Teratogenic Effects of Retinyl Palmitate during Early and Late Gestation Periods in Rats

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Abstract

Retinyl palmitate or vitamin A palmitate has been associated with dose-related developmental toxicity when administered orally to mice, rats, rabbits, and monkeys during critical stages of embryonic development. We report a study to determine the teratogenic effects of retinyl palmitate in pregnant Sprague Dawley rats during early and late gestation periods and to observe the toxic effects of retinyl palmitate in dams. Forty sexually mature fertile female Sprague Dawley rats were divided into 4 groups: Early control, Late control, Early gestation (Early) and Late gestation (Late) groups. Control groups were given a placebo of maize oil while treatment groups were given the same dosage of retinyl palmitate. Pregnant females were randomly assigned to the different groups and treated with retinyl palmitate during early pregnancy on gestation day (GD) 1-7 for Early group and GD 8-14 for Late group. The results obtained showed that retinyl palmitate treated groups had no significant difference in maternal body weights compared to control groups. Maternal kidney weights in early treated group showed significant difference ($p<0.05$) compared to early control group while liver weights had no significant difference in both control and treatment groups. Fetuses from both early and late treated groups showed a significant decrease in weight compared to control groups. For fetal skeletal anomalies, treatment with retinyl palmitate in Early and Late groups showed malformed wavy ribs and thoracic vertebrae, additional ribs, lumbar vertebral defect and extra ossification center. This preliminary experiment suggests that retinyl palmitate show significant teratogenic effects when fed to pregnant Sprague Dawley rats during early and late gestation periods.

Keywords: Retinyl palmitate, teratogenesis, gestation, female rodents.

Introduction

Vitamin A is a term which designates any compound possessing biological activity of retinol. Retinol belongs to retinoids which is a family of chemical compounds. The basic structure of the retinoid molecule consists of a polyene side chain with alternating double bonds, a polar end group and a cyclic end group (Sommer, 2008). In animal tissues, retinyl palmitate, the ester of retinol and palmitic acid with formula $C_{36}H_{60}O_2$ is the predominant form of Vitamin A. Functions of vitamin A in the body are maintenance of normal vision, growth, repair, cell differentiation, pregnancy, fetal development and gives protection against infection. After its absorption into the skin, retinyl palmitate is converted to retinol, and ultimately to retinoic acid (Bailly *et al*., 2000). An excess of vitamin A has a severe effect on pregnancy where high doses can be harmful to an unborn child (Higdon, 2003). Vitamin

A has the ability to transcend the placenta (Eckhoff *et al*., 1989) and has been shown to be teratogenic in all species it was tested upon (Ritchie *et al*., 1998). However most teratogens can be species-specific, e.g., aspirin and corticosteroids are teratogenic in mice and rats but appear to be safe in humans (Shepard, 1979). Therefore it is wise to do a reproductive screening test for certain plants before consumption by humans to see possible teratogenecity effects (Yao *et al*., 2006).

Female rats exhibited estrous cycle and ovulation every 4 to 5 days with a gestation period of 21 to 23 days (Kohn and Clifford, 2002). Rats had a haemochorial type of placenta, gave birth usually at night and exhibited placentophagia behavior. A litter of 10 to 12 pups was considered normal. The gestation period of rats was divided into two stages whereby day 1 to 6 was blastocyst development, preimplantation and implantation while day 7 to 15 was organogenesis (Brinster, 1993).

Materials and Methods

Preparation of retinyl palmitate

Retinyl palmitate was purchased commercially from Fisher Scientific Malaysia. It was dissolved in maize oil before feeding to the pregnant rats. According to Collins *et al*. (1994), the dose for retinyl palmitate to give effect was 90 mg/kg per day. For this experiment, we prepared 30000USP/kg after converting the unit according to the international unit system.

Animals

Forty mature fertile female Sprague Dawley rats weighing 200-250g were obtained from the Laboratory Animal Unit, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia with ethics approval from the Animal Ethics Committee, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (ACUC). The animals were fed on standard pellet diet and water was given *ad libitum*. The animals were also maintained in standard environmentally controlled rooms (temperature 25±2 ℃) and light (12-h light/12-h dark cycle). Rats were given seven days to adapt to the environment and to undergo two consecutive estrous cycles. The rats were randomly assigned into 4 experimental groups: Early gestation control (EGC), Late gestation control (LGC), Early gestation treated (EGT) and Late gestation treated (LGT) groups. EGC and EGT groups were treated from gestation day (GD) 1 to 7 while LGC and LGT groups were treated from GD 8 to 15.

Experimental procedure

Each female rat was mated with a male rat and kept overnight in their cages with a tray at the bottom of the cage for the purpose of collecting vaginal plugs and for easy observation of appearance of the plug. In the morning, when a vaginal plug was detected then that day was considered as day 1 of pregnancy and day 1 post-coitum. The mated females were randomly assigned to the four experimental groups. The treatment consisted of ingestion by gavage of 30000 USP/kg per day of retinyl palmitate or maize oil (EGC and LGC groups on Day 1 to 7 (early gestation) and Days 8 to 15 (late gestation). During pregnancy, the rats were observed closely twice a day for survival, changes in appearance, behavior, and signs of vaginal bleeding and food and water consumption. The dams were also weighed daily to monitor toxicity throughout the experiment. The maternal weight gain was recorded during the entire pregnancy (total weight gain) and during the treatment

period. On day 21 of pregnancy, the dams were killed with chloroform overdose and their uteri removed by Caesarean section. Livers and kidneys of dams were weighed. The number of fetuses was recorded and examined for obvious external malformations before subsequent processing. The fetuses underwent fetal staining to detect skeletal malformations. For skeletal examinations, the number of skeletal elements was counted and any malformations or variations were recorded according to standard skeletal malformation procedures (Dicke, 1989; Wangikar *et al*., 2005)

Fetal staining

Fetuses were washed with tap water after been kept in 10% formalin solution. The abdominal region was partially incised and all organs were eviscerated. The fat pad between the scapula and the vertebrae was removed through a dorsal midline incision from the base of the skull to the midthorax and penetrated down to the spinal column, but not into it. Each fetus was placed into individual clean bottles which consisted of 1:4 (diethyl ether: methanol) and left for a week. The solution was later discarded and replaced with 0.3% Alizarin and 10% KOH. Once the Alizarin red colour was absorbed by the fetus, the solution was changed into 1:1 (glycerin: 75% ethanol) and left for 24 or 48 h. Finally, all fetuses were stored in pure glycerin.

Statistical analysis

The data obtained were analyzed with SPSS (Statistical Package for Social Sciences, version 10). Two way ANOVA and Tukey's Test were used to determine significant differences among experimental groups. A p value of less than 0.05 was considered to indicate a significant difference. The results were expressed as means ± SEM.

Results and Discussion

The effect exerted by the oral administration by gavage of retinyl palmitate at dose 30000 USP/kg is illustrated in Figure 1. Maternal body weights of EGC group and LGC group showed no significant difference when compared to EGT and LGT groups even though there was a decrease in maternal body weights of EGC group when compared to EGT group. The mean weights of EGC, EGT, LGC and LGT were 304.4, 208.1, 271.6 and 297.7 g, respectively. There was a significant ($p<0.05$) increase in maternal kidney weight in EGT compared to EGC group, however there was no significant difference between LGT and LGC groups. The means for kidney weights for all groups are illustrated in Figure 2. For fetal weight analysis, there was a significant difference in fetal weight between treatment groups with a significant decrease between EGC and EGT groups and between LGC and LGT groups. The mean fetal weights of EGC, EGT, LGC and LGT groups were 55.4, 25.9, 56.5 and 32.1 g, respectively. The means for fetal weight for all groups are illustrated in Figure 3.

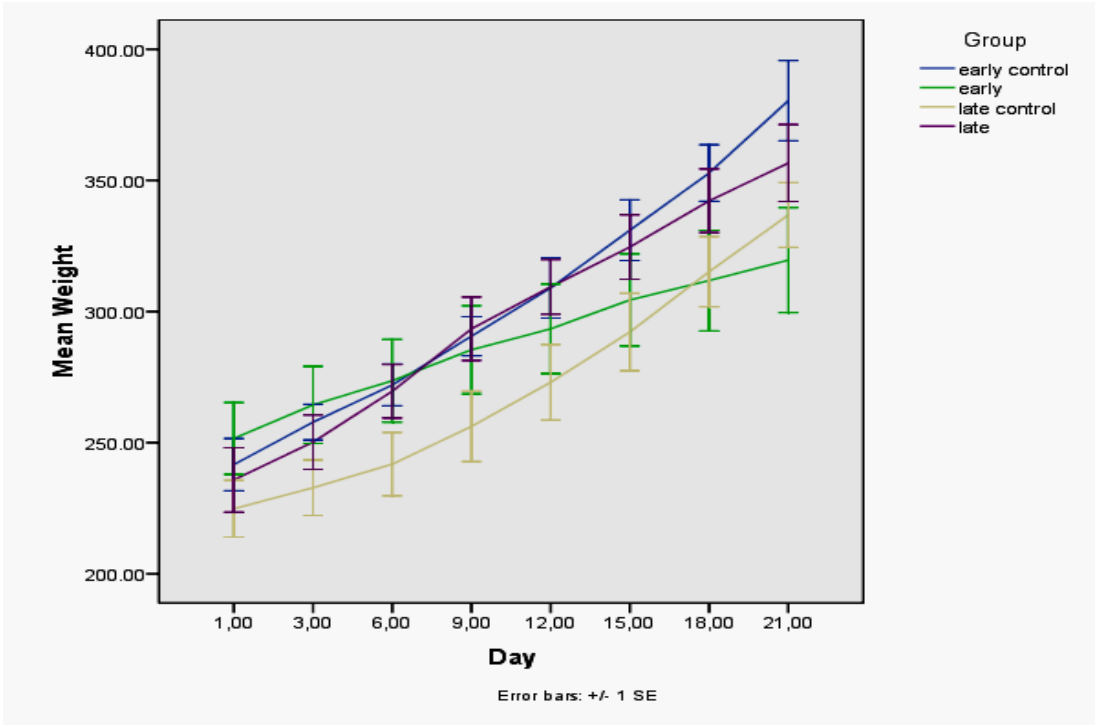

Figure 1. Changes in mean body weight of dams treated with retinyl palmitate during early and late gestation periods

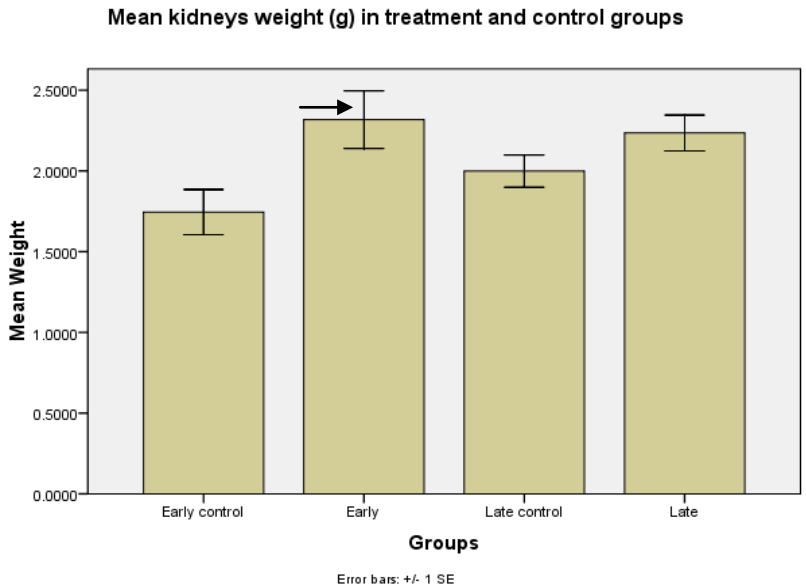

Figure 2. Mean kidney weight of dams in treatment and control groups in early and late gestation periods.
Arrow shows significant difference ($p<0.05$) of EGT compared to EGC.

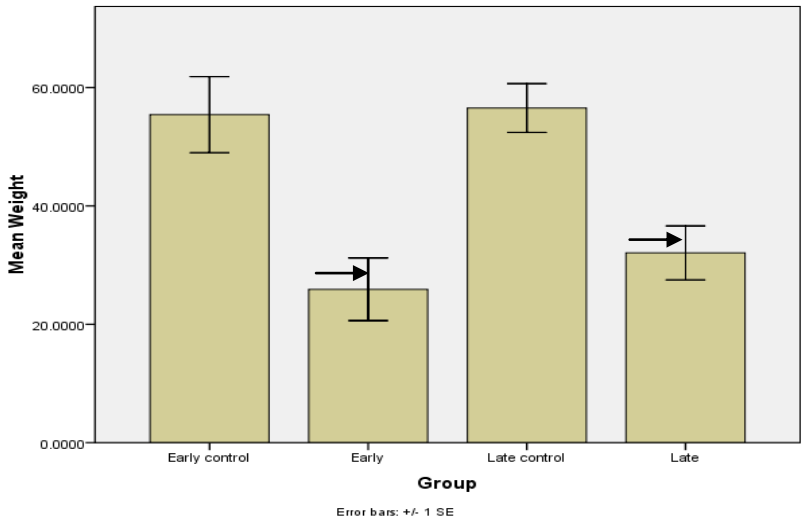

Figure 3. Mean fetal weight (g) in retinyl palmitate treatment and control groups in early and late gestation treatment periods. Arrows show significant difference ($p<0.05$) between treatment groups compared to control groups.

The weight gain of dams during pregnancy was altered by retinyl palmitate in EGTas compared to EGC group and weight loss was considered to be a good indication of toxicity. This suggests that retinyl palmitate has a toxic effect on the dams in the early gestation period. Consumption of noxious chemicals can damage liver and kidney. According to Johns (2000), as the primary function of the liver is detoxification while the kidney filters impurities from the blood for elimination from the body, these organs may be damaged from performing these functions due to harmful effects of teratogens such as retinyl palmitate. The liver enzymes also hydrolyse retinyl palmitate to be non toxic (Harrisons and Daz, 1989). Significant increase in the weight of kidneys from Early group compared to Early Control group suggested a possible accumulation of the retinyl palmitate in large amounts which was toxic to the kidney. Preformed vitamin A is rapidly absorbed and slowly cleared from the body. Therefore, toxicity from preformed vitamin A may result acutely from high-dose exposure over a short period of time or chronically from a much lower intake (Higdon, 2003). There was also a significant decrease ($p< 0.05$) in weight of fetuses in EGT and LGT groups compared to EGC and LGC groups. Visual observation of fetuses from treatment groups showed that they were smaller in size compared to the control groups. The decrease in weight of fetuses suggested that retinyl palmitate might have affected growth. A normal supply of vitamin A particularly retinoids is critical for embryonic development and proves to be a requirement for brain development; particularly in its early stages (Willhite *et al.*, 1989). Retinol binds and is converted effectively into retinoic acid (RA) at the neural plate and an excess of retinoic acid on the CNS can be teratogenic (Sommer, 2008). The number of affected fetuses with skeletal abnormalities is shown in Table 1

while Figures 4, 5, 6 and 7 show various skeletal abnormalities (as labeled) such as malformed wavy ribs and dumb-bell shaped vertebrae, in both Early and Late groups. These figures show evidence of teratogenic effects of retinyl palmitate in fetuses from dams fed retinyl palmitate compared to dams given placebo.

Figure 4: A normal fetus from EGC group

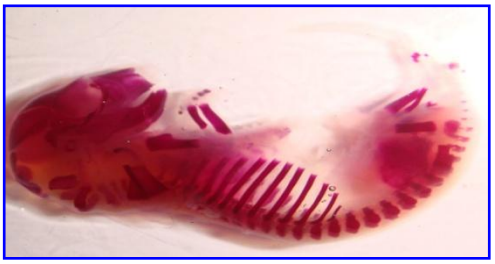

Figure 5: A normal fetus from LGC group

Figure 6: A fetus from EGTgroup.
The arrow shows malformed wavy ribs

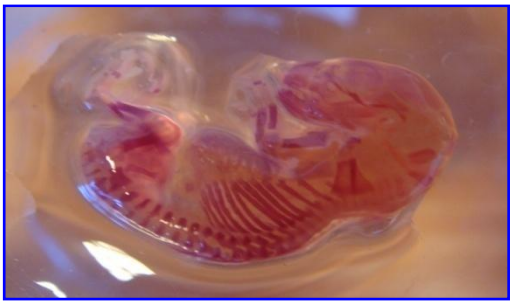

Figure 7: A fetus from LGT group.
The arrow shows dumb-bell shaped vertebrae

Table 1. Number of external and skeletal malformations in fetuses of treatment and control groups

	Group			
	Early Control (EGC)	Early Gestation (EGT)	Late Control (LGC)	Late Gestation (LGT)
Total no. of fetuses	53	34	48	48
External malformations (small in size)				
Affected fetuses	0	25	0	32
Skeletal malformations				
Vertebral anomalies	0	21	0	15

Conclusions

Retinyl palmitate had teratogenic effects on the fetuses and caused acute toxicity in dams by reducing the weight of dams in early gestation compared to early control group. Retinyl palmitate given during early and late gestation periods did not show any effects on organ weights except for kidney weight. There was evidence to indicate higher retinyl palmitate effect when administered during early gestation than late gestation periods. We suspect possible embryotoxic effects as there were some uteri that did not have fetuses suggesting early abortion, fetal resorption or non-implantation. Based on the external and skeletal malformation in fetuses observed, we can conclude that retinyl palmitate has teratogenic effects. It is also recommended that visual observation for 24 hours be performed to observe placentophagia and abortion.

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