TOXICOLOGICAL STUDY OF *SEMECARPUS ANACARDIUM* NUT EXTRACT

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Summary : Toxicological study was carried out in rats with chloroform-soluble fraction of the nuts of *Semecarpus anacardium* to determine its safe non-toxic dose. The fraction produced toxicity at all levels tested (50-400 *mg/kg*) but the extent of toxicity was found dose-dependent. At lower doses this fraction induced partial growth inhibition over 36 days and higher doses proved fatal within 6 days. It was observed that 230 *mg/kg* caused 50% mortality in rats and this value is 1380 *mg/m*^{2} when expressed for body surface area. This work will be of some use in the cancer chemotherapy study of the fraction.

Key words : *Semecarpus anacardium* toxicity plant product intraperitoneal lethal dose 50

INTRODUCTION

Semecarpus anacardium Linn. (Fam. Anacardiaceae) commonly known as marking nut or Bibha or Bhallataka is a deciduous tree distributed in the sub-Himalayan and tropical parts of India. The nut of this Indian plant is known to have medicinal properties and is reported to be highly efficacious in Ayurvedic medicine for the treatment of neuritis, arthritis, leprosy, warts and rheumatism (5). Bose *et al.* (1) using the extracts of the nuts reported an action similar to known histamine liberators. The oily extract from this nut is found to have anti-inflammatory activity (14,15) as well as anti-hook worm effect (13). The anacardic acid isolated from the nuts and its sodium salt are reported to have anthelmintic, antimicrobial and antiamoebic activity (2,3,6).

Considerable work has been conducted in the past 15 years concerning the anti-tumour activity of extracts of the nuts in experimental models (8,9) as well as in human tumors (10,16,17,18). More recently, it is observed that the extracts of *S. anacardium* are active in mice bearing ascitic L 1210 leukemia, B 16 melanoma and glioma-26 (4). In view of these experimental and clinical results, a further careful scientific study is warranted. The present paper reports data on the toxicity of this extract which may help to determine a safe non-toxic dose.

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MATERIAL AND METHODS

Animals :

Wistar strain rats (male) of 8 weeks age (body weight 90-110 *g*) from the Animal Colony of Cancer Research Institute were used.

Preparation of chloroform extract :

Whole nuts of *S. anacardium* (pericarp and seeds) were crushed and extracted extensively in boiling chloroform. The solvent was distilled off under reduced pressure and the thick viscous extract (yield 20% W/W) was used for toxicity testing. This extract easily dissolved in refined ground nut oil which was used as a vehicle for administration. To the refined ground nut oil was added vitamin E (1 *mg/ml*) for enhancing the stability of the preparation (Nigudkar - Personal communication). This extract was obtained from Bombay Pharmaceutical Works Pvt. Ltd., Bombay as a gift.

The extract was administered by intraperitoneal route.

Parameters :

The parameters used for drug toxicity were animal behaviour, weight gain or loss, diarrhoea, micturition and death of the animals.

General :

The present study was carried out in two phases :
In the first phase, the thick viscous extract was mixed with sterile (dry sterilization) refined ground nut oil to final concentrations of 5,10,20,40 and 80 *mg/ml*. A group of 6 rats was used; each rat was subjected to single 1 *ml* intraperitoneal injection of each dilution. The control rats received 1 *ml* of sterile refined ground nut oil per rat. Observations were made for a period of 36 days. The weights were recorded prior to injection and on alternate day thereafter.

In the second phase of experiments, the extract was administered at doses of 200, 250, 300, 350 or 400 *mg/kg* body weight of rat. Six rats were used for each dose. Solutions in oil were so prepared that each rat received its dose in 1 *ml* of the ground nut oil. Control rats received 1 *ml* of only ground nut oil. Weight record was kept as in the first phase of experiment. The experiment was terminated after 90 days. The mortality data at the end of 90 days were used to calculate LD_{50} value.

RESULTS

In general, administration of chloroform soluble extract produced toxicity at all dose levels tested and the extent of toxicity was found dose-dependent (Fig. 1). After

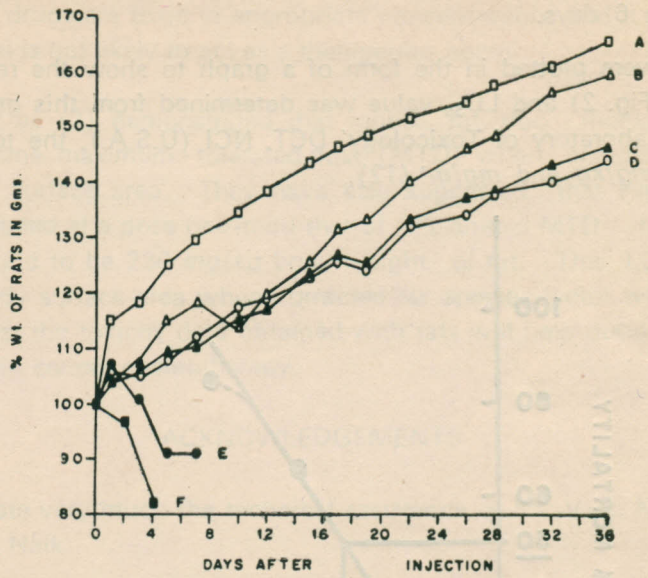

Fig. 1 : Effect of extract of *S. anacardium*(SA) nuts on body weight of the rats :

A — Oil control;
B — 5 *mg*/rat;
C — 10 *mg*/rat;
D — 20 *mg*/rat;
E — 40 *mg*/rat;
F — 80 *mg*/rat.

24 hrs of administration of this extract at dose levels of 80 and 40 *mg*/rat, rats appeared sluggish. They suffered from diarrhoea and frequency of micturition. Urine was relatively more yellowish in colour. Abdomen was distended. All the rats injected with 80 and 40 *mg*/rat died before 4 and 7 days respectively. At autopsy, peritoneal cavity was found to be filled with 3-5 *ml* fluid containing few floating oily droplets of extract. No cells were observed. No other gross abnormality was observed in viscera. Lungs were haemorrhagic and slightly congested. All rats receiving either 5, 10 or 20 *mg* of extract/rat survived till 36 days; at that stage the experiment was terminated. These results clearly indicated that LD_{50} value for extract in rats probably ranged between 40 *mg*/rat where all rats died within 7 days and 20 *mg*/rat, where all animals lived upto 36 days.

In the second set of experiments, the above doses are converted to 400 *mg/kg* body weight and the doses arranged from 200 to 400 *mg/kg* body weight of rat. The

experiment was terminated after a period of 90 days and the data were used for the calculation of toxicity effect. The dose of 200 *mg/kg* body weight produced 33% (2/6) mortality. The mortality rates at dose levels of 250 and 300 *mg/kg* body weight were 66% (4/6) and 83% (5/6) respectively. Doses of 350 and 400 *mg/kg* body weight produced 100% mortality in 6 days.

The data were plotted in the form of a graph to show the relationship between dose and effect (Fig. 2) and LD_{50} value was determined from this graph (11). As per the protocol of 'Laboratory of Toxicology' DCT, NCI (U.S.A.), the toxicity doses are to be expressed as *mg/kg* and *mg/m²* (12).

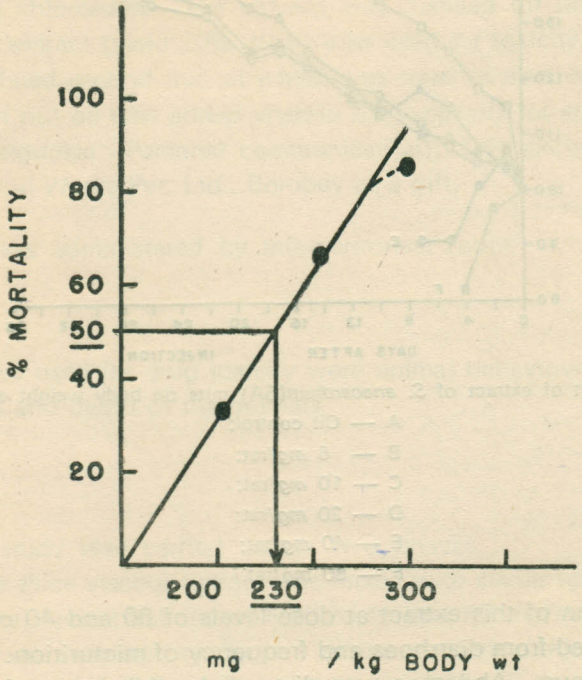

Fig. 2 : Dose *Vs.* per cent mortality graph : *S. anacardium*(SA) extract.

The (dose in *mg/m²*) = (dose in *mg/kg*) x (S) where (S) is a species factor; S = 3 in mice, 6 in rats, 7 in guinea pigs, 11 in rabbits, 12 in monkeys, 20 in dogs and 37 in man (7). It was observed that a dose of 230 *mg/kg* body weight caused 50% of the mortality in rats and this value is 1380 *mg/m²* when expressed for body surface area.

DISCUSSION

The toxicity studies in general will provide the dose that produces death in animals and it gives an idea of the margin of safety of the drug. The toxicity is expressed as "Lethal

Dose" LD_{50} which is obtained by interpolation, using several doses of the drug. In single dose studies which were used in the present experiment, the chloroform soluble extract was found to be toxic at all doses tested as evincible by impaired growth (Fig. 1). However, the degree of this toxicity was found proportional to the dose. It is important to note that all the known drugs are toxic in appropriate concentrations and it may be stated that a non-toxic material is not likely to act as a therapeutic agent.

Freireich *et al.* (7) demonstrated that mouse, rat, dog, monkey and man have essentially the same maximum tolerated dose (MTD) when compared on the basis of *mg/sq.m.* of body surface area. They have also suggested that Phase I clinical trials could be safely initiated at a dose one-third that of the animal MTD. In the present study LD_{50} value was found to be 230 *mg/kg* body weight of rat. This LD_{50} value becomes 1380 mg/m^2 of body surface area when corrected for species factor which is six for rats (7). It is likely that the toxicity data obtained with rats will help during the clinical study of this preparation in cancer chemotherapy.

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