inhibit thiaminase I enzyme responsible for thiamin catabolism through acting as enzyme substrate leading to thiamin deficiency in castles [124, 125].

Loline dichorohydrate as well as N-benzoyl iodomethylate derivatives have been found to decline coronary blood flow as well as blood pressure in mammalians such as cats and doges. In this context, loline alkaloids are reported to exhibit cardiac arrest subsequent to their negative inotropic effect at 0.1 and 1 mM concentration, however, cardiac arrest in froge model. Where as, at 10 mM level they have demonstrated instant cardiac arrest while diastole. In addition, intravenous administration of loline dihydrochloride at doses of 1-60 mg/Kg have been reported to cause dose-dependent reduction of blood pressure as well as respiratory deepening for 2-15 minutes [126]. Moreover, (Hammouda, et al., 1988) have reported that the loline alkaloids of the seeds ethanolic extract of *Lolium temulentum* exploit fast onset/short duration reversible cardiac arrest via depressing the predilective inotropic rather than the chorotropic function of the atrium in rabbit heart model along with very weak antagonistic effect against the tone as well as contractility of isolated rabbit aortic spiral strip vascular smooth muscle treated with epinephrine as compared to loline dihydrochloride which lacks such effect despites the later higher adrenaline tissue sensitizing influence. Furthermore, against the GIT, they have demonstrated very potent muscle tone depression on rabbit's jejunum smooth muscles model greater than their effect on their rhythmic contractility. However, on guinea pig ilium smooth muscles, the lower concentration of the loline alkaloids containing ethanolic extract have found to potentiate acetylcholine activity while, opposite activity is observed in the higher concentration along with uniform histamine effect antagonism at both concentrations explained by the total loline alkaloids synergistic influences. Meanwhile, loline dihydrochloride has exhibited no considerable effect on muscle tone on the same model while great enhancement of ilium contractility. However, both loline dihydrochloride as well as ethanolic extract total loline alkaloids have lacked any considerable influences against the tone of smooth muscle. Yet, the extract's total alkaloid have exploited resembling partial antagonistic action against Ach and histamine-induced contractions, but, loline dihydrochloride has elicited a selective histaminergic contraction antagonistic influence in ilium model. Moreover, the total alkaloids extract have elicited less Ach blood level influencing effect than loline since, the later exhibits more potent serum pseudocholine esterase inhibitory influence explaining its ability to provoke the transmission of partially irreversible neuromuscular blocking impulse in a perfused diaphragm rat's phrenic nerve model. On the CNS, the ethanolic extract total loline alkaloids have demonstrated CNS depression accompanied by hind limbs paralysis as well as ataxia hence, ultimately have brought about complete motor incapacitation, total skeletal muscle hypotonia along with postural reflexes/external stimuli response in mice model which have been found to be significantly greater than that to loline dihydrochloride. In addition, both ethanolic extract alkaloids as well as loline dihydrochloride have exploited dose-dependent time prolongation of barbiturate based hypnoptic activity [84]. Furthermore, (Putnam, et al, 1991) have reported that feeding of pregnant mares on endophytes infected *Acremonium coenophialum* tall fescue containing loline alkaloid derivatives 1610 μg/g NAL plus NFL as well as 0.39 μg/g of ergovaline plus ergovalinine has caused abortion or after lethality of birth fetus during pregnancy, after birth or during lactation in 90% of these fetuses. However, this toxic effect is not known whether due to a direct or indirect influences on mammalians [58].

Remarkably, N-acetylloline has been reported to exploit an in vitro mitogenic on the stationary smooth muscles of the blood vessels 10–1000 pM concentration while prohibit cell growth of others at 100– 1000 pM concentration [105]. In addition, (Petroski et al., 1994) have been reported that loline alkaloids as well as their semisynthetic derivatives of 8-12 carbon acyl chain substituting C1 amino group have significant antineoplastic influences against solid tumor in brine-shrimp model of 0.274 μg/ml mean ED_{50} against human lung carcinoma A-549, breast carcinoma MCF-7, and colon adenocarcinoma HT-29, yet, through an unknown mode of action. However, N-Acyllolines of >12 carbon length have a non-significant with weak cyctotoxicities as what is observed for the parent congener loline, yet, those with 12-18 carbon atom length acyl substitutions have exploited significant cytotoxic influences although much weaker than that of the anticancer drug adriamycin. Interestingly, the most active N-acylloline congener is N-Oleoylloline which has exhibited some degree of selectivity against HT-29 human colon adenocarcinoma [127]. Whereas, in vivo studies have demonstrated that the metabolism of the naturally occurring pyrrolizidine alkaloids including loline alkaloids gives rise to non-cytotoxic metabolites that can't interact with the intracellular macromolecules [128].

Moreover, the *Lolium* species indole-diterpene neuro-active mycotoxin alkaloid, lolitrem B rich in seeds of endophyte-infected ryegrass seed have been reported to exhibit an anti-mammalian influences involving neurological symptoms of hyperexcitability, muscle tremors and ataxia. Yet, it develops clonic seizures then death in sever cases of toxicity in male mice model as it has been detected in liver, kidney, cerebral cortex and thalamus as they are presumed to be its primary site of influence, but not in the cerebellum as well as brain stem which also encountered in sheep and cattle [95, 129, 130]. It mode of action may involve alteration in the metabolic pathways of essential neurotransmitters like caticholeamines as well as amino acids like tyrosine as their profile have been found to be significantly altered with time. Thus, lolitrem B exhibits its toxic influence in the CNS via regions specific manner particularly in the cerebral cortex that involves the emotion, mental and cognition functions of the brain as it accumulate in this tissue within a short period of time through perturbation of neurotransmitters metabolism leading to tremors and behavioral alterations in both high and low doses in a dose dependent mode. Lolitrem B, can disturb the metabolism of branched amino acids leading to their accumulation in the brain, hence, it deregulates the sedative neuromodulatory/catecholamine pathways leading to enhance its tremorgenic and non-tremorgenic excitatory influence in the forebrain [130]. Centrally mediated, Loliterm B, prolonged/reversible tremorgenic influence have been also reported in sheep and murine models [90, 131-136] which may have a pharmacological, discovery as well as drug design importance [132]. The mode of action of loliterms is reported to undergo structure dependent large conductance calcium-activated potassium channels potent blockage [90, 137, 138, 139] that maintain its neurological, emotional, behavioral as well as motor activity disturbance [90, 133, 137] besides, explaining its potential pharmacological action [138]. Although others have reported that tremorgenicity is indirectly related to the blockage of these channels besides, not seldom mechanism contributing to its symptoms of toxicity [138]. However, their metabolic alteration in vivo declines their activity due to the resulted structural alteration as it have observed in animal models [137-139]. However, stereochemistry have a role in the determining loliterm termjorgenic influence. For example, its natural stereoisomer lolitrem F. of A ring of alpha-phase is of no such influence [140] Remarkably, loliterm B binds to the charybdotoxin binding allosteric site located in the pore of the channel [141]. However, due to its lipophilicity, large molecular weight and non-volatility, lolitrem B tends to accumulate in the fatty tissues including the brain tissues [142-144] which is detected in fatty tissues of lactating and mature animals [143-147] as in case of sheep [142, 143]. Authors have speculated that loliterm as well as its biosynthetic intermediates including paxilline and terpendole C induce its termorgenic influence via close molecular mechanisms in mice model [92, 131]. In addition, a contractile tension inducing synergistic influence on the sheep distal colon smooth muscle longitudinal preparation have been reported for it combination with ergotamine leading to diarrhea [148], while its combination with ergovaline leads to decline bovine milk production [149-151]. In addition, in sheeps its GIT influence against duodenum via interfering the acetylcholine release [152]. It is reported that interpertoneal loliterm B tremorgenic influence lasts longer than other indole-diterpene alkaloids like aflatrem while much potent than paxilline [131, 145]. Remarkably, both lolitrem B as well as its intermediate metabolite, 31-epi-lolitrem B, significantly attenuate the production of IL-6 and TNFα cytokines production in murine macrophages, whereas, no cytotoxic influence have been observed against the viable cells even at 100-250 folds higher levels making them an excellent candidates for designing immune modulator drugs [153]. It is noteworthy to know that lolitrem is structurally related to the other lolium indole-diterpene alkaloid tremorgen, paxilline [154], however, loliterm B, maximum tremorgenic influence is exhibited at a dose of 8.0 mg/Kg of body weight [131]. While other *Lolium* metabolites such as lolilline and lolitriol have no tremorgenic influence [155, 156]. Nevertheless, despite that both of Lolitrem A, B and ergovaline are of the major mycotoxins in endophyte-infected perennial ryegrass and tall fescue respectively [147, 157], however, the level of lolitrem B is five to ten folds than ergovaline knowing that in lamb as well as lactating ews the required toxic threshold of ergovaline [140, 158-161] is much lower that of lolitrem B [147, 162, 163] of 1800-2000 μg/kg in cattle and sheep [164].

Furthermore, un like loline alkaloids which has weaker influence as well as peramine which has no influence on mammalians than ergot alkaloids and other indole-diterpene alkaloids, ergot alkaloids of lolium specie including ergovaline exhibit their potent toxicities through prohibiting CNS neurotransmitters metabolism besides, endocrine function counteraction influence potentially mediated through dopaminergic pathway interaction mode of action indicated by the low blood melatonin and prolactin levels [39]. In this context, (Larson, et al. 1995) have reported that ergovaline exploits an elevated affinity to the dopaminergic receptors, in addition to, its intestinal vasoactive peptide as well as cAMP production stimulants

[106]. In fact, ergovaline is the most toxic and abundant ergopeptide alkaloid in the infected tall fescue exhibiting similar neurological/motor symptoms of lolitrem B [165, 166]. However, in lactating ewes both ergovaline in particular and lolitrem B exhibit mild activity against certain drug-metabolizing enzymes [147, 162].

Moreover, the other *Lolium* species indole-diterpene alkaloid, paxilline of weak tremoergenic influence [29, 95] induce GIT smooth muscles various stimulation responses particularly in sheep duodenum [156] which is reported by (McLeay, et al. 1999) to be coincided with its skeletal muscles directed tremoring influence, hence, disturbing digestion as encountered with lolitrem B [132]. In murine model, its tremorgenic influence prolongs for many hours with LD_{50} of 150 mg/kg body weight [167]. In fact, paxilline is a powerful smooth muscle high conductance calcium-activated BKca channel blocker [138] in very low Ka value range of (2-10) nM as encountered in bovine aortic smooth muscle channels [168, 169]. In addition, (Selala, et al. 1991), have also reported their contribution to smooth muscles contractions in guinea-pig ileum model via blocking these BKca channels along with enhancing acetylcholine release [170]. Interestingly, paxilline along with its novel congeners pyrapaxilline and 21-isopentenylpaxilline, have been reported to prohibit nitrous oxide NO production in murine RAW264.7 cell line, nevertheless, they elecited their influences at 30 mg/ml and 10 mg/ml concentration respectively. The later compound greater activity is related to its additional dihydropyran ring [171]. Further more, paxilline has been also reported to attenuate the macrophages lipopolysaccharides-induced singnaling of the IkB-a/NF-Kb signaling pathway [172]. In addition, other remarkably unexpected antiviral against H1N1 influenza virus is reported for paxilline as well as other related congeners, 21-isopentenylpaxilline, paspaline, and dehydroxypaxilline [173].

In addition, paxilline at 0.1-10 μM level has been reported to incline rodents urinary bladder, and duodenum spontaneous tension which can not be reversed by atropine, while, treachea spasm in guinea-pig in a dose dependent manner via blocking the high conductance Ca^{2+}-activated potassium channel, despite, its inactivity against their isolated portal vein and aortic rings at 1-10 μM concentration. Yet, authors have expected stimulation may happens at concentration higher than 10 μM. However, at 10 μM paxilline inclines the integrated myogenic of the bladder by (9.6 ± 2.8) folds of their basal level [138] via promoting acetylcholine release from nerve terminals [170] although it dose not include muscarinic receptors agonistic influence. In addition, at concentration of 10 μM paxilline induces treacheal spasm to an extent around one quarter of the maximum influence of carbacol similar dose within 20 min. In deed, (DeFarias, et al. 1996) have spectulated that paxilline through blocking the BKca channel conductance prolongs the action potential hence, inclines the intracellular calcium inflex to the sarcolemma while the excitation-contraction coupling process as the molecular mechanism for its smooth muscle stimulation influence on the rodent bladder as well as that of GIT. Besides, they have concluded that paxilline synergistically potentiates charybdotoxin stimulatory influence on guinea-pig bladder [174]. Earlier, (Knaus, et al. 1994) have reported that paxilline enhances the binding of charybdotoxin to the BKca channel through paxilline binding to an allosteric site that enhances the receptor, located in the channel pore, affinity to charybdotoxin, although paxilline by itself is a powerful channel blocker as it permeate through the affected cell plasma membrane, hence, exploiting full blocking influence [138]. Remarkably in sheeps both paxilline, and lolitrem B have been also reported to stimulate skeletal muscles while, both stimulate and inhibits duodenum smooth muscles although their stimulatory effect can be partially antagonized by atropine [175, 176]. Furthermore, since, paxilline's BKca channel blockage is calcium dependent thus, this blockage effect is declined with the incline of calcium ion level that enhances the channel conductance [138, 177, 178]. Paxilline have been reported to be detected in rats brain membrane besides, inhibiting the GABA-induced chlorine influx into microsacs through binding to $GABA_A$receptors as it can pass the blood brain barrier passing rapidly into the synapse by mean of their characteristic lipophilicty, hence, eliciting its central influence [179, 180]. Thus, (Gant, et al. 1987) have postulated that brain GABA receptors is its major site for electing its tremorgenic influence [179]. As compared to lolitrem B that elicit its maximum tremorgenic influence at 8 mg/kg dose [131], paxilline is considered as a weaker tremorgenic agent as it elicit an intermittent tremorgenic influence at 35 mg/kg while a sustained influence at 227 mg/kg intraperitoneal dose in murine model [181]. However, in sheep it exhibits extensive tremorgenic influence at 1.2 mg/kg body weight intravenous dose [182]. Through comparing both paxilline and lolitrem B pharmacological/toxic influence, it is obvious that a tiny structural difference modifies these mycotoxines binding properties to the calcium-activated BK channels as what is observed in wild and genetically modified mice models, yet, lolitrem B is still much more potent/longer acting blocker to these channels of motor functions than paxilline in vitro as well as in vivo [183-185]. Nevertheless, in contrast to lolitrem B, paxilline has more rapid onset/shorter duration of action [145]. In this context, brief tremorgenic influence at 4-80 mg/kg dose of complete inhibition BK/Maxi or hSlo Channel at 1 μM concentration in mice, while, 70% channel blockage at 1.0 mg/kg dose and 10 nM concentration in sheep exploited as moderate to strong tremor 2 minutes after administration which disappear within an hour [132, 138, 140, 186]. Finally, it is necessary to not that *Lolium* species alkaloid biological influence is pH dependent as it has its impact on their chemical structure [187].

PHARMACOKINETICS OF LOLINES AND INDOLE-DITERPENS ALKALOIDS:

Loline alkaloids are speculated to be retained intact in the blood after ingestion, however, in lambs it is reported that little fraction of loline is absorbed via passive diffusion mechanism while the majority of loline quantity is absorbed by other mechanism due to its molecule hydrophilicity, small molecular weight along with neutral charge, making this compound easily cleared out of the GIT mucosa.Hence, it could be an excellent potential anthelmintic agents of local GIT action for pharmaceutical investigation [107, 188,

189], although it is reported to have good bioavailability in the blood/gastric mucosa of horses and sheep next to oral intake [188, 190] as it is reported to be readily absorbed beside, rapidly excreted renally in hours as well as bovine models [87, 191, 192]. Loline congeners including loline base, N-acetylloline and N-formylloline can cross passively across all of the GIT cross-section tissues of epithelium particularly ileum that exhibit the maximum 5% capacity. However, the greatest detected level in the blood was of loline base followed by N-formylloline followed by N-acetylloline, while, solely loline metabolites is detected in the liver and kidney tissues in lambs model as it suffers rapid metabolism. Yet, only small amount of N-formylloline are detected in these two organs and blood as compared to loline base metabolites availably in abundance [188] while some N-acetylloline and N-formylloline are located in hours blood [190]. Interestingly, (Seawright, et al. 1991) have reported that these pyrrolizidine alkaloids metabolites bind to the hemoglobin's globulin thiol groups of the hours's red blood cells [193].

In addition, these four loline alkaloids are renally excreted 2 hrs next to dosing in lamb model in addition to the metabolites of loline base as well as N-formylloline [194]. In this context, (Froehlich, 2020) have speculated that N-formylloline is the active form of loline metabolites while the simple loline base is un effective due to its rapid metabolism to an un effective metabolites while parasites counteracting N-formylloline metabolite is of good oral bioavailability while of poor urine excretion, thus remains in the blood for several hours [188, 194]. Some have supposed that loline alkaloids are absorbed, metabolized and excreted quickly, hence, exerts no symptoms of poisoning [194, 195]. Some of the loline alkaloids are metabolized in the intestinal mucosa in sheep, while, loline alkaloids are detected in urine in cow urine where over 50% of the absorbed loline is renally excreted followed by N-formylloline (bout 20%) followed by N-methylloline in sheep model meaning that their renal excretion is fast process occur within 15 minutes post dosing along with slow metabolism [194]. However, N-formylloline and N-acetylloline are excreted in hair as reported in hours model [196]. Nevertheless, (Ruan, et al.) have reported that the metabolites of these unsaturated pyrrolizidine alkaloids, particularly the platynecine type, are readily excreted without any binding to renal tissues protein adducts [128].

The *Lolium* species indole-diterpene, Loliterm B, is a lipophilic molecule insoluble in water, however, after oral administration, it has been reported to exhibit poor oral bioavailability due to poor GIT absorption [146, 197]. However, unlike, paxilline which is detected in murine brains at very low levels, high intravenous (75 μg/kg BW) dose has exploited fast clearance from the systemic circulation despite the observed long term termorgenic influence in sheep model [145] as well as in lactating goat treated with (23 μg/kg BW) dose [197]. It is hypothesized that loliterm B is trapped in certain body compartments then gradually released to the systemic circulation to find its way to the brain. This hypothesis is explained by the rapid blood clearance along with long term tremorgenic influence and encouraged by its detection in goat milk 32 hours and 75 hours post 23 μg/kg BW IV dosing of 3% excretion rate and 100 μg/kg BW oral dosing of slower excretion rate respectively [197, 145]. Remarkably, a resembling long term detection of loliterm B in bovine milk is reported by (Finch, et al. 2013) [146]. Finally, both paxilline and loliterm is metabolically oxidized hepatically into a detoxification more polar metabolites excreted billary [198].

TOXICOLOGY OF LOLIUM SPECIES AND THEIR ALKALOIDS

Human as well as animal toxicities happens in certain instances due to food, medicine and herbal products contaminated with plant toxins particularly pyrrolizidine alkaloids found in 3% of the flowering plants including grass that, regardless their long term consequences on health, have bring about fatalities in animals and human globally. Several poisoning cases have been encountered in case of using herbal preparations and teas of these alkaloids [26, 49]. Most of these alkaloids are hepatotoxic or even carcinogens; however, others are non-toxic or targets organs other than liver by their toxicity [26, 199]. Lolium species toxicity mostly known as ryegrass toxicity of often resembling causative toxin, clinical outcomes, case development as well as toxicological mechanism. In mammalians including humans and cattle the most common clinical manifestation include neurological toxicity expressed as tremor, diarrhea, loss of appetite, endocrinal outcomes expressed by reduction of milk production, and late manifestations including jaundice as well as photosensitivity. The most characteristic clinical manifestation, tremor happens via blocking the CNS inhibitory pathways through allosteric binding to GABA receptor chloride channel as well as chloride and calcium channel. However, despite no marked histological findings of their toxicities, yet, signs of adipose stores loss as well as emaciation are reported [26]. Several determinant factors related to the enzymatic as well as structural aspects lies behind these compounds' toxicity. The structural factors is related to their necine core basic or acidic structure and their substitutions nature/position mostly the ester one which are non-toxic unless they are bio-transformed into active metabolites such as pyrrolic esters [200, 201]. However, their bio-activation is hepatically performed, rather than detoxification, via consecutive series of oxidation-reduction as well as conjugation reactions [201-203] although chemically different metabolites are obtained from the metabolism of different classes of these pyrrolizidines alkaloids. The detoxification of the two classes retronecine and otenecine which are with single unsaturation at C1, C2 positions of the necine basic ring leads to their oxidative activation via cytochrome P450 hepatic enzymes into extensively reactive unstable pyrrolic esters (dehydropyrrolizidine) hepatotoxins that interact with thiol group of essential intracellular biomolecules such as enzymes or other proteins forming toxic pyrrole-protein adducts that targets the liver, lung besides other organs even acting as carcinogens [9, 128, 204]. However, these pyrrole active metabolites if interact with DNA may lead to genotoxicity bringing about carcinogencity [128]. In addition, if these reactive metabolites can be endogenously detoxified via binding to endogenous glutathione forming glutathione-pyrrole inactive water soluble adduct that us easily excreted [200, 201, 204, 128] on one hand. On the

other hand, platynecines class of the pyrrolizidines alkaloids, which are of saturated necine bases nucleus are not hepatotoxic compounds as the previously mentioned classes [201, 202] although they pass a resembling oxidative hepatic metabolic fate via cytochrome P450 enzymes, yet, the resulted pyrrolic esters formed (dehydropyrrolizidine) is stable, un reactive, water soluble carboxylic acid that can not undergo conjugation reaction with thiol groups due to the absence of the necine base unsaturation, thus, needs no glutathione for their excretion [200]. The proposed metabolic pathway of *Lolium* species pyrrolizidine alkaloids is illustrated in figure (4) adapted from (Ruan et al. 2014) report [200]. However, (Stegelmeier, et al. 2013) consider pyrrolizidine alkaloids are generally non-toxic while, some of them are of toxicities other than hepatic one. In fact, (Stegelmeier, et al. 2013) have reported that although N-oxidation hepatic detoxification reaction of pyrrolizidine alkaloids along with increasing its solubility, these N-oxide metabolites are readily reduced in the GIT to re-establish their toxicity [26].

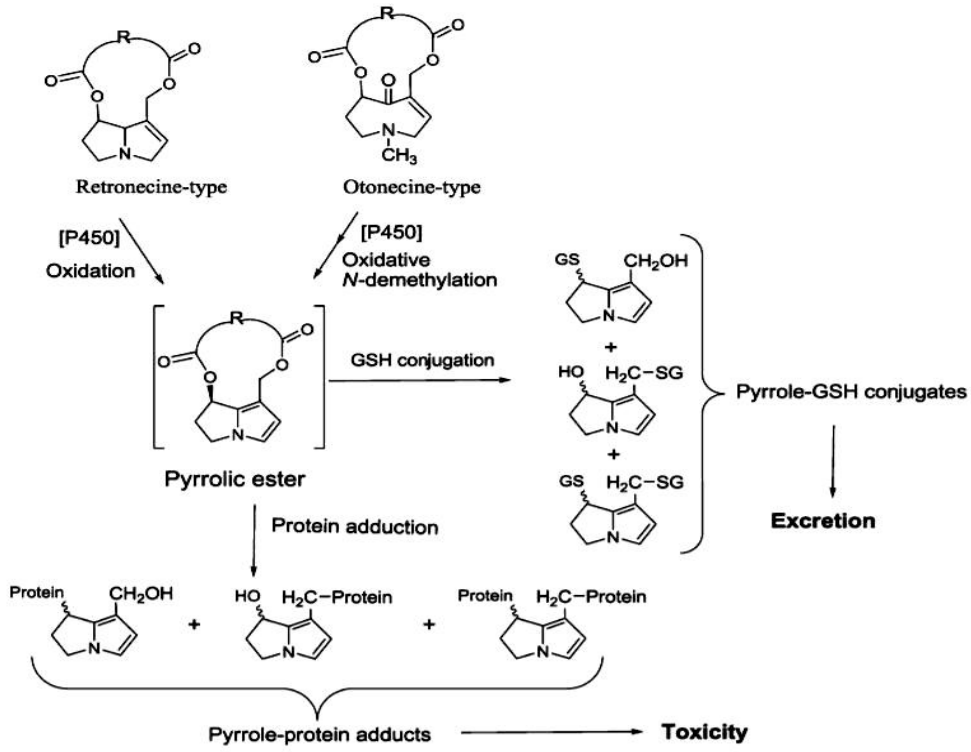

Figure (4): Metabolism of pyrrolizidine alkaloids proposed by (Ruan et al. 2014) [200].

In general loline alkaloids are with no necine ring α,β-unsaturation, with C2 and C7 oxygen bridge or without C1 or C7 ester substitution thus they lack the common pyrrolizidine alkaloids cytotoxicity [9, 65, 205], nevertheless, unlike oral administration, IV administration of loline alkaloids do exert toxic influences on mammalians [205]. While, other authors considers loline alkaloids have no anti-mammalian influence exhibited by other pyrrolizidine alkaloids particularly those related to genotoxicities [65-67] although liver cytochrome P450 oxidative enzymes have the ability of production of different genetic materials cross linking active pyrrolizidine alkaloids metabolites [66]. Generally, dimethylated or 1-amino functionality aceylated loline alkaloids are not/ non-conclusively mammalian poisoning substances [1, 48]. Remarkably, in mice models loline alkaloids have been reported to exploit immunosuppressive as well as anorexic influence in rodents model [107, 205]. In addition, (Wang, et al. 2019) have reported that pyrrolyzidine alkaloids is also detected in honey [206], while, other reported their detection in bovine milk contributing to toxic influence against lactating infants although in both cases their concentration bellow the toxic thresholds of adults indicating low toxicity to humans consuming animal meat, fats and products as reported in Germany [142, 146, 207]. However, number of reports have been issued regarding the incidence of several human poisoning cases due to ingestion of wheat products contaminated with the *L. temultentum* seeds [208, 209]. In this context, pure loline dihydrochloride have exhibited no toxicity up to 200 mg/kg body weight dose administered intraperitoneally, lethal at 400 mg/kg IV dose while nontoxic at oral 100mg/kg dose in murine model [14, 17, 108]. Besides, it has no interaction with α_1-adrenergic receptor [108], similarly with serotonergic receptors [109], cholinergic nicotinic/muscarinic receptors [110, 111] or benzodiazepine receptors in calf brain [112], thus, *L. temulentum* or *L. arundinaceum*, is likely no associated with loline alkaloid as earlier believed [1]. Probably, the poisonous influence of *L. temulentum*seeds is exhibited by a mixture of its loline alkaloids, loline, lolinine, lolinidine, temuline and temulentine [16, 209] although (Bush, et al. 1983) have reported that loline analogues, N-formylloline, N-acetylloline, N-methylloline, norloline, N-acetylnofloline and N-formylnofloline are not hepatotoxines, although they may promote the biological membranes penetration as well as toxicity of other *Lolium* species toxins like ergot alkaloids, hence, exploiting indirect toxicity in large excess

concentration [9]. Nevertheless, other authors have reported that lolines including N-acetyl loline, N-acetyl norloline, N-methyl loline, and loline base are generally not toxic to human beings or other mammalians as compared to other infected lolium alkaloids like ergot alkaloids [1, 9, 192, 194], despite, some have believed that N-formylloline and N-acetylloline are involved in equine fescue oedema [190, 196]. In general, lolines obtained from leaves, stems and head stems of *L. temulentum* are less toxic than nicotine in animal model [210]. However, in certain reports toxic/ lethal doses of loline alkaloids are specified. For example, the lethal dose of festucine (loline base) is 400 mg/Kg when administered IV, while, it is safe up to 1000 mg/kg oral dose [14] as what is encountered in murine models [17, 211, 212]. In addition, daily oral administration of N-formylloline, N-acetyl loline, N-acetyl norloline, N-methyl loline, and loline base mixture in a dose of 415 mg/kg has exhibited no obvious pathological, histological, hematological influence besides, no apparent influences on heart rate, blood pressure or motor coordination in murine model, although anorexia influence as well as cessation of weight gain have been reported particularly for N-formylloline [205, 212]. Nevertheless, (Jackson, et al. 1996) have correlated between N-formylloline and growth-stimulating factor for its anorexic influence although they have reported that it has no influence on testes, hypothalamus and corpus striatum mass or on , prolactin and alkaline phosphatase levels [205]. Furthermore, as a dominant component (45.46%) of the in the total alkaloids (2.7%) seeds alcoholic extract of *Lolium temulentum*, loline has been reported to exhibit acute toxicity in rodents (mice and rats) model post oral as well as intraperitoneal dosing manifested as CNS depression that is deteriorated to coma then death due to respiratory failure. Yet, lethality of the total alkaloids toxicosis is greater in mice model by 1.58 and 1.35 for the two routs respectively while, lethality after oral intraperitoneal rout is 30 folds greater than oral routs mostly due to the total alkaloids neuroleptic influence, rather than loline alone, that starts to affect animals behavior, without interfering the learning capability in a dose dependent manner. Remarkably, at doses of 280 and 440 mg/kg doses loline lacks fatal acute toxicities in mice and rat models respectively [40]. It is reported that like classical ergotism, N-Acetylloline in fescue exploits its pituitary gland directed prolactin release inhibition, reproductive abnormality issues, hyperthermia and dry gangrene of extremities due to vasoconstriction inducing influence toxicity characteristics in animal models [32, 117]. Moreover, in Pakistan, *Lolium temulentum L* seeds consuming toxicity are rarely lethal to humans, nevertheless, the toxicity characteristics of diarrhea, gastroenteritis, vomiting, ataxia, nausea. giddiness, apathy and mydriasis are reported to be attributed to Cynoide as well as loline alkaloids like temuline and loliine [21]. Interestingly, toxicity case of endophyte-infected tall fescue, containing 1610 pg/g (N-formylloline and N-acetylloline) combination in addition to 0.39 μg/g of ergovaline plus ergovalinine combination, ingestion by pregnant mares have caused teratogencity so that only 3 of 11 fetus have been delivered alive while solely one of them passed the natal stage to lactate despite the two classes of alkaloids are bellow the toxic concentration [58]. However, other loline metabolites like lolilline and Lolitriol are also exhibit nontremorgenic toxicity at doses of 8 mg/kg and 20 mg/kg respectively in murine model [155] on one hand. On the other hand, lolitriol have been reported exhibit its influence via targeting BK/Maxi or hSlo ChannelIC_{50} = 196 nM as compared to IC_{50} of 43 nM of loliterm B [139, 140]. However, the indole-diterpene alkaloid of *Lolium* species, paxilline has its tremorgenic toxicity on vertebrate that need further investigations for its neurological effect on K^+ channels [90, 213], as it is metabolically converted in vivo into the other indole-diterpene alkaloid, loliterm B that mediate most of the neural transmission disturbance fescue poisoning symptoms attributed to the alkaloid-promoted thiamine deficiency in animal model [9]. In this context, (Miles, et al. 1992) have reported that Lolitriol plus β-Paxitrol (16 mg/kg and 100 mg/kg) combination have exhibited lethal toxicosis at doses of 200 μL dosage post initial lethargy period [140]. As compared to loliterm B, paxilline is a weaker termorgenic toxine [129]. In fact, loliterm B, is considered the most toxic indole-diterpene alkaloid of *Lolium* species, particularly in perennial ryegrass, that primarily contribute to their motor/neurological (tremorgenic) toxicities [129, 157] as well as in any other plant seeds containing loliterm B [157, 214], via binding to BK channels, however, the duration/severity of its toxicity beside its excitatory influences are location dependent. In addition, these influences/toxicity are also dose, lipophilic character and metabolic fate dependent [215]. Moreover, (Craig, et al. 2014) have reported that loliterm B exhibits its toxic effect at threshold level > 1.8 μg/g dry weight of lolium plants in cattle [216], while, sever/prolonged tremor toxicity is exhibited by loliterm B, at dose range of 0.5-8.0 mg/kg due to inhibition of BK/Maxi or hSloChannel with IC_{50} of 4 nM at 70 μg/kg dose [131, 135, 185]. Furthermore, both of ergot alkaloids including ergovaline of anti-vertibrate/invertebrate toxicity and loliterms of solely antivertibrate toxicity are the active poisonous alkaloids responsible for endophytes infected *Lolium* species, in which they are detected, toxicities including *L.perenne* [5, 13] to which most of toxicity cases in mammalians are reported [100, 217-219]. In this context, both of Lolitrem B and ergovaline are responsible these grasses toxicity when available in concentrations of 1.8 μg/g and 0.3 μg/g dry weight of the plants [89, 91, 220]. Yet, trace amount of ergot alkaloids including Ergovaline and related ergopeptines in tall fescue is also associated with its toxicity, characterized by reduce weight, hypethermia, blood flow restriction, reduced reproduction/milk production [1, 150]. In addition, like loline alkaloids, peramine is of weak toxicological characteristics against mammalians [215, 221]. In this context, peramine hydrochloride, at oral dose of 1000 mg/kg body weight exhibit highest toxicity level in murine model manifested by sluggish motor activity along with acute liver damage as revealed by autopsy, while, not influencing food intake, behavior, growth rate at dose of 50 μg/g [221]. In murine model, infected ryegrass seeds consuming CNS toxicity manifestations involves hyperexcitability as well as nervousness [222]

Regarding the Lolium plants toxicities like perennial ryegrass (*Lolium perenne*) motor/neurological toxicity,

characterized by tetanic muscle spasms that leads to severe incoordination as well as hypersensitivity to external stimuli, is to be a reversible case in animals in Australia and North America [26, 223], however, no human toxicities are reported. While, toxicity of annual ryegrass (*Lolium rigidum* Gaud.) can be lethal manifested as neurological disorders as commonly reported in in Australia, South Africa, but, rarely reported in North America [26]. In Germany, symptoms of stiffness and movement disorders are reported in horses ingested perennial ryegrass due to ergot alkaloid [224]. L. temulentum seeds of the loline alkaloid temuline, toxicity is manifested as CNS as well as GIT symptoms [68, 225]. Other fescue poisoning manifestations involve interference with energy metabolic processes due to thiamine deficiency [226], interference with brain/hypothalamic functions including, gamma-aminobutyrate (GABA), glutamine serotonergic and melatonin pathways [227]. However, (Watt and Breyer-Brandwijk, et al. 1962) have reported that lolium plants human intoxication symptoms are mutually similar to alcoholic sedation characterized with headache, dizziness, vertigo, mental confusion, difficulty in speech, inability to walk, vomiting, hypothermia and generalized shivering although a decoction of these plants is traditionally used in Moroccans folokloric medicine for haemorrhage and urine incontinence. In addition, other traditional medicine use of the powdered plant seeds for suppress the psychological and vasomotor disturbances associated with menopause when taken orally besides, being used topically for various skin disorders [209].

CONCLUSIONS:

The genus *Lolium* belonging to the family Poaceae or Gramineae involves around seven species, of poisonous grass plants grown globally particularly in Asia in corps especially wheat fields, however, in Iraq are called "rewatta". Their toxicity is mostly related to their characteristics alkaloids, the pyrrolizidine; lolines, indole-diterpenes (ergots, loliterms, and paxillines) as well as peramine alkaloids mostly concentrated in their seeds, for which endophytes symbiosis/infection are involved in their synthesis particularly *Acremonium, Neotyphodium or Epichloe*¨species although these plants are capable of producing of amino-pyrrolizidine alkaloids like lolines individually without the need for fungal infection. These alkaloids are described to be contributing to both neuro- and non neuro-toxicities. The levels of these alkaloids ranging from 0.2-1 mg/g inclines to their optimum levels in the areal parts as well as seeds during later summer and autumn reaching up to 10 folds in dry plant and constitute 45% of *Lolium cuneatum* Nevski of the total alkaloids (0.23%) of the chloroform extract. Chemically, the core nucleus of their pyrrolizidine alkaloids is necine composed of two fused saturated heterocyclic pentagonal rings with a nitrogen atom at one of the bridgehead with C1 amine group substitution which is characteristic to their loline alkaloids, including loline base, loline, Norloline, N-acetylloline, N-formylloine, N-acetylnorloline and N-formylnorloline. besides, a third exocyclic ring structure due to exocylic oxygen bridge occurs between C2 and C7. The difference between loline and norloline alkaloids is the existence of N-methyl group substitution at C1 amino group while the acetyl, methyl as well as formyl derivatives of loline are results of C1 amino group acylation or alkylation. Nevertheless, other dimeric loline alkaloids like lolidine in addition to other tricyclic alkaloid perloline have been isolated from *Lolium temulentum* L. both of the indole-diterpene alkaloids paxilline and ergovaline are reported to be the precursor of the most toxic lolium species alkaloid loliterms including loliterm B of levels ranging 3-6 mg/g dry plant weight, which are biosynthesized with aid of endophytes symbiosis especially in the perennial ryegrass. In general, lolium species loline alkaloids are considered as relatively polar molecules as compared to the other lipophilic indole-diterpene alkaloids paxillines, loliterms and ergovaline which are the actual indicators of these plants toxicity. In some mammalians loline alkaloids are of poor oral bioavailability due to limited passive absorption although it is spectualted to remain unchanged within systemic circulation, thus, local intra-lumen pharmacological influences of these alkaloids are expected while in horses for example loline base, N-acetylloline and N-formylloline have exhibited good oral bioavailability potentially due to absorption mechanisms other than passive one particularly along with rapid renal excretion as in bovines. Only loline metabolites are detected in mammalian liver and kidney tissues as they suffer extensive/rapid metabolism, hence, loline base level > N-acetylloline > N-formylloline in their blood blinding to hemoglobin SH groups. For such kinetics the solely systemically active form of loline alkaloids is N-formylloline due to poor renal excretion, while, loline base is inactive due to fast first pass metabolism as well as rapid renal excretion since bovines for example excrete 50% of the absorbed loline renally. However, the indole-diterpene alkaloids have poor GIT absorption due to extensive lipophilicity as well as poor brain tissues accumulation in murine model due to rapid clearance from the systemic circulation through entrapment in body fat depots that may contributes to their prolonged influence. In general, this type of lolium alkaloids are detoxified through metabolic N-oxidation to a more polar metabolites excreted through the biliary rout. Regardless some reported traditional uses from Africa, the loline alkaloids of these plants have been reported to exploit diverse neuronal/motor as well as nonneurological influences. The neurological influences primarily demonstrated as a depressive activity, exhibited via affecting the central nervous system through interacting the α1-adrenergic, α2-adrenergic, D_2 dopamine, cholinergic, serotonergic and benzodiazepine receptors in the hypothalamic and cerebral cortex regions leading to tremorgenic as well as anorexic influences. Nevertheless, along with these neurological effects they affect the pituitary function leading to decline prolactin production. In the brain loline alkaloids particularly targets the dopaminergic recepotrs. However, peripherally, they have influenced the α_2-adrenergic, D_2 dopamine, or serotonergic receptors in the blood vasculatures smooth muscles leading to vasoconstriction and blood vessels thickening. While, in rabbits, cats and doges loline causes hypotension that could be accompanied by cardiac arrest at diastole due to its negative inotropic effect along with declining coronary blood flow. Moreover, loline alkaloids exhibits a remarkable immunosuppressive influence in murine model.

N-acetylloline, N-formylloline and N-methylloline have no central pituitary influences as they don't target brain's dopaminergic receptors. While, in excessive dose these loline alkaloids affects the energy production via affecting thiamine metabolism leading to thiamine deficiency. GI influences are also reported for lolines including increasing intestinal smooth muscles tone and contractility leading to diarrhea via promoting acetylcholine release and along with blocking histaminergic receptors. Moreover, both loline alkaloids as well as ergot alkaloids causes miscarriage as well as teratogenic influences in horses that causes 90% mortalities in fetuses. Although, semisynthetic derivatives of 8-12 carbon acyl chain substituting C1 amino group have significant antineoplastic influences against solid tumor in brine-shrimp model against human lung carcinoma A-549, breast carcinoma MCF-7, and colon adenocarcinoma HT-29. Similar CNS directed tremorgenic as well as GIT directed stimulatory influences have been reported to the indoel-diterpene alkaloids paxilline and loliterm B that could fatal for loliterm overdoses exhibited through high conductance calcium-activated BKca channel blockade in addition to similar endocrinal as well as peripheral influences. In the context of lolium alkaloids toxicity, these with C1 and C2 necine nucleus unsaturation are with hepatotoxicity, genotoxic as well as carcinogenic as they are activated metabolically through oxidation into a very reactive N-oxide metabolites, while, those with no unsaturation are not. However, most of lolines are of saturated necine nucleus thus they share with other indole-diterpene alkaloids particularly loliterm B and paxilline CNS toxicity, diarrhea, endocrinal as well as photosensitivity through allosteric binding to GABA receptor chloride channel as well as chloride and calcium channel determined by structural aspects particularly the their necine core basic or acidic structure and their substitutions nature/position for loline alkaloids. Finally, toxic influences of lolium alkaloids are function of their biological influences mostly exhibited via resembling molecular mechansims centrally as well as peripherally. Unfortunately, an extensively little is reported regarding their pharmacological, toxicological as well as kinetics in humans despite many spectulations for pharmacological benifits, some traditional uses as well as some biological activities of their acyl derivitives are reported which requires future investigations.

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