Pregnancy Outcome in Rats Following Exposure to A Palm Vitamin E

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Abstract – Palm vitamin E, an extract from palm oil contains high amount of tocotrienol. It has been shown to have no toxicological effects to young albino mice and Sprague-Dawley rats when administered with 250mg/kg body weight (b.w.) to 2500mg/kg b.w. To date, there is no report of toxicological evaluation of tocotrienol intake on fetal and newborn development in rats. The present study therefore was undertaken to investigate the effect of palm vitamin E on fetal and newborn development in rats following maternal feeding with various doses of palm vitamin E. Pregnant rats were treated orally with 100, 250, 500 and 1000mg/kg b.w. palm vitamin E from day 1 until day 13 of gestation. Number of implantation observed were between 9 to 11 embryos per mother, which corresponded to the number of corpora lutea released. No significant differences were observed on the implantation rate between control and treated rat. It shows that no adverse effect on the reproductive performances upon giving high dosages of palm vitamin E to pregnant rat. Feeding palm vitamin E does not alter their gestational period and number of pups delivered. Pups from mother rat supplemented with palm vitamin E showed no abnormalities in their birth weight as compared to control. Therefore, it is suggested that maternal intake of palm vitamin E up to dose 1000mg/kg b.w. by normal pregnant rats have no adverse effects on perinatal development.

Key Words – Newborn, palm vitamin E, reproductive performance, Sprague-Dawley rats.

1 Introduction

Vitamin E is a generic term that refers to family of compounds that are further divided into two subgroups called tocopherols and tocotrienols which share a common general structure i.e. an aromatic chromanol head and a 16-carbon tail. Tocotrienol differs from tocopherol by the presence of saturated tail. Each group comprises of four different isomers i.e. α, β, γ, and δ which all have different biological activity (Azzi & Stocker, 2000). In general, tocopherols and tocotrienols are considered to have beneficial health effect. The main sources of vitamin E in dietary are vegetables oils and their products. These include wheat germ oil, soybean oil, palm oil, corn oil and cottonseed oil (Bramley et al., 2000).
Palm vitamin E is extracted from palm oil, contains largely of tocotrienol (70%) and only 30% of tocopherols. Both types of vitamin E have been shown to possess high antioxidant activity (Soelaiman et al., 2004; Asmadi et al., 2005; Yoshida et al., 2005). In addition, tocotrienol is also claimed to have anticancer (Nesaretnam et al., 2000; Iqbal et al., 2003; Wada et al., 2005) and anti-angiogenic activities (Inokuchi et al., 2003; Miyazawa et al., 2004), as well as potent inhibitory effect on 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, a rate limiting enzyme of cholesterol biosynthesis (Raederstoff et al., 2002; Iqbal et al., 2003).

Vitamin E used as a therapeutic agent may have a beneficial role in newborns. It has been reported to improve conditions associated with oxidative stress in neonates such as retinopathy (Brion et al., 2004), jaundice (Gross, 1979; Rudenko et al., 1990) and bronchopulmonary dysplasia (Ehrenkranz et al., 1979). It is also one of the essential nutrients for immunity (Coquette et al., 1986) and reproduction (Cederberg et al., 2001). Vitamin E is directly involved in antenatal growth and indispensable for the proliferation and function of the placenta (Jishage et al., 2005). Previous animal studies showed that the deficiency of vitamin E may affect reproduction, resulting in fetal death and embryonic resorption (Nelson, 1980). Many pharmacokinetic studies on the effect of tocopherol in foetus or neonates have been documented (Hidiroglou et al., 2001; Hidiroglou, 2003; Pressman et al., 2003) however, studies involving tocotrienol are very limited.

Therefore, the present study is conducted to evaluate the toxicity effect of palm vitamin E on female reproductive and its potential in inducing labor in rats.

2 Materials and Methods

2.1 Animal

This research was approved by the Animal Care and Use Committee, Faculty of Medicine, University Malaya and was conducted at Department of Anatomy, Faculty of Medicine, University of Malaya.

A total of 50 female Sprague-Dawley rats aged 3-6 months, weighing 160-240g were obtained from Animal Experimental Unit, University Malaya. Animals were housed individually in plastic cage under standard animal house condition; humidity 50 -55%, temperature 25±2 ºC and 12 hour light/12 hour dark. Animals had free access to rat chow (pellet) and drinking water (ad libitum). Animals were divided in five groups; 10 rats per group. Pregnancy was obtained by individually pairing female rat overnight with a fertile male rats and the presence of mucus plug on the following day was indicated as day 1 of pregnancy.

2.2 Animal exposure towards palm vitamin E

Palm vitamin E extract, a courtesy from the Malaysian Palm Oil Board (MPOB), contains 18.43% α-tocopherol, 14.62% α-tocotrienol, 32.45% γ-tocotrienol and 23.93% δ-tocotrienol. The doses used were 100, 250, 500 and 1000 mg/kg. The palm vitamin E was given by oral gavage. The control group was given super olein, a vehicle for palm vitamin E. Daily doses of palm vitamin E and control were given for entire pregnancy period.
2.3 Effects of palm vitamin E on embryonic development

Pregnant rats were divided into 6 groups of 10 rats per group. Rats of Group A (negative control) were maintained standard diet and water ad libitum throughout experiment period. Rats of Group B (positive control) received 10 mL super olein. Rats of group C, D, E and F were given 10 mL palm vitamin E of 100, 250, 500 and 1000mg/kg body weight respectively throughout gestational period. On day 15 of pregnancy, the rats were anaesthetized with chloroform and laparotomized under aseptic conditions. The uteri were examined in situ for the presence and location of resorption sites. The appearance and the number of corpora lutea in each ovary also recorded.

2.4 Effects of palm vitamin E on pregnancy outcome

Pregnant rats were divided into 6 groups of 10 rats in each as follows. Rats of Group A (negative control) maintained on standard diet and water ad libitum throughout experiment period. Rats of Group B (positive control) received 10mL super olein. Rats of group C, Group D, Group E and Group F received 10mL palm vitamin E at 100, 250, 500 and 1000mg/kg body weight respectively. Upon delivery, the number of viable and/or stillborn pups was recorded, and their body weights were measured. All pups were evaluated for any external congenital abnormalities (apalpebralia, exophthalmos, microphthalmos, microstomia, astomia, incomplete mandible, abnormal auricle, micromelia, ectrodactylia, syndactylia, microcaudate, acaudate). Pups’ mortality upon delivery, the day of eye opening and appearance of fur were recorded.

Base on the data obtained, the following indices were analyzed (Jayatunga et al., 1998); quantal pregnancy (number of pregnant dams/number mated) × 100, implantation index (total number of implants/number mated) × 100, birth index (number of pups born/number of implantations) × 100, fetal survival ratio (number of surviving pups/number of implantations) × 100, live birth index (number of live born pups/total number of pups born) × 100 and gestation index = (number of live pups/number of pregnant dams) × 100.

2.5 Statistical analyses

The means of all parameters were calculated and shown with standard error of mean (SEM). Statistical analysis was performed using the Students’t test. Statistical significance was established as p < 0.05. All statistical analysis was performed using IBM SPSS Statistic 13.0 software.

3 Results

Oral administration of palm vitamin E up to 1000mg/kg dose during gestational period has no adverse effect on reproductive and litter outcome; no mortality or treatment-related overt signs of maternal toxicity, stress or abnormal behavioral changes were observed. None of the pregnant rats showed vaginal bleeding or expulsion of products of conception.

Results on reproductive parameters are presented in Table 1. The administration of all dosage of palm vitamin E during gestational period shows no significant (P > 0.05) changes in the gestational duration and the percentages of pre-implantation losses compared to the control group. The results obtained regarding the litters are presented in Table 2. Neither parturition related maternal deaths nor stillbirths were observed. There were no significant (P > 0.05) changes on the number of pups delivered,
viability index, birth index, live birth index, lactation index and average pups body weight between treated doses groups compared to the control group.

Table 1: The Effect of Palm Vitamin E on Female Rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=10)</th>
<th>Super Olein (n=10)</th>
<th>Palm Vitamin E 100mg/kg (n=10)</th>
<th>Palm Vitamin E 250mg/kg (n=10)</th>
<th>Palm Vitamin E 500mg/kg (n=10)</th>
<th>Palm Vitamin E 1000mg/kg (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantal pregnancy (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Gestational duration (days)</td>
<td>22.2 ± 0.2</td>
<td>22.2 ± 0.1</td>
<td>22.2 ± 0.2</td>
<td>21.9 ± 0.2</td>
<td>21.9 ± 0.2</td>
<td>22.0 ± 0.2</td>
</tr>
<tr>
<td>Gestation index (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Fertility index (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Implantation sites/litter</td>
<td>10.0 ± 0.9</td>
<td>10.1 ± 1.1</td>
<td>9.1 ± 0.6</td>
<td>10.4 ± 0.8</td>
<td>9.7 ± 0.6</td>
<td>10.0 ± 0.7</td>
</tr>
<tr>
<td>% Pre-implantation loss</td>
<td>8.8</td>
<td>9.0</td>
<td>5.9</td>
<td>4.8</td>
<td>4.0</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, P>0.05 compared to control; statistically not significant

Table 2: The Effect of Palm Vitamin E on Litters Born from Treated Female Rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=10)</th>
<th>Super Olein (n=10)</th>
<th>Palm Vitamin E 100mg/kg (n=10)</th>
<th>Palm Vitamin E 250mg/kg (n=10)</th>
<th>Palm Vitamin E 500mg/kg (n=10)</th>
<th>Palm Vitamin E 1000mg/kg (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of pups born</td>
<td>9.6 ± 1.0</td>
<td>7.9 ± 0.8</td>
<td>9.7 ± 0.7</td>
<td>8.0 ± 1.2</td>
<td>8.1 ± 1.1</td>
<td>8.4 ± 2.0</td>
</tr>
<tr>
<td>Parturition index (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Viability index (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Lactation index (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Pups weight on day 3 postnatal (g)</td>
<td>8.6 ± 0.3</td>
<td>8.9 ± 0.3</td>
<td>8.9 ± 0.3</td>
<td>8.3 ± 0.2</td>
<td>8.7 ± 0.3</td>
<td>8.8 ± 0.2</td>
</tr>
<tr>
<td>Pups weight on day 7 postnatal (g)</td>
<td>14.9 ± 0.4</td>
<td>15.0 ± 0.6</td>
<td>13.8 ± 0.4</td>
<td>13.9 ± 0.8</td>
<td>13.0 ± 6.6</td>
<td>14.3 ± 0.4</td>
</tr>
<tr>
<td>Live birth index (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Birth index (%)</td>
<td>96</td>
<td>78.2</td>
<td>106</td>
<td>76.9</td>
<td>78.6</td>
<td>84.0</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, P>0.05 compared to control; statistically not significant
At birth, the pups are without fur, toothless, and have short limbs and tails. Fur starts to grow when they were 6 days old, and their eyelids separation generally occurs by 12 days old. No abnormalities were observed in any newborns pups of all control and treated groups (Table 3).

Table 3: Gross Observation on External Features of Newborn Rats.

<table>
<thead>
<tr>
<th>Abnormalities Observation</th>
<th>Control</th>
<th>Super Olein</th>
<th>Treatment Doses (100, 250, 500, 1000mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Apalpebralia</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>2) Exophthalmos</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>3) Microphthalmos</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>4) Microstomia</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>5) Astomia</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>6) Incomplete Mandible</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>7) Abnormal Auricle</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>8) Micromelia</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>9) Ectrodactyla</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>10) Syndactyla</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>11) Microcaudate</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>12) Acaudate</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
</tr>
</tbody>
</table>

n.d: not detected

4 Discussion

The motherly reproductive performance is a very important parameter for the analysis of perinatal toxicity of drugs (Lemonica et al., 1996). Thus, considering that corpora lutea are the main source for progesterone secretion (Kato et al., 1979) and it is shown that their growth are deeply related to the increase in the progesterone. The analysis of corpora lutea is important in determining the health during gestational period (Waynforth, 1971). In the existence of a correlation between the number of corpora lutea and the number of ovulations, it is normal that the number of corpus lutea is also correlated to the number of embryonal implantations, since in each ovulation, an oocyte that can be fecundated is released and turn into a pre-embryo (Ortiz et al., 1979).

Corpora (body) lutea (yellow) is named by Marcello Malphigi (1628-1694) and described precisely by Regner de Graaf (1641-1673) through encountered of globular shape of coitus bodies in the rabbit’s ovary. Corpora lutea is important in the secretion of progesterone, a crucial substance in maintaining the pregnancy. Corpus lutea are formed by the residues of granulosa cells and internal theca of the ovary (Niswender et al., 2000). The corpus luteum persisted even after labour (Niswender et al., 2000). De Graaf discovered that the number of corpora lutea is in concomitant with the number of pups born (Short, 1977).
During follicular phase, where the development of primary follicle to the Graafian follicle occurs, the level of progesterone is low. Simultaneously, estrogen (estradiol) up-regulates luteinizing hormone (LH) production through induction of hypothalamus and pituitary gland. The increasing level of LH is needed for the development of follicle to Graafian follicle thus, prepare the follicle for ovulation (Lucy et al., 1992).

Soon after ovulation, the formation of corpus luteum commence under the influence of LH, which the corpus luteum will secretes progesterone. The increasing level of progesterone will down-regulates the secretion of LH through suppression of gonadotropin releasing hormone (GnRH) in hypothalamus (Attardi & Happe, 1986). Continuous secretion of progesterone, with the help of LH will prevent the growth of new follicle. When the level of LH regress, corpus luteum will degenerates and undergoes luteolisis.

Further development of corpus luteum depends on the fertilization of the oocyte released. If the fertilization fails to occur, the corpus luteum reaches decreases in size through degeneration and desorbs. If the oocyte is fertilized, degeneration of the corpus luteum is prevented. In human, corpus luteum continues to secrete progesterone until after twelve weeks of pregnancy. Thereafter, it slowly regresses. Nevertheless, pregnancy can still continue with the help of abundant secretion of progesterone by the maternal placenta (Raman et al., 1997).

During degeneration and regression of corpus luteum, reactive oxygen compound such as superoxide, hydrogen peroxide and lipid peroxide were produced (Sawada & Carlson, 1989). The presence of these reactive agents may interfere the physiology of cells, membranes and enzymes (Pryor, 1982) and in this case, may lead to the failure of luteal function (Sawada & Carlson, 1989), by damaging the luteal cell membrane and interference of progesterone production (Gatzuli et al., 1991; Vega et al., 1995). It is well documented that there are detoxification and defense mechanism towards the reactive agents in the ovary such as catalase and superoxide dismutase (Agrawal & Laloraya, 1977), Vitamin E and C (Aten et al., 1992) as well as carotenoid and lutein (Chew et al., 1984).

It has been discovered that vitamin E level is increased in the ovary as the corpus luteum develops and regresses (Aten et al., 1992). Vitamin E acts as a defense agent, protecting components in the ovary from free radical activities. It also prevents peroxidation of lipid during steroidogenesis (Aten et al., 1994).

In the current study, palm vitamin E used does not interfere with the process of Graafian follicle formation. Administration of vitamin C together with vitamin E supplement may prevent the reduction rate of ovulation of aging rats (Tarin et al., 1998), while another study showed that regression of Graafian follicles were interfered in vitamin E deficient rats (Das and Chowdhury, 1999). It shows that anti-oxidant activity in the vitamin E may play a role in the maintenance of fertility by maintaining the ovarian normal function.

Our study showed that no significant alterations observed in the number of corpora lutea of all animals exposed to palm vitamin E. It is suggested that palm vitamin E administered to female experimental animals may not interfere with the process of follicular phase (before ovulation) and luteal phase (after ovulation). In contradiction, it gives extra protection to the ovary from free radicals activities and prevents lipid peroxidation in maintaining normal ovarian function.

In general, the present study showed that the exposure of palm vitamin E does not have any adverse effect towards the implantation process in comparison to that of the control. In addition, it is also
indicated that the exposure towards palm vitamin E does not modify the rate of pre-implantation losses, in concomitant with the finding of previous study.

Hurley et al. (1983) revealed that the administration of vitamin E (22312.5 IU/kg diet) does not influence the number of implantation in pregnant animals supplied with low vitamin E diet. The same finding is also obtained by Norfilzar et al. (2001), that is, the exposure of 60mg/kg palm vitamin E does not interfere with the implantation as well as embryonic development. Vitamin E is a dietary supplement beneficial to pregnant experimental rats (Putnam & Comben, 1987). However, there is no document published on toxicity or teratogenic effect of vitamin E on rats’ embryos and foetuses (Hurley et al., 1983).

A study by Kaempf-Rotzoll et al. (2002) showed that α-tocopherol plays a role in the process of implantation via the action of α-TPP (α-tocopherol transfer protein). Failure of pregnancy is demonstrated by the absence of implantation site on the uterus (Hazarano et al., 1996) While early abortion in rabbits show little implantation site (Feussner et al., 1992).

Palm vitamin E perhaps plays a role in the preparation of uterus for implantation. This is in concomitant with the finding of Ledee-Bataille et al. (2002), which showed that the administration of vitamin E (1000 IU/day) for sixty days increased pregnancy rates of subject with thin endometrium by increasing the thickness of the endometrium as a preparation for implantation.

Based on our data, it is concluded that experimental doses have no effect on the number of embryos. It shows that the administration of palm vitamin E does not hinder pregnancy in rats. Palm vitamin E may act by protecting the embryos from teratogenic effects. Mokhtar et al. (2008) study in rat treated with nicotine showed that palm vitamin E did not affect the females’ reproductive performance. It is also concluded that high content of tocotrienol in palm vitamin E is capable to reverse the retardation effect of nicotine on embryogenesis and consequently pregnancy loss in rats.

The present findings indicated that palm vitamin E has no adverse effect on embryogenesis, implantation process and gestational period. Prolonged duration of pregnancy could be related with the delay of implantation or during the cleavage phase (Card & Mitchell, 1979; Hammer & Mitchell, 1979). Lower dose of palm vitamin E (60mg/kg b.w.) has been reported to have no effect on the duration of the pregnancy of the rat (Rajikin et al., 2001).

Present study suggested that palm vitamin E does not have any effect on the number of pups delivered. A similar result was reported by Norfilzar et al. (2001). Observations made on the development of the pup show that palm vitamin E exposed to the pregnant dams do not affect the body weight and development. Study by Martin and Hurley (1977) demonstrated that exposure of vitamin E (22.5 to 2252 mg/kg/day) on pregnant rats has no abnormal or teratogenic effects on the pups born.

Vitamin E plays a role in supporting normal embryonic development from early embryogenesis. The percentage of abnormal pups born would be high if embryogenesis is disturbed. There are numerous studies regarding the action of vitamin E in protecting the embryos from free radicals and oxidative pressure. These free radicals have the potential to attack the embryos’ chromosomes that may leads to malformation of the pups (Young, 1992). Study by Rajikin et al. (2001) revealed that the palm vitamin E (60mg/kg b.w.) has protective effect against nicotine (source of free radicals) in pregnant rats. This has increased the status of fertility and percentage of normal pups born from the nicotine exposed dams. It is known that vitamin E is a potent anti-oxidant that protects the embryos from free radicals (Arouma, 1999).
5 Conclusion

In summary, palm vitamin E in this study (100 to 1000mg/kg b.w.) does not pose any significant reproductive toxicity or complication in pregnancy, delivery and early pup growth in rats.

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