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Toxicology and Health Benefits of Plant Alkaloids

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5.1 Introduction

Alkaloids are a class of natural products containing carbon (C), hydrogen (H), nitrogen (N), and usually oxygen (O). These compounds have been categorized into different classes based on their sources, pharmacokinetics, and chemical structure. They include isoquinoline alkaloids, indole alkaloids, pyrroloindole alkaloids, piperidine alkaloids, aporphine alkaloids, pyridine alkaloids, methylxanthine derivatives, vinca alkaloids, lycopodium alkaloids, indole beta-carboline, and erythrine by-products [1, 2]. Alkaloids are derived from plants (especially in

certain flowering plants) [1, 2], microorganisms, and animals and are widely found in products such as herbal preparations, beverages, well-cooked foods, and tobacco smoke. Some alkaloids, such as beta-carbolines, are naturally present in body fluids and tissues [3, 4]. Most alkaloids are available in many plant species that are used for human and animal nutrition [5]. Most of the plant species contain a limited number of alkaloids, but others in families such as Solanaceae (nightshades), Papaveraceae (poppies), Ranunculaceae (buttercups), and Amaryllidaceae (amaryllis) contain several kinds of alkaloids [1, 2]. Alkaloids have a variety of psychopharmacological, biochemical, and behavioral effects in both humans and animals [3, 4]. Plant alkaloids exist as the salts of organic acids such as malate, acetate, and citrate or in combination with other molecules such as tannins. Alkaloids are mostly basic and lipophilic, which makes them soluble in polar organic solvents.

Some alkaloids, such as pyrrolizidines, are derived from ornithine as well as insects that consume alkaloids; these insects then use the alkaloids in their fight against predators [6]. Alkaloids mostly occur as monoesters, diesters, or macro-cyclic diesters and are formed by an amino alcohol (necine base) and (a) necic acid(s) (mono- or dicarboxylic aliphatic acids), which then give them their structural diversity. Alkaloids rarely exist as a free form of the pyrrolizidine base; usually, they exist as tertiary bases or pyrrolizidine alkaloid *N*-oxides (PANO) [6, 7].

Pyrrolizidines are an important source of amino alcohols. Pyrrolizidines have a core composed of at least two five-membered rings attached by a nitrogen. Sometimes, they acquire a double bond at positions 1 and 2, and this may be responsible for the frequently reported enhanced toxicity in pyrrolizidine alkaloids (PAs) [8]. In addition, a single alcohol can be formed at C1, while a second and less often third alcohol can be formed at positions C7 (di-hydroxylated) and C2 or C6 (tri-hydroxylated), respectively [9–11]. Furthermore, an esterification reaction can occur at C7 and/or C9 [11]. Several approaches have been made to partially or fully synthesizing many naturally occurring PAs and related non-natural analogs [12, 13].

Most PAs can be ingested orally and absorbed from the gastrointestinal tract; they are excreted in urine, feces, and milk. Passage to the placenta is limited because they have high lipophilicity [14]. The majority of the compounds are bioactivated or biotransformed in the liver, and this makes the liver the organ most susceptible to the toxicity of PAs [15]. The other most susceptible organ is the lung, followed by the kidney, as the PAs pass through these organs on their way to their targets, metabolic processes, excretion through blood circulation. Excretion and toxicity of the PAs occur only after biotransformation [16].

The activation of the PAs occurs metabolically through three principal pathways. These include hydrolysis, *N*-oxidation, and oxidation, which lead to the formation

of necic acids and necines, PANO, and dehydropyrrolizidine alkaloids (DHPAs) or pyrrolic esters, respectively. Hydrolysis is also responsible for detoxification through the promotion of clearance of the compounds [17, 18]. *N*-oxidation also facilitates detoxification by facilitating excretion of the compounds through the formation of easily conjugated PANO, which is then easily excreted [16].

Several alkaloids exhibit both toxicity and potent pharmacological activities. Many alkaloids have been used both lawfully and illicitly as pharmaceuticals, stimulants, and narcotics. Concentrations or amounts of these alkaloids in foodstuffs have not been a cause for concern in acute poisoning. However, the concentrations have been sufficient enough to cause chronic toxicity because the alkaloids are either consumed from different sources of food in a day or consumed from one foodstuff that is frequently ingested in a day, such that they exceed the maximum daily intake suggested by authorities, which poses a risk of development of chronic disease [5].

5.2 Pharmacological Properties of Alkaloids

Alkaloids have demonstrated several health benefits in humans and animals. Notable ones in humans include antimicrobial activity, anti-inflammatory activity, anticancer activity, anti-human immunodeficiency virus (HIV) activity, and acetylcholinesterase (AChE)-inhibiting activity, as described below.

Alkaloids show significant antimicrobial activity, the defensive role that is expected of this class of secondary metabolites in plants [19]. Monocrotaline, usaramine, and azido-retronecine are examples of PAs that have shown antimicrobial activity against some bacteria species [20]. Analysis of usaramine activity against the effective formation of biofilms in *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* has shown that it reduced the formation of biofilms by *S. epidermis* by about 50% at 1 mg/ml, but had no activity against the formation of biofilms by *P. aeruginosa*. Monocrotaline and azido-retronecine had activity against *Trichomonas vaginalis* (concentrations up to 1 mg/ml), being lethal to 70% and 85% of bacterial cells, respectively, but had no toxicity toward *T. vaginalis*. They also exhibited desirable selective toxicity but did not interfere with vaginal epithelial cells, which is an important trait for compounds under consideration for development into drugs such as topical antimicrobial agents [5].

Anti-inflammatory activity has also been elucidated for alkaloids. The inflammatory process is a physiological response of the body aimed at eliminating, neutralizing, and/or destroying stimuli from infection or tissue damage [21, 22]. Also, the inflammatory process involves the upregulation of inducible synthase of nitric oxide (NO) as a consequence of proinflammatory mediators, such as cytokines, that lead to increased levels of NO, which is important for mediation of

the inflammatory response [6, 23]. Therefore, the regulation of its production in tissues may be important for the treatment of inflammation [5]. Alkaloids nervosine I–VI and the PAs lindelofidine and labumine have been found to exhibit inhibitory capacity toward NO production by lipopolysaccharide-challenged macrophages from the RAW 264.7 cell line. The results indicated that the molecules that were effective in this model had half-maximal inhibitory concentration (IC_{50}) values ranging from 2.16 to 38.25 μM [24].

Anticancer activities have also been reported for many types of cancers. For example, alkaloids have been used in the treatment of pediatric cancers such as acute lymphoblastic leukemia with indicine-*N*-oxide at two dose levels (2000 and 2500 mg/m^2/day) for five consecutive days. However, the therapeutic index was very narrow and the dose–response curve was very steep and registered mild acute hepatotoxicity at the doses tested. Treatment with indicine-*N*-oxide in some patients has led to liver failure. Indicine-*N*-oxide that was obtained from *Heliotropium indicum* (L.) inhibited the proliferation of cancers in various human cancer cell lines (breast, cervical, prostate, and cervical squamous) with IC_{50} values ranging from 46 to 100 μM. At these concentrations, cell cycle arrest at mitosis was detected without noticeable changes in the organization of the spindle or interphase microtubules [5, 25, 26].

Anti-HIV activity of polyhydroxylated PAs has also been tested and the results have shown that these PAs are able to affect the progression of HIV. For example, 10 mM alexine in conjunction with 0.1 mM australine, obtained from *Castanospermum australe* A.Cunn. and *Alexa Leiopetela* Sandwith, respectively, inhibit glycosidase activity, especially for the HIV glycosylation process that is linked with nitrogen. The activity is attributed to reduced fusion of cells with virions, which leads to restricted syncytium formation. Another alkaloid in the class of PAs and alexine from *C. austral* and *A. leiopetala*, respectively, also had inhibitory activity against HIV-1. Activity was obtained with 7,7a-diepialexine and an IC_{50} of 0.38 mM was found. The anti-HIV activity results correlated positively with the inhibition of pig kidney α-glucosidase 1 and the diminished cleavage of the precursor HIV-1 glycoprotein gp160 [5].

Inhibition of AChE has also been reported for alkaloids. AChE is involved in the catalysis of acetylcholine (ACh) and other esters that act as neurotransmitters. AChE is also important for neural function. It is mainly present in the synaptic gaps of the peripheral and central nervous systems and is responsible for terminating nerve impulses. When ACh is overstimulated, this can lead to disorders such as depression, while lower levels of ACh can lead to other diseases such as Alzheimer's disease and myasthenia gravis [27, 28]. Therefore, because of these factors, inhibitors of AChE are exploited as therapeutic targets [27]. Four PAs from *Solenanthus lanatus*, including 7-*O*-angeloylechinatine-*N*-oxide, 3′-*O*-acetylheliosupine-*N*-oxide, heliosupine-*N*-oxide, and heliosupine, had AChE inhibitory activity, with IC_{50} values ranging between 0.53 and 0.60 mM.

Another study of 7-*O*-angeloyllycopsamine-*N*-oxide, echimidine-*N*-oxide, and 7-*O*-angeloylretronecine, obtained from *Echium confusum*, against AChE showed IC_{50} values of 0.275–0.769 mM [3, 17].

The effects of an alkaloid extract of *Senecio brasiliensis* (Spreng) leaves on rats and mice showed the potential of using PAs in the treatment of stomach pain and ulcerogenic disease. Results showed that lesions significantly decreased by 32.9%, 42.5%, and 66.8%, respectively, with concentrations of PA extract of 12.5, 25, and 50 mg/kg (containing integerrimine, senecionine, retrorsine, seneciphylline, and usaramine). Similarly, a dose of 12.5 mg/kg of the same PA extract in the same study was shown to ameliorate non-steroidal anti-inflammatory drug-induced gastric ulcers [29, 30].

Alkaloids have also shown promising antidepressant effects [31, 32]. For example, strictosidinic acid isolated from *Psychotria myriantha* showed antidepressant-like effects on 5-hydroxytryptamine (5-HT) in a rat hippocampal system [9, 33]. Another alkaloid, berberine, resulted in a significant increase in mobility and climbing behavior in the forced swim test in rats [34, 35]. Additionally, alkaloids isolated from *Annona cherimola*, which included anonaine, 1,2-dimethoxy-5,6,6a,7-tetrahydro-4H-dibenzoquinoline-3,8,9,10-tetraol, nornuciferine, and liriodenine, also showed antidepressant-like activity in mouse tests [36, 37]. Beta-carboline alkaloids such harmine, harmane, and nor-harmane also increased the mobility time in a mouse forced swim test, thus producing the effects of an antidepressant [38, 39]. Tetrahydrosecamine, akuammidine, and rhaziminine from *Rhazya stricta* also showed a significant antidepressant effect in experimental animals on administration of a lyophilized extract. Mitragynine, a bioactive agent from *Mitragyna spicosa*, which grows in Malaysia, administered as an injection, also significantly reduced the immobility time of mice in both the tail suspension test and the forced swim test without any significant effect on locomotor activity. Mauritine A, an active compound from *Ziziphus apetala* collected in the People's Republic of China, showed strong activity against 11-β-hydroxysteroid dehydrogenase inhibition in an in vitro assay. The diterpene alkaloids songorine, napelline, mesaconitine, and hypaconitine from *Aconitum baicalens* have also demonstrated antidepressant effects in a depression animal model. The alkaloid punarnavine, isolated from *Boerhaavia diffusa* (L.), showed significant antidepressant activity in unstressed and stressed mice. Evodiamine, isolated from *Evodia fructus*, favored the increases in sucrose preference, 5-HT, and sodium level as well as increasing immobility time [40, 41]. Several other alkaloids such as mesembrine from *Sceletium tortuosum*, which grows in the USA; piperine from *Piper nigrum*, which grows in Thailand; leatispicine, an amide alkaloid from *Piper laetispicum*, which grows in China; *Dactylicapnos scandens* Hutch.; and the non-ergoline alkaloid pramipexole showed significant clinical efficacy in an antidepressant activity model [32].

5.3 Toxicological Properties of Alkaloids

Different species are affected by alkaloid compounds differently. This is attributed to the balance between the formation of DHPA and the formation of detoxification compounds such as necines, necic acids, and PANO. DHPA is formed through necine base hydroxylation at the C3 and C8 positions in the case of heliotridine and retronecine types. For otonecine, oxidative *N*-demethylation is required. After the formation of the highly reactive metabolites, they are bound to glutathione (GSH) to form GSH conjugates and are eliminated in the process. This is why conjugation to GSH is considered to be a route of detoxification. Similarly, pyrrole esters can bind to DNA and other proteins, later forming adducts. These metabolites can also be hydrolyzed and transform to dehydronecines, which are also toxic metabolites but are less reactive than the previously mentioned forms [5].

DHPA has been recognized as the main toxic mechanism of PAs, and is concerned particularly with binding the groups containing sulfur, nitrogen, and oxygen present in proteins to form adducts, such as 2,3-dihydro-1H-pyrrolizine-protein [5], mainly at the site of formation. Pyrroles are also capable of penetrating the nucleus and reacting with DNA, consequently causing DNA cross-links and DNA-to-protein linkages with poor functions, which cause damage, mainly in hepatocytes. They can pass into the sinusoidal lumen and attack sinusoidal cells. The injury caused by the toxic metabolites in hepatocytes and in hepatic vein walls leads to veno-occlusive disease (VOD), which is also known as hepatic sinusoidal obstruction syndrome (HSOS) [16]. This proposed mode of activity has been supported by other subsequent studies [42–44].

5.4 Acute and Chronic Toxicities

As already stated, the liver is the main target of toxicity. HSOS is the clinical manifestation most frequently found, being considered a marker for PA intoxication, and causing symptoms such as vomiting, enlargement of the liver, and bleeding diarrhea [17, 18]. PA intoxication can be acute, subacute, or chronic. Each type of intoxication exhibits its own symptoms. Acute intoxication is recognized by symptoms such as hemorrhagic necrosis, hepatomegaly, and ascites. Subacute intoxication is characterized by the blockage of hepatic veins, which causes HSOS (primary sinusoidal damage and parenchymal cell dysfunction) [1, 15, 45]. Additionally, chronic PA exposure manifests in the form of necrosis, fibrosis, cirrhosis, and proliferation of the bile duct epithelium [21, 25], while liver failure and death occur at the highest level of toxicity [1].

5.4.1 Genotoxicity and Tumorigenicity

Some alkaloids have been shown to be capable of affecting genes and inducing tumors. For example, retrorsine was reported to be capable of inducing lung, pancreatic, skin, liver, bladder, spinal cord, brain, and gastrointestinal tract tumors in vivo [14]. The mechanism of formation of the tumor is believed to be as a result of DNA adduct formation in the form of DHPA [46, 47], and high levels of DHPA-induced DNA adducts were associated with the appearance of tumors. Hence, these can be used as tumorigenicity biomarkers caused by PAs [29]. Furthermore, studies have shown that the tumors can also be caused by reactions between the compounds and proteins that cause chromatid exchange, DNA cross-linkages, and the aberration of chromosomes [11, 17, 18].

Alkaloids in the PA family are also linked to skin cancer owing to their ability to undergo photosensitization in animals after consumption and metabolism [48, 49]. It is believed that, normally, a porphyrin (phylloerythrin) produced as a result of chlorophyll damage by microorganisms in the gastrointestinal tract travels via the blood circulation through the liver to the bile. However, when the liver is compromised by a PA, it fails to eliminate the phylloerythrin, which accumulates in the skin and blood. When subjects are then exposed to sunlight, the metabolites produced can trigger oxidative stress and lipid peroxidation in skin tissues, which may facilitate the development of tumors [49, 50].

Although there are no reports of cancer in humans as a direct consequence of PA consumption, it is reasonable to conclude that such PAs may be tumorigenic and genotoxic to humans. This is because riddelliine metabolism in rodent liver microsomes is similar to that in humans, which includes DNA adduct formation; also, PAs induce liver tumors in rodents through the formation of DNA adducts [51, 52]. Similarly, the US National Toxicology Program asserted that riddelliine has the potential to cause cancer [5].

Some studies have already been done to assess the potential of PAs in causing, for example, cancer, liver diseases, congenital anomalies, and pulmonary hypertension [34]. These alkaloids are genotoxic and initiate such diseases slowly. This may be difficult for clinicians to identify since they cannot know about patients' dietary exposure to the alkaloids. Researchers have therefore attempted to define two pointers that can indicate a dietary dehydrogenase plant alkaloid etiology: cirrhosis, mainly one associated with HSOS and/or copper accumulation in the liver, and congenital anomalies and/or cancers where evidence of asymptomatic or overt HSOS, bone deformities, pulmonary arterial hypertension or immunological deficiencies is available. The presence of several of these indicators affirms that it is possible that dietary exposure to PAs is involved in disease etiology [5].

5.4.2 Lung Toxicity, Neurotoxicity, and Teratogenicity

The lungs are one of the target organs for alkaloid toxicity because DHPA can move from the liver into pulmonary arterioles to cause damage similar to HSOS. When the alkaloid reaches the organ, occlusion and inflammation may occur as a result of thrombi forming in the vessels and thickening in their walls. Overall, the combined effects of these phenomena consequently stimulate pulmonary hypertension and subsequent congestive heart failure. This was confirmed in a hooded Wistar rat study which showed that PAs can cause lung lesions (intravascular accumulation of mononuclear cells) as a result of low-level (0.025 mmol/ kg body weight) and long-term exposure to PAs. This may lead to venous occlusion and extravascular alteration, whereby alveolar septa are thickened and the number of cells increased. The study results also led to the conclusion that rats developing lung lesions always also presented with chronic liver lesions [5].

Neurotoxicity has also been reported for alkaloids. For example, tricodesmine causes encephalitis, which is associated with headaches and vertigo, which can cause derilium and consciousness loss. Teratogenicity has also been reported as one of the consequences of consuming alkaloids. This is based on the finding that some alkaloids are capable of crossing a placenta from a mother to an unborn baby (fetus). For example, a hepatic HSOS case has been reported in the newborn of a mother who had taken herbal tea extracted from *Tussilago farfara* and the consumption of *Senecio madagascariensis* Poir. by a mare in Australia was reported to have led to hepatic failure [5]. Furthermore, clivorine from *Ligularia hodgsonii* Hook., in concentrations between 10 and 100 μM, showed that this PA can induce DNA fragmentation, which is compatible with apoptosis, in a human fetal hepatocyte cell line and mouse hepatocytes with an IC_{50} value of 40.8 μM [51, 53].

5.5 Factors that Influence the Toxicological Profile of Alkaloids

Both chemical and biological factors influence the toxicological effects of PAs. For example, the presence of a 1,2 double bond and/or one or two hydroxyl groups attached to the pyrrole ring can play a significant role in the toxicity of alkaloids; the presence of this functional group in retronecine, heliotridine, and otonecine types has been associated with their toxic effects. Furthermore, the presence of a methyl group at C1, two esterified groups, and branching in at least one of the carboxylic acids have also been implicated in the toxicity of alkaloids. Based on the reasons above, it is concluded that cyclic diesters, open chain diesters, and monoesters exhibit the highest, intermediary, and lowest levels of toxicity, respectively. To illustrate the relationship between the esterification level and toxicity, macrocyclic DHPA was found to be more toxic than open chain diesters [14–16].

Among the important factors that affect the toxicity of alkaloids are age, sex and differences activating metabolism within and between species. It is reported that men are at higher risk of toxicity than women, and children and fetuses are even more vulnerable to the toxicity of alkaloids than men [1]. There are also variations in toxicological vulnerability between distinct PAs within a species and of the same PA in different species [31, 54].

Finally, the presence of bacteria and metals influences the toxicity of alkaloids. This was demonstrated when exposure to low doses of the alkaloid monocrotaline, which has no toxicity risk normally, elicited hepatotoxicity in the presence of bacteria [42, 55]. In this case, centrilobular and midzonal liver lesions were registered. In addition, coadministration of retrorsine and copper led to more serious liver damage than retrorsine alone; a result that was confirmed by Moreira et al. [5].

Therefore, with the increasing consumption of herbal medicines and the toxicities widely reported in the literature, alkaloid poisoning has been increasingly considered as a public health problem. Some countries such as the USA, the Netherlands, and Austria are now establishing regulations around alkaloids in foodstuffs [36, 38, 40, 46].

5.6 Conclusion

Alkaloids are a very important class of compounds, particularly in drug discovery. They are mostly found as bases in nature because of the presence of a nitrogen group. Nitrogen significantly contributes to toxicity and other important pharmacological properties in drug design. Alkaloid forms of phytotoxins are not widely exploited in research. There is a need for more studies into the individual properties of common alkaloids as well as their characteristics in terms of toxicity.

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