ACUTE TOXICITY STUDY OF ETHANOLIC EXTRACT OF SOLANUM INCANUM. L FRUIT

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ABSTRACT

Solanaceae is a large plant family containing two thousand and three hundred, nearly half of which belong to single genus, Solanum. Solanum incanum is a species of nightshade that is native to north western Africa and the Middle East (USDA, 2006). The leaves are consumed as leafy vegetables, while the fruits are extensively used in Kenya for the treatment of cutaneous mycotic infections and other pathological conditions. This study was aimed to determine the toxicity profile of the ethanolic extract of Solanum incanum fruit by determining its effects after administration in female mice. The acute toxicity studies were carried out based on OECD guidelines 423 and fixed dosage studies was adopted where the limit dose is 2000mg/kg body weight of test animal. The animals were orally administered a single dose of 100, 250, 500, 750, 1000 and 2000 mg/kg body weight. Signs of toxicity and mortality were noted after 1, 4 and 24h of administration of the extract for 14 days. The highest dose administered (2000mg/kg body weight) did not produce mortality or changes in general behavior of the test animals. These results indicate the safety of the oral administration of ethanol extract of Solanum incanum.

Keywords: Acute toxicity, Solanum incanum, ethanolic extract, mice.

INTRODUCTION

In the plant family Solanaceae (night shadow plants) the genus Solanum is a very large group of about 1400 species found throughout in the temperate and tropical regions of the world. These are the most prominent plants because they are growing or cultivated worldwide and/or are used commercially (1). All green and unripe parts contain steroid glycosides, in form of glycoalkaloids. In the genus Solanum they are important, both ecologically and commercially. They are widely regarded as defensive allelochemicals of the plants against pathogens and predators. (2). Different extracts from S. incanum berries were tested in vitro for antimicrobial activity against three Gram-positive two Gram-negative, and five human fungal pathogens. S. incanum methanol extract of fruits showed a very strong inhibition (3). Solamargine, purified from Chinese S. incanum possessed a potent cytotoxicity to human hepatocytes and normal skin fibroblasts leading to cell apoptosis by changes of cell morphology and DNA content (4).

The main aim of our study was to evaluate the extracts for their toxic effects before it can be used for applications that are of importance to the public. Hence the ethanolic extract, of Solanum incanum were analysed for their acute toxicity profile with reference to behavioural aspects, in Swiss Albino mice. The limit test dose of 2000mg/kg body weight was used following OECD guidelines (5, 6).

MATERIALS AND METHODS

Plant Material

Solanum incanum was collected from Sathyamangalam hills, Coimbatore, Tamilnadu. The plant was identified by Dr.G.V.S.Murthy, Scientist F& Head of Office, Botanical survey of India, Southern Regional Centre TNAU Campus, Coimbatore-03 with the number BSI/SRC/5/2/2012-13/Tech312. The fruits were collected from the plant and it was washed with water thoroughly to free from debris. The fruits were sliced and shade dried for 20 days. The dried fruit was ground coarsely and stored for further use.

Extraction of the plant

Solanum incanum (20gm) was extracted successively with ethanol (100ml) for 48hrs in a soxlet and the extract was kept in vacuum evaporator to get dried and that was used for the study.

Experimental animals

Acute oral toxicity test was performed as per Organization for Economic Co-operation and Development (OECD) guidelines 423(7). The institutional ethical committee of KMCH College of Pharmacy, Coimbatore Tamilnadu, India approved the protocol for these experiments under number RMCRET/Ph.D/09/2013-2014. Experiments were performed using healthy young adult female Swiss albino mice, nulliparous, non-pregnant and weighing 25-30 g. Female rats were chosen because of their greater sensitivity to treatment(8).

Assignment of animals

The animals were randomly divided into six groups each containing six mice. They were identified by the markings using a yellow stain. One mouse was unmarked and the others were marked on head, body, tail and head and body, tail and to ease the observation.

Housing and Diet

The animals were housed in polypropylene cages (55 x 32.7 x 19 cm), with sawdust litter in a temperature controlled environment (23 ± 2°C). Lighting was controlled to supply 12 h of light and 12 h of dark for each 24-h period. Each cage was identified by a card. This card stated the cage number, number and weight of the animals it contained, test substance code, administration route and dose level. The animals were fed with standard laboratory animal food pellets with water ad libitum.

Mode of administration

The test substance was administered in a single dose by gavage using specially designed mice oral needle. Animals were fasted 3 h prior to dosing (only food was withheld for 3 h but not water). Water is used as vehicle for administration of test substance.

Administration Dose

Following the period of fasting, animals were weighed and test substance was administered orally at a dose of 100, 250, 500, 750, 1000 and 2000 mg/kg. After the administration of test substance, food for the mice was withheld for 2 h.

Test substance administration volume
The administration volume was 1ml/kg body weight of the animal. Based on the body weight of the animal on the day of treatment, the quantity of the test substance was calculated.

**Observation period**

Animals were observed individually after at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h, 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.

**Signs recorded during acute toxicity studies**

Direct observation parameters include tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. Skin and fur, eyes and mucous membrane, respiratory, circulatory, and autonomic and central nervous systems, somatomotor activity and behaviour pattern are the other parameters observed. The time of death, if any, was recorded. After administration of the test substance, food was withheld for further 1-2 h. The number of survivors was noted after 24 h and then these were maintained for a further 14 days with a daily observation.

**RESULTS**

The present study conducted as per the OECD guidelines 423 revealed that the said extracts did not produce any mortality throughout the study period of 14 days even when the limit dose was maintained at 2000mg/kg body weight. The oral LD50 was indeterminable being in excess of 2000mg/kg body weight. So, testing the extracts at a higher dose may not be necessary and the extracts were practically non-toxic.

Table 1: indicates the parameters observed before and after the administration of the test substance for the three extracts of Solanum incanum. The writhing reflex was observed immediately up to 15 min after administration of the test substance at all administered doses for the extracts of Solanum incanum whereas all the other parameters observed were normal even at the highest dosage of 2000mg/kg body weight of the test animal. This clearly indicated that the above extracts of Solanum incanum do not produce oral toxicity. The medium lethal dose (LD_{50}) of the extracts is higher than 2000 mg/kg body weight and hence, in a single dose administration, the plant extracts had no adverse effect.

**DISCUSSION**

The non-toxic nature of ethanol extract of Solanum incanum is evident by the absence of mortality of the test animals at oral treatment of 2000mg/kg body weight. Solanum incanum contains saponin steroids, in particular glycoalkaloids, which are found in all parts of the plant, but in highest concentrations in the fruit. The main glycoalkaloid is solasonine (9). Alkaloids such as solasodine are used commercially as precursors for the production of steroidal compounds for medicinal use, mainly as contraceptives. Flavonoids and chlorogenic acid, a phenolic derivative, have also been isolated. Solamargine has shown promise for treatment of liver, lung and breast cancer (10). In 1999 it was reported that SM promoted apoptosis of hepatic cancer cells and lung cancer cells with consequential observations of nuclear condensation, DNA fragmentation and Sub-G1 peak appearances, confirming the earlier observations (11). It was found that apoptosis of cancer cells caused by SM was achieved by activating the TNFRs and Fas of cancer cells resulting in apoptosis caused by the cellular hydrolytic enzymes Caspase-8 and Caspase-3. By combining SM with cisplatin, the effective killing of cisplatin resistant cancer cells can be enhanced, particularly lung cancer cells (4). SM was shown to be much more effective than taxol, cisplatin or gemcitabine in killing lung cancer cells. The non-toxic nature of ethanol extract of Solanum incanum reveals the non-toxic nature of the foresaid phytochemicals at the tested dosage. Hence ethanol extract of Solanum incanum may be exploited for its use in product application like pharmaceuticals/ nutraceuticals/ cosmeceuticals. The oral non-toxic nature of the plant and the use of this plant go hand in hand with scientific evidence provided by the study.
CONCLUSION

The non-toxic nature of ethanol extract of Solanum incanum is evident from the acute oral toxicity conducted as per OECD guidelines. The normal behaviour of the test animals during a period of 14 days suggests the non-toxic nature of the foresaid extracts. Hence Solanum incanum could be safe up to the dose of 2000 mg/kg body weight of the animal. Further studies are warranted for determining chronic toxic symptoms.

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