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Lack of Teratogenicity of Liv.52*

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ABSTRACT

In our experiments involving chronic exposure to Liv.52 in three generations of mice, we encountered no adverse effects on such parameters as fertility, pregnancy and growth of the fetus in utero. The data further confirms the lack of any teratogenicity due to Liv.52 and establishes its safety of administration even during pregnancy.

INTRODUCTION

Since time immemorial, man has made use of plants in the treatment of disease. The history of medicinal plants dates back to the Rigveda and Ayurveda era (about 2500 BC), which gives a detailed account of many drugs.

In view of the extensive usage of such plan extracts over a long period, it may be argued that toxicity and teratogenicity tests in animals are superfluous. On the other hand, there is growing awareness or consciousness among the general public about the ill effects due to chemical, environmental and teratogenic factors. Teratogenicity is a study of the effects of intrinsic and extrinsic factors, which cause permanent structural and functional deviations during embryogenesis. A teratogenic agent can either induce or increase the incidence of congenital abnormalities.

There is a dearth of data on teratogenicity or toxicity of herbal preparations, as they are believed to be completely safe for human use. In spite of the established safety of Liv.52 on its long-term use, it was decided to study the effect of Liv.52 on the fetus and thus establish its complete safety and lack of any teratogenic property.

MATERIALS AND METHODS

Laboratory bred rats of original Wistar strain were housed in a room at constant temperature, to which they were acclimatized. They were exposed to the natural day and night cycle and fed a synthetic diet. The experiments were carried out on the 15^{th} in-bred generation of sexually active female rats. They were weighing between 200 to 230 grams and were 3 months old.

The female rats were kept for 1:2 mating with males of the same age and weight. The males were removed three days later.

Thirty female mice were divided into three groups of 10 each. Group I served as negative control receiving only the vehicle for 20 days. Group II animals received Liv.52, 1 gm/kg in the form of suspension, once a day orally for 10 days form the first day of conception. Group III animals received 1 gm/kg of Liv.52 once a day orally for 20 days from the first day of conception.

A daily record of their general behavior, activity, weight and food intake was maintained. All the animals in the three groups were allowed to deliver naturally and the numbers of live births were noted.

They were observed daily and weighed twice a week for three months.

The rats of the 16th generation were inbred further without drug administration by a 1:2 mating to produce the 17th generation rats. Female rats of the 17th generation were divided again into 3 groups and drug administration was carried out as before, after successful mating, from the day of conception. After mating the male rats were dissected to see if there were any abnormalities in this generation of rats following administration of Liv.52 to the 15th generation. On day 20 after drug administration the female rats of the 17th generation were also dissected to count the fetuses in the uterine horns. The number of live and dead fetuses were noted, and resorption if at all, was also recorded.

The following parameters were then recorded:

1. Fertility Index = $\frac{\text{No. of pregnant animals}}{\text{No. of animals with successful copulation}} \times 100$

2. Gestation Index = $\frac{\text{No. of females with live newborns}}{\text{No. of pregnant animals}} \times 100$

3. Viability Index = $\frac{\text{No. of newborns alive on day 4}}{\text{No. of live newborns}} \times 100$

4. Weaning Index = $\frac{\text{No. of live weanlings}}{\text{No. of newborns alive on day 4}} \times 100$

5. Birth Index = $\frac{\text{No. of live fetuses}}{\text{No. of implantations}} \times 100$

The fetuses were then observed for gross and skeletal abnormalities using the alizarin red stain technique.

RESULTS

Tables 1 and 2 depict the mean weight profiles (in gm) of animals in the three groups and belonging to the 15th and 16th generations.

Although there was no appreciable difference in the weights between the three groups at 3,

Table 1: Showing the mean weight profiles (in gm) in mice of the 15th generation (n=10)

Age	Group I (-ve control)	Group II (Liv.52 for 10 days)	Group III (Liv.52 for 20 days)
3 months	233.30 ± 6.66	220.20 ± 9.71	215.00 ± 4.22
6 months	246.60 ± 7.02	241.22 ± 10.31	234.30 ± 6.29
9 months	238.80 ± 8.24	248.80 ± 11.95	241.42 ± 8.50
10 months	281.00 ± 15.74	275.00 ± 14.45	270.0 ± 11.45

'n' refers to the number of mice used.

6 and 9 months intervals, there was a significant increase in the weights of all the three groups at the end of 12 months, which was related to natural growth.

Fertility and the ability to sustain the pregnancy were not effected by Liv.52 treatment, as shown by the fertility and gestation indices. The viability index, which indicates the number of fetuses alive, was higher in the Liv.52 treated groups, as compared to the control group. The weaning and birth indices were similar in all the groups (See Tables 3, 4 and 5).

The fetuses and their skeletal structures in the control as well as the Liv.52 treated animals did not reveal any abnormalities.

Table 2: Showing the mean weight profiles (in gm) in mice of the 16th generation

Age	Group I (-ve control) n=7	Group II (Liv.52 for 10 days) n=9	Group III (Liv.52 for 20 days) n=9
3 months	215.70 ± 5.71	236.70 ± 9.10	220.00 ± 7.81
6 months	249.28 ± 11.62	272.50 ± 9.54	232.70 ± 4.79

'n' refers to the number of mice used.

Table 3: Showing the percentage indices of the 15th generation female rats natural delivery

Parameters	Group I (-ve control)	Group II (Liv.52 for 10 days)	Group III (Liv.52 for 20 days)
Fertility index	100%	100%	100%
Gestation index	44%	55.5%	63%
Viability index	50%	74%	47%
Birth index	–	–	–
Weaning index	100%	100%	100%

DISCUSSION

It is estimated that about 10% of humans are born with congenital anomalies due to known environmental, chemical or teratogenic factors[2]. Fifty to seventy thousand chemicals currently exist either as untested drugs, industrial by-products or as environmental pollutants. These are supplemented by the addition of 700-1000 new ones every year. Such numbers cause great concern whether so many agents can be accurately and efficiently tested for potential hazards to the conceptus.

Table 4: Showing the percentage indices of the 16th generation female rats natural delivery No vehicle or drug administration

Parameters	Group I (-ve control)	Group II (Liv.52 for 10 days)	Group III (Liv.52 for 20 days)
Fertility index	100%	100%	100%
Gestation index	85%	66%	44%
Viability index	46%	61%	46%
Birth index	–	–	–
Weaning index	100%	100%	100%

Prior to 1961, indications of potential or actual induction of congenital malformations in man have been observed with a number of agents like nitrogen mustard, androgenic hormones etc[3,4]. However, it was not until the relationship between phocomelia and thalidomide ingestion during pregnancy was recognized, that regulatory agencies insisted on the inclusion of teratogenicity studies in the battery of toxicological studies required prior to release of drugs[5].

Table 5: Showing the percentage indices of the 17th generation female rats

Parameters	Group I (-ve control)	Group II (Liv.52 for 10 days)	Group III (Liv.52 for 20 days)
Fertility index	100%	100%	100%
Gestation index	75%	100%	70%
Viability index	–	–	–
Birth index	–	–	–
Weaning index	100%	100%	100%

The primary aim of testing in animals is to reduce hazards in man. The conventional testing method involves treating pregnant laboratory animals with the test agent during the period of organ

formation in the embryo. This evaluation confirms whether the agent is a developmental toxicant or not. Rats have been frequently used (rodent species) for teratogenicity testing, as their placental and hormonal characteristics are somewhat similar to those in man[4]. The gestation period between 21 to 22 days and the time of copulation could be easily determined by vaginal smear techniques.

In our experiments involving chronic exposure to Liv.52 in three generations of mice, we encountered no adverse effects on such parameters as fertility, pregnancy and growth of the fetus *in utero*. The data further confirms the lack of any teratogenicity due to Liv.52 and establishes its safety of administration even during pregnancy.

Liv.52 is a well-known herbal remedy for many liver ailments. Liv.52 is a general tonic with a record of safety attested by its continuous, extensive and universal use over several years.

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