Potential impact of bee pollen administration during pregnancy in rats

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Abstract: Although bee pollen was recommended as a supplemental diet for its nutritionally beneficial components, it is warning for its usage during pregnancy. In this study bee pollen (PB) water extract with different doses (2.5 & 5 and 10 g/kg b.w./day) was delivered to pregnant rats orally from day 1 to day 21 of gestation to address the physiological relevance of bee pollen rich in proteins and phytoestrogen during pregnancy in rats and to examine whether bee pollen administration modifies the serum steroid hormones involved in fetal outcome. The results revealed that bee pollen administration at high doses (5 &10 g/kg/day) during pregnancy has an adverse effect on mothers and fetal outcome manifested by dams death, failure in implantation processes, resorbtion of fetuses, reduction in fetal numbers, retardation in fetal and placental weights. Lipid oxidation markers such as MAD and GSH levels were changed on day 21 of gestation in bee pollen treated rats referring to incidence of imbalance of oxidant/antioxidant system. Circulating profile of estradiol (E_2), testosterone and progesterone were changed at selected time intervals (7,12,17 and 21) of gestation. Bee pollen had no apparently effect on cholesterol value and decreasing effect on triglyceride, HDL-cholesterol and LDL-cholesterol values through gestational period, it produced hypercholestermia and hyperlipidemia on day 21 of gestation especially at high doses. On determining the concentration of total protein and albumin, it was showed a significant increase particularly, in the second half of pregnancy pertaining to the groups administered bee pollen at a dose of 5 & 10 mg/kg b.w./day. The present results revealed that supplemental of pregnant rats with bee pollen throughout gestational period had harmful effect to a great extent on mothers and fetuses life. [Journal of American Science 2010;6(5):44-53]. (ISSN: 1545-1003).

Key Words: pollen- pregnancy - rats

1- Introduction

In modern times perception-that bee pollen as apicultural product is focused for human diet because of its nutritionally beneficial compounds has increasingly come under attack. Bee pollen (BP) is flower pollen collected from selected flower species by the honey bee, is known to be of particular essentiality for the reproduction and survival of these creatures. It is well documented that bee pollens are rich in essential amino acids, protein, unsaturated fatty acids and also contains many vitamins, minerals and trace elements which contribute to the health effects **(Campos et al., 2003)**. Because of its nutrient-rich components, it has been used as a folk medicine for centuries, to alleviate or cure conditions such as cold, flu, ulcer, premature aging, anemia, colitis, enteritis, and allergic reactions **(Šarić et al., 2009)**.The main active ingredients of bee pollen are primarily phytoestrogens including isoflavones, flavonols and lignans, otherwise known as plant hormones, compounds with well-documented hormonal benefits for both men and women **(Kristoffersen et al., 1997 and Moon et al., 2006).** Also, the phenolic components of bee pollen were reported to exhibit high levels of antioxidant and radical scavenging activity **(Carpes et al., 2007 and Eraslan et al., 2009).** Although its precise mode of action is still obscure, evidence gained from experiments suggested that the extract's lipid soluble fraction contains the material responsible for inhibiting the biosynthesis of prostaglandins and leukotrienes **(Šarić et al., 2009)**. Accordingly, BP is one alternative therapy utilizing plant-derived products (phytotherapy) as the treatment of choice for many chronic conditions and can be for developing preventive and therapeutic agents for various estrogen-mediated diseases **(Šarić et al., 2009).**

Though nutritional benefit has been noted with this dietary supplement, one study only, up to author knowledge, suggested that bee pollen could improvement nutrition without affecting normal fetal development, noting pollen to be a practical and effective nutrient during pregnancy but they warned from its usage **(Xie et al., 1994).** To asses the manifestations effect of bee pollen on pregnancy, oral injection to pregnant rats was performed to study the adverse effects resulting from bee pollen administration. Furthermore and more important, the aim of the present study was to demonstrate the effect of bee pollen on fetal outcome associated with

maternal hormonal pattern changes. The present study bears originality with regard to the inexistence of any previous study in which bee pollen was used as nutritional diet during pregnancy.

2. Material and Methods

Bee Pollen Extracts:

The pollen was collected from clover (*Trefoil Alexandrinum*) using special pollen traps from bee hives located at Sharkia Governate, Egypt. Firstly, the bee pollen was ground and this ground was stored continuously at 2–8 °C in desiccators. Microscopic examination demonstrated a purity of greater than 97%, with less than 0.5% foreign bee pollen and less than 0.9% plant parts. The powder of bee pollen (5g) was suspended in distilled water (20 ml) and mixed vigorously for 2 h. Following filtration and centrifugation (10,000×g, 20 min), the combined solution was concentrated and finally freeze dried. For use in experiments, the bee pollen of extract was dissolved in distilled water **(Masayoshi et al., 2007).**

Animals and Treatments:

Rats were kept under an environmentally controlled room (19 C, range 18-21^{o}C, relative humidity 64%). Food pellets and tap water were freely available. Adult rats were housed in groups of two females and one male in a plastic cage measuring 36 × 23 × 20 cm during a 4-day period. Vaginal smears were collected each day, with the first day of detectable sperm designated as embryonic day 1. On the first day of gestation, females were isolated in individual cages and randomly divided into four groups, each consisted of 10 rats.

The water-solubilized extract of bee pollen was orally administered at doses of 2.5,5 and10 gm/kg b.w./day to pregnant rat groups through a stomach tube from day 1 to day 21 of gestation. Control rats were received distilled water orally.

Pregnancy assessment and blood samples collection:

Maternal survival and mortality were recorded throughout gestational period and the dead animals were compensated by others to obtain the desired numbers of animals (n=10) in each group. All animals were fasted over night prior to scarifying. Blood samples were collected from orbital venous plexus of pregnant rats at time intervals of 2, 7, 12, 17 and 21 days of gestation and centrifuged at 3000 rpm for 10 min to separate plasma and serum. Subsequently, the animals were killed on day 21 of gestation with an overdose of ether and the uteri were removed with their contents to determine the number of live, dead and resorbed fetuses. The uterus of apparently non pregnant rats was stained with a 10% solution of sodium sulphide and evaluated for evidence of early resorption or implantation sites **(Chahoud and Paumgartten, 2005)**. Live fetuses and their placentas were weighed to the nearest milligram.

Hormonal analysis:

Serum estradiol (E_2), progesterone and testosterone concentrations were quantified by radioimmunoassay according to the method of **Burtis and Ashwood (1994), Smith (1985)** and **Wilson and Foster (1992),** respectively.

Measurement of oxidative stress markers and biochemical parameters:

Plasma malondialdehyde (MDA) levels and blood reduced glutathione concentration were determined as lipid oxidation markers according to the methods described by **Yoshioka et al. (1979)** and **Beutler et al., 1963**, respectively. Serum total cholesterol, triglycerides and HDL-cholesterol levels were measured by kinetic method using commercial kits according to the method of **Stein (1986), Walleyed (1974)** and **Wieland and Seidel (1981),** respectively and LDL was calculated. Also, serum protein and albumin contents were measured according to the method **of Dumas and Biggs (1975).**

Statistical analysis:

All results are presented as mean ± standard error of the mean. Statistical significances of the differences between the mean of the two groups of samples were assessed using Student's *t* test. Differences were considered to be statistically significant at $p < 0.05$ and 0.01.

3. Results

The adverse effect of bee pollen supplementation to pregnant rats firstly appeared on the inability of dams for continuity to survive. Since, there are 30% dams died on day 11 and 30% on day 13 of gestation in groups which received 5 &10 g/kg/day bee pollen, respectively.

Dissection of the uteri revealed that, in the females with uniform-sized uterine swellings, pregnancy appeared normal and general appearance of the fetuses. Opening of the uterine horns of the females with several sizes of uterine swellings revealed that normal viable fetuses were associated with the largest swellings, whereas the smaller one contained disintegrating or no fetal tissue. The uterine swellings that appeared abnormally small contained no fetal

tissue at the sites of implantation or placentation, and resorptions were considered to be completed at these sites. Resorbing fetuses within the closed uterine compartments showeddifferent degrees of disintegration. The uterine lumen was open between viable fetuses, whereas uterine compartments isolated the sites with disintegrating or no fetal tissue. The endometrium within these compartments was extensively folded, greatly enlarging the luminal surface. Placentas were smaller at sites where no fetal tissue remained than that of viable fetuses, but the general appearance of it was similar in two cases.

As shown in Table 1, bee pollen administration to rats over a period of gestation reduced fetal number at full term and this effect was dependent on the administered dose. Fetal weights were lighter in all groups administered bee pollen than controls, and this was accompanied with similar reductions in placental weight. Among 30 pregnant rats treated with bee pollen, 11 cases showed red spots in the uterine segment as a signs of implantation with no fetal tissues and 11 cases showed sign of resorptions.

Table 1: Uterine implantation, fetal resorbtion, live fetuses, fetal and placental weights of control and bee pollen groups on day 21 of gestation in rats

Groups / Parameters	Control group	Bee pollen administered groups		
		2.5 g/kg	5 g/kg	10 g/kg
Live fetal numbers	7.1± 1.44	6.5 ±1.16	4.4 ± 0.44**	2.4 ± 0.14**
Live fetal weights	4.1 ±0.40	3.8 ± 0.28	3.1 ± 0.14*	3.1 ± 0.20*
Placental weights	0.54 ±0.03	0.49 ±0.07	0.45 ± 0.23*	0.39 ±0.04**
Implanted sites Recorded cases No. of implanted sites No. of live fetuses	Zero	2 7 6	4 13 5	5 24 Zero
Resorbed fetuses Recorded cases No. of resoebed fetuses No. of live fetuses	Zero	4 8 10	4 8 12	3 8 10

Values are represented as means ±S E for the recorded cases; *P< 0.05, **P<0.01

In table (2), administration of bee pollen extract with high doses (5 &10 g/kg b.w.) through gestational period to pregnant rats appeared to increase significantly plasma MDA associated by significant decrease in blood GSH value on day 21 of gestation as compared to controls. It, however, showed non significant change in either MAD or GSH values in animals received the lower dose (2.5 g/kg b.w.) of bee pollen.

Table 2: plasma MDA and blood GSH of control and bee pollen groups on day 21 of gestation in rats.

Groups / Parameters	Control group	Bee pollen administered groups		
		2.5 g/kg	5 g/kg	10 g/kg
MDA (nmol/ml)	7.45±0.34	9.0±0.76	10.5**±0.66	12.0** ± 1.01
GSH (mg/dl)	61.47±2.0	58.8± 1.44	53. 6*± 2.61	50.0*±2.11

Values are represented as means ±S E for the recorded cases. * P< 0.05, ** P<0.01

Among all treated animals, bee pollen extract appeared modifying effect in E_2, testosterone and progesterone patterns at selected time intervals through gestational period and this effect was dose dependent. Since, bee pollen administration caused a significant decrease in circulating E_2 level on days 7 (fig 1) concomitant with non significant change in testosterone (fig 2) and progesterone (fig 3) levels followed by a sharp increase in E2 and testosterone levels only on day 12 of gestation. The three hormonal levels showed sharp decrease on day 17 of gestation than control remaining to day 21 except progesterone level, increase significantly at term.

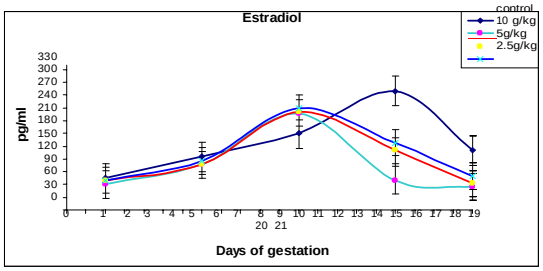

Fig 1: Serum estradiol pattern in control and bee pollen groups

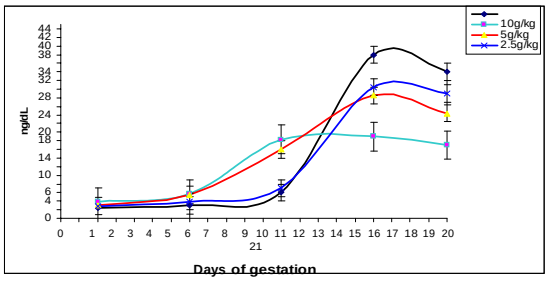

Fig 2: Serum testosterone pattern in control and bee pollen groups.

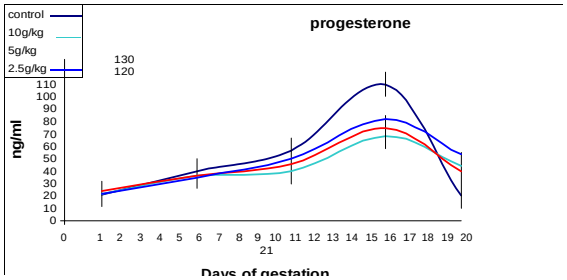

Fig 3: Serum progesterone pattern in control and bee pollen groups.

Neither serum cholesterol nor triglyceride concentrations differed among the four groups (control and treated groups) on day 2 of gestation. Thereafter, levels of cholesterol were maintained within control range among all BP treated animals until day 21 of gestation where it increased only in animals receiving 10g/kg b.w/day. BP. But triglyceride levels decreased significantly in animals receiving 5 and 10 g/kg BP on days 7 and 12 of gestation returning to approach control value on day 17 and increased sharply on day 21 of gestation. Whereas, the animals that received 2.5 g/kg BP, the triglyceride level recorded significant decrease on day 12 and 17 of gestation and returned to control level on day 21 (Fig 4 &5).

Animal group receiving 10g/kg of BP, showed significant decrease in HDL-cholesterol and LDL-cholesterol levels from day 7 till day 21 of gestation. Whereas, the animals received 5g/kg b.w. of bee pollen showed significant decrease in HDL-cholesterol and LDL-cholesterol levels from day 12 to day 21 of gestation. HDL-cholesterol and LDL-cholesterol in animals administered 2.5 g/kg of BP were slightly variable but not statistically differ than control (Fig 6 &7).

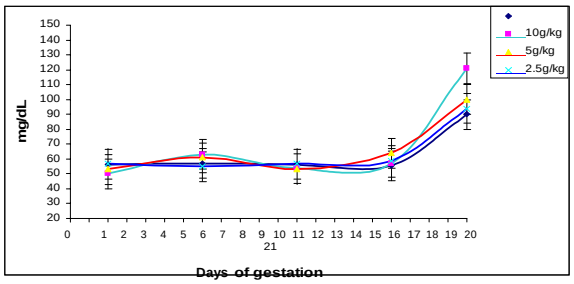

Fig 4: Serum cholesterol pattern in control and bee pollen group

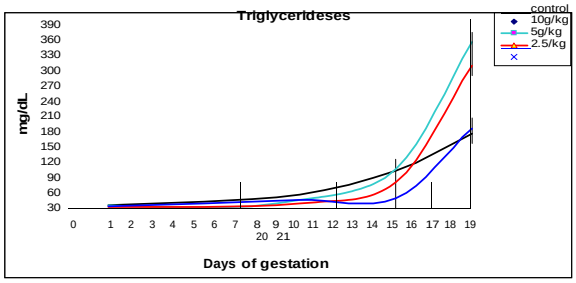

Fig 5: Serum triglyceride pattern in control and bee pollen groups.

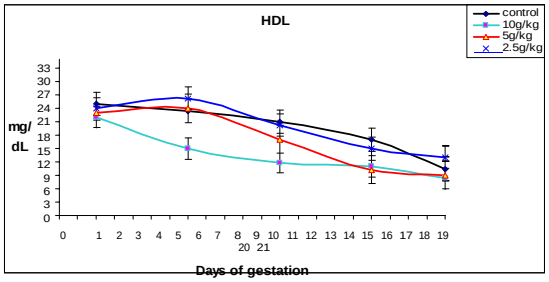

Fig 6: Serum HDL-cholesterol concentration in control and bee pollen groups.

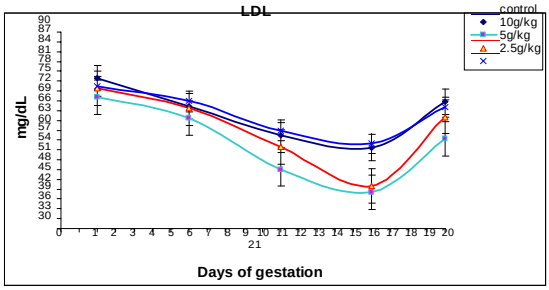

Fig7: Serum LDL-cholesterol concentration in control and bee pollen groups.

Administration of BP extract (10g/kg b.w./day) to pregnant rats caused significant increase in total protein concentration from day 7 till day 21 of gestation and this increase was dose dependent. Relative to control, little gradual increase was observed in protein concentration of animals that received 2.5 and 5 g/kg BP on days 7 and 12

followed by significant increase on days 17 and 21 of gestation. These changes were contaminant with noticeable variable and non significant increase, especially on the second half of gestation, in albumin concentration among all animals administered BP.

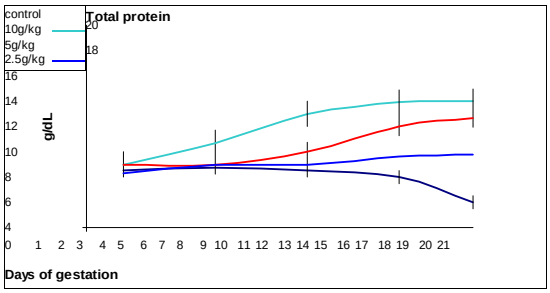

Fig 8: Serum total protein pattern in control and bee pollen groups.

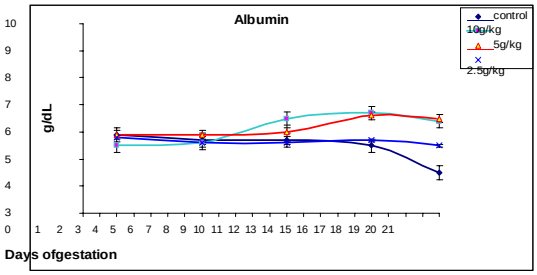

Fig 9: Serum albumin pattern in control and bee pollen groups.

4. Discussions

The quality of diet has long been known to affect the fetal condition, a better maternal diet being associated with better condition of the offspring. Extremes of diet are well recognized as adversely affecting the outcome of pregnancy (Law et al., 2000). However, inadequate nutrition during fetal development can alter aspects of morphologic and physiologic development increasing the predisposition in adult life to metabolic diseases such as diabetes mellitus and cardiovascular disease (Gillman, 2002). Fetal nutrition depends on the concentration of nutrients in the maternal bloodstream, placental perfusion and the transfer of nutrients through the placenta (Pisani et al., 2008).

In the current study, about 30% of dams administered the high doses of bee pollen (5 & 10g/kg b.w.) died in mid-pregnency. The mortaliry of dams may incident as a consequent to ingestion of certain toxins intake of bee pollen into the body. Bee pollen may constitute *ochratoxin* A **(Medina et al., 2004)** and bacterial spores of *Clostridium botulinum* **(Compas et al., 2008).** The analysis of ready-to-eat bee pollen samples have revealed contamination with potential mycotoxin producing species, including *Penicillium verrucosum*, *Aspergillus niger* aggregate, *Aspergillus carbonarius*, *Aspergillus ochraceus*, *Aspergillus flavus*, *Aspergillus parasiticus* and *Alternaria* spp. **(Gonzalez *et al.*, 2005)**, heavy metals **(Jablonski *et al.*, 1995; Leita, 1996; Conti and Botre, 2001)** and pesticides (**Fleche *et al.*, 1997; Kubik *et al.*, 1999**) originating from the environment and from agricultural practices are also considered the main contaminants of bee pollen but the bacterial contamination is a greater problem than pesticide or heavy metal contamination **(Bogdanov, 2006).**

Based upon the previous evidence, it could be suggested that the contamination of bee pollen which was provided into the pregnant rats was the main reason of alteration observed in MDA and GSH concentrations. Another important factor may be involved is, that the administration of bee pollen with high concentration of flavones constitute could be harmful because in condition in which a state of oxidation is not induced, flavonols can itself behave as an oxidant agent, inducing liver damage and a high production of NDA **(Okuda, 1999**). Also, after extensive flavonoid intake, flavonoid concentration in plasma may be insufficient to exert systemic antioxidant effects *in vivo,* probably as a result of flavonoid metabolites (methylated, sulphated or glucuronidated forms) which tend to have decreased antioxidant activity **(Dudov and Starodub, 1994).**

In bee pollen treated rats, the embryonic uterine implantation did not correspond to the numbers of fetuses at term, since a high degree of uterine reabsorption occurred. By examination of dam uteri, it was revealed that decreased litter size was due to increased prenatal mortaliry, predominantly at the post-implantation stage, and that the ovulation rate had not changed associated with placental growth retardation, particularly within groups with highest embryonic mortality. Accordingly, the number and weight of viable fetuses and their placentas recovered in the treated pregnant rats at term were less than in control. Such fecundity defects is usually considered to result from a toxic substance-induced prenatal growth retardation **(Eriksson et al., 2002).** It appeared that the consumption of bee pollen may constitute an important risk factor not only for mothers but also for the fetuses concerning the presence of mycotoxins **(Compas et al., 2008)** associated with oxidative stress which reduced the placenta retained **(Mandang et al., 2007)**. On the other hand, consumption of bee pollen rich in phytoestrogens **(Janeczko and Skoczowski, 2005**

and Moon et al., 2006) may lead to changes in hormonal levels and/or ovarian sensitivity to hormones with subsequent changes in maternal estrogen/progesterone ratio required for pregnancy maintenance **(Ruhlen et al., 2008)**. Because estrogens administration at any stage of gestation can affect various aspects of fetal development **(Ruhlen et al., 2008)**, phytoestrogens consumption during pregnancy represent a potential risk factor for abnormal development **(Janeczko and Skoczowski, 2005).** Phytoestrogen administration during rat pregnancy decreased placental blood flow **(Mahendroo et al., 1997)**, lowered fetal weights **(Becker et al., 2005 and Ruhlen et al., 2008)** and is also lethal to the embryo. Depending upon the modern-day analyses, many of the plants contain phytoestrogens were historically noted for their ability to prevent pregnancies or cause miscarriages **(Ruhlen et al., 2008)**. Although there are a wide variety of effects reported for rats that were exposed to phytoestrogens during gestation, there is inconsistency within the literature which suggests that the amount and administration route may be critical and effective **(Nagao et al., 2001, Dlclos et al., 2001, Lewis, et al., 2003, Kouki et al 2003 and Nikaido et al., 2004)**.

In the current study, the adverse effect of bee pollen on fetal outcome was associated with a change in circulating maternal hormonal patternthrough gestational period. Estrogen is essential for ovarian progesterone production throughout pregnancy **(Rothchild, 1983)** and the placenta may be the source of testosterone substrate for ovarian estrogen formation during the second falf of rat pregnancy **(Bartholomeusz et al., 1999)**. It is pertinent the serum estradiol level accumulated in all pregnant rats administered bee pollen with different doses was lower in the first half of pregnancy (days 2&7 of gestation) than control, reaching its peak on day 12 and returned back to sharp decrease on days 17 and 21 of gestation and this change was dose dependent. However, estradiol is among the powerful of estradiol hormones in mammals, and even slight changes in the estrogen response system during gestation can affect fetal development **(Collins, 1996)** or fetal death because the endogenous control of E2 secretion must be carefully regulated to maintain optimum conditions for fetal development **(Bartholomeusz et al., 1999)**. Stimulation of estrogen production by phytoestrogens in trophoblast cells is probably due to estrogen receptor blocking effects of phytoestrogens. Trophoblast cells seem to compensate blocking of its estrogen receptors by higher estrogen production **(Richter et al., 2009)**.

The alteration in estradiol pattern in bee pollen administered dams was concomitant with a similar pattern change in the testosterone level. Since, the significant changes in testosterone value starting from day 12 till day 21 of gestation. Such changes may be related to specific growth-retarding effect of estradiol on the placenta which secondarily limits fetal growth and the ability of estradiol to inhibit placental production of testosterone **(Legrand et al., 1984)**, essential precursor for its own synthesis by the ovary **(Jackson and Albrecht, 1986).** Maternal circulating testosterone in bee pollen treated rats was also responsible for pregnancy disruption because testosterone can affect fetal growth and size through modifying maternal energy homeostasis and decreasing the nutrient supplies to the placenta and fetus **(Steckler et al., 2005)**. Alternatively, testosterone may modify placental function and reduce the capacity for transport of nutrients to the fetus or cross the placenta and exert a direct effect on fetal growth and/or energy homeostasis **(Carlsen et al., 2006).** On the other hand, the decrease in estrogen related to testosterone concentration in rats receiving bee pollen may be due to inhibitory effect of phytoestrogen content on aromatase activity, the enzyme that converts androgen to estrogen whether as a result of competition for its active site or by reducing enzyme expression regulating aromatase activity **(Oh et al., 2000 and Burton and wells, 2002).** There is, however, increasing evidence that phytoestrogens may bind to aromatase and/or 17β-hydroxysteroid dehydrogenase (HSD) and thereby reduce the availability of these enzymes for the production of estrogen from androgen precursors and/or the production of estradiol from weak estrogens **(Le Bail *et al*., 2000).**

With regard to progesterone, growth and development of the embryo and fetus are unaffected over a wide range of progesterone concentrations in the maternal plasma. The present results revealed a marked change in progesterone concentration till term, which implies that bee pollen affects the ovarian function and the uterine environment resulted again on the very vunreable hormonal cycle. However, bee pollen may disturb the production of 20α hydroxysteroid dehydrogenase (20α HSD), the enzyme that converts intraluteal progesterone to its inactive metabolite 20α-dihydroprogesterone **(Arosh *et al.* 2004)**, in which circulating progesterone level elevated at term **(Piekorz *et al.* 2005)** referring to delay of parturition**.** On the other hand, the plant estrogens inhibit enzymes involved in dteroidogenesis because these compounds are estrogenic per se and may thus replace endogenous estrogens. Also, phytoestrogens sufficiently reduce progesterone production in term trophoblast cells **(Jefferson et al., 2006).** Because blockade of

progesterone is a possible mechanism involved in initiation of labor, thus the high doses of phytoestrogens at the feto-maternal interphase could play a negative role in maintenance of pregnancy. There is some concern that bee pollen when used orally might have uterine stimulant effects; avoid using **(Leung and Foster, 1996).**

The present findings are consistent to great extent with the reports of many previously workers such as, **Jefferson et al., (2006)**, who suggested that the plant estrogens are inhibit enzymes involved in steroidogenesis because these compounds are estrogenic per se, and may thus replace endogenous estrogens. Also, phytoestrogens sufficiently reduce progesterone production in term trophoblast cells. Because blockade of progesterone is a possible mechanism involved in initiation of labor, thus the high doses of phytoestrogens intake through bee pollen consumption at the feto-maternal interphase could play a negative role in maintenance of pregnancy **(Richter et al., 2009)**. In another study, **Kao and P;Eng (1995)** revealed the ability of phytoestrogens to interfere in estrogen negative feedback by binding to estrogen receptors in interior pituitary or hypothalamus and indirectly alter ovarian steroidogenesis.

Although the administration of bee pollen had no apparently effect on cholesterol value and decreasing effect on triglyceride, HDL-chlesterol and LDL-cholesterol values through gestational period, it produced hypercholestermia and hyperlipidemia on day 21 of gestation especially at high doses. Perhaps the best approach explanation is the definition of the functional capacity of the placenta to perform metabolic alterations of certain lipids. In most studies the degree of transport across the placenta and the degree of fetal or placental synthesis of lipids varies with gestational age **(Herrera et al., 2006).** Thus, placental size, architecture, developmental and pathological processes, and interaction with the fetus cooperate with transport and metabolic mechanisms to affect placental-fetal nutrient exchange **(William and Hay 1994).** Apparently, earlier in pregnancy, there is more dependence on maternal lipids to provide placental and fetal lipids. The placenta has the capacity of altering those lipids presented to it by selective transport and inter conversions mediated transfer of lipid uptake from lipoproteins, metabolic alteration in the placenta, and release into the fetal plasma **(Hussain et al., 2003).** Also, most lipid classes are synthesized *de novo* in the placenta **(Herrera et al., 2006).** In this study, phytoestrogen content in bee pollen could exert damage on placental cells **(Janeczko and Skoczowski, 2005)** leading to restriction in placental blood supply to carry lipids to meet the high demand of the developing fetus **(Wadsack et al., 2003)** resulting in accumulation of lipid fractions in maternal circulation.

The concentration of total proteins and albumin values showed significant increase particularly, in the second half of pregnancy pertaining to the groups administered bee pollen at a dose of 5 & 10 mg/kg b.w./day. The elevation in protein and albumin concentrations may be due to the high contents of protein and amino acids supplemented in bee pollen (**Xie et al., 1994** and **El-Missiry, 1999),** that provided amino acids greatly in excess of the requirements of the fetuses and the transfer of these amino acids might have been limited by the functional capacity of the placenta **(William 1994).** Interestingly, combustion of pregnant rats to dietary protein caused elevation of estradiol level earlier in pregnancy and a significant decrease in E2 concentrations near term **(Wilson et al., 2000).** It was suggested that a protein rich diet was associated with an increased incidence of premature deliveries, babies with low birth weights and neonatal death **(Malhorta and Sawers 1986).**

One must consider that the present results, although consistent and significant, are not sufficient to a further discussion. The data obtained are suggestive to inspire future research, one can speculate that the bee pollen at a high doses acted mainly during implantation stage, a susceptible period when toxic action of any substance leads to embryonic death. Bee pollen did not acted during organogenesis, because in doing so, it could lead to fetal malformations.

The current work provides evidence that bee pollen toxicity was high enough to affect both mother and embryo. Decline in size and death of litters, death of mothers, as well as pregnancy failure were all observed. The results obtained also show that bee pollen extract toxicity affects embryonic implantation resulting in significant reduction in implantation process.

References

1 Arosh JA., Banu SK., Chapdeiane P., Madore E., Sirois J. & Fortier MA. (2004). Prostaglandin biosynthesis, transport, and signiling in corpus leutum: a basis for autoregulation of luteal function. Endocrinology, 145, 2551-2560.

2 Becker L A., Kunkel A J., Brown MR., Ball EE. And Williams MT (2005). Effects of dietary phytoestrogen exposure during perinatal period. Neurotxicology and Teratology, 27 (6): 825-834.

3 Bartholomeusz RK., Bruce NW and Lynch AM (1999). Embryo survival and fetal mortality and placental growth following elevation of maternal

estradiol blood concentrations. Biology of reoroduction, 61, 46-50.
4 Beutler, E., Duron, O. and Kelly, B. M. (1963). Improved method for determination of blood glutathione. Lab Clin. Med. 61(5); 882-885. Bogdanov, S (2006). Contaminants of bee products. Apidologie, 38; 1-18.
5 Burtis CA., Ashwood ER., editors. (1994). Tietz textbook of clinical chemistry. 2nd ed. Philadelphia; WB. Saunders.
6 Burton JL., Wells M. (2002). The effect of phytoestrogens on the female genital tract. Journal of Clinical Pathology ;55:401-407.
7 Campos MG, Webby RF, Markham KR, et al. (2003). Age-induced diminution of free radical scavenging capacity in bee pollens and the contribution of constituent flavonoids. J Agric Food Chem;51(3):742-745.
8 Campos MGR., Bogdanov S., Ligia Bicudo de Almeida-Muradian,Teresa SzczesnT.,Yanina Mancebo Y., Frigerio C., Ferreira F. (2008). Pollen composition and standardization of analytical methods. Journal of Apicultural Research and Bee World, 47(2): 156–163.
9 Carlsen, SM., Jacobsen G. and Romundstad P. (2006). Maternal testosterone levels during pregnancy are associated with offspring size at birth. European J of Endocrinology, vol 155 (2): 365-370.
10 Carpes S T., Begnini R., Matias de Alencar S., Masson M. L. (2007). Study of preparations of bee pollen extracts, antioxidant and antiradical activity. Ciênc. agrotec., Lavras, v. 31 (6), p. 1818-1825.
11 Conti, M E, Botre, F (2001). Honey bees and their products as potential bioindicators of heavy metal contamination. Environmental Monitoring and Assessment 69(3): 267–282.
12 Chahoud F. and Paumgartten J.R. (2005). Relationships between fetal body weight of Wistar rats at term and the extent of skeletal ossification. Braz J Med Biol Res, 38(4) 565-575.
13 Collins P (1996). Vascular aspects of estrogen. Maturitas, 23, 217-226.
14 Delclos KB, Bucci TJ, et al. (2001). Effects of dietary genistein exposure during development on male and female CD (Sprague-Dawley) rats. Reproductive Toxicology 15(6): 647-63.
15 Dumas BT and Biggs HG. (1975). In Standard Methods of Clinical Chemistry, Vol 7, Academic Press, New York, p 175.
16 Dudov IA, Starodub NF. (1994). Antioxidant system of rat erythrocytes under conditions of prolonged intake of honeybee flower pollen load. Ukr Biokhim Zh 66: 94–96.
17 Eriksson CA., Forsen T., Tuomilehto J., Jaddoe VW., Osmond C.& Baker DJ. (2002). Effect of size at birth and childhood growth on the insulin resistance syndrome in elderly individuals. Diabetologia, 45: 342-348.
18 Eraslan G, Kanbur M, Silici S, Liman B C, Altınordulu S and Sarıca Z S. (2009). Evaluation of protective effect of bee pollen against propoxur toxicity in rat Ecotoxocology and environmental safety, 72 (3), 931-937.
19 El-Missiry, M. A. (1999). Bee-pollen as antioxidant for the control of radiation- induced oxidative stress in male rats. J. Egypt. Ger. Soc. Zool. 29(A), 121-132.
20 Fleche, C; Clement, M C; Zeggane, S; Faucon, J P (1997). Contamination of bee products and risks for human health: the situation in France. Revue Scientifique et Technique de l'Office International Des Epizooties 16(2): 609–619.
21 Gillman M W (2002). Epidemiological challenges in studying the fetal origins of adult chronic diseases. Int J Epidemiol 31, 294-299.
22 Gonzalez G, Hinojo MJ and Mateo R. (2005). Occurrence of mycotoxin producing fungi in bee pollen. Int J Food Microbiol;105 (1):1-9.
23 Hussain, M. M., J. Shi, and P. Dreizen. (2003). Microsomal triglyceride transfer protein and its role in apoB-lipoprotein assembly. *J. Lipid Res.* 44: 22–32.
24 Herrera E., E. Amusquivar E., López-Soldado I., Ortega, H.(2006). Maternal Lipid Metabolism and Placental Lipid Transfer Horm Res; 65 (Suppl. 3):59-64.
25 Jackson JA, Albrecht ED. (1986). Estrogen regulates placental androstenedione production during rat pregnancy. Endocrinology, 119: 1052-1057.
26 Jablonski, B; Koltowski, Z; Marcinkowski, J; Rybak Chmielewska, H; Szczesna, T (1995). Contamination of nectar, honey and pollen collected from roadside plants. Pszczelnicze Zeszyty Naukowe. 40: 129–144.
27 Janeczko A. and Skoczowski A (2005). Mammalian sex hormones in plants. Folia Histochemica et cytobiologica, vol., 43 (2): 71-79.
28 Jefferson WN, Newbold R, Padilla-Banks E, and Pepling M. (2006). Neonatal Genistein Treatment Alters Ovarian Differentiation in the Mouse: Inhibition of Oocyte Nest Breakdown and Increased Oocyte Survival. Biology of Reproduction 74(1):161-168.
29 Kristoffersen K, Thomsen BW, Schacke E, Wagner HH. (1997). Use of natural medicines in women referred to specialists]. Ugeskr Laeger. 159(3): 294-6.

30 Kubik, M; Nowacki, J; Pidek, A;Warakomska, Z; Michalczuk, L; Goszczynski, W (1999). Pesticide residues in bee products collected from cherry trees protected during blooming period with contact and systemic fungicides. Apidologie
30(6): 521–532.
31 Kouki T, Kishitake M. (2003). Effects of neonatal treatment with phytoestrogens, genistein and daidzein, on sex difference in female rat brain function: Estrous cycle and lordosis. Hormones and Behavior 44(2):140-145.
32 Kao PC. and P'Eng F K. (1995). How to reduce the risk factors of osteoporosis in Asia.
Zhonghua Yi Xue Za Zhi (Taipei) 55(3):209-213.
33 Leita, L; Muhlbachova, G; Cesco, S; Barbattini, R; Mondini, C (1996). Investigation of the use of honey bees and honey bee products to assess heavy metals contamination. *Environmental Monitoring and Assessment* 43: 1–9.
34 Le Bail, J.C., Champavier Y., Chulia, A.-J. and Habrioux, G. (2000). Effects of phytoestrogens on aromatase, 3and 7-hydroxysteroid dehydrogenase activities and human breast cancer cells. *Life Sci.*, 66, 1281–1291.
35 Legrand C., Marie J. and Maltier JP (1984).
Testosterone, dihydrotestoterone, androtestosterone and dehydroepiandrosterone concentrations in placentae, ovaries and plasma of the rat in late pregnancy. Endocrinol. 105(10):119-125.
36 Lewis, P. M., Dunn M. P, McMahon J. A., Logan M., Martin J. F., St-Jacques B., and McMahon A. P. (2003). Cholesterol modification of sonic hedgehog is required for long-range signaling activity and effective modulation of signaling by Ptc1. Cell. 105: 599–612.
37 Law J M G, Zhang K L, Osmond C, Stein C and Barker D. (2000). Effects of infant birth weight and maternal body mass index in pregnancy on components of the insulin resistance syndrome in China. Ann Inten Med, 132, 253-260.
38 Masayoshi Y., Reiko H., Safoshi U, Kaori I and Ken H. (2007). Preventive effects of bee pollen cistus ladaniferus extract on bone loss in Streptozotoc in diabetic rats in vivo. *J. Health. Sci.* 53, pp. 190–195.
39 Malhorta A. and Sawars R S (1986). Dietry supplementation in pregnancy. Br. Med. J., 203 (6545): 465-466.
40 Mahendroo MS, Cala KM, Landrum DP and Russell DW (1997). Fetal death in mice lacking 5-reductase type 1 caused by estrogen excess. Mol Endocrinol, 11: 917-927.
41 Medina A, Gonzalez G, Saez JM. (2004). Bee pollen, a substrate that stimulates ochratoxin A production by Aspergillus ochraceus Wilh. Syst Appl Microbiol.,27(2):261-267.
42 Mandang S, Manuelpillai U and Wallace E M (2007). Oxidative stress increases placental and endothelial cell activin A secretion *Journal of Endocrinology* 192, 485-493.
43 Nagao T and Yoshimura S. (2001). Reproductive effects in male and female rats of neonatal exposure to genistein. Reproductive Toxicology
15(4):399-411
44 MoonYJ., Wang X. and Morris ME (2006).
Dietary flavonoid effects on xenobiotic and carcinogenic metabolism. Toxicol.Vitro 20, 187-210.
45 Nikaido Y, Yoshizawa K. (2004). Effects of maternal xenoestrogen exposure on development of the reproductive tract and mammary gland in
female CD-1 mouse offspring. Reproductive Toxicology 18(6):803-811.
46 Rothchild, I (1983). Role of progesterone in initiatingand maintaining pregnancy.
Progesterone and progestins, edited by C. Wayane Bardin, Edwin Milgrom, and Pierre Mauvais-Jarvis. Raven Press, New York.
47 Oh S-M, Choung S-Y, Sheen Y-Y, *et al*. (2000).
Quantitative assessment of estrogenic activity in the water environment of Korea by the E- SCREEN assay. *Sci Total Environ*;263:161–96
48 Okuda, T. (1999). Antioxidants in herbs: polyphenols, In: Packer, L. Midori, H., Toshikazu, Y. (Eds), Antioxidant food supplements in human health. Academic Press, USA, pp.393-410.
49 Pisani L P, Oyama L M, Bueno A A, Biz C, Albuquerque K T and Riberiro E B. (2008). Hydrogenated fat intake during pregnancy and lactation modifies serum lipid profile and adipokine mRNA in 21-day-old rats. Nutrition
24(3):255-261.
50 Piekorz RP., Ginger S., Hoffmeyer A., Ihie JN. & Weinsyein Y. (2005). Regulation of progesterone levels during pregnancy and parturition by signal transducer and activator of transcripition 5 and 20 alpha hydroxsteroid dehydrogenase. Molecular Endocrinology 19,
431- 440.
51 Ruhlen R. L., Howdeshell K.L., Mao J., Taylor J. A., Bronson F. H., Newbold R. R., Welshons W. V. and Vom Saal F. S (2008). Low Phytoestrogen Levels in Feed Increase Fetal Serum Estradiol Resulting in the "Fetal
Estrogenization Syndrome" and Obesity in CD-1

Mice. Environmental Health Perspectives, Vol 116 (3).
52 Robertson A and Sprecher H. (2008). Human placenta lipids metabolism. III Synthesis and hydrolysis of phospholipids Acta pædiatrica, 57 (13); 1-19.
53 Richter D U., Mylonas I.,Toth B, Christoph Scholz C., Briese V., Friese *K* and Udo Jeschke U.(2009). Effects of phytoestrogens genistein and daidzein on progesterone and estrogen (estradiol) production of human term trophoblast cells *in vitro* Vol. 25, (1), Pages 32-38.
54 Stein EA. (1986). In: Textbook of Clinical Chemistry, NW Tiez, Ed W. B. Saunder, Philadelphia, pp. 886, 1818-1829.
55 Steckler T., Wang J., Bartol FF., Roy SK. & Padmanabhan V. (2005). Fetal programming: a prenatal testosterone treatment causes intrauterine growth retardation, reduces ovarian reserve and increases ovarian follicular recruitment. Endocrinology, 146, 3185-3193.
56 Šarić A, Balog T, Sobočanec S, Kušić B, Šverko V, Rusak G, Likić S, Bubalo D, Pinto B, Reali D and Marotti T. (2009). Antioxidant effects of flavonoid from Croatian *Cystus incanus* L. rich bee pollen Food and Chemical Toxicology, Vol., 47 (3), 547-554.
57 Smith RL. (1985). Effect of serum-separating gels on progesterone assays.Clinical Chem., 31; 1239.
58 Wahleyeld, AW (1974). In: Methods of Enzymatic Analysis, Vol 5, HU Bergmeyer, Ed. Academic Press, New York, pp 1831-1835.
59 Wilson JD., Foster DW, editors (1992). Williams textbook of endocrinology. Philadelphia: Saunders, 923-926.
60 Wadsack, C., A. Hammer, S. Levak-Frank, G. Desoye, K. F. Kozarsky, B. 60- Hirschmugl, W. Sattler, and E. Malle. (2003). Selective cholesteryl ester uptake from high density lipoprotein by human first trimester and term villous trophoblast cells. Placenta. 24: 131–143.
61 Wieland H and Seidel D (1981). Artzt Lab. 27, 141-154.
62 William W. Hay, Jr. (1994) Placental Transport of Nutrients to the Fetus. Vol. 21 (3), 180-184.
63 Wilson TA, Garner, SC and Anderson J.J.B. (2000). Dietary protein source and serum estradiol concentrations of rats during pregnancy, lactation and post-lactation. Nutririon research, vol. 20 (12), 1735-1747.
64 Xie Y, Wan B and Li W. (1994). Effect of bee pollen on maternal and fetal growth. Hua xi Yi Ke Da Xue Bae, 25 (4):
65 Yoshioka, T., Kawada, K., Shimada, T. and Mori, M (1979). Lipid peroxidation in material and cord blood and protective enzyme against activated oxygen toxicity in the blood. Am J. Obstet. Gynecol., 135(3): 372- 376.