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Embryotoxic effect of low doses of caffeine

Ocena embriotoksycznego działania niskich dawek kofeiny

Caffeine is one of the most commonly ingested substances, mainly found in coffee, tea, cola and other soft drinks. Because of its own analgesic effect caffeine is frequently used in various over-the--counter combination medications containing antipyretic and analgesic components such as aspirin, paracetamol, propyphenazone, and others (11).

Many experimental studies have shown fetal toxicities as well as congenital malformations to be associated with the administration of high doses of caffeine during pregnancy (1, 5, 6, 10, 16). However it is controversial whether caffeine has an adverse effect on human development.

OBJECTIVE

The purpose of our study was to evaluate the embryotoxic effect of low doses of caffeine.

MATERIAL AND METHODS

Wistar rats, weighing about 200 – 250 g, were obtained from commercial breeder. Standard rat chow and top water were available *ad libitum*. The animals were maintained in a study room, which provided a 12 hours light/dark cycle. After two weeks' acclimatisation period, the rats were mated and the day on which spermatozoa were found in vaginal smears was considered the first day of pregnancy. The positive smear females were pooled in experimental groups. Some females were not pregnant despite the presence of spermatozoa.

The caffeine (Azienda Chemica E Farmaceutica SPA) was grounded with 0.1% poysorbate 80 (Tween 80 by Sigma) and then diluted with distilled water. The suspension was administered orally, once a day, throughout the whole second trimester of pregnancy (8–14 day of pregnancy). Caffeine was administered in three different doses: C1 – 0.7 mg/kg body weight (b.w.)/day, C2 – 7.0 mg/kg b.w./day, C3 – 70.0 mg/kg b.w./day.

Two control groups were designed. The negative control group (T) was given a volume of 10 ml/kg/day of suspending vehicle alone. In group UT – the females, which did not received any substances (untreated control).

The dames were sacrificed by decapitation on day 21st of pregnancy. The ovaries and uterus were removed by caesarean section, and the number of corpora lutea, fetuses, resorptions, and total implantations were recorded. Each fetus was externally examined, weighed, and the length of body and tail were checked.

The experiment was done with approval of the University Ethic Commission.

The data were analysed using the two–tailed Student t–test and Mann–Whitney test with 5% considered significant (α=0.05). Analyses were conducted on a Toshiba microcomputer.

RESULTS

During the entire experiment, no maternal deaths were recorded in any of the groups.

There were not any statistical differences between both control groups (T, UT); because of this, we decided to combine them into one common control group (CON) to minimise observation error.

Table 1. Checked parameters in experimental groups and the common control group

	CON MD ± SD	C1 MD ± SD	C2 MD ± SD	C3 MD ± SD
Fetal weight (g)	3.80 ± 0.42	3.89 ± 0.39	3.52 ± 0.30**	3.84 ± 0.20
Fetal length (mm)	38.55 ± 1.09	37.88 ± 1.41	37.60 ± 1.20*	3814 ± 0.94
Tail length (mm)	11.85 ± 0.51	11.85 ± 0.51	11.81 ± 0.38	11.70 ± 0.12
Placental weight (g)	0.59 ± 0.06	0.58 ± 0.04	0.56 ± 0.06	0.52 ± 0.04*‡
No. of corpora lutea	15.13 ± 0.43	15.00 ± 1.92	15.11 ± 2.71	13.43 ± 1.90*
No. of fetuses	14.31 ± 2.25	14.14 ± 2.03	14.22 ± 2.86	12.28 ± 1.79*
No. of resorptions	0.55 ± 0.68	0.57 ± 0.53	0.55 ± 0.52	0.85 ± 0.69
Preimplantation mortality	2.08 ± 4.37	1.91 ± 3.02	2.56 ± 3.64	2.37 ± 3.74
Postimplantation mortality	3.40 ± 4.19	4.00 ± 3.58	3.67 ± 3.44	6.22 ± 4.20
No. of subcutaneous ecchymois	0.20 ± 0.41	0.42 ± 0.78	0.44 ± 0.72	0.28 ± 0.48

MD – mean, SD – standard deviation, * $p < 0.05$ corresponding to CON,
** $p < 0.05$ corresponding to C3, ‡ $p < 0.05$ corresponding to C1

A summary of data is listed in Table 1. The body weight of fetuses showed no difference among the groups ($p > 0.05$) corresponding to the common control groups. However, the fetal weight of group C2 was less that of group C3 ($p < 0.05$).

A significant decrease of the length was found in group C2, when compared with the control group. A decrease of this parameter in other treated group did not cause significant alternations. The length of the tail was not statistically different. Placental weight differences were observed only in the group C3, when compared with the common control group, as well as with the group C1. Analysis of the number of corpora lutea and the number of fetuses showed a significant decrease only in the group C3, when compared with the group CON. However the preimplantation mortality factor in all the experimental groups were not statistically different. A mean number of resorption and postimplantation mortality factor was not statistically different.

An external exam showed no significant increase ($p>0.05$) of the number of the subcutaneous ecchymosis among the groups. The most often localisations were on the left cervical triangle and interscapular region.

DISCUSSION

The decrease in the length of the fetal body observed in the group C2, but did not confirm in the highest dose. Considering that fetal body weight and the tail length did not present any statistical differences, it could be interpreted as occasional changes.

A significant decrease of placenta weight was observed in the litters of the dames that received the highest doses of caffeine. However, without any other expression of the embryotoxicity, the placental weight cannot be the basis to find fault with (4).

The pre– and the postimplantation mortality factors, in spite of significant drop of the number of corpora lutea and the number of fetuses in the highest doses, were not statistically different. The lesser number of fetuses can be interpreted as a consequence of the lesser number corpora lutea, not as a side effect of the tested substance, the more so as administrations were after the postimplantation period.

The observed subcutaneous ecchymosis, as in the other study, was located more often on the left side of fetal body (1) However, in opposition to Bartel's results we did not observe statistical differences between the groups. The dose used by the Łódź team was higher (400 mg/kg b.w.) and caffeine was administered in a single intraperitoneal injection.

The previous investigation shows decreases in fetal body weight as a result of caffeine administered intravenously in doses 37.5 mg/kg b.w/day and higher to the pregnant rats (6) In doses higher than 112.5 mg/kg b.w. decreases in the fetal length of the tail were observed.

In another investigation, caffeine administered in doses of 30 and 60 mg/kg b.w. to rats was shown to cause significant fetal growth retardation (1).

Scott (14) administered caffeine intraperitoneally on 11th and 12th day of gestation to the pregnant mouse showing embryotoxic effect only at 250.0 mg/kg b.w. The middle doses (175.0 – – 250.0 mg/kg b.w.) caused only a minimal increase of resorptions.

Female monkeys exposed to caffeine throughout the whole pregnancy in doses of 10.0 – – 30.0 mgl/kg b.w. per day, had an increased incidence of miscarriages and stillbirths (5). The weight and length of the fetuses were decreased compared to the control group.

Several authors have not associated caffeine consumption with decreased fertility, increased incidence of spontaneous abortion, low birth weights, and congenital malformations (7, 8, 12). Unfortunately Martin et al. (9) showed that the risk of having a small baby was increased in women consuming high doses of caffeine (>300 mg/day) compared with women without caffeine intake.

A reduction of the fetal body weight were recorded in the group using caffeine in doses greater than 300 mg and smoking 15 or more cigarettes daily (2). In this group of women the placental weight was significantly decreased. The other parameters such as fetal head circumference and fetal body length were not affected by caffeine consumption.

In the other study, moderate to heavy consumption (151 mg or more of caffeine per day) was associated with twofold increased risk of late 1st and 2nd trimester spontaneous abortion (15) Consumption of greater than 200 mg/day did not increase this risk. In women who had a spontaneous abortion in their last pregnancy, light caffeine use (less than 150 mg/day) was associated with a fourfold increase in late pregnancy loss.

In the end it is worth noticing that caffeine, as a cofactor, could increase the embryotoxic effect of the other xenobiotics such as acetazolamide, acetaminophen, isopropylantipyrin, alcohol etc (3, 11).

CONCLUSIONS

Caffeine administration for the whole second trimester did not cause embryotoxic effects.

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STRESZCZENIE

Kofeina występuje w większości popularnych napojów, a także w preparatach leczniczych. Celem pracy była ocena wpływu niskich dawek kofeiny na rozwój embrionalny i płodowy, ze szczególnym uwzględnieniem działania embriotoksycznego. Ciężarnym samicom szczura białego szczepu Wistar podawano dożołądkowo, jeden raz dziennie, przez II trymestr ciąży, kofeinę w wodnej zawiesinie Tweenu 80, w trzech dawkach: C 1 – 0,7 mg/kg masy ciała (m.c.), C2 – 7,0 mg/kg m.c., C3 – 70,0 mg/kg m.c. W 21 dniu ciąży cesarskim cięciem wydobywano płody. Liczono ciałka żółte obu jajników, płody, resorpcje i miejsca implantacji. Mierzono długość płodu, masę płodu i łożyska. Oceniano umiejscowienie i liczbę wylewów krwawych w tkance podskórnej. Analizę statystyczną przeprowadzono przy pomocy testu T–Studenta i testu Mann–Whitneya. Wykazano istotny ($p < 0,05$) spadek masy łożyska, ilości ciałek żółtych i płodów w grupie C3, a także spadek długości płodów w grupie C2 w porównaniu z kontrolą.