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Acute toxicity studies of two different varieties of finger millet (*Eleusine coracana* - Ragi) and its formulation in albino rats

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Abstract
Medicinal plants is the source for the search of many novel therapeutic compounds in developing countries. 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 ethanol extracts of *Eleusine coracana* varieties (powder of C014, C015 and flake of C014, C015) and its formulation (1:1 ratio of C014, C015 powder and 1:1 of ratio C014, C015 flake) were carried out *in vivo* on albino rats. Seven groups of albino rats were treated with five different doses of the ethanolic extracts of *Eleusine coracana* varieties (powder of C014, C015 and flake of C014, C015) and its formulation (1:1 ratio of C014, C015 powder and 1:1 of ratio C014, C015 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 finger millet powder and formulation extracts treated rats groups. So the LD_{50} values of the tested finger millet extracts were more than 2000 mg/kg bwt.

Keywords: finger millet, *eleusine coracana* varieties, acute toxicity, behavioral studies

Introduction

Nowadays, the usage of products of natural origin such as those derived from plant, animal, or marine sources as health supplements, revitalizers, and agents in disease prevention is in rise. Since 1950, a vast number of plant-derived agents are used to treat various diseases (Kinzler and Vogelstein, 2002) [1]. Plants contain a large profile of secondary metabolites that are mainly responsible for their cytotoxic activity. In the development of anti-cancer agents, the isolation of vincristine and vinblastine from vinca and podophyllotoxins from Podophyllumhexandrum are considered as milestones (Newman *et al*., 2003) [2]. Various traditional plants are used by a majority of the people in developing countries to treat a number of diseases and ailments (Liu, 2011) [3]. Plant based medications are often considered to be safe as they are natural and free from side effects (Lopes *et al.*, 2000) [4]. 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) [5]. 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) [6].

No drug should be used clinically without its clinical trials and toxicity studies (Anisuzzaman *et al.*, 2001) [7]. 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) [8]. 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) [9, 10]. In the present study, the ethanol extracts of *Eleusine coracana* (Finger millet) varieties (powder of C014, C015 and flake of C014, C015) and its formulation (1:1 ratio of C014, C015 powder and 1:1 ratio of C014, C015 flake) were tested for its acute toxicity *in vivo*.

Materials and Methods

Collection of finger millet and preparation of powder and flakes

Finger millet varieties as C014 (Bill No. 077950) and C015 (Bill No. 077951) purchased from Tamil Nadu Agricultural University, Tiruvannamalai, Tamil Nadu, India. Before the experiment, dried whole grain of finger millets were ground into powder using a blender. In preparation of flakes, 200gms of powdered finger millet mixed with green chilies (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 finger millet powder and flakes

The powdered finger millet were separately weighed by sensitive digital weighing balance and a total of 20g of each variety finger millet (powder of C014, C015 and flake of

C014, C015) and its formulation (1:1 ratio of C014, C015 powder and 1:1 ratio of C014, C015 flake) were macerated with ethanol (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 using 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 at 4°C until used.

Animals

Acute toxicity study carried out accordance with The Organization for Economic Cooperation and Development (OECD) 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±2ºC 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 ethanol extract of *Eleusine coracana* variety C014 powder, Group 3 treated with ethanol extract of *Eleusine coracana* variety C015 powder, Group 4 treated with ethanol extract of formulation in C014 and C015 (Ratio of 1:1) powder, Group 5 treated with ethanol extract of *Eleusine coracana* variety C014 flake, Group 6 treated with ethanol extract of *Eleusine coracana* variety C015 flake, Group 7 treated with ethanol extract of formulation in C014 and C015 (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 refex, tremors, convulsion, salivation, diarrhea, lethargy if any, and also monitored for any mortality during the course of toxicity study.

Results and discussion

Agents from phytotherapeutic products are many times, mistakenly believed to be safe as they are natural (Gesler, 1992) [11]. But there are evidence that these products contain several bioactive principles that had the potential to cause adverse toxic effects (Bent and Ko, 2004) [12]. 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) [13].

Appropriate animal is used as models in toxicity studies. These animal models are commonly used to assess the potential health risks in humans (Schulz *et al*., 2001) [14]. Determination of acute oral toxicity is the first step in the screening and evaluation of toxic potentials of pharmacological compounds (Akhila *et al.*, 2007) [15]. 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) [16]. 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) [17]. 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) [18].

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) [19]. On the 14 days treatment of *Eleusine coracana* varieties (powder of C014, C015 and flake of C014, C015) and its formulation (1:1 ratio of C014, C015 powder and 1:1 of ratio C014, C015 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 finger 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 refex, tremors, convulsion, salivation, diarrhea and lethargy if any when compared to control group (Table 1).

Table 1: Acute toxicity study of extract in wellness parameters of rats

Observations	Response						
	Control rat	Extract (2,000 mg/kg body wt)					
		C014 Powder	C015 Powder	C014: C015 Powder	C014 Flake	C015 Flake	C014: C015 Flake
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 finger millet variety extracts treated groups. Any pharmaceutical drug or compound with the oral LD_{50} higher than 1000 mg/kg bwt could be considered safe and low toxic. Oral LD_{50} 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) [20]. Following the administration of ethanolic extracts of finger millet varieties, there was no noticeable change in food and water intake. This showed that the oral administration of ethanolic extracts of finger 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) [21]. Acute oral toxicity effects of ethanolic extracts of finger 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 (LD50) of tested extracts in rats was estimated to be higher than 2000 mg/kg (Table 2). On the basis of acute toxicity studies, the $1/10^{th}$ (200mg/kg) dose from the LD_{50} has taken as perform the animal studies.

Table 2: Shows the LD_{50} of tested finger millet extracts

S. No.	Finger millet varieties and formulation	Stating dose (mg/Kg)
1	C014 Powder	2000mg/kg
2	C015 Powder	2000mg/kg
3	C014: C015 Powder formulation	2000mg/kg
4	C014 Flake	2000mg/kg
5	C015 Flake	2000mg/kg
6	C014: C015 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 ethanolic extracts of finger miller varieties (powder of C014, C015 and flake of C014, C015) and its formulation (1:1 ratio of C014, C015 powder and 1:1 of ratio C014, C015 flake) up to the tested dose level of 2000 mg/kg bwt. The LD_{50} values of all the tested groups were considered to be more than 2000 mg/kg bwt. On the basis of acute toxicity studies, the $1/10^{th}$ (200mg/kg) dose from the LD_{50} has taken as perform the animal studies.

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