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Research Article

# ACUTE TOXICITY STUDIES OF AQUEOUS SEED EXTRACT OF *VIGNA UNGUICULATA* IN ALBINO RATS

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**ABSTRACT**

**Objective:** Increased usage of traditional folklore medicines by the public has led to scientific evaluation of the safety of the herbs thereby providing the physicians the data required to employ them in the management of ailments. Seeds of *Vigna unguiculata* are commonly consumed as vegetables and as a culinary dish in most parts of Asian subcontinent. The present study was carried out to screen phytochemical constituents, evaluate acute toxic effects, and determine lethal dose ($LD_{50}$) of aqueous seed extract of *V. unguiculata.*

**Methods:** Phytochemical screening was carried out as described by Kokate. An acute oral toxicity study was carried out based on Organization for Economic Co-operation and Development guideline 423, and a limit test at a dose of 2000 mg/kg body weight was carried out in female Wistar rats. The extract was orally administered in animals at a single dose of 2000 mg/kg body weight. Signs of toxicity and mortality were noted after 30 min, 1 hr, 2 hrs, 4 hrs, 8 hrs and 24 hrs of administration of the extract for 14 days.

**Results:** Phytochemical screening of the extract revealed the presence of flavonoids, alkaloids, and proteins. No mortality and no significant changes were observed in physical observations, behavioral observations, autonomic effects, sensory responses, reflexes, respiratory effects, and somatomotor activity in animals which reveal the safety of the extract at dose of 2000 mg/kg body weight.

**Conclusion:** Conclusively, the results suggest that the aqueous extract is not acutely toxic to the rats and $LD_{50}$ was found to be higher than 2000 mg/kg body weight

**Keywords:** Acute toxicity, Safety, *Vigna unguiculata*, Seeds, Organization for Economic Co-operation and Development guideline 423.

## INTRODUCTION

Many indigenous plants have been in the use of man since time immemorial for alleviating various ailments, and there is also an emerging increase in the consumption of herbal formulations by the public [1]. Herbal medicine being natural is considered safe lacking any adverse effects as compared to conventional allopathic medicine, without the actual knowledge of their toxic potential [2]. However, herbal preparations may contain heavy metals, aflatoxins and pathogenic microbes due to the manner, in which they are obtained or prepared [3]. Traditional herbal medicines are not often explored for their toxicity and properly documented when compared to allopathic drugs, which are properly researched and developed [4]. Moreover, herbal medicines have no standardized doses often resulting in overdose leading to toxicity [5]. Hence, there is a strong need to evaluate the toxicity of herbal medicines as per globally accepted guidelines to prove their safety and standardize the clinical doses employed.

*Vigna unguiculata* which is most commonly called as "cow pea" is an ancient, annual edible herbaceous legume of the family Fabaceae. Resembling many other legumes, seeds are the most economically valued part of cowpea and are popular due to their accredited nutritional and medicinal properties. Known to be an exceptional source of protein, cowpea is also rich in vitamins, minerals, and soluble and insoluble dietary fiber [6]. Among the rural people in Indo-Pakistan subcontinent, cowpea fresh young leaves, immature pods, seeds are commonly used as vegetables and seeds are also used in culinary dishes, after processing such as soaking, dry heating, followed by cooking along with cooked rice [7]. In different classical texts of Ayurveda, seeds of *V. unguiculata* are being recommended as both drug and diet. *Sapthasara Vata Churna* contains seeds of *V. unguiculata* along with other 7 herbs, used in the management of constipation, spleenomegaly, colics, spasms, and prostatic hypertrophy. *Dhanvantra Tailam* also contains seeds of *V. unguiculata* used in the treatment of paralysis, neuritis, and neurasthenia [8]. Apart from this, the seeds of the plant are claimed to have astringent, laxative, diuretic, anthelmintic, antibacterial, appetizer, aphrodisiac, and galactagogue properties [7]. But, though used as a source of both drug and diet, it was reported as a major causative factor of acid peptic disorder (Amlapittajanana) by Charaka in Charakasamhita [9]. Hence, aim of the present study was to carry out acute toxicity studies of seeds of *V. unguiculata*, as per Organization for Economic Co-operation and Development (OECD) 423 guideline in experimental rats along with estimation of lethal dose ($LD_{50}$). Aqueous extract of seeds of *V. unguiculata* (AVU) was employed in the present study as mostly boiled seeds are consumed commonly.

## MATERIALS AND METHODS

### Collection of plant materials

The dry seeds of the plant *V. unguiculata* were collected from in and around local markets of Tirupati. They were identified and authentified by Professor B. Sitaram, Department of Dravyaguna, S.V. Ayurvedic College, Tirupati. (Reg. No. of the certificate PARC/2011/990).

### Preparation of aqueous seed extract

The AVU was prepared by the following method with slight modifications [10]. The dry seeds obtained were subjected to size reduction to obtain coarse powder using a grinding mill. 100 g of powder was mixed with 1000 ml of distilled water, and mixture was kept for 48 hrs in an orbital shaker at room temperature. Then, it was filtered separately through five folds of muslin cloth, and the filtrate was collected in screw glass vials. The supernatant was collected and filtered by using Whatman's Filter No. 1 and this formed the stock solution of 99 mg/ml. The filtrate was kept in refrigerator at 4°C for further use.

**Phytochemical screening**
A preliminary phytochemical screening of aqueous seed extract was performed as described by Kokate [11].

**Acute oral toxicity study**

*Experimental animals*

Acute oral toxicity test was performed as per OECD guideline 423 [12]. All experiments and protocols described in the study were approved by the Institutional Animal Ethical Committee (IAEC) of Sri Padmavathi School of Pharmacy, Tiruchanoor, Tirupati, and with permission from Committee for the purpose of Control and Supervision of Experiments on Animals (SPSP/CPCSEA/IAEC-1016/a/2014/001), Ministry of Social Justice and Empowerment, Government of India. The study was performed using healthy young adult female rats, nulliparous, non-pregnant, and weighing 125-130 g. Female rats were selected because literature surveys of conventional $LD_{50}$ tests show that usually there is little difference in sensitivity between sexes, but in those cases where differences are observed, females are generally slightly more sensitive [13]. The animals were randomly selected and kept in their cages for 5 days before dosing to allow for acclimatization to the laboratory conditions. The animals were housed individually in clean polypropylene cages. Room temperature and humidity were maintained at 22°C (±3°C) and 55-65%, respectively. Artificial lighting was provided, the sequence being 12 hrs light, 12 hrs dark (light from 06:00 am to 06:00 pm). Clean paddy husk bedding was provided to the animals. The animals were fed with commercially available standard pellet chow and unlimited supply of filtered drinking water.

*Methodology*

Paragraph 22 of OECD guideline 423 suggests two types of acute oral toxicity tests, i.e., limit test and main test. The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity below regulatory limit doses. However, in those situations where there is little or no information about its toxicity, or in which the test material is expected to be toxic, only the main test should be performed. As the literature survey of this seeds indicate that it was commonly consumed as a culinary dish from age-old times, limit test was performed.

*Procedure for limit test*

Before dosing, animals were fasted overnight and aqueous seed extract was orally administered in a single dose. The volume given was not more than 2 ml/100 g body weight. Following the period of fasting, the fasted body weight of each animal was determined, and the dose was calculated according to the body weight. AVU was administered orally at a dose of 2000 mg/kg (Fig. 1) and after administration food was withheld for further 3-4 hrs. If no mortality was observed in the animals, then the extract was administered in another set of three animals to confirm the same. If test substance-related mortality is produced, further testing at the next lower level may need to be carried out.

*Observation period*

Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hrs, with special attention given during the first 4 hrs, and daily thereafter, for a total of 14 days. All the rats were observed at least twice daily with the purpose of recording any symptoms of ill-health or behavioral changes.

**Observations recorded**
Physical observations, behavioral observations, autonomic effects, sensory responses and reflexes, respiratory effects, and somatomotor activity were monitored and recorded. The time of death, if any, was recorded.

**RESULTS**

**Preliminary phytochemical screening of AVU**
Phytochemical screening of the aqueous seed extract revealed the presence of flavonoids, alkaloids, proteins and the absence of triterpenoids, resins, steroids, carbohydrates, saponins, and tannins.

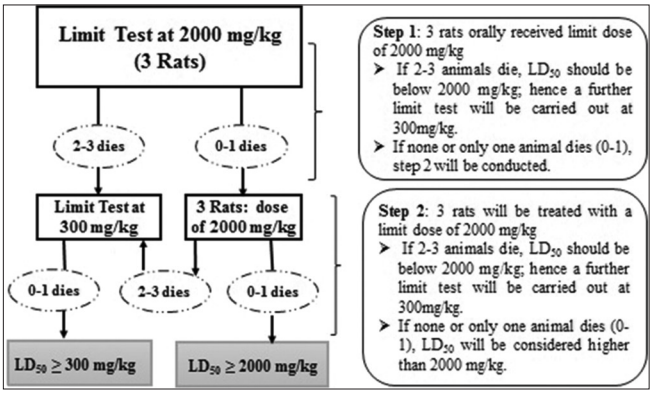


**Fig. 1: Procedure for limit test [12]**

**Acute toxicity study**
The present study conducted as per the OECD guideline 423 revealed that the AVU did not produce any mortality throughout the study period of 14 days even when the limit dose was maintained at 2000 mg/kg body weight. A similar observation was made in the second set of female rats treated with 2000 mg/kg of the extract. Therefore, the approximate acute $LD_{50}$ of *V. unguiculata* seed extract in female rats was estimated to be higher than 2000 mg/kg. No significant changes were observed in parameters used for evaluation of toxicity (Tables 1-6).

**DISCUSSION AND CONCLUSION**

Phytotherapeutic products from medicinal plants have become universally popular in primary healthcare, particularly in developing countries because of their efficacy, easy availability, and low cost [14,15]. Thus, the growing public interest and awareness of natural medicines have led the pharmaceutical industry and academic researchers to pay immense attention toward phytotherapeutic products [16].

In view of numerous therapeutic potentials of seeds of *V. unguiculata* as an alternative medicine effective for a extensive range of diseases, as known from traditional literature and number of reported scientific papers, it is only appropriate that a safety profile of the seeds of the plant be established as a guide for the management of its applications and usage in herbal preparations [17-19]. As seeds of *V. unguiculata* are commonly consumed as a culinary dish, these studies should serve to prevent exposing human subjects to potential toxicity-related health risks. Potential health risks in humans are commonly assessed by toxicity studies in appropriate animal models. Such toxicity studies assess the hazard and determine the risk level by addressing the probability of exposure to that particular hazard at certain doses or concentrations [20].

Hence, the present study was particularly designed to investigate the toxicity of AVU using acute oral toxicity analysis. The absorption might be slow through the oral route, but this method costs less and is painless to the animals. Since the crude extracts are administered orally, the animals should be fasted before taking the dose because food and other chemicals in the digestive tracts may affect the reaction(s) of the compound. The animals were administered single dose of 2000 mg/kg of AVU. All the procedures were performed based on the OECD guideline 423.

Table 1 shows that there were no variations in physical parameters such as body temperature, color of skin, fur, eyes, and urine in animals during the study. Body temperature was measured rectally in animals, and no increase in body temperature may indicate that the extract is non-pyrogenic. Behavioral effects were displayed in Table 2 and effects observed were mood, central nervous system (CNS) excitation, and depression. Animals have shown normal exploratory activity, grooming, alertness, and reactivity toward environment without restlessness and irritability specifying that administration of extract had not influenced

**Table 1: Physical observations for the limit test at 2,000 mg/kg body weight of *V. unguiculata* aqueous seed extract in rats**

| Observations | 0 minute | 30 minutes | 1 hr | 2 hrs | 4 hrs | 8 hrs | 24 hrs | 7th day | 14th day |
|---|---|---|---|---|---|---|---|---|---|
| Body temperature | N | N | N | N | N | N | N | N | N |
| Skin colour | N | N | N | N | N | N | N | N | N |
| Fur colour | N | N | N | N | N | N | N | N | N |
| Eyes colour | N | N | N | N | N | N | N | N | N |
| Urine colour | N | N | N | N | N | N | N | N | N |

N: Normal, *V. unguiculata: Vigna unguiculata*

**Table 2: Behavioural effects of aqueous seed extract of *V. unguiculata* in experimental rats at a dose of 2,000 mg/kg body weight**

| Observations | 0 minute | 30 minutes | 1 hr | 2 hrs | 4 hrs | 8 hrs | 24 hrs | 7th day | 14th day |
|---|---|---|---|---|---|---|---|---|---|
| Mood | | | | | | | | | |
| Alertness - exploratory activity | N | N | N | N | N | N | N | N | N |
| Eyes opened/closed | N | N | N | N | N | N | N | N | N |
| Grooming | N | N | N | N | N | N | N | N | N |
| Restlessness | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |
| Irritability | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |
| Reactivity (environment) | N | N | N | N | N | N | N | N | N |
| CNS excitation | | | | | | | | | |
| Tremors | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |
| Twitches | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |
| Convulsions | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |
| CNS depression | | | | | | | | | |
| Sedation | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |
| Sleep | N | N | N | N | N | N | N | N | N |
| Catatonia | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |
| Ataxia | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |

*V. unguiculata: Vigna unguiculata*, N: Normal, CNS: Central nervous system

**Table 3: Autonomic effects of aqueous seed extract of *V. unguiculata* in experimental rats at a dose of 2,000 mg/kg body**

| Observations | 0 minute | 30 minutes | 1 hr | 2 hrs | 4 hrs | 8 hrs | 24 hrs | 7th day | 14th day |
|---|---|---|---|---|---|---|---|---|---|
| Defecation | N | N | N | N | N | N | N | N | N |
| Lacrimation | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |
| Urination | N | N | N | N | N | N | N | N | N |
| Salivation | N | N | N | N | N | N | N | N | N |
| Piloerection | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |
| Mydriasis | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |
| Miosis | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |
| Emesis | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |
| Diarrhea | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |

N: Normal, *V. unguiculata: Vigna unguiculata*

**Table 4: Sensory responses and reflexes of aqueous seed extract of *V. unguiculata* in experimental rats at a dose of 2,000 mg/kg body**

| Observations | 0 minute | 30 minutes | 1 hr | 2 hrs | 4 hrs | 8 hrs | 24 hrs | 7th day | 14th day |
|---|---|---|---|---|---|---|---|---|---|
| Sensory responses | | | | | | | | | |
| Touch response | N | N | N | N | N | N | N | N | N |
| Pain response | N | N | N | N | N | N | N | N | N |
| Reflexes | | | | | | | | | |
| Pinna | N | N | N | N | N | N | N | N | N |
| Corneal | N | N | N | N | N | N | N | N | N |

*V. unguiculata: Vigna unguiculata*, N: Normal

any changes in mood of the animals during the study. CNS stimulatory effects such as tremors, twitches, convulsions, and CNS depressant effects such as sedation, catatonia, and ataxia were also not observed in animals. Hence, this shows that single dose administration of AVU has no deleterious effects on CNS.

Table 3 depicts autonomic effects of AVU. Animals have shown normal defecation, urination, salivation with the absence of lacrimation, piloerection, mydriasis, miosis, emesis, and diarrhea which may indicate that there was no excessive stimulation or depression of sympathetic and parasympathetic nervous system. Table 4 illustrates that sensory responses such as touch, pain response and sensory reflexes such as pinna, corneal reflexes were not altered by the single dose administration of AVU in rats. Animals had not shown any signs of apnea or dyspnea indicating that there were no ill effects of extract on respiratory system (Table 5). Righting reflexes and body posture of the extract administered animals were normal demonstrating absence of any abnormal somatomotor effects (Table 6). No mortality was observed in the extract administered animals during the study and hence the extract was administered at the same dose in another set of 3 animals as per the guideline, and the same trend was observed indicating that extract is safe at a dose of 2000 mg/kg.

**Table 5: Respiratory effects of aqueous seed extract of *V. unguiculata* in experimental rats at a dose of 2,000 mg/kg body**

| Observations | 0 minute | 30 minutes | 1 hr | 2 hrs | 4 hrs | 8 hrs | 24 hrs | 7th day | 14th day |
|---|---|---|---|---|---|---|---|---|---|
| Apnea | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |
| Dyspnea | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |

*V. unguiculata: Vigna unguiculata*, N: Normal

**Table 6: Somatomotor effects of aqueous seed extract of *V. unguiculata* in experimental rats at a dose of 2,000 mg/kg body**

| Observations | 0 minute | 30 minutes | 1 hr | 2 hrs | 4 hrs | 8 hrs | 24 hrs | 7th day | 14th day |
|---|---|---|---|---|---|---|---|---|---|
| Motor indication | | | | | | | | | |
| Abnormal gait | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |
| Righting reflex | N | N | N | N | N | N | N | N | N |
| Body posture | | | | | | | | | |
| Body position | N | N | N | N | N | N | N | N | N |
| Limb position | N | N | N | N | N | N | N | N | N |

*V. unguiculata: Vigna unguiculata,* N: Normal

The absence of acute toxic effects of the AVU can be attributed to the phytochemicals present in the extract. Flavonoids, alkaloids, and proteins present in the extract are metabolites are generally used in various pharmaceutical and cosmetic preparations which are an indication that these metabolites may be non-toxic [21].

In conclusion, this study reckoned that *V. unguiculata* aqueous seed extract does not cause acute toxicity effects and an $LD_{50}$ value >2000 mg/kg. In principle, the limit test method is not intended for determining a precise $LD_{50}$ value, but it serves as a suggestion for classifying the crude extracts based on the expectation at which dose level the animals are expected to survive [22]. According to the chemical labeling and classification of acute systemic toxicity recommended by OECD, the AVU can be placed under category 5 or unclassified category of globally harmonized system, which was the lowest toxicity class. Further studies are warranted for determining sub-acute and chronic toxicity to support safety and folklore use of the seeds of the plant.

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