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Potential Risk of Cyclopiazonic Acid Toxicity in Kodua Poisoning

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ABSTRACT

Kodo millet (*Paspalum scrobiculatum L.*) is minor millet extensively nutritious and is used as a staple food in some parts of North India. Although millet is cultivated in dry and semi-arid regions, sometimes environmental conditions like spring and summer strike as being suitable for a certain kind of poisoning which leads to greater economic crop loss. Millets are more prone to fungal infection followed by bacterial and viral; these infections adversely affect the grain and fodder yield. Ergot is a parasitic fungal endophyte that grows in the ear heads of various blades grass, most frequently on kodo millet, consumption of such Kodo grains is often found to cause poisoning. The grains of kodo millet are frequently infested with Aspergillus and penicillum, which produces a considerable amount of a mycotoxin, cyclopiazonic acid (CPA). This review reveals the fungal pathogens associated with the secretion of CPA and its toxicity on animals and humans, both by itself or in combination with other mycotoxins, and its biocontrol strategies employed to overcome the production of mycotoxin and storage methods to be practiced after the postharvest of kodo grains for better utilization.

Keywords: Kodo Poisoning; Cyclopiazonic Acid (CPA); Mycotoxin; Toxicity; Paspalum Scrobiculatum L

Introduction

Millets are a group of small cereal grains produced from annual hardy monocot plants that are widely cultivated in semi-arid and dry land parts of the world. It can grow in dry hot and humid climatic areas where the possibility of wheat and rice growing is considerably low [1]. Millets are grown world-wide but are mostly intense in central, southern, and western parts of India and in a few states in north India including the Himalayan Mountain regions [2]. Millets are highly nutritious grains with rich protein content, fat and fiber, several bioactive polyphenolic compounds [3] but their net carbohydrates content is lower than that of other cereal grains like wheat and rice. Global production of millets has been rapidly increased from 26.7 to 30.0 million metric tons in 2012-2022 [4]. as it is farmers, environment friendly and it is a direct source of proteins.

Kodo millet (*Paspalum scrobiculatum L*.) is also known as Kodra and Varagu in India. It is a good source of polyphenols, flavonoids, and antioxidant compounds [5]. Due to its significant nutritional parameters and health benefits, Kodo and other little millets are produced widely and consumed in various states of India, which shows that millets are gaining their importance and people are trying to the eat right food. The phytochemicals and phytates present in *P. scrobiculatum* make it anti-cancerous and help to reduce body weight and knee and joint pains/arthritis [6,7]. In addition, the antioxidant potential of kodo millet extracts as well as whole grain has shown positive results in reducing the risk of various other age-related ailments, such as Alzheimer's, diabetes, heart disease, stroke, and cataracts [8].

There are also immunostimulant reports on and immunomodulatory responses in little and kodo millet, respectively, in RAW 264.7 cells [9]. Apart from all these bioactive compounds and health promoting affects, P. scrobiculatum is involved in causing intoxication and poisoning by fungal pathogens in moist and humid conditions. Generally, kodo millet grains are infested by Claviceps purpurea, Penicillium cyclopium, P. patulum, P. viridicatum, P. crustosum, P. camemberti and Aspergillus like A. flavus, A. versicolor, *A. tamarii.*, which produce a neurotoxin, α -cyclopiazonic acid (CPA), it is a low molecular weight compound, resembles the structure of indole tetramic acid (Figure 1) and is highly stable at elevated

temperatures. Cyclopiazonic acid (CPA) is produced by several molds that commonly occur on agricultural commodities and is known to be hepatocarcinogenic mycotoxins [10]. Many strains of Aspergillus flavus can produce this toxin in a variety of commodities and foods and as a result, CPA has been found as a natural contaminant in many products including cheeses, figs, maize, rice, peanuts [11], millet, feeds [12,13] and chicken meat [14]. CPA may also have been a major contributor to the original outbreak of "Turkey X" disease, which led to the discovery of the aflatoxins [15]. Our study emphasizes the toxic effects of mycotoxin, cyclopiazoinic acid and to minimize the risk of kodo poisoning upon consumption of a superfood.

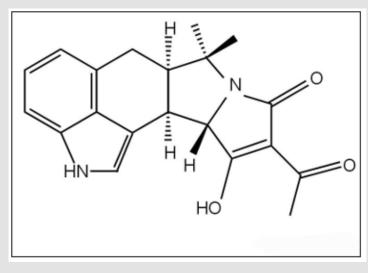


Figure 1: Structure of Cyclopiazonic Acid (CPA).

Fungi Associated with Cyclopiazonic Acid

Cyclopiazonic acid or α -cyclopiazonic acid (CPA) is chemically an indole-tetramic acid. Mycotoxin is produced as a secondary metabolite by the fungal genera Aspergillus and Pencillium. These fungal genera are omnipresent in nature and thrive on several seeds, grains and processed foods like cheese and meat [16-20]. CPA was first isolated from the Penicillium cyclopium Westling, hence the toxin is named Cyclopiazonic acid [21]. Later, it was noticed that P. cyclopium or its synonym P. aurantiogriseum [22] are not actual producers of CPA, and the species of Pencillium producing CPA was recognized as P. griseofulvum Dierckx [23]. P. griseofulvum, P. camemberti, P. urticae and *P. commune* are some of the species of *Penicillium* which are the persistent producers of CPA [24]. Several other species of Penicillium like P. chrysogenum, P. nalgiovense, P. crustosum, P. hirsutum and P. viridicatum are noted to contribute to the production of CPA but their taxonomical confirmation is yet to be done [25]. Apart from producing aflatoxin by species of Aspergillus they are also known producers of CPA like A. flavus, A. oryzae, A. fumigatus, A. versicolor, and A. tamarii

[26-28]. Further in a review by Vinokurova et al. stated that 30% of *Aspergillus fumigatus* and *Aspergillus phoenicis* strains produce CPA whereas only one strain of *A. versicolor* is involved in CPA production [29]. Another study revealed that *A. versicolor Tiraboschi* was initially known to produce CPA and was later recognised as *A. oryzae* [30]. Isolates of *A. minisclerotigenes* producing CPA are found in the United States (Texas) [31] and Europe (Italy, Portugal, and Spain) [32-34].

Causes of Poisoning

CPA (Cyclopiazonic acid) is one of the major mycotoxins associated with the kodo millet seeds causing kodo poisoning which was first recognised during the mid-eighties [35]. The kodo millet seed infected by *Aspergillus flavus* and *A. tamarii* and both mycoflora have been found to be involved in mycotoxicosis. The infected kodo millet sample containing CPA was isolated and identified which showed the symptoms of kodo poisoning in humans. Further, the extract of the toxic grain was injected into the mice, it showed symptoms of depression and complete loss of mobility. It was the first report that showed the implications of CPA, a mycotoxin with kodo poisoning [35]. The Studies suggest that consumption or feeding of infected/ polluted kodo millet is a serious health threat to humans and animals because of the possibility of exposure to CPA formed by the toxigenic fungi in kodo millet. Besides, fungal growth in fodder also causes a decline in nutritional value and results in health hazards.

Kodo Poisoning Antiquity

Kodo poisoning occurs mainly due to the consumption of kodo grains, when maturing and harvesting if the grains had encountered with rainfall, resulting in fungal infection leading to 'poisoned kodo' which is locally known as 'Matawna Kodoo' or 'Matona Kodo' in northern India [36]. Hence in certain places where kodo is cultivated, farmers believe that kodo millet is poisonous after rain. Kodo poisoning mainly affects the nervous and cardio-vascular systems and the chief symptoms include vomiting, giddiness, and unconsciousness, small and rapid pulse, cold extremities, shaking of limbs, tremors, and resistance to outside interference. Further, it had developed poisonous properties under certain unknown conditions. Yet, no cases of death were detected. Poisoned persons could be recovered by washing out the stomach, by giving stimulants, hot tea or milk and warmth to the extremities [37]. The signs and symptoms of kodo poisoning persisted for one to three days followed by recovery [38]. In some illustrations, it produces derilium with violent tremors of voluntary muscles and detrimental effects that even lead to death in very extreme cases. Normally, the husk and leaves get poisonous character generally due to heavy rain falls [38,39].

Toxic Effects on Other Food Stuff

CPA can be found in a variety of foodstuffs, including grains, fruits, and meats. The toxic effects of CPA can vary depending on the dose and duration of exposure, as well as the type of foodstuff. In grains, CPA contamination can cause a range of effects such as reduced grain yield, poor seed quality, and reduced germination rates [40]. CPA contamination in fruits can cause fruit rot and reduced shelflife [41]. In meats, CPA contamination can cause necrosis, resulting in meat quality degradation [24].The house keeping role of sarco/ endoplasmic reticulum calcium ATPase (SERCA) helps to manage the calcium gradient that is a higher percentage of calcium in the endoplasmic reticulum and lower in the cytosol, as this gradient is very much crucial for controlling cell proliferation, differentiation, and death. Studies suggest that CPA obstructs the calcium entry channels and attenuates the transmembrane ATPase and causes a range of toxic effects in animals and humans [42].

Effect and Symptoms of CPA on Human and Animal Health

As CPA is the prime constituent of kodo poisoning, the symptoms of kodo poisoning are like CPA toxicity. The CPA toxicity study has been carried out on many animals. The CPA toxicity study results in degeneration, necrosis, and liver dysfunction [43], lesions of the myocardium, by affecting calcium signalling in the heart, leading to cardiomyocyte damage and impaired heart function [44]. In addition, it has neurotoxic effects through the alteration of calcium homeostasis and cellular transduction processes in many animal species [45,46] and varies the ion transport across the cell membranes [47]. Furthermore, CPA toxicity has been reported in various animals as loss of mobility, depression, and reluctance to move in mice [35]. Acute hepatotoxicity in rats [10]; and weakness, inactivity, anorexia, rough hair coats, reduced body weight, gastric ulcers, mucosal hyperemia, and hemorrhage in pigs [48]. CPA was one of the liable mycotoxins in the turkey 'X' disease out-break in 1960 [49]. CPA damages DNA and promotes the growth of cancerous cells, resulting in the carcinogenic effect [50]. CPA-induced toxicity in dogs was found to affect gastrointestinal tract and kidneys [51]. The oral administration of CPA, isolated from Aspergillus tamarii allied with Kodo millet cause acute hepatotoxicity and preneoplastic alternations in rat liver [10]. A study found that CPA can cause gastrointestinal disorders in animals and can elevate the production of reactive oxygen species (ROS) in the intestine, which can lead to inflammation and damage to the gastrointestinal tract [52]. According to a report by the World Health Organization (WHO), CPA has immuno toxic effects which suppress the immune system, making the body more susceptible to infections. The report highlighted that CPA could impair the production of cytokines, which are important signaling molecules for immune cells [53].

Bio Control Strategy to Overcome the Toxicity and to Ensure Food Security

Currently, farming or cultivation has suffered a very significant financial/commercial decrement due to its little production or being smashed by various factors, the most common being are the illnesses and plagues. The "natural enemies," such as: parasites, predatory, and pathogens [54]. The tools and mediators used to combat and guard the harvests of this loss are mostly belong to products of synthetic origin or chemicals [55]. Nevertheless, in previous years, pesticides and herbicides usage has caused dispute since it has adverse effects on consumers, by affecting their health, and on the environment. Further, when these chemicals are used in long term it has negative effects like carcinogenic potential and teratogenic effect [56]. on food and health of both humans and animals. Hence, the usage of these pesticides and herbicides in cultivation is highly reduced and has paved the way for the use of bio control agents (BCA). Bio control agents are nothing but the use of an organism to fight against another organism. It can be done either by using a native organism or by incorporating some distinct organisms that have the capacity to fight against the pathogens of the crop. Many microbes have been used to minimize fungal development and mycotoxin secretion as a possible substitute which help in controlling plant diseases through one or more mechanisms by developing resistance to host or producing antimicrobial compounds or by its antagonistic property and competition with pathogens [57,58]. Trichoderma is commonly

known to inhibit mycotoxin production in paddy by Fusarium spp. Like F. culmorum and F. graminearum [59] and the strain Streptomyces corchorusii UCR3-16 was also found to inhibit other fungal pathogens of rice [60]. Trichoderma viride, T. harzianum and Pseudomonas spp. showed promising results both in laboratory and field studies in controlling grain molds in sorghum [61]. Furthermore, a biological control strategy that appears to be effective is inoculating the fields for many years with competitive, non-toxigenic strains of the same fungi proved to reduce in aflatoxin contamination and cyclopiazonic acid by 30 - 90% and 85.7% respectively [62]. Regrettably, once infected, feed or food containing mycotoxins are condemned since these toxins are strong and stable against thermal, physical, and chemical treatments during food processing. As kodo poisoning is caused by different strains of storage fungi Aspergillus and Penicillum the above practices may be beneficial and can be practiced reducing the contamination of mycotoxin, CPA. Moreover, bio control alone will not be enough but should be used in combination with good agricultural practices coupled with good postharvest management like sorting and proper storage, preferably in hermetic/airtight devices to significantly decrease mycotoxins [63]. In kodo millet, the fungi spreads so quickly in the moist environment as the early harvest continues in the field for drying which gets rain at times hence, harvested heaps must be sheltered from the rain. The old practice of threshing by moistening the plants before threshing should be stopped and only dried harvest should be threshed. Removing the infected grains also helps in reducing the spread of the disease. Research and development on these formulations should not stop/end so as to confirm the sustainability and ensure food safety.

Conclusion

Kodo millet is a superfood gaining importance as nutricereal in recent years but its production and consumption have declined due to Kodo poisoning. CPA is a mycotoxin that can contaminate a variety of foodstuffs, including grains, fruits, and meats. The consumption of CPA-contaminated foods can pose a health risk to humans and animals, and the prevention of CPA contamination is critical for ensuring food safety. The measures that can be taken to control CPA contamination include good agricultural pre and post-harvest management, such as proper crop rotation and storage practices, the use of fungicides, and the use of effective detoxification methods.

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