

Acute Oral Toxicity of the Combined Mixture of *Embelica officinalis* Fruits and *Trigonella foenum-Graecum* Seeds

Ashok Kumar CK*, Satheesh Kumar D, Narotham Reddy K, Jaya Sree P, Mamatha J and Satish Babu Kotha.

Sree Vidyanikethan College of Pharmacy, A. Rangampet, Tirupathi, Andhra Pradesh, India

*Corresponding Author: E-Mail: ashokkumarck@yahoo.com

ABSTRACT

The aim of the present investigation was evaluate the acute oral toxicity of the combined mixture of *Embelica officinalis* fruits and *Trigonella foenum-graecum* seeds in Wistar rats. No mortalities or evidence of adverse effects have been observed in Wistar rats following acute oral administration of combined mixture of *Embelica officinalis* fruits and *Trigonella foenum-graecum* seeds upto the dose of 2000mg/kg. In addition, no significant differences were noticed in the body and organ weights between the control and treated groups. These results state that mixture of combined mixture of *Embelica officinalis* fruits and *Trigonella foenum-graecum* seeds is toxicologically safe by oral administration.

Keywords: OECD, *Embelica officinalis*, *Trigonella foenum-graecum* and Acute Oral Toxicity.

1. INTRODUCTION

Medicinal plants play a very significant role in health care needs of rural populations in Indian and other world countries especially in treatment of diseases. *Emblca officinalis* Gaertn. (commonly known in India as Amla, Syn. *Phyllanthus emblica* L.) which belong to the family Euphorbiaceae. It is regarded as "one of the best rejuvenating herbs" in the Ayurveda, an Indian traditional medicinal science. Several recent reports revealed that fruit extract of *Emblca officinalis* protect against radiation [1], antiatherosclerosis [2], possess antidiabetic activity [3,4], inhibits aging process [5], gastroprotective [6], cytoprotective and immunomodulatory [7] properties. Fenugreek (*Trigonella foenum-graecum* Linn.) commonly known as methi which belong to the family Leguminosae. It is used as food [8]. The seeds can inhibit cancer, lower blood cholesterol levels and also have posses antidiabetic potential [9]. In the past decade, an antidiabetic effect [10] have been reported. Hence, the aim of the present investigation was evaluate the acute oral toxicity studies of a combined mixture of *Embelica officinalis* fruits and *Trigonella foenum-graecum* seeds in Wistar rats, which will be helpful for further evaluation for its combined antidiabetic property.

2. MATERIAL AND METHODS

2.1. Collection and Identification of Plant materials

The *Embelica officinalis* fruits and *Trigonella foenum-graecum* seeds were collected

from Sree Vidyanikethan International School region in Tirupathi, Andhra Pradesh. The taxonomical identification of the plant was done in Department of Botany, Sri Venkateswara University, Tirupathi, Andhra Pradesh. The *Embelica officinalis* fruits and *Trigonella foenum-graecum* seeds were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve. The powdered *Embelica officinalis* fruits and *Trigonella foenum-graecum* seeds were mixed in the ratio of 1:2 respectively.

2.2. Acute Toxicity Study

2.2.1. Animals

In-breed Wistar rats (150-250) of either sex were obtained from the animal house in Sree Vidyanikethan College of Pharmacy, Tirupati. The rats were maintained in a well ventilated room with 12/12 hr light/dark cycle in polypropylene cages. Standard pellet feed (Hindustan lever limited, Bangalore) and drinking water was provided *ad libitum* through out experimentation period. Rats were acclimatized to laboratory conditions one week prior to initiation of experiments ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Committee for the Purpose of Control and Super- vision of Experiments on Rats)

2.2.2. Acute Oral Toxicity Study

Three female and three male Wistar rats were selected randomly and housed separately in white polypropylene cages in an experimental animal room. The cages were marked for individual

identification and provided with husk as bedding material. The acute oral toxicity of mixture of powder from *Embelica officinalis* fruits and *Trigonella foenum-graecum* seeds in Wistar rats was carried out as per OECD (Organization of Economic Co-operation and Development) guidelines 423 [11]. The mixture of powder from *Embelica officinalis* fruits and *Trigonella foenum-graecum* seeds was administered to individual rats at the dose of 2000mg/kg body weight, in 0.5ml of distilled water. The rats were observed carefully for signs of toxicity in the first four hours after the treatment period, and daily thereafter for a period of 14 days. Parameters such as mortality, sign of illness, injury, pain, distress, allergic reactions, changes of outer appearance, behavioral alterations (i.e. ataxia, hyper activity, hypoactivity) and general stimulation or sedation were observed twice daily, whereas feed intake, water intake and body weight were recorded weekly once during the study period of 14 days, after the oral administration of mixture of powder from *Embelica officinalis* fruits and *Trigonella foenum-graecum* seeds. The observations were recorded systematically; individual records were maintained for each rat. On 15th day study all surviving rats were sacrificed as per CPCSEA guidelines and detailed necropsy was carried out. The results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of chemicals which cause acute toxicity.

3. RESULTS AND DISCUSSION

Experimental screening method is imperative in order to establish the safety and efficacy of traditional and herbal products and also to set up the active components of the herbal products [12]. Investigation of acute toxicity is the first step in the toxicological analysis of herbal drugs. Present study was undertaken to determine acute oral toxicity and safety parameters of combined mixture of *Embelica officinalis* fruits and *Trigonella foenum-graecum* seeds in Wistar rats. No mortality was reported in experimental rats when the mixture of powder of *Embelica officinalis* fruits and *Trigonella foenum-graecum* seeds was administered orally at 2000mg/kg body weight (Table 1). Also there were no significant changes observed in behavior (i.e. ataxia, hyper activity, hypoactivity) in any of the rat, nor did they show variations in the general appearance throughout the 14th day study period. Body weight gain, feed intake and water consumption were found to be normal during the course of the study.

Table -1: Results of general behavioral studies in rats on administration mixture of powder of *Embelica officinalis* fruits and *Trigonella foenum-graecum* seeds at the dose of 2000mg/kg

Parameters	Result
Motor activity	Absent
Tremors	Absent
Convulsion	Absent
Straub reaction	Absent
Pile erection	Absent
Loss of light reflex	Absent
Sedation	Absent
Muscle relaxation	Absent
Hypnosis	Absent
Analgesia	Absent
Ptosis	Absent
Lacrimation	Absent
Diarrhoea	Absent
Change in skin colour	No change

As no mortality, no adverse changes in behavior of animals as well as no abnormalities were detected at necropsy in experimental rats at the dose of 2000mg/kg body weight, the combined mixture of powder of *Embelica officinalis* fruits and *Trigonella foenum-graecum* seeds was assigned to class 5 (LD₅₀ > 2000mg/kg), which were recommended by OECD.

4. CONCLUSION

Therefore, it is concluded that the administration of combined mixture of powder from *Embelica officinalis* fruits and *Trigonella foenum-graecum* seeds could be regarded as safe in experimental rats. Further toxicity study over longer period of time involving detection of effects on vital organ functions would ensure that these plants are safe for human consumption. The present study will be helpful for further evaluation for its combined antidiabetic property and also any pharmacological activities in the combination of *Embelica officinalis* fruits and *Trigonella foenum-graecum* seeds.

5. REFERENCES

1. Jindal A, Soyal D, Sharma A and Goyal PK. Protective effect of an extract of *Embelica officinalis* against radiation-induced damage in mice. Integr Cancer Ther., 2009; 8: 98–105.
2. Kim HJ, Yokozawa T, Kim HY, Tohda C, Rao TP and Juneja LR. Influence of amla (*Embelica*

- officinalis Gaertn.) on hypercholesterolemia and lipid peroxidation in cholesterol-fed rats. *J Nutr Sci Vitaminol Tokyo*. 2005; 51: 413–8.
3. Kusirisin W, Srichairatanakool S, Lertrakarnnon P, Lailerd N, Suttajit M and Jaikang C. Antioxidative activity, polyphenolic content and anti-glycation effect of some Thai medicinal plants traditionally used in diabetic patients. *Med Chem.*, 2009; 5: 139–47.
 4. Suryanarayana P, Saraswat M, Petrash JM and Reddy GB. *Emblica officinalis* and its enriched tannoids delay streptozotocin-induced diabetic cataract in rats. *Mol Vis.*, 2007; 13: 1291–7.
 5. Yokozawa T, Kim HY, Kim HJ, Okubo T, Chu DC and Juneja LR. Amla (*Emblica officinalis* Gaertn.) prevents dyslipidaemia and oxidative stress in the ageing process. *Br J Nutr.*, 2007; 97: 1187–95.
 6. Al-Rehaily AJ, Al-Howiriny TA and Al-Sohaibani MO. Rafatullah S. Gastroprotective effects of 'Amla' *Emblica officinalis* on in vivo test models in rats. *Phytomedicine.*, 2002; 9: 515–22.
 7. Sai Ram M, Neetu D, Yogesh B, Anju B, Dipti P and Pauline T. Cyto-protective and immunomodulating properties of Amla (*Emblica officinalis*) in lymphocytes: an in vitro study. *J Ethnopharmacol.*, 2002; 81: 5–10.
 8. Chadha YR. The wealth of India, A dictionary of Indian Raw Materials and Industrial products. CSIR, New Delhi, Vol. X., 1976: 299.
 9. Alarcon-Aguilara, FJ, Roman-Ramos R, Perez-Gutierrez S, Aguilar-Contreras A, Contreras-Weber CC and Flores-Saenz JL. Study of the anti-hyperglycemic effect of plants used as antidiabetics. *J Ethnopharmacol.*, 1998; 61: 101-110.
 10. Chevallier A. The Encyclopedia of Medicinal Plants. Dorling Kindersley., London, 1996.
 11. OECD (Organization for Economic Co-operation and Development) 423: Harmonized Integrated Hazard classification System for Human Health and Environmental Effects of Chemical Substances. Paris: OECD; 2001.
 12. Mythilypriya, Rajendran., Shanthi, Palanivelu, Sachdanandamand Panchanatham. Oral acute and Subacute toxicity studies with Kalpaamruthaa, a modified indigenous preparation on rats. *Journal of Health Science*, 2007; 53 (4): 351-358.