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Safe Use of Salajeet During the Pregnancy of Female Mice

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Abstract: The effect of Salajeet on development of mice embryo was studied. A total of 71 pregnant female mice were given Salajeet (250 and 500 mg kg^{-1}) orally via needle tube, daily from day 8-12 of pregnancy. All the treated and control animals showed no differences in the number of the litter size, the placenta and the body weight of the embryos and the number of resorped embryos at day 17 of gestation. However few abnormalities were observed in both treated and control groups.

Key words: Salajeet, shilajit, pregnancy, embryo and development

Introduction

Salajeet (or Shilajit) is a blackish brown organic mass found in the steep rocks of the Himalayan belt ,of Pakistan, Afghanistan, China, Bhutan, Nepal, Kashmir and USSR (Ghosal ***et al.***, 1989). It is also available in Saudi Arabia known as momia imported from Yemen or India. Very little is known about its chemical nature. It is reported to contain resins, fatty acids, benzoic acid, hippuric acid and albuminods. Ghosal and his coworkers, reported three different classes of organic compounds, triter penes and sterols, aromatic carboxylic acid, elegiac acid and 3, 4 benzcoumarins and amino acids in Shilajit collected from the Kumaon region of Uttar Pradesh, India (Ghosal ***et al.***, 1976). They separated a mixture of high merhumic acid and fulvic acid and several fulvic acid equivalents consisting of H- rich aromatic moities. It was observed that the interaction of plants and micro-organisms produced the major mass of Salajeet (Aiken ***et al.***, 1985). The Salajeet possibly has similar compounds from different origins. Similar compounds are observed in plants of same area showing the contribution of vegetation in the formation of Salajeet (Ghosal ***et al.***, 1988a). It has been used for ages in traditional medicines in the treatment of bronchial asthma, diabetes, genito-urinary infection, wound healing, nerve disorder (Chopra ***et al.***, 1976). Several studies have verified antirheumatic, antiulcer and mast cell protecting effect of Salajeet. People of those areas use it to combat cold, stress and as tonic (Ghosal ***et al.***, 1988b; Goel ***et al.***, 1999); Qureshi ***et al.*** (1994) proved that Salajeet did not increase the incidence of micro nucleated polychromatic erythrocytes (PCE) in the bone marrow cell of mice. A mild reduction in RNA contents follow by slight decrease in PCE/NCE (normochromatic erythrocyte) ratio. Salajeet treatment reduced the increase in micro nucleated PCE, caused by cyclophosphamide. Fulvic acid and 4-methoxy-6-carbomethoxybphenyl, two major organic compounds isolated from Shilajit were screened for anti-ulcerogenic effect (Qureshi ***et al.***, 1994). Transina (TR), an Ayurvedic herbal formulation comprising of ***Withania somnifera***, ***Tinospora***

cordifolia*, *Eclipta alba*, *Ocimum sanctum*, *Picrorrhiza kurroa and Shilajit, had little per SE effect on blood sugar concentration and pancreatic islet super-oxide dismutase activity in euglycaemic rats in the dose of 100-200 mg kg^{-1}, p.o. administered once daily for 28 days (Bhattacharya ***et al.***, 1997).

There is evidence that at least two of the constituent of (TR) namely Shilajit and *W. somnifera*, can increase tissue free radical scavenging activity (Bhattacharya ***et al.***, 1995). There is no data about the effect of Shilajit on reproduction and embryonic development. The present study was designed to observe the effect of this versatile drug during the pregnancy and the development of mice embryo.

Materials And Methods

Salajeet (or Shilajit)

It was purchased from Riyadh city open market (as a paste, originated from India) and stored at 6°C. The fresh aqueous solution was prepared in distilled water for each experiment (250 mg kg^{-1} and 500 mg kg^{-1} body weight of the female). Shilajit solution was administered orally (via force feeding tube) for five days starting from day eight of pregnancy.

Animals

Groups of female mice (SWR strain) brought from animal house at College of Pharmacy, King Saud University, were housed with male (4+1) for mating in plastic cages in an environmentally controlled room with 25°C, 10 hour light and 14 h dark. The animals were fed with Pilsbury's food and had free access to water. Pregnancy was confirmed by presence of vaginal plug daily. The pregnant females were separated from others and that day was counted as day zero of pregnancy as described elsewhere (Al-Himaidi and Umar, 1999.)

Treatment

The pregnant female mice were divided into three groups of two treated and one control group. The first group (24 females) were given 250 mg kg^{-1} of Shilajit solution. The second group (20 female) were given 500 mg kg^{-1} of Shilajit solution. The third group (27 females) of controls were given water only (varying from 0.02-0.05 ml as volume) The treatment were administered orally (via force feeding tube) for five days starting from day 8 to 12 of pregnancy. The females were dissected at day seventeen of pregnancy and number of Litters, weight with placenta, number of resorped embryos and the gain of weight in the females from day 8-17 of pregnancy were studied. The embryos were checked for any morphological abnormality in developments.

Statistical analysis

The statistical analysis of the data was done by Chi-square analysis (X2) (2X2 contingency table) to compare between each two means of each treatment. ANOVA and least square of means were used to compare between all means of data .All calculations were done by computer using program Instat.

Table 1: Mean±SE of Salajeet treatments of the pregnant females mice from day 8- 12 of pregnancy on the embryos

Treatment dose dose (mg kg^{-1})	Female total No.	Body wt. gain gm	placenta wt. gm	Embryo resorption	Died embryo	Embryo Wt. (gm)	Litter No.
Control	27	17.55±0.94	0.1081±0.0027	o.82±0.23	0.333±0.832	1.07±0.034	11.52±0.66
250	24	15.52±1.15	0.1075±0.0027	1.41±0.32#	0.125±0.337	0.98±0.036	11.75±0.66
500	20	17.04±0.91	0.1979±0.0044	0.70±0.0.24	0.100±0.447	1.07±0.038	11.75±0.53

*The females were dissected on day 17 of pregnancy. ** The body gain weight from day 8-17 of the pregnant females. # Significantly different form the others at the same column ($p < 0.05$)

Results

A total of 71 pregnant females mice were studied. All the treated and control group of animals showed no differences in the mean number of the litter size. The control females sired a mean of 11.52 embryos/female, the first treated group (250 mg kg^{-1}) sired a mean of 11.75 embryos/female and the second treated group (500 mg kg^{-1}) sired 11.75 embryos/female. The placenta, the body weight of the embryos and the number of died embryos at day 17 of gestation (the day of dissections) showed no differences between the treated and control group as shown in Table 1. The treated females with 250 mg kg^{-1} show higher rate (at $p<0.05$) in the resorped embryos (1.41 embryo/female) (one of the female had 15 embryos and six of them were resorped) compared to the control (0.82 resorped embryo/female) and the 500 mg kg^{-1} treated females showed a mean of 0.7 resorped embryo/female Table 1. Also there was no difference in the female body weight gain (from day 8 to day 17 of gestation) between the treated groups varying from (15-17 gm/ female) and control group (17.5 gm/female). Regarding the abnormal development of the embryos the treated group (250 mg kg^{-1}) sired one hernia and one microcephaly embryos, the (500 mg kg^{-1}) treated group showed only one embryo with abnormal eyes ,while the control group showed one ecto-pregnancy, one excencephally and one hernia embryos.

Discussion

The results of this study add support to the safe use of Shilajit. Due to the large molecular weight of the Shilajit compound it may be prevented to reach the embryo by the placental barrier and it confirmed the idea that the Shilajit to be deviod of clastogenic activity (Bhattacharya ***et al.***, 1995). It did not increase the body weight of the pregnant females compared to the result of Gupta ***et al.*** (1966) studies, where it showed an increased in the body weight of the treated new born rats. The differences that appear in the resorped embryos of the (250 mg kg^{-1}) treated group was that one of the female of this group was sick and did not gain weight and it had 13 embryos; six of them were resorped and this might showed the differences from the control and the (500 mg km^{-1}) treated females. Both treated and control group showed some abnormal developed embryos. This might have occurred normally, or due to some genetically- abnormalities or other factors but not due to the use of the Shilajit itself. Otherwise such effect would have shown up in the treated animals as well. The findings of the present study agree with Ghosal ***et al.***, 1989) that appraises for the of use of Shilajit for reactions treatment in Ayurvedic medicine with no side effect on pregnancy and reproduction. Also, as indicated by Schliebs ***et al.*** (1997), that Shlilajit treatment increases in cortical muscarinic

acetylcholine receptor capacity that might partly explain the cognition-enhancing and memory-improving effects.

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