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Evaluation of the Role of Liv.52 Treatment in Swiss Albino Mice during Pre- and Post-natal Development

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ABSTRACT

Liv.52 drops were given orally to experimental pregnant mice at a dose of 0.25 ml/day/animal through days 0 to 17 of gestation, while control animals received an equal volume of tap water in a similar manner. There was a significant increase in the weights of 18-day old foetuses treated with Liv.52 in utero as compared to the control group. An improvement in the weights was also observed in litter born to Liv.52-administered mice during pregnancy. Resorbed and dead embryos, external malformations in foetuses/offspring and skeletal anomalies in foetuses were absent in these groups. Moreover, foetuses born to Liv.52-treated mothers showed better ossification in the skull bones than the control group. No significant alteration in litter size and sex ratio of the foetuses was noticed in any of these groups. There was no significant variation in weights of the various organs of six-week old animals born to mothers of either group.

INTRODUCTION

Various drugs, heavy metals and chemicals have been found to be teratogenic in laboratory animals and human beings. Most of the Ayurvedic preparations are not studied to evaluate their Teratogenic or toxic effects, even though-herbal preparations have been extensively used since 2500 B.C. Liv.52 is an indigenous remedy used in India and other countries for treatment of various liver disorders and other diseases[1,2,4,12].

Liv.52 has been reported as a powerful detoxifying herbal agent[6,5,7,11] and also as a protective drug against radiation damage[8-10]. This study assesses the effect of Liv.52 on Swiss albino mice during embryogenesis and postnatal development.

MATERIALS AND METHODS

Swiss albino mice (7-9 weeks old, 25 ± 1 g body weight) were selected from an inbred colony, maintained on standard mice feed (procured from Hindustan Lever Ltd.) and were provided with water *ad libitum.* Females and males were placed in a 4:1 ratio in each cage overnight for mating, and the following morning, mice with vaginal plugs were separated. Indication of vaginal plugs was considered day zero of pregnancy. These females were divided into two groups of ten: experimental (Liv.52-treated) and control (non-drug treated). Liv.52 drops received from The Himalaya Drug Co., were administered orally to the pregnant animals at a dose level of 0.25 ml/day per animal, through days 0 to 17 of gestation, while animals of the control group received an equal volume of tap water.

On day 18 of gestation, 50% of the dams from each group were sacrificed by cervical dislocation to determine their reproductive status, while pregnancy in the remaining 50% of animals from each group was allowed to continue. The uteri of all the sacrificed mice were examined. The number of

implant sites was counted, and the conceptus at each site *in utero* was classified as either being resorbed, dead or alive. The foetuses (alive or dead) were sexed, blotted dry, weighed individually and examined for external alterations. A randomly selected group of 60% of foetuses (alive or dead) were fixed in 95% ethanol, cleared in KOH, stained with alizarin red S and examined for skeletal alterations.

The size and sex ratio of the litter born to the remaining 50% of mice of the control and experimental groups were recorded at birth. They were also examined for the development of physiological markers like pinna detachment, eye opening, morphological anomalies and postnatal mortality, if any. Their weekly weights were also recorded up to the age of six weeks. Autosies were performed on these six-week old animals by cervical dislocation to record the weights of the various organs.

RESULTS

Prenatal Studies

The average number of implant sites were found to be almost equal in each uterine born, both in the Liv.52-treated and control groups. Resorbed and dead embryos were absent in either group. The mean foetal weight was found to be significantly higher 1.46 ± 0.04 g) in the prenatally Liv.52-treated group as compared to the control group (1.20 ± 0.30 g) (p<0.001). No alteration in sex ratio was observed. Further, no external malformations or skeletal anomalies were detected in either group (Figs. 1,3a,b). However, foetuses of mice treated with Liv.52 showed more ossified skull bones as compared to the controls (Figs. 2, 3b).

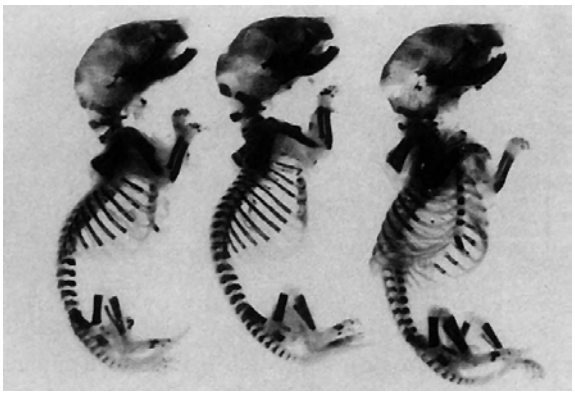

Figure 1: Showing normal skeleton elements (lateral view) of foetuses born to control females

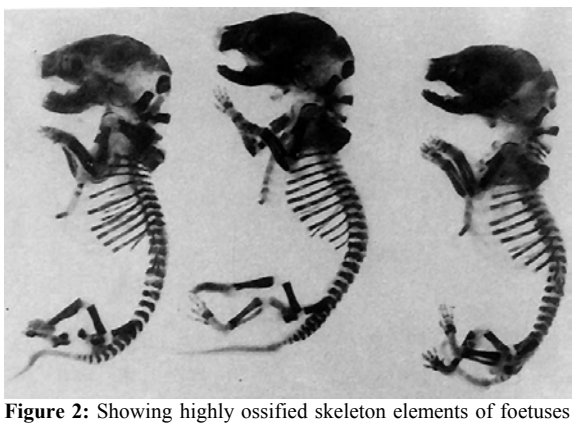

Figure 2: Showing highly ossified skeleton elements of foetuses born to Liv.52-treated mothers (lateral view).

Postnatal Studies

The average litter size in both the groups was 8.86, and there was no deviation in sex ratio in either of them. Weekly weights of 47 offspring of the Liv.52-treated group and 44 of the control group were recorded up to the age of six weeks (Table 1a). During this period, no postnatal mortality and macroscopic anomalies were seen in the offspring of both groups, and all were found to be normal in morphology. The postnatal growth

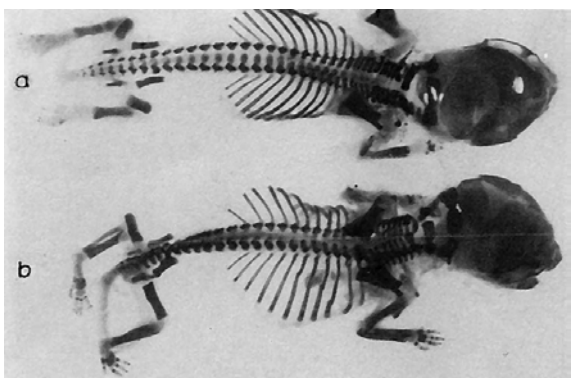

Figure 3 a-b: Showing dorsal views of normal skeletons of control and experimental foetuses respectively.

rate in these two groups was compared by weight analyses. The results of the present study showed that the mean weekly postnatal weights of offspring of the experimental group were higher during infancy as compared to those in the control group, but the difference was statistically nonsignificant. The ears of the offspring exhibited complete unfolding on day 3, body hair appeared on day 5, and the eyelids opened on day 12 of postpartum age respectively in both the groups. The weights of the various organs (testis, brain, liver, lung, kidney, spleen and thymus) of both groups were also recorded but were not significantly different (Table 1b).

Table 1a: Mean weekly body weights (g) of mouse offspring (±SE)

Group	Postpartum age (week)					
	1	2	3	4	5	6
Control (non-drug treated)	4.00 ± 0.13	6.30 ± 0.13	11.22 ± 0.48	15.82 ± 0.29	18.46 ± 0.58	21.35 ± 0.51
Experimental (Liv.52-treated)	4.80 ± 0.25	7.80 ± 0.31	11.42 ± 0.41	16.21 ± 0.55	18.84 ± 0.53	21.72 ± 0.38

Table 1b: Mean organ weight of six-week old mouse offspring (±SE)

Group	Testis (mg)	Brain (mg)	Liver (g)	Lung (mg)	Heart (mg)	Kidney (mg)	Spleen (mg)	Thymus (mg)
Control (non-drug treated)	106.8 ± 2.5	414.6 ± 2.9	1.668 ± 0.090	192.0 ± 8.9	144.6 ± 4.9	164.6 ± 5.8	113.2 ± 4.8	64.3 ± 1.6
Experimental (Liv.52-treated)	112.0 ± 3.0	422.6 ± 4.0	1.748 ± 0.060	214.2 ± 2.9	160.0 ± 6.0	160.0 ± 4.2	123.0 ± 1.6	67.6 ± 1.4

DISCUSSION

In the present study, there was a significant increase in the weights of 18-day old foetuses of mice treated with Liv.52, as compared to the control group. Such improvement in weight of mice offspring born to the Liv.52-treated mothers has been reported earlier. For instance, Srinivasan and Balwani (1968) have shown that Liv.52 is a growth-promoting agent, and the weight gain is due to increased food consumption as well as more efficient food utilization. Similarly, increased food consumption by the Liv.52-treated mothers during pregnancy resulted in the increased mean foetal body weight in the present study.

The increase in body weight is of great significance. It is well known that infants of low birth weight are more prone to develop physiological jaundice. It has been also suggested that the lower the weight of the infant at birth, the higher the risk of developing severe hyperbilirubinaemia. This results in physiological jaundice[14]. It is in vogue to give Liv.52 to the neonates suffering from physiological jaundice. However, it is also desirable to adopt preventive measures. One such measure would be to administer Liv.52 to pregnant females. In the present study, Liv.52 has also been shown to be non-foetotoxic. Similar results have also been reported[3]. Therefore, Liv.52 can be safely used by pregnant females. Thus, Liv.52 emerges as a general tonic with a safety record testified by its continuous administration to females throughout pregnancy in this study.

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