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Therapeutic Use of Liv.52 in Beryllium-induced Reproductive Toxicity in Rats

Mathur, S., Prakash, A.O. and **Mathur, R.**
School of Studies in Zoology, Jiwaji University, Gwalior, India.

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ABSTRACT

The toxic effects of beryllium salts have been studied on the reproductive organs of cyclic adult female albino rats; simultaneously an attempt has been made to overcome these effects by using an Ayurvedic remedy Liv.52 (The Himalaya Drug Co., Bombay). Liv.52-primed rats (1 ml/rat/d for 15 days) were exposed to beryllium nitrate intravenously and were sacrificed at different time intervals. At autopsy, the ovary, uterus, cervix and vagina were processed for histopathological examination. Histoarchitecture of these revealed severe necrotic changes with beryllium nitrate per se treatment. A significant improvement was observed in the histology of these organs when beryllium was injected in Liv.52-primed animals. At the end of 30 days of beryllium and Liv.52 treatment histoarchitecture of these organs was normal.

INTRODUCTION

Beryllium is not a normal constituent of living matter and it exhibits disease producing potential when industrial workers and laboratory animals are exposed to it[1,2]. A number of diseases such as bronchitis, pneumonitis, dermatitis, acute pneumonitis and chronic pulmonary granulomatosis are reported due to beryllium intoxication[2-6]. High incidence of beryllium diseases was reported in pregnant and non-pregnant female workers[7]. Women were found to be more susceptible to beryllium-induced diseases and showed weight loss, cough, dyspnoea and abnormal chest X-ray findings[8-10]. Mathur *et al.,*[11] have reported toxic effects of beryllium nitrate on the early and late pregnancy in rats. Although toxic effects of beryllium are well documented by previous workers in the females, the exact mechanism of this differentiation still remains obscure. Previously, a number of chemical agents, viz., BAL, salicylic acid, ATA, EDTA were tried to overcome beryllium's toxic action but failed in clinical trials[12-14]. No drug of plant origin has been tested against beryllium toxicity. An Ayurvedic remedy Liv.52 (The Himalaya Drug Co., Bombay, India) is known to restitute various biochemical and histological parameters to normal against chemical toxicity[15,16]. Saini *et al.,*[17] reported that with Liv.52 treatment normal number of pups were delivered in mice when exposed to 2.5 Gy gamma rays. The present investigation deals with the histopathological alterations in the reproductive organs of adult female albino rats when exposed to beryllium nitrate. Simultaneously the role of an ebullient medicine Liv.52 has been tested for its therapeutic action in regulating beryllium toxicity.

MATERIALS AND METHODS

Adult healthy female albino rats (150 ± 10 g) of Swiss strain were selected from the Defence Research Laboratory, Gwalior. All the animals were maintained under uniform husbandry conditions of light and temperature and were given "Hindustan Lever" rat pelleted diet and water *ad libitum.* The dose of beryllium nitrate was prepared at a concentration of 0.316 mg/kg ($1/10^{th}$ of

LD_{50}) and injected intravenously once only. Animals showing normal growth rate were selected and were divided into four groups of fifteen each. The animals of groups 1 and 3 were kept as such and received vehicle only, whereas rats of groups 2 and 4 were primed with Liv.52 for 15 days (1 ml/rat/d) orally prior to the experiment. To begin with, the animals of group 1 served as control and continued to receive vehicle only. Each rat of groups 3 and 4 was injected with beryllium nitrate intravenously at a dose of 0.316 mg/kg body weight once only. The rats of groups 2 and 4 additionally received Liv.52 syrup daily till the last day of experiment. All the rats were sacrificed after 2, 10 and 30 days of beryllium administration. Control and experimental rats showed oestrus phase at the time of autopsy. The organs viz., ovary, uterus, cervix, vagina and adrenals were excised out, freed from adhering tissue, blotted, weighed and processed for histology. Small pieces of each tissue were fixed in alcoholic Bouin's fluid for 7-8 hours. The material was processed for the preparation of paraffin blocks through the alcoholic series using the methyl benzoate method. Later haematoxylin-eosin stained slides were observed for changes in the cellular organization.

RESULTS

The histopathology of the various organs under different treatments is described as follows:

(i) *Ovary*

Liv.52 per se: Histoarchitecture of the ovary under Liv.52 treatment showed normal features.

Beryllium nitrate per se: The ovarian histoarchitecture revealed necrotic changes after 2, 10 and 30 days of treatment. After 2 days of exposure developing follicles showed degenerative changes. Growing follicles showed accumulation of oedematous fluid accompanied with total disorganization of the follicular cells (Fig. 1). After 10 days of beryllium exposure the medullary portion of the ovary showed severe damage. The number of matured follicles had decreased. In some follicles ova had decreased. In some follicles ova had undergone atrophy and looked very much deformed and granulated (Fig. 2). After 30 days of intravenous administration of beryllium the ovarian histoarchitecture showed less damage as compared to the previous durations. Some unspecific vacuolated cells were also observed with darkly

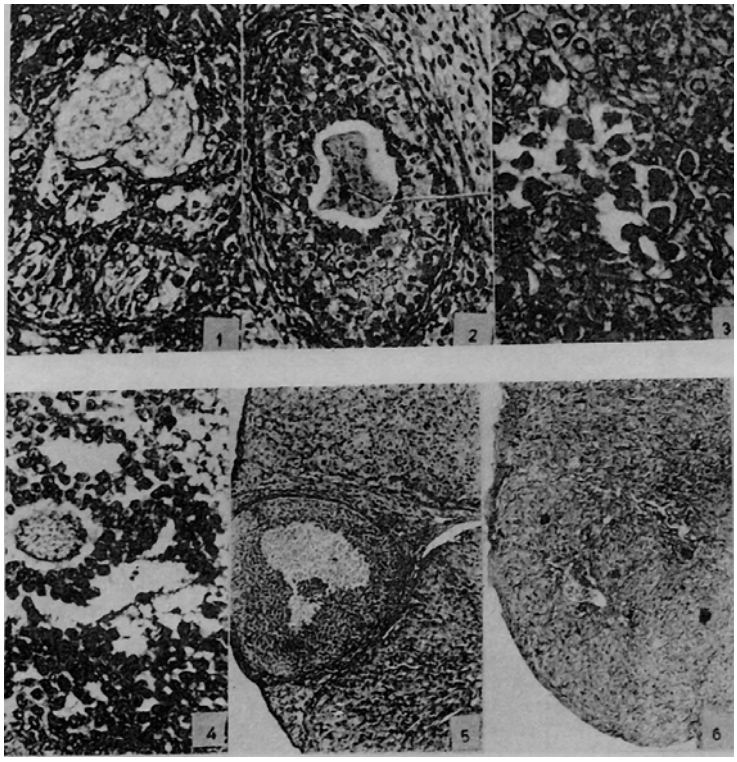

Plate 1

Figs. 1-6: Photomicrographs of rat ovary exposed to beryllium nitrate.

Fig. 1: After 2 days of treatment. Note accumulation of oedematous fluid in growing follicles (X 400). **Fig. 2:** After 10 days of treatment. Note atrophied and shrunk ova (X 400). **Fig. 3:** After 30 days of treatment. Note some unspecific vacuolated cells in the stroma (X 400). **Fig. 4:** Ova of the Graafian follicle show granulation and shrinkage (X 400). **Fig. 5:** After 10 days with Liv.52. Note normal features in the ovarian follicle. **Fig. 6:** After 30 days with Liv.52, corpora lutea show more or less normal structure (X 400).

stained nuclei in the stroma (Fig. 3). Ova of the Graafian follicles showed granulation and shrinkage, whereas follicular cells were darkly stained and showed disturbed cell arrangement (Fig. 4).

Beryllium nitrate and Liv.52-treated rats: Ovarian histoarchitecture revealed improvement after beryllium and Liv.52 treatment. After a 2-day regimen follicles were less affected and the follicular cells were generally in their normal position. The stroma was more or less compact with normal connective tissue. After 10 days of beryllium and Liv.52 treatment the ovarian histological features showed improvement in comparison to beryllium nitrate *per se* (Fig. 5). Newly formed corpora lutea were observed with normal structure. The mature Graafian follicles were observed with almost normal structure. Stroma was rather loose with prominent vascularity. After 30 days of beryllium and Liv.52 treatment the developing follicles showed better organization with normal follicular cells and ova. Freshly ovulated Graafian follicles were present. Corpora lutea showed normal structures (Fig. 6).

(ii) Uterus

Liv.52 per se: Histoarchitecture of the uterus under Liv.52 treatment showed normal structure (Fig. 7).

Beryllium nitrate per se: Uterine histoarchitecture revealed considerable alteration after 2 and 10 days' duration. After 2 days of beryllium exposure mild toxic changes were observed. Uterine endometrium showed liquefaction and proliferation of columnar epithelial cells and these cells were multinucleated (Fig. 8). The stroma was loose and fibrocellular. After 10 days of beryllium treatment severe necrotic changes were observed. Stroma showed severe fibrosis and uterine glands were damaged and few in number. Nuclei present were mostly pyknotic (Fig. 9). After 30 days of treatment similar histological features were observed. Comparatively, the alterations were less significant as compared to the earlier durations. Multiple nuclei were present at the base of columnar

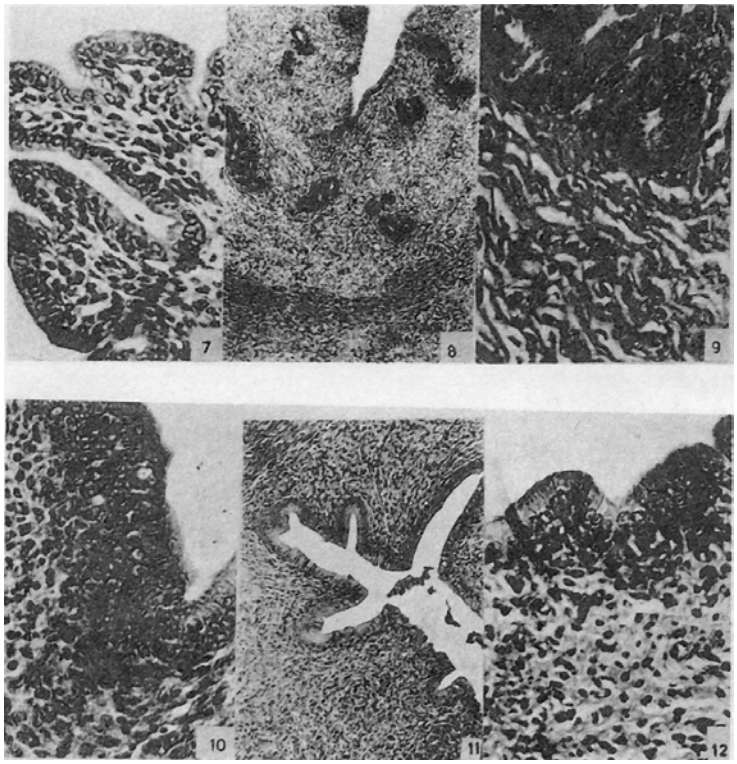

Plate 2

Figs. 7-12: Photomicrographs of rat uterus exposed to beryllium nitrate.

Fig. 7: With Liv.52 *per se* control rat showing normal features (X 400). **Fig. 8:** After 2 days of treatment. Note vacuolations and disruption in columnar cells (X 400). **Fig. 9:** After 10 days of treatment. Note severe fibrosis and vacuolations in the stroma (X 400). **Fig. 10:** After 30 days of treatment. Note multiple nuclei and aggregation in the epithelial cells (X 4000). **Fig. 11:** After 2 days of treatment with Liv.52. Note normal uterine histoarchitecture (X 120). **Fig. 12:** After 10 days of treatment with Liv.52. Normal epithelium with compact stroma (X 400).

epithelium (Fig. 10).

Beryllium nitrate and Liv.52-treated rats: Uterine histoarchitecture revealed improvement with conjoint treatment of Liv.52 and beryllium nitrate. The endometrium was lined with simple columnar epithelium and most of the nuclei were basally located (Fig. 11). After 10 and 30 days' treatment the uterus revealed comparatively less alteration when compared with the beryllium *per se* treated one at the same duration (Fig. 12). The endometrium showed reduced height and nuclei were basally placed.

(iii) Cervix

Liv.52 per se: The histological structure of the cervix under Liv.52 treatment showed normal features (Fig. 13).

Beryllium nitrate per se: Cervical epithelium showed mild necrosis after 2 days of exposure and mucosa showed normal plicae palamatae. Stroma exhibited mild degree of fibrosis and the number of darkly stained nuclei was increased (Fig. 14). After 10 and 30 days' exposure the cervical epithelium showed normal features, although covered with thick mucus. In the stroma fibrosis was increased as compared to the previous duration and some stromal nuclei were pyknotic. The cervical glands were few in number and showed disorganized structures (Fig. 15).

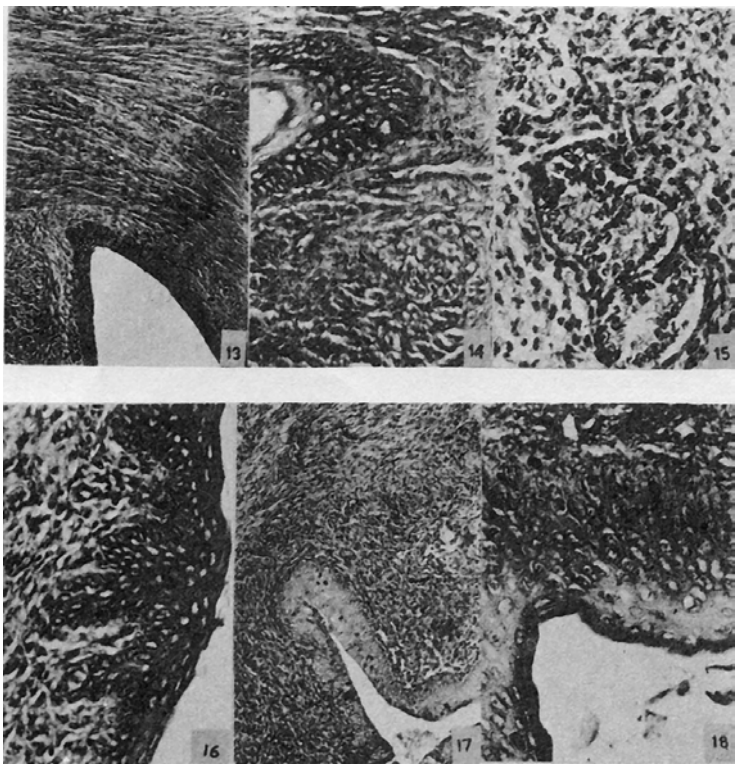

Plate 3

Figs. 13-16: Photomicrographs of rat cervix exposed to beryllium nitrate.

Fig. 13: Liv.52 control showing normal features at oestrus stage (X 120). **Fig. 14:** After 2 days of treatment. Note cervical epithelium is normal but stroma is fibrotic (X 400). **Fig. 15:** After 10 days of treatment. Note cervical glands show disorganized structure and nuclei lie free in the lumen (X 400). **Fig. 16:** After 10 days' treatment with Liv.52. Note cervical epithelium is loose and stroma is rather compact (X 400).

Figs. 17-20: Photomicrographs of rat vagina exposed to beryllium nitrate.

Fig. 17: After 10 days' treatment. Note cornification in vaginal epithelium (X 120). **Fig. 18:** Note some darkly stained nuclei and vacuolation (X 400).

Beryllium nitrate and Liv.52-treated rats: Cervical histoarchitecture showed improvement with Liv.52 therapy in beryllium-exposed rats. There was no significant alteration in cervical histoarchitecture after 2, 10 and 30 days of exposure. After 2 days' regimen the stroma remained more or less compact with normal stromal nuclei. Cervical epithelium and plicae palamatae showed normal structures. At 10 and 30 days' schedule, the

cervical epithelium was more or less normal and mucosa showed normal plicae palamatae. However, stroma was compact with few darkly stained nuclei (Fig. 16).

(iv) *Vagina*

Liv.52 per se: The vagina of Liv.52-treated rats revealed no significant alteration.

Beryllium nitrate per se: After 2 days' schedule vaginal epithelium showed binucleate and multinucleate cells at base and also desquamation of superficial cells. Stroma showed degenerative changes and appeared flat. After 10 days of treatment, vaginal stratified squamous epithelium revealed cornification (Fig. 17), whereas superficial epithelial cells showed some darkly stained nuclei and perinuclear vacuolations (Fig. 18).

After 30 days of treatment stratified squamous epithelium revealed cornification; however, rugae remained normal. Stroma was loose and revealed fibrous structure (Fig. 19).

Beryllium nitrate and Liv.52-treated rats: When beryllium was administered conjointly with Liv.52, the vaginal histoarchitecture revealed more or less normal features at all the schedules. At 10 and 30 days' schedules all the histoarchitectural changes resembled that of the control group indicating thereby complete reversibility (Fig. 20).

(v) *Adrenals*

Liv.52 per se: Histoarchitecture of the adrenals under Liv.52 treatment showed normal features.

Beryllium nitrate per se: After intravenous administration of beryllium nitrate maximum changes were observed after 2 and 10 days. At 2 days' duration the zona fasciculata showed heavy granulation and vacuolation (Fig. 21). At 10 days' regimen hyperplasia of zona glomerulosa and pyknotic nuclei were observed. In the zona fasciculata cytoplasm showed severe granulation and vacuolation. At 30 days'

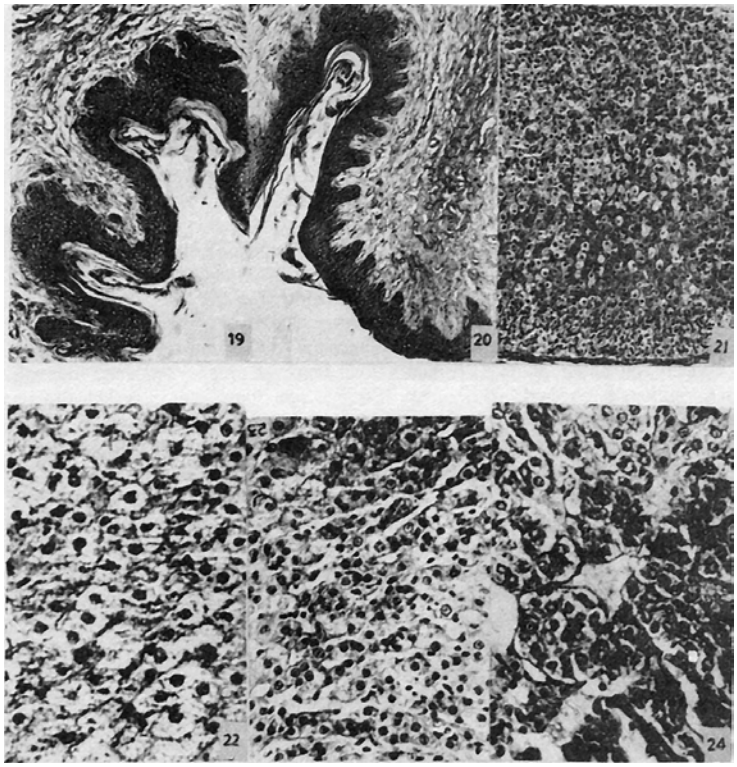

Plate 4

Fig. 19: After 30 days' treatment. Note cornification in vaginal epithelium and loose stroma (X 120). **Fig. 20:** After conjoint treatment with Liv.52. Note normal rugae and stroma almost normal (X 120).

Figs. 21-24: Photomicrographs of adrenals exposed to beryllium nitrate.
Fig. 21: After 2 days' treatment. Note hyperplasia of zona glomerulosa and severe vacuolation and granulation in fasciculata (X 120). **Fig. 22:** After 30 days' treatment. Zona fasciculata shows darkly stained nuclei of various sizes and shapes (X 400). **Fig. 23:** After 10 days with Liv.52. Zona glomerulosa reveals normal structure (X 400). **Fig. 24:** After 30 days with Liv.52. Medulla shows better organization (X 400).

regimen the zona fasciculata had more severe effects as nuclei of different sizes and shapes were observed (Fig. 22). In some cells the cytoplasm was vacuolated and granular. Leucocytic infiltration was also observed.

Beryllium nitrate and Liv.52-treated rats: With conjoint treatment of Liv.52 and beryllium, the adrenal histoarchitecture revealed significant improvement after 2, 10 and 30 days of exposure. The capsule wall was normal. The adrenal histoarchitecture showed normal features after 10 days, except in the reticularis where mild degeneration was observed. Zona glomerulosa seemed to be less disturbed as compared to that in the beryllium *per se* treatment group (Fig. 23). At 30 days of Liv.52 treatment in beryllium-exposed rats the medullary region showed better organization (Fig. 24).

DISCUSSION

A number of toxic compounds, xenobiotics, are known to affect the ovary and other reproductive organs. In the present study the ovary and uterus showed necrotic changes at various durations with beryllium nitrate *per se*. Similarly the histological alterations associated with the cervix and vagina showed degenerative changes at various durations. These changes may be related to the reduced glycogen level and other physiological changes in these organs as reported during beryllium intoxication[18].

Aldridge *et al.,*[19] reported changes in the adrenal histoarchitecture accompanied by shrinkage of the zona glomerulosa. They associated these alterations with the physiological response to damaged tissues elsewhere. Clary *et al.,*[20] proposed a mechanism for beryllium toxicity and later on the basis of their experiments provided significant evidence in support of the fact that adrenal function was related with the onset of chronic beryllium disease in mice. Present findings in adrenals further confirm the view of Clary *et al*.,[20] that onset of beryllium toxicity is due to imbalance in adrenocorticoid hormones. Zona glomerulosa has been severely affected with beryllium treatment. Since zona fasciculata and zona reticularis also showed degenerative changes, it is suggested that glucocorticoid and androgen synthesis is also affected with beryllium treatment. A number of antidotes, for example ATA, 2-3-dimercapto-I-proponal (BAL), salicylic, sulfosalicylic and steroid therapy have been tested against beryllium[12-14]. The therapeutic approach of these chelators did not prove successful as they failed in clinical trials. Moreover, these chemical agents have their own toxic effects in the living organism[21]. An indigenous plant preparation Liv.52 is known to combat the toxic insult and to regulate the liver cells owing to its unique hepatoprotective reputation. It is a well tolerated, non-toxic, efficacious remedy with outstanding therapeutic features of a powerful hepatic stimulant and cholerectic[15,16]. The prophylactic action of Liv.52 against various hepatotoxins, viz., drugs, alcohol, carbon tetrachloride and antibiotics is well documented[21,23]. LD_{50} of beryllium salts is known to increase many fold with Liv.52 treatment[24]. Congruously, various haematological parameters and liver histoarchitecture revert to normal with the prophylactic action of Liv.52[25,26]. Saini and Saini[27] reported that Liv.52 protected the vital organ against radiation-induced changes. The protective effect was manifested in the form of early recovery as indicated by the absence of pathological changes like cytoplasmic granulation, loss of nuclei from many cells and other abnormal architecture.

In the present study when beryllium nitrate was injected to Liv.52-primed animals significant improvement was observed in the histological pictures of the ovary, uterus, cervix, vagina and adrenals at 2 days through 30 days of treatment when compared with beryllium *per se* treated animals. At 30 days of conjoint Liv.52 and beryllium treatment the histoarchitecture of these organs was normal.

Therefore, on the basis of the present study it is concluded that the administration of beryllium nitrate causes severe toxic lesions in the female genital tract and reproductive organs. However, these toxic changes are not met within animals treated with Liv.52. It is reported that liver is the target organ in beryllium toxicity and its damage further causes necrotizing lesions in different organs[25]. Some portion of beryllium also reaches these organs and affects them directly as indicated by almost parallel changes in most of the organs. Protective doses of Liv.52 have brought about recovery from beryllium *per se* induced toxic effects[25]. This observation clearly indicates that the severity of toxic effects has been increased by liver dysfunction. When the liver is protected by Liv.52 treatment the reproductive organs also showed less toxic effects. However, to know the exact protective mechanism of Liv.52 in the reproductive organs of beryllium-induced rats, further pharmacokinetic studies are in progress.

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