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Toxic Behaviour of Naturally Occurring Pyrrolizidine Alkaloids

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Abstract

The Pyrrolizidine Alkaloids (PAs) are the chemicals which are found in various plant species throughout the world. Pyrrolizidine alkaloids (PAs) are secondary metabolites those have been evolved as a powerful means in the plant defensive system against herbivores. Several studies are being performing to detect presence of PAs in the food and feed which are obtained from plants through direct or indirect sources. Biosynthesis of PAs is dependent upon plant's growth and biomass production. In this review, chemistry, biosynthesis and metabolism of pyrrolizidine alkaloids have been summarized. Pyrrolizidine alkaloids are toxic in nature so the presence of PAs in food is undesirable. PAs are reported for their hepatotoxicity, carcinogenicity and genotoxity. PAs first target liver after consumption for their toxicity. PAs intoxication develops acute, sub acute and chronic stage of the liver which leads liver failure and ultimately death.

Keywords: *Pyrrolizidine alkaloids, chemistry, metabolic pathways, biosynthesis, intoxication*

1. Introduction

Pyrrolizidine alkaloids consist of a number of natural products which have been used in many studies. PAs have various biological applications in the treatment of cancer, diabetes, and viral infections such as HIV. Some PAs also have application in agriculture industry due to their antifeedant activity against several insects. Antifeedant activity of a chemical is to repel insects without being toxic to the insect or the plant. Pyrrolizidine alkaloids (PAs) which possess a 1, 2 double-bond in their base moiety (necine) are hepatotoxic, carcinogenic, genotoxic, teratogenic and sometimes pneumotoxic. PAs have also been found in about 3% of all flowering plants[1]. Pyrrolizidine alkaloids occur in plants varies widely, depending on the plant species and the part of the plant, and is also influenced by other factors (e.g. climate, soil properties). Due to their potentially harmful effects, particularly 1, 2-unsaturated pyrrolizidine alkaloids (PAs) are not suitable for food and feed. High doses of these compound cause acute liver damage.

From the last years, it has been well known that humans can also be affected by PAs intoxication [2,3,4,5]. The main source of PAs intoxication was found contaminated grain and bread. PAs mainly occur in species of the plant families Asteraceae, Fabaceae and Boraginaceae. In animal studies, some PAs have proven to be genotoxic carcinogens. Pyrrolizidine alkaloids (PAs) are a group of natural toxins produced by various plants, some of which may be known to be highly hepatotoxic and also shown to be carcinogenic in rats. They have been associated with a number of livestock diseases. They also involved with cases of human poisoning following consumption of herbal remedies or after contamination of staple foods. PAs also may be transferred to other food products such as honey, milk, and eggs. Several European countries concerning phytopharmaceuticals have regulated the use of these preparations [6]**.** Recently, PAs have been studied to be found frequently in retail honey and food supplements containing bee pollen[6,7]. Additionally, some studies showed a more complex occurrence of PAs in the human food chain, through PA contaminated plants in retail packed salads [8] or contaminated fodder for livestock [9]. Numerous studies demonstrated an essential role of PAs in the life cycle of specialized adapted herbivorous insects. These insects are depending on the PAs in their food supply for protection against predators[10].

2. Chemistry of pyrrolizidine alkaloids

Chemical structure of pyrrolizidine describes two-fused 5-membered rings with a nitrogen atom at the bridgehead. This motif is the central structure of a variety of Pyrrolizidine Alkaloids. The study about alkaloids began with the isolation of two compounds from *Senecio latifolius* by scientist Watt in year 1909. In 1911, Cushny

tested these alkaloids for toxicity in frogs, cats, rats and rabbits and stated that these alkaloids were responsible for the diseases in domestic animals caused by the eating of *Senecio* spp in South Africa and in the other places. Till to date, several hundred PAs have been described [11,12] and the continuously are being discovered. However, not all of these PAs are hepatotoxic. The PA molecule contains two five membered rings, tending towards each other and share nitrogen for both rings at position 4. Most of the PAs which are found in nature are the derivatives of 1- methyl pyrrolizidine. Some of them are esters of 1-hydroxymethyl pyrrolizidine unsaturated on the position at 1 and 2 can be named as esters of 1-hydroxymethyl 1, 2- dehydropyrrolizidine. These esters of unsaturated hydroxymethyl PAs are hepatotoxic. The central structure of hepatotoxic pyrrolizidine alkaloids conatains an unsaturated 3-pyrroline ring, one or two hydroxyl group which are attachedto the pyrroline ring through one carbon atom, esterified hydroxyl group and branched chain acidic group. These all are minimal structural requirement for being toxic in nature have been shown in **figure 1**[12]**.**

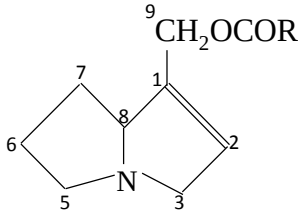

Figure 1 The basic structure of the hepatootoxic pyrrolizidine alkaloids

Esterification may be present at C9 and C7 position. Necine is an amino alcohol and when this become esterified at the C9 position or C7 position or both position is called. Pyrrolizidine alkaloids may be present as mono or diester form in closed and open cyclic structure. N-oxide form of these alkaloids are less toxic but when ingested they are converted into to the base alkaloid. This form of N-oxide becomes more toxic. The variety of plants contain these pyrrolizidine alkaloids which are hepatotoxic as Supinine, Echimidine, Indicine,Retrorsine. These alkaloids are monoester or diester which have active centre at C9 position or C7 position or both position [12].

3. Biosynthesis

The biosynthesis of PAs has been reported from polyamines as putrescine and spermidine. Putrescine acts as a substrate for secondary metabolism in plants. This putrescine is common polyamine which is present in eukaryotes and also in bacteria. On the other hand spermidine is a universal cell growth factor which concerned with various physiological processes in eukaryotes[13,14]. Both putrescine and spermidine are derivatives of arginine. *Spermidine* is biosynthesized from putrescine in the presence of spermidine synthase enzyme. In this reaction, an aminopropyl group is transferred from decarboxylated SAM [15]. In animals, putrescine may alsobe synthesized from ornithine by ornithine decarboxylase. In pants, Ornithine decarboxylase is absent so putrescine is synthesized through arginine [16]. The first specific step in pathway is homospermidine formation which is catalyzed by homospermidine synthase (HSS). The HSS play role in transferring aminobutyl group from spermidine to putrescine in presence of NAD+ coenzyme [17,18,19,20]. The reaction has been shown by Sander and coworkers [20] in the root culture of *Senecio* species using radioactive precursors. Homospermidine forms necine base through oxidative deamination and get converted into senecionine. The final product N-oxide, forms alkaloid. Location of Biosynthesis may vary in the species to species for example in *Heliotropum indicum* (Boraginaceae) the biosynthesis takes place in shots but in *Symphytum officinale* it occurs in roots only [21]. It may also happen that biosynthesis occur in more than one places for example in asteraceae family, biosynthesis takes place in the roots and next conversion occurs in the leaves and inflorescens. The difference in biosynthesis site may affect quality and quantity of alkaloids which generate species specific type of alkaloid. The transformation of alkaloids consist one or two step reactions of metabolism (hydroxylation, dehydrogenation, epoxidation, acetylation etc.). The PAs can be moved in plants from one place to another and distributed in whole plant. They can be accumulated in one place as in the inflorescense (*Seneciosp.*) or flower buds (*Phalenopsis* sp.)[22,23,24]. The accumulation of PAs in different organ also provides protection in plants against herbivores. The lolines compounds are structurally similar to the PA, which are produced by grasses which are infected by endophytic fungal symbionts[25]. The biosynthesis of lolines increases tolerant of plant from biotic and abiotic stress. Its structure resembles with necine ring also a tertiary amine. The lolines are insecticidal but not hepatotoxic for mammalian herbivores. Lolines are synthesized from L-proline and L-homoserine amino acids[26,27].

4. Metabolism of pyrrolizidine alkaloids

PAs are ingested and absorbed in the small intestine and carried to the liver. The PAs and their N-oxides which are hydrophilic in nature are excreted in urine to a large extent without any change within a day. PAs can be hydrolysed into necines and necic acids by the action of esterases enzymes. Necic acid has a branched chain structure due to this structure it prevent its hydrolysis. This ester in the liver is acted by liver microsomal oxygenases enzyme. There necine based alkaloids become changed into N-oxides or C3 or C8 hydroxyl derivatives. C3 and C8 hydroxyl derivatives are not stable

and convert into didehydropyrrolizidine alkaloid and pyrrole with loss of water and intramolecular rearrangement. Otonecine alkaloid become demetylated at N atom and hydroxylated at C8 and form didehydropyrrolizidine alkaloid. N-oxides are excreted in urine in as such form and cannot be metabolized. Didehydropyrrolizidine alkaloid are called primary toxic metabolites and hydrolysed into consequent pyrrolic alcohol within the liver cells in presence of liver microsomal enzymes. Pyrrolic alcohols are now called secondary metabolites. These pyrrolic metabolites counter with cellular macromolecules and strongly attach with sulphydryl groups. They also bind with amino groups of nitrogenous bases of nucleic acids and proteins. C7 or C9 of pyrroles reacts with exocyclic nitrogen of guanosine of the DNA. The main point of interaction of pyrroles with DNA is the reaction of the C7 or C9 of the pyrrole with the exocyclic nitrogen of guanosine[28,29]**.** These all reactions of PAs cause initial hepatocellular damage. Pyrroles metabolites can also keep on unreactive for a long time in aqueous medium. PAs which are formed by persistent primary metabolites can cause lung damage as well as liver damage. These primary metabolites permeate from hepatocyte into the adjacent sinusoids. In sinusoid, these metabolites counter with the endothelial cells linked hepatic vein. They can bind on red cells passing down the sinusoids. They can also access to the lungs and heart[30]. Pyrrolic alcohol are more stable secondary metabolites which can cause extensive extrahepatic injury predominantly in young animals, which affects mainly rapidly growing tissues of the body[31]. PAs also have been observed to damage chromosomes in various biological forms as in blood cells of children affected with Veno-Occlusive Disease. Various alkaloids are also studied for inducing DNA repair synthesis and sister chromatids exchange. Primary and secondary metabolites of alkaloids can also induce an anti-mitotic effect and linked with mutation in one or more cell cycle genes.

5. Toxicity of pyrrolizidine alkaloids in humans

The presence of PAs in the herbal raw material is highly unwanted due to toxic activity of pyrrolizidine alkaloids. There are numerous reports which suggested hepatotoxicity, carcinogenicity and genotoxicity of PAs[32,33]. The pyrrolizidine alkaloids are responsible for toxicity in human, due to the ingestion of contaminated food and consumption of medicinal plants in the form of dietary supplements. PAs of *Senecio sp* are esterified alkaloids derived mainly from the necines, retronecine and otonecine. They are carcinogenic, mutagenic, genotoxic, fetotoxic and teratogenic in nature. PAs intoxication process is well investigated and found that PAs are less toxic in nature but during metabolism intoxication occurs in liver due to modification of PAs.

PA toxicity in human can be progressed from an acute to sub acute and also to a chronic state. Acute and sub acute toxicity can be determined by haemorrhagic necrosis, ascites and hepatomegaly. In chronic cases liver necrosis and liver dysfunction can cause liver failure or circulatory obstruction which is due to chronic pathological changes developed in the liver after intake of low doses of alkaloids over a period of weeks and months which ultimately cause death [34,35]. Hepatic veins occlusion takes place due to endothelial proliferation, which leads veno-occlusive disease (VOD). This is specific histological sign for PA toxicity. Occlusion of the central and sub-lobular veins is called veno-occlusive disease (VOD). These are the major hepatic lesion as well as histologically and functionally. Due to occlusion vessel may be cannulated and the perivenular fibrosis may develop non-portal cirrhosis. This also causes centrilobular congestion, necrosis, fibrosis and liver cirrhosis which is the end-stage of chronic PA intoxication.

The conventional symptoms and signs of human PA toxicosis are abdominal pain and quickly developed ascites. The other symptoms as vomiting, nausea, anorexia, diarrhoea, oedema, emaciation, hepatomegaly, splenomegaly and mild jaundice have been seen[32].

Conclusion

Till to date, several new pyrrolizidine alkaloids have been identified and also have been synthesized to find the new compounds with potential pharmacological activities or the new important economical value. The pyrrolizidine alkaloids are still interesting and promising group of secondary metabolites. The various studies are paying attention on their biosynthetic pathways and detection of control mechanisms which permit to produce the valuable and safe raw material or to find the new applications of plants containing pyrrolizidine alkaloids.

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