



## Evaluation of safety assessment of two different varieties of pearl millet (*Pennisetum glaucum*-Kambu) and its formulation in albino rats

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### Abstract

The concept of food as medicine has evolved and been in practice a long time ago. Before used as medicine, drugs from plant origin must be ensured as safe. The present work focused to study the *in vivo* toxicity effects of the petroleum extracts of *Pennisetum glaucum* (Pearl millet) varieties (powder of C010, Nattu kambu and flake of C010, Nattu kambu) and its formulation (1:1 ratio of C010, Nattu kambu powder and 1:1 of ratio C010, Nattu kambu flake) were carried out *in vivo* on albino rats. Seven groups of albino rats were treated with five different doses of the petroleum extracts of *Pennisetum glaucum* varieties (powder of C010, Nattu kambu and flake of C010, Nattu kambu) and its formulation (1:1 ratio of C010, Nattu kambu powder and 1:1 of ratio C010, Nattu kambu flake) orally for 14 days. General appearance and behavior were observed for 14 consecutive days. Acute toxicity studies results showed that up to the tested dose of 2000 mg/kg bwt in extract treatments, throughout the 14 days of treatment, the extract does not produced any toxicity symptoms. No toxicity related symptoms were observed in doses of Pearl millet powder and formulation extracts treated rats groups. So the LD<sub>50</sub> values of the tested Pearl millet extracts were more than 2000 mg/kg bwt.

**Keywords:** pearl millet, *Pennisetum glaucum* varieties, acute toxicity, behavioral studies

### Introduction

The concept of food as medicine has evolved and been in practice a long time ago. Likewise, natural product remedies were the most popular and reliable method among traditional people in the disease prevention and treatment, as evident from ancient literature. It is truly universal as could be seen in different regions of the world using the approach of natural product therapies, but under different names which are mainly the Traditional Chinese Medicine (TCM), Ayurveda, Siddha, and Unani. Even though the names are different, they all rely on a general platform of using materials from nature in the cure and protection against diseases (Newman and Cragg, 2012) [1]. As a continuum, more scientific research was directed at identification and isolation of bioactive principles from natural sources. Despite the development of successful drugs from natural products, most of the synthesized drugs could be coupled with distress caused by the side effects. This has catalyzed the exploration and usage of natural resources for their medicinal properties. Among the natural sources, medicinal plants are the main contributing class (Gregory *et al.*, 2015) [2].

Worldwide, millets are regarded as a significant grain, however, they are the least exploited. Millet grain is abundant in nutrients and health-beneficial phenolic compounds, making it suitable as food and feed. The diverse content of nutrients and phenolic compounds present in finger and pearl millet are good indicators that the variety of millet available is important when selecting it for use as food or feed (Shweta, 2015) [3]. The increase in the popularity of plant remedies and the limited number of

scientific works on their safety and efficacy, toxicity and adverse effects related to plant remedies are widely recognized (Saad *et al.*, 2006) [4]. There are growing evidence that support the toxicity of medicinal plants towards their users. Though various studies of the pharmacological potential of medicinal plants have been carried out in the past, works investigating their potential toxicities are very limited (Wojcikowski *et al.*, 2004) [5]. Acute oral toxicity studies of herbal medicines are essential to identify the safety and the determination of dose level that could be used subsequently. It also helps in the investigation of the therapeutic index of drugs and xenobiotics (Rang *et al.*, 2001) [6]. No drug should be used clinically without its clinical trials and toxicity studies (Anisuzzaman *et al.*, 2001) [7]. In particular, millet that are rich in phenolic phytochemicals are gaining increasing attention due to their potential health benefits and their leading role as a staple food in the human diet (Venn and Mann, 2004; Kim *et al.*, 2011) [8, 9]. However, the chemical constituent profile and toxicity data of such product are not available yet. An acute toxicity study is a preliminary, yet an important study which could shed light on the general safety of a substance. Generally, a single high dose of the material is administered to animals and they are observed for behavioral, motor-neuronal changes and mortality (Kennedy Jr *et al.*, 1986) [10]. The median lethal dose (LD<sub>50</sub>) could be calculated so as to decide the safety window for usage. In the present study, the petroleum extracts of *Pennisetum glaucum* (Pearl millet) varieties (powder of C010, Nattu kambu and flake of C010, Nattu kambu) and its formulation (1:1 ratio of C010, Nattu kambu powder and

1:1 ratio of C010, Nattu kambu flake) were tested for its acute toxicity *in vivo*.

## Materials and Methods

### Collection of Pearl millet and preparation of powder and flakes

Pearl millet varieties as C010 (Bill No. 077963) and Nattu kambu (Bill No. 077964) purchased from Tamil Nadu Agricultural University, Tiruvannamalai, Tamil Nadu, India. Before the experiment, dried whole grain of Pearl millets were ground into powder using a blender. In preparation of flakes, 200gms of powdered Pearl millet mixed with green chillies (10gms), small onion (20 gms), cumin seeds (5gms) and curry leaves (10gms). Required amount of salts were added. Further the mixed sample was grinded, cooked and then kept in overnight. After the overnight, 20ml of buttermilk was added and mixed well. Finally put in the mould and dried the sample under sunlight to make a flake.

### Extraction of Pearl millet powder and flakes

The powdered Pearl millet were separately weighed by sensitive digital weighing balance and a total of 20g of each variety Pearl millet (powder of C010, Nattu kambu and flake of C010, Nattu kambu) and its formulation (1:1 ratio of C010, Nattu kambu powder and 1:1 ratio of C010, Nattu kambu flake) were macerated with petroleum ether (20 g in 150 mL) in Erlenmeyer flask for 24hrs at room temperature (25–27°C). The extraction process goes on for 24 hours facilitated by using shaker. After 24 hrs, the extract was separated from the marc using gauze and further filtered by Whatman filter paper No. 1. The obtained filtrates were concentrated in a water bath set at 40°C. After drying, the amount of dry extract obtained was harvested and the dried extract was transferred into airtight bottles and stored in a refrigerator until used.

### Animals

Acute toxicity study carried out accordance with The Organization for Economic Cooperation and Development (OECD, 1992) <sup>[11]</sup> guidelines for the Testing of Chemicals. Male albino rats of Wistar strain approximately weighing 180-200gms were used in this study. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27±2°C and 12 hrs light / dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water *ad libitum*. They were acclimatized to the environment for 1 week prior to experimental use. All the animal experimental protocols were approved (Approval number: 265/CPCSEA) by the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

### Acute toxicity studies

Male Albino rats were randomly assigned into seven groups of each six rats. Group 1 is control group, fed daily with only normal laboratory diet and water. Group 2 treated with petroleum extract of *Pennisetum glaucum* variety C010 powder, Group 3 treated with petroleum extract of *Pennisetum glaucum* variety Nattu kambu powder, Group 4 treated with petroleum extract of formulation in C010 and Nattu kambu (Ratio of 1:1) powder, Group 5 treated with

petroleum extract of *Pennisetum glaucum* variety C010 flake, Group 6 treated with petroleum extract of *Pennisetum glaucum* variety Nattu kambu flake, Group 7 treated with petroleum extract of formulation in C010 and Nattu kambu (Ratio of 1:1) flake. All the groups were treated with respective extract at a dose of 2,000 mg/kg body weight for 14 days through an oral needle following a period of 10-h fasting. All animals were maintained on standard laboratory diets with water *ad libitum*. After administration of the extract, animals were monitored continuously for every two hours for a day to detect acute changes in behavioral responses, spontaneous activity, irritability, corneal reflex, tremors, convulsion, salivation, diarrhea, lethargy if any, and also monitored for any mortality during the course of toxicity study.

## Results and Discussion

Paracelsus, known as the father of toxicology stated that “All substances are poisons; there is none which is not a poison. It is the right dose that differentiates a remedy from poison” (Hunter, 2008) <sup>[12]</sup>. Agents from phytotherapeutic products are many times, mistakenly believed to be safe as they are natural (Gesler, 1992) <sup>[13]</sup>. But there are evidence that these products contain several bioactive principles that had the potential to cause adverse toxic effects (Bent and Ko, 2004) <sup>[14]</sup>.

The assessment of the toxic nature of plant extracts is useful to define the intrinsic toxicity of the plants and the effects of an acute overdose. It was also indispensable to consider a treatment as safe. Rats are sensitive to toxic components present in plants. The dosing of the plant extracts in increasing amounts helps to evaluate the toxicity limits (Parra *et al.*, 2001) <sup>[15]</sup>. Acute toxicity studies using animal models provide important preliminary data that helps to select natural remedies with potential health benefits for future work (Rosenthal and Brown, 2007) <sup>[16]</sup> and commonly used to assess the potential health risks in humans (Schulz *et al.*, 2001) <sup>[17]</sup>. Determination of acute oral toxicity is the first step in the screening and evaluation of toxic potentials of pharmacological compounds (Akhila *et al.*, 2007) <sup>[18]</sup>. Toxicity effects of natural remedies in animals and humans are analyzed using some physiological parameters like behavior, body weight, food intake, biochemical, hematological and histological analysis (Ahmad *et al.*, 2013) <sup>[19]</sup>.

### General appearance and behavioral observations

The clinical signs and symptoms exerted by drugs on vital body organs are considered as principal observations among toxicity indicators (Subramanion *et al.*, 2011) <sup>[20]</sup>. On the 14 days treatment of *Pennisetum glaucum* varieties (powder of C010, Nattu kambu and flake of C010, Nattu kambu) and its formulation (1:1 ratio of C010, Nattu kambu powder and 1:1 of ratio C010, Nattu kambu flake), the rats in all groups were survived throughout the entire study period. No treatment-related toxic symptoms or mortality were observed after oral administration of tested Pearl millet variety extracts. None of these rats had shown any abnormal behavioral responses in any dose range. There was no change in behavioral responses, spontaneous activity, irritability, corneal reflex, tremors, convulsion, salivation, diarrhea and lethargy if any when compared to control group (Table 1).

**Table 1:** Acute toxicity study of extracts in wellness parameters of rats

Observations	Response						
	Extract (2,000 mg/kg body wt)						
	Control rat	C010 Powder	Nattu Kambu Powder	C010: Nattu Kambu Powder (F)	C010 Flake	Nattu Kambu Flake	C010: Nattu Kambu Flake (F)
Consciousness	+	+	+	+	+	+	+
Grooming	-	-	-	-	-	-	-
Touch response	+	+	+	+	+	+	+
Sleeping duration	+	+	+	+	+	+	+
Movement	+	+	+	+	+	+	+
Gripping strength	+	+	+	+	+	+	+
Righting re flex	+	+	+	+	+	+	+
Food intake	+	+	+	+	+	+	+
Water consumption	+	+	+	+	+	+	+
Tremors	-	-	-	-	-	-	-
Diarrhea	-	-	-	-	-	-	-
Hyper activity	-	-	-	-	-	-	-
Pinna reflex	+	+	+	+	+	+	+
Corneal reflex	+	+	+	+	+	+	+
Salivation	+	+	+	+	+	+	+
Skin color	+	+	+	+	+	+	+
Lethargy	-	-	-	-	-	-	-
Convulsion	-	-	-	-	-	-	-
Morbidity	-	-	-	-	-	-	-
Sound response	+	+	+	+	+	+	+

**Note:** + indicate normal - indicate absent

No major differences were observed between control and Pearl millet variety extracts treated groups. Any pharmaceutical drug or compound with the oral LD<sub>50</sub> higher than 1000 mg/kg bwt could be considered safe and low toxic. Oral LD<sub>50</sub> values for acute toxicity as per OECD are as follows: <5 very toxic, >5 < 50 mg/kg bwt, toxic, >50 < 500 mg/kg bwt - harmful and > 500 < 2000 mg/kg bwt - no label (Walum, 1998) [21]. Following the administration of petroleum extracts of Pearl millet varieties, there was no noticeable change in food and water intake. This showed that the oral administration of petroleum extracts of Pearl millet varieties did not induce any suppression in appetite

and had no deleterious effect on food and water intake. This indicated that the metabolism of carbohydrate, protein, and fat are not affected (Klaassen, 2001) [22]. Acute oral toxicity effects of petroleum extracts of Pearl millet varieties on rats were studied and no animal deaths in rats receiving 2000 mg/kg of extract. No sign of toxicity was observed in the wellness parameters during the 14-day observation period. Therefore, the approximate acute lethal dose (LD<sub>50</sub>) of tested extracts in rats was estimated to be higher than 2000 mg/kg (Table 2). On the basis of acute toxicity studies, the 1/10<sup>th</sup> (200mg/kg) dose from the LD<sub>50</sub> has taken as perform the animal studies.

**Table 2:** Shows the LD<sub>50</sub> of tested Pearl millet extracts

S.No.	Extracts of Pearl millet varieties and formulation	Stating dose (mg/Kg)
1.	C010 Powder	2000mg/kg
2.	Nattu kambu Powder	2000mg/kg
3.	C010: Nattu Kambu Powder formulation	2000mg/kg
4.	C010 Flake	2000mg/kg
5.	Nattu kambu Flake	2000mg/kg
6.	C010: Nattu Kambu Flake formulation	2000mg/kg

## Conclusion

For the control and treatment of many ailments, there has been a growing interest in the study of therapeutic potentials of natural products derived from plants. In the present work, the results of *in vivo* acute toxicity study clearly showed the nontoxic nature of petroleum extracts of pearl miller varieties (powder of C010, Nattu kambu and flake of C010, Nattu kambu) and its formulation (1:1 ratio of C010, Nattu kambu powder and 1:1 of ratio C010, Nattu kambu flake) up to the tested dose level of 2000 mg/kg bwt. The LD<sub>50</sub> values of all the tested groups were considered to be more than 2000 mg/kg bwt. On the basis of acute toxicity studies, the 1/10<sup>th</sup> (200mg/kg) dose from the LD<sub>50</sub> has taken as perform the animal studies.

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