

CHAPTER 5

TOXICITY STUDIES

5.1 INTRODUCTION

Toxicology tests are used to examine finished products such as pesticides, medications, food additives, packing materials or their chemical ingredients. Most tests involve testing ingredients rather than finished products.

The substances to be tested toxicologically are applied to the skin or dripped into the eyes; injected intravenously, intramuscularly or subcutaneously, inhaled either by placing a mask over the animals and restraining them, or by placing them in an inhalation chamber; or administered orally, through a tube into the stomach, or simply in the animal's food. Doses may be given once, repeated regularly for many months, or for the lifespan of the animal.

Toxicology tests, includes acute, sub-acute, and chronic toxicity. Acute toxicity is studied by using a rising dose until signs of toxicity become apparent. Acute toxicity tests must be carried out in two or more mammalian species covering at least two different routes of administration [70]. There are several different types of acute toxicity tests. The LD₅₀ ("Lethal Dose 50 %") test is used to evaluate the toxicity of a substance by determining the dose required to kill 50% of the test animal population. This test was removed from

OECD international guidelines in 2002, replaced by methods such as the fixed dose procedure, which use fewer animals and cause less suffering [71].

Acute toxicity refers to the effects on the whole body of a single dose of a chemical (or several doses within a 24-hour period), usually manifested over a period of 14 days.

Acute toxicity data are used mainly to:

- i) Identify lethal/toxic doses of chemicals for humans (primarily for the regulatory purposes of classification and labelling)
- ii) Indicate the mode of toxicity in humans, including the susceptibility of key target organs
- iii) Provide a rough guide for dose selection in repeat-dose tests in animals.

ATS were carried out according to guidelines by the Organization for Economical Co-operation and Development (OECD 2001). Acute toxicity testing for the MELA extract follows Fixed Dose Procedure, OECD Guideline 420.

5.1.1 Fixed Dose Procedure, Guideline 420

Principle

The Fixed Dose Procedure (FDP) is a method for assessing acute oral toxicity that involves the identification of a dose level that causes evidence of non-lethal toxicity (termed *evident* toxicity) rather than a dose level that causes lethality. *Evident toxicity* is a general term describing clear signs of toxicity following administration of test substance, such that an increase to the next highest fixed dose would result in the development of severe toxic signs and probably mortality.

The method was first suggested by the British Toxicology Society in 1984 [72] as an alternative to the traditional acute toxicity methods, with the aim of reducing both the numbers of animals and the level of pain associated with acute toxicity testing. The stimuli for the development of the FDP were a combination of ethical and scientific concerns regarding the traditional methods that use lethality as the key endpoint.

Experimental Design

Animals were randomly assigned to five groups of three (n = 3) each. Fixed doses of Methanolic Extract of *Limonia acidissima* (MELA) viz., 5, 50, 300, 2000 and 5000 mg /Kg was given orally.

Acute oral toxicity of MELA was determined using nulliparous, non-pregnant female mice. Albino mice were fasted for 3 h prior to the experiment and were administered single dose of MELA dissolved in water and observed for mortality up to 48 h. Based on the short term toxicity, the dose of the test animals were determined by fixed dose procedure as per OECD guidelines 420. All the animals were observed for lethal or toxic signs up to 2000mg/kg. The animals were monitored for changes in autonomic and behavioural responses continuously for first 6 h and thereby for 48 h in all dose levels. The observation was continued for another 14 days [73].

5.2 RESULTS

The methanol extract of *Limonia acidissima* (MELA) at dose of 5,50,300,2000 and 5000 mg/kg orally for every 24 h for 28 days did not produce any toxicity in tested animals. No sign of observable toxicity was detected during the experimental period according to the OECD Guidelines at different doses of 5, 50, 300 and 2000 mg/kg when administered orally. All the physiological functions and animal behavior were normal at higher dose

levels up to the dose level of 2000 mg/kg. No sign of mortality was seen hence, it could be concluded that the estimated LD50 of MELA is above 2000 mg/kg bodyweight when given orally. Thereby the therapeutic dose for the pharmacological evaluation by MELA was 1/10th of the maximum tolerated dose which was then fixed to be 200 and 400 mg/kg of the experimental animal.

The acute toxicity studies were conducted as per the OECD guidelines 420, where the limit test dose of 2000 mg/kg used. No test substance- related mortality was observed at 2000 mg/kg so, testing at higher dose may not be necessary and the extract was said to be practically non-toxic. The presence of flavonoids, terpenoids, tannins etc., in MELA as shown by phytochemical tests would have contributed to the less toxicity associated with the extract. The basic principle involved in the usage of crude plant extract in traditional medicine was that the adverse effect of one component will be nullified by the protective effect of the other components without interfering with their therapeutic properties.