Chapter 4

Acute toxicity study
For centuries, natural products, such as medicinal plants have been the basis for the treatment of various ailments. Screening of natural products for the pharmacological activity, assessment and evaluation of the toxic characteristics of extract, fraction, or compound are usually an initial step (Yuet et al., 2013). However, it must be noted that not all medicinal plants are safe for consumption in the crude form. Some level of toxicity may arise as a result of potential toxic compounds they contain and pesticide application during cultivation. The therapeutic properties of medicinal plants used by traditional medical practitioners may be due to one or more of the many compounds of the plant material (Saidul et al., 2010). These phytochemicals include complex carbohydrates, alkaloids, glycopeptides, terpenoids, tannins, cyanogens, peptides and amines, steroids, flavonoids, lipids, coumarins, sulphur compounds and inorganic ions among numerous others. Some of these compounds may be toxic and thus the plants containing them, when consumed could confer varied levels of toxicity to the individual (Humphrey and McKenna, 1997). The growing interest in herbal medicine therefore demands toxicity risk assessment of the various indigenous preparations used in the treatment of diseases (Yuet et al., 2013).

**Acute Toxicity Study**

Pharmaceutical industries routinely perform toxicity studies in their investigation of new drugs or molecules. The toxicity studies involved are acute, sub acute and chronic toxicity tests. In the acute toxicity test the main objective is the determination of LD$_{50}$ (the dose which has been lethal to 50% of the animals in the experiment). It is an initial assessment of toxic manifestation of the compound under investigation. It also helps to decide the dose of a test compound to be administered safely in an animal. It was developed in 1920’s and called “classical LD$_{50}$” involved 100 animals for 5 dose-groups, later in 1981 it was modified by the Organization for Economic Co-operation and Development (OECD) and reduced number
upto 30 for 3 dose-groups. Due to excess of animal sacrifice we should go to alternative methods which minimize the number of animals required. FRAME (Fund for the Replacement of Animals in Medical Experiment) believes that the lethal dose test is unnecessarily cruel and scientifically invalid. Several countries, including the UK, have taken steps to ban the oral LD$_{50}$. The OECD, the international governments’ advisory body abolished the requirement for the oral test in 2001. Three alternative methods and these are: Fixed Dose Procedure (FDP)-OECD TG 420, Acute Toxic Class method (ATC)-OECD TG 423, Up-and-of death. Signs recorded during studies like; increased motor activity, anaesthesia, tremors, arching and rolling. Alternative methods save numbers experimental animals (Deora et al., 2010).

**Fixed Dose Procedure (FDP)-OECD TG 420**

This method does not use death as an end point; instead it uses the observation of clear signs of toxicity developed at one of a series of fixed dose levels to estimate the LD$_{50}$. Groups of animals of a single sex are dosed in a stepwise procedure using the fixed doses of 5, 50, 300 and 2000 mg/kg. The initial dose level is selected on the basis of a sighting study as the dose expected to produce some signs of toxicity without causing severe toxic effects or mortality.

**Acute Toxic Class (ATC) Method- OECD TG423**

This method does not use death as the only end point; it also uses signs of toxicity in its stepwise approach to estimating the LD$_{50}$. It is based on the Probit model. The ATC method is a sequential testing procedure using only three animals of one sex per step. Depending on the mortality rate three but never more than six animals are used per dose level. This approach results in the reduction of numbers of animals used in comparison to the LD$_{50}$ test by 40–70%.
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**Up and Down Procedure (UDP)-OECD TG425**

This method does still use death as an end point, but doses animals one at a time to see if the dose needs to be put up or down to achieve an estimate of the LD$_{50}$ therefore giving the minimum number of animals a lethal dose of the test substance. In the up-and-down procedure, animals are dosed one at a time. If an animal survives, the dose for the next animal is increased; if it dies, the dose is decreased. Each animal is observed for 1 or 2 days before dosing the next animal. Surviving animals monitored for delayed death for a total of 7 days.

**Method of toxicity study**

The method followed for acute toxicity was Up and Down Method following OECD guidelines TG425. 5 healthy swiss albino mice were used for the experiment. These pre-acclimatized fasted animals were received sequentially with dose of 2000 mg/kg body weight of MEZA with each animal were observed for 48 hrs for safety. The animals were scrutinized for increased motor activity, anaesthesia, tremors, arching and rolling, clonic convulsions, tonic extension, lacrimation, Straub reaction, salivation, muscle spasm, writhing, hyperesthesia, loss of righting reflex, depression, ataxia, stimulation, sedation, blanching, hypnosis, cyanosis and analgesia.

**Result**

Oral administration of methanol extract of *Zanthoxylum alatum* (MEZA) upto 2000 mg/kg body weight did not produce any significant alteration in the behavior, breathing, cutaneous effects, sensory nervous response or gastrointestinal effects. During the toxicity study no deaths occurred which inferring that MEZA is safe upto dose 2000 mg/kg body weight.
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References


