Liv.52 Protection against Beryllium Toxicity in Female Albino Rats

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ABSTRACT
Toxic effects of beryllium salts on the reproductive organs of cyclic adult female albino rats have been studied. An attempt was made to overcome these effects using an Ayurvedic medicine Liv.52 (The Himalaya Drug Co., Bombay). Liv.52-primed rats (1 mL/rat/day for 15 days) were exposed to beryllium nitrate intravenously and were sacrificed at different time intervals. At autopsy ovary, uterus, cervix, and vagina were processed for biochemical and histopathologic examination. Histoarchitecture of the ovary, uterus, cervix and vagina revealed severe necrotic changes with beryllium nitrate treatment. Tissue glycogen content and the activity of alkaline phosphatase were inhibited significantly after beryllium treatment. Total and esterified cholesterol levels increased significantly in these organs when exposed to beryllium salts. However, a significant improvement was observed in the biochemical parameters and histoarchitecture of these organs when beryllium was injected into Liv.52-primed animals.

Key Words: Beryllium, Female reproductive toxicity, Uterus, Ovary, Vagina, Cervix, Liv.52.

INTRODUCTION
Intoxication with various compounds may lead to infertility and may cause adverse effects on the developing fetus. Various salts of beryllium cause a high incidence of disease in pregnant and nonpregnant female workers. Eisenbud et al. have reported berylliosis in females who lived 2 miles from a beryllium plant and were exposed to beryllium dust while washing the clothes of their husbands who were employed in the plant. A similar case is reported of a mother who attributed her beryllium disease to dust that her daughter brought home from the plant. De Nardi has reported 20 cases of beryllium poisoning; interestingly, 80% of them were females who showed weight loss, cough, dyspnea, and abnormal chest X-ray findings. Mathur et al. have reported toxic effects on early rat pregnancy of beryllium injected at day 10 or 11 of pregnancy (critical period), as the implantation sites were resorbed. They also reported that when beryllium was injected at the time of placenta formation, the fetus survived.

A number of chemical agents, namely, BAL, salicylic acid, auriintricarboxylic acid (ATA) and EDTA have been tested as protectants against beryllium toxicity but have failed in clinical trials. However, no medicinal plant or herbal drug has been tested against beryllium toxicity. An Ayurvedic medicine Liv.52 (The Himalaya Drug Co., Bombay, India) has been used in many liver diseases. It is well known for its hepatoprotective properties in restituting various biochemical and histologic parameters. Saini et al. reported that after Liv.52 treatment normal numbers of pups were delivered in mice exposed to 2.5 Gy gamma rays. The present investigation also deals with the biochemical and histopathologic alterations in the reproductive organs of adult female albino rats when exposed to beryllium nitrate. The role of Liv.52 is tested for its possible therapeutic action in modifying beryllium toxicity.
MATERIALS AND METHODS
Adult healthy cyclic female albino rats (150 ± 10 g) of Swiss strain were selected. All the animals were maintained under uniform husbandry conditions of light and temperature. They were given “Hindustan Lever” rat pelleted diet and water ad libitum. All the animals were weighed daily for 20 days to record their normal growth rate.

Beryllium nitrate was dissolved at a concentration of 0.316 mg/ml in pyrogen-free distilled water and was injected intravenously in each rat of Group 3 and 4 once only at a dose of 0.316 mg/kg body weight (10% of the LD\textsubscript{50} dose). Liv.52 was procured from The Himalaya Drug Company, Bombay, India. Each mL of Liv.52 contained the extracts of the plants, Capparis spinosa (17 mg), Cichorium intybus (17 mg), Solanum nigrum (8 mg), Cassia occidentalis (4 mg), Terminalia arjuna (8 mg), Achilla millefolium (4 mg), and Tamarix gallica (4 mg). The selected animals were divided into 4 groups of 18 each and were treated as follows:

Group 1 was given 1 mL distilled water by gavage as a vehicle, at the same time the treated animals were given Liv.52 treatment in Group 2 and 4. This group served as a vehicle control.

Group 2 was primed with Liv.52 (1 mL/rat/day orally) for 15 days prior to the experiment and thereafter received Liv.52 daily until the 30\textsuperscript{th} day of experimentation. This group served as the Liv.52 control.

Group 3 was administered beryllium nitrate intravenously once at a dose of 0.316 mg/kg at a time designed as 0 h.

Group 4 was first primed with Liv.52 (1 mL/rat/day orally) for 15 days as in Group 2 and then was given beryllium nitrate 0.316 mg/kg intravenously. These animals continue to receive a daily dose of Liv.52 until the 30\textsuperscript{th} day of experimentation.

Throughout the experiment, the vaginal smear of each rat was checked daily at a regular interval of 24 h. At the end of the experiment the record of the different stages of the estrous cycle of each rat was analyzed. The rats were sacrificed 2, 10 and 30 days after beryllium administration.

At autopsy the ovaries, uterus, cervix, and vagina were excised, free from adhering tissue, blotted on a filter paper, and weighed. Fresh tissue was processed for the estimation of glycogen, and isotonic buffered homogenate was used for the estimation of total proteins, acid and alkaline phosphatases, and total and esterified cholesterol. Small pieces of these tissues were fixed in alcoholic Bouin’s fluid for histopathologic studies. Groups were compared for statistically significant differences using analysis of variance.

RESULTS
Effect of beryllium nitrate and Liv.52 on the estrous cycle
Our observations of the estrous cycles in the treated animals showed no alterations as compared to those of the control animals. The stages of the cycles, namely, proestrus, estrus, metestrus, and
diestrus, revolved in a cyclic clockwise direction in all the groups (Figure 1). The percent phase duration of each stage remains static irrespective of treatment.

**Alterations in biochemical parameters**

*Glycogen*: Glycogen contents of various body organs of control and Liv.52-control rats did not show any alterations during 30 days of experimentation (Figure 2). Beryllium nitrate treatment caused significant alteration in glycogen content of the ovary, uterus, cervix, and vagina 2 and 10 days after treatment. At 30 days post-treatment these values showed less alteration, and maximum depletion was observed 10 days after beryllium administration. When beryllium was administered in Liv.52 primed animals, there was a significant initial rise in tissue glycogen content compared to treatment with beryllium nitrate alone.

*Total proteins*: Beryllium nitrate treatment did not significantly change the protein concentration in the reproductive organs (Figure 3). Similarly, when beryllium nitrate was administered to Liv.52 primed rats, there was insignificant change in the values as compared to control groups.
Acid phosphatase: Administration of Liv.52 (Group 2) at 2 days through 30 days of treatment did not provoke significant alteration in the enzymatic activity of the reproductive organs (Figure 4). With beryllium nitrate treatment, acid phosphate activity in the ovary, uterus, cervix, and vagina showed insignificant change at different durations after treatment.

Alkaline phosphatase: Activity of alkaline phosphatase in controls and Liv.52 controls did not register significant change during the experiment (Figure 5). Administration of beryllium nitrate caused significant inhibition in the activity of alkaline phosphatase in all organs. Maximum inhibition was observed 10 days after exposure in the enzymatic activity of the ovary, uterus, cervix, and vagina. With Liv.52 treatment, the fall in alkaline phosphatase activity was prevented at all the durations.

Total cholesterol: The control values in groups 1 and 2 were not statistically different from one another (Figure 6). With beryllium nitrate treatment, the cholesterol level was elevated in all the organs at all the durations. Maximum elevation was observed 10 days after treatment. When beryllium was administered to Liv.52 primed rats, the cholesterol content remained lower as compared to group 3 at all the durations.

Esterified cholesterol: The tissue esterified cholesterol level of group 1 and group 2 remained virtually unchanged throughout the experimental regimen (Figure 7). Following administration of beryllium nitrate there was a sharp increase in the cholesterol content at the subsequent time intervals in all the organs. Conjoint treatment of Liv.52 and beryllium nitrate significantly reduced the cholesterol level in all the organs.

Histopathology
Ovary: Histoarchitecture of the ovary under Liv.52 treatment showed normal features (Figure 8a). Ovarian histoarchitecture revealed necrotic changes 2, 10 and 30 days after beryllium treatment. Two days after exposure, developing follicles showed degenerative changes. Growing follicles showed accumulation of edema fluid accompanied by total disorganization of the follicular cells. Some of the nuclei were hyperchromatic and showed clear cytoplasmic space (Figure 8b). Ten days after beryllium exposure, the number of mature follicles had decreased. Primary follicles showed...
granular cytoplasm and shrinkage of the ovum. Developing follicles showed hypertrophy, granulation, and vacuolations in the follicular cells. Ovarian histoarchitecture 30 days after beryllium exposure showed less damage as compared to the previous durations. However, ova of the graafian follicles were divided by the thecal cells so that the antrum of the follicle looked divided into two chambers (Figure 8C). Ovarian histoarchitecture revealed improvement after beryllium and Liv.52 treatment. After 2 days, follicles were less affected and the follicular cells were generally in their normal position. Ten days after beryllium and Liv.52 treatment, the ovarian histologic features showed improvement in comparison to beryllium nitrate alone (Figure 8d). Newly formed corpora lutea were observed at day 30 with normal structure. The mature follicles were observed with almost normal structure. Stroma was rather loose with vascularity.

**Uterus:** Histoarchitecture of the uterus under Liv.52 treatment showed normal structure (Figure 9a), however, it revealed considerable alteration 2 and 10 days after beryllium treatment. Two days after beryllium exposure, mild toxic changes were observed. The

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**Figure 6:** Effect of beryllium nitrate on total cholesterol (a) of ovary (—) and uterus (– - -) and (b) of cervix (—) and vagina (– - - -). ● = Control; □ = Be(NO₃)₂; ▲ = Liv.52; O = Liv.52 + Be(NO₃)₂; p<0.05 as compared with parallel control group 1. *p<0.05 compared with parallel Be(NO₃)₂ group 3. Values are expressed as mean ± SEM of 6 rats.

**Figure 7:** Effect of beryllium nitrate on esterified cholesterol (a) of ovary (—) and uterus (– - -) and (b) of cervix (—) and vagina (– - - -). ● = Control; □ = Be(NO₃)₂; ▲ = Liv.52; O = Liv.52 + Be(NO₃)₂; p<0.05 as compared with parallel control group 1. *p<0.05 compared with parallel Be(NO₃)₂ group 3. Values are expressed as mean ± SEM of 6 rats.

**Figure 8:** (a) Photomicrograph of rat ovary after Liv.52 treatment showed normal histoarchitecture (X 103). (b) Rat ovary after 2 days of beryllium treatment showed hyperchromatic nuclei and clear cytoplasmic spaces (X 344). (c) Thirty days after beryllium treatment follicles were divided by thecal cells so that the antrum of the follicle appeared to consist of two chambers (X 103). (d) Ten days after beryllium and Liv.52 treatment ovarian histological features were more nearly normal (X 103)
endometrium showed liquifaction and disruption of columnar epithelial cells, and these cells were multinucleated. The stroma was loose and fibrocellular. Ten days after beryllium treatment, severe necrotic changes were observed. Columnar cells of the epithelium were thin and elongated when compared to normal cells. Nuclei were elongated with variable positions. There was severe hypertrophy of the luminal epithelium (Figure 9b). Uterine glands showed cytoplasmic vacuolations and fibrotic stroma (Figure 9c). Thirty days after treatment, similar histologic features were observed, although alterations were less significant when compared to the earlier durations.

Uterine histoarchitecture revealed improvement with conjoint treatment with Liv.52 and beryllium nitrate. The endometrium was lined with simple columnar epithelium, and most of the nuclei were basally located (Figure 9d).

Cervix: The histologic structure of the cervix under Liv.52 treatment showed normal features. Cervical epithelium was more or less normal 2 days after exposure to beryllium, and the mucosa showed normal plicae palmatae. The stroma appeared flat, and the number of darkly stained nuclei was increased (Figure 10a). After 10 and 30 days, the cervical epithelium showed normal features, although it was covered with thick mucus. In the stroma, fibrosis was increased as compared to the previous duration, and some stromal nuclei were pyknotic. There was
hyperchromasia of the nuclear membrane of cervical epithelial cells (Figure 10b). Cervical histoarchitecture showed improvement with Liv.52 therapy in beryllium-exposed rats. There was no significant alteration in cervical histoarchitecture 2, 10 and 30 days after exposure. After 2 days, the stroma remained more or less compact with normal stromal nuclei. Cervical epithelium and plicae palmatae showed normal structure. At 10 and 30 days, the cervical epithelium was more or less normal, and the mucosa showed normal plicae palmatae.

**Vagina:** The vagina of rats treated only with Liv.52 revealed no significant alteration. Two days after beryllium treatment, the vaginal epithelium showed binucleate and multinucleate cells at the base and also showed desquamation of superficial cells. The stroma showed degenerative changes and appeared flat. Ten days after treatment, the vaginal stratified squamous epithelium revealed cornification, and superficial epithelial cells showed some darkly stained nuclei and perinuclear vacuolations (Figure 11a). When beryllium was administered conjointly with Liv.52, vaginal histoarchitecture revealed more or less normal features at all the durations (Figure 11b).

**DISCUSSIONS**

A number of toxic xenobiotics are known to affect the ovary and other reproductive organs. The psychoactive compound $\Delta^9$-tetrahydrocannabinol may impede the reproductive process by inhibiting the secretion of gonadotropins at the hypothalamic level. The use of alcohol during pregnancy can injure the fetus and may also cause menstrual disorders, frequent abortion, and infertility. Arthur and Eaton have observed reduced egg production in the fathead minnow treated with a sublethal dose of chloramine. Khosa and Chandrasekahr have observed that administration of copper acetate to fish resulted in the early maturation of ova and also increased vitellogenesis. Kumar and Pant evaluated histopathologic alterations in the gonads of the teleost, *Puntius conchonius* Ham after exposure to copper, zinc, and lead. All these metals induced significant atresia in the ovary. However, zinc damaged mainly the younger oocytes, whereas copper and lead were more toxic to relatively older oocytes. In the present study, the ovary, uterus, cervix, and vagina of rats exposed to beryllium nitrate revealed significant alterations in various biochemical and histologic parameters. However, these changes were less significant in animals that also received the Ayurvedic medicine Liv.52.

Disturbance of carbohydrate metabolism is one of the most notable consequences of beryllium toxicity. Further, it has been reported that disruption of glycogen storage is associated with dysfunctional and dystrophic changes in the liver and other organs due to inhibition of key enzymes in carbohydrate metabolism such as hexokinase, glucokinase, and phosphoglcomutase. This is supported by the present findings in which administration of beryllium nitrate reduced the glycogen
content of the reproductive organs. The presence of glycogen in the reproductive organs is of importance for many reproductive phenomena such as formation of deciduomata and uterine proliferation, implantation, nidation, uterine receptivity, vaginal cornification, and sperm transport in the female genital tract.

In the present study, administration of beryllium nitrate also resulted in the inhibition of alkaline phosphatase activity; however, no significant change was observed in the activity of acid phosphatase. Previous authors have reported inhibition of alkaline phosphatase activity in vitro, and Du Bois et al. held that cobalt, nickel, and magnesium antagonized the inhibitory effect of beryllium on serum alkaline phosphatase. Inhibition of the activity of alkaline phosphatase encountered during beryllium toxicity was attributed to the displacement of magnesium (Mg++) ion by beryllium ion. During chronic beryllium poisoning, the activity of acid phosphatase increased together with functional incompetence of the liver. The activity of acid phosphatase in the reproductive organs showed no significant decrease after beryllium treatment in the present study. The authors do not find any reason for this difference in the activity of acid phosphatase in the reproductive and vital organs.

The presence of cholesterol in the genital tract in rats has been reported by many workers. The level of uterine cholesterol remains unchanged during different phases of the estrous cycle. In the present investigation administration of beryllium nitrate significantly increased the total and esterified cholesterol level in all the reproductive organs. In the ovary, the enhanced level of total cholesterol might be due to a reduction in its rate of utilization for estrogen synthesis. The authors have also observed a reduction in the wet weight of the ovary, which further suggests diminished gonadotropin release. Singh and Singh have reported that synthesis of ovarian hormones was decreased while the ovarian cholesterol level was greatly increased in response to cythion and hexadrin treatment. Higher cholesterol levels observed in the reproductive organs may also be due to necrotic changes. It is difficult to ascertain the mechanism involved in the suppression of steroidogenic activity at this stage as several enzymes are involved in estrogen biosynthesis.

Beryllium has been reported to bind with specific proteins in serum. Tepper reported that beryllium, unlike other hepatotoxins, does not inhibit protein synthesis. Our findings show no specific alteration in total protein concentration of various tissues, which is consistent with previous reports. It is possible that beryllium binds with specific proteins and in doing so alters them without changing measures of total protein contents.

In the present study the ovary and uterus showed necrotic changes at various durations after beryllium treatment. Similarly the cervix and vagina showed degenerative changes at various durations. These changes may be related to the biochemical alterations observed in these organs. However, these alterations were less severe in animals cotreated with Liv.52.

A number of antidotes, for example ATA, 2-3-dimercapto-1-proponal (BAL), salicylic acid, sulfosalicylic acid, and steroid therapy have been tested in beryllium toxicity. These chelators did not prove successful in clinical trials. An indigenous plant preparation, Liv.52, is believed to be hepatoprotective. A prophylactic action of Liv.52 against hepatotoxins such as acetaminophen, alcohol, carbon tetrachloride, and antibiotics is well documented. Liv.52 is known to restitute
various biochemical parameters. Its administration stimulates the liver cells to convert more glucose into glycogen and increases the activity of alkaline phosphatase. A decrease in SGOT and SGPT values suggests that Liv.52 limits further necrotic changes. Liv.52 also restores the disturbed blood cellular picture toward normal in various diseases. The LD$_{50}$ of beryllium salts is known to increase manifold with Liv.52 treatment. Various hematologic parameters and liver histoarchitecture remain normal with prophylactic treatment with Liv.52. Liv.52 promotes regeneration of the hepatic parenchyma and increases functional efficiency of the liver after exposure to hepatotoxins. In carbon tetrachloride poisoning, Liv.52 given prior to treatment prevented impairment of liver function. When given after carbon tetrachloride, liver function was restored. Saini and Saini reported that Liv.52 protected the vital organs of albino mice against radiation-induced changes. The protective effect was manifested in the form of early recovery as indicated by the absence of pathologic changes such as cytoplasmic granulation, loss of nuclei from many cells, and other abnormalities of tissue architecture.

In the present study, when beryllium nitrate was given to Liv.52 primed animals significant improvement was observed in the biochemical and histologic pictures in the ovary, uterus, cervix, and vagina at 2 through 30 days after treatment when compared to beryllium only treated animals. At 30 days after conjoint Liv.52 and beryllium treatment, histoarchitecture and biochemical parameters were normal.

It is concluded that the administration of beryllium nitrate causes severe toxic lesions in the female reproductive organs. However, these toxic changes are not as severe in animals cotreated with Liv.52. It is possible that the liver is the target organ in beryllium toxicity and its damage further causes necrotizing lesions in other organs. Some beryllium also reaches these organs and affects them directly as indicated by the almost parallel changes in most of the organs. In order to define the exact mechanism of the protective effect of Liv.52 in the reproductive organs of beryllium-treated rats, further studies are in progress.

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