Studies on the Effect of Caffeine on Kidney during Fetal Development in Albino Rats

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Caffeine, a potential teratogen, was administered in various doses to pregnant albino rats and the effects were observed at the cellular level in kidney of developing embryos. It has been observed that caffeine when administered too pregnant mothers, causes significant damage to the renal tissue in the developing embryos.

Key words: Teratogen, Kidney, Caffeine, Pregnant albino rats.

That some drugs can cause congenital malformations was first brought to light in 1961-62[1,2,3]. Caffeine was suspected to be a teratogen but its status has not yet been established conclusively.

Although teratogenic effect of caffeine on various organs has been studies by various persons. Among other thing caffeine has been shown to experimental animals to reduce fertility, increase the incidence of premature deliveries, reduce the litter size and increase in the incidence of digital defects. It was also shown to be responsible for lowering the birth weight, delayed ossification and enlarged liver and kidney[6,7]. Caffeine can also cause an increase in neurotransmitters, blood glucose and non-estrified fatty acid, while it reduces the level of aromatic and branched amino acids[8]. When taken orally caffeine can penetrate placenta and blood-brain barrier and can cause respiratory stimulation and a decrease in thyroid stimulating hormone and glucocorticoid[9].

The teratogenic effects of caffeine are exaggerated with taken in combination with ethanol[10,11,12] or nicotine[13,14]. Caffeine has been regarded as potential teratogen in human beings since 1960 but no malformations were attributed to caffeine. It was however, shown that intake by large amounts of tea/coffee by pregnant women can cause increase in abortion rate, an increase in abortions, higher incidence cleft palate and neural plate defects[15,16].

It has been pointed out that in pregnant women taking more than 7 cups of tea/coffee per day, there is an increase in the incidence of spontaneous abortion, preterm labour and still birth[14,15,16].

No work has been done on the effect of caffeine on the cells of various tissues in developing embryos.

Materials and Methods

For this research work 60 female and 24 adult male albino rats were obtained from Veterinary Research Institute, Lahore. The animals were kept in well-ventilated and illuminated room under identical conditions in Animal House of the Anatomy Department of King Edward Medical College, Lahore. for 15 days animals were kept without treatment to allow for acclimatization and were provided with chick feed No.3 and tap water adlibitum.

Mating was started after 2 weeks by placing 6 female (age 8-12 weeks, weight 150-200gm) and 2 male sexually mature albino rats in one cage.

The pregnant female rats were divided into 4 groups A, B, C, D. The group A was kept as control (non-treated group) comprising of six pregnant rats. The other 3 groups B, C and D contained 18 pregnant rats and served as experimental (treated) groups. The animals in these groups were given intra-muscular injection of caffeine during first, second and third week of pregnancy respectively.

The experimental groups were further divided into 3 sub-groups B1, B2, B3, C1, C2, C3 and D1, D2, D3, comprising of six animals each. Sub-groups B1, C1 and D1 were given 100mg/kg of caffeine daily during 1st, 2nd and 3rd weeks of pregnancy, respectively. Sub-groups B2, C2 and D2 were given 150mg/kg of caffeine daily during 1st, 2nd and 3rd weeks, respectively. Whereas sub groups B3, C3 and D3 were given 200mg/kg of caffeine daily during 1st , 2nd and 3rd weeks respectively.

Intramuscular injections were prepared by dissolving 50mg of caffeine in one ml of distilled water. Injections were given in gluteal region.

Throughout pregnancy, the animals were closely watched for increase or decrease in food and water intake, behavioral changes and ante-partum haemorrhage. On the twenty fifth day the animals were weighed and then sacrificed after giving ether anaesthesia. A midline abdominal incision was given, uterus together with fetuses was removed and fixed in Bouin's solution for forty-eight hours. The fetuses were then dissected and their liver removed. These were separately weighed and were put in 70% alcohol with several changes till no more Bouin's fluid came out of them. Tissues were then processed embedded, sectioned at 5-6μm and stained with Ehrlich's Haematoxylin, Eosin and PAS.

Observation

Group A Control

Mean weight of single kidney – 64.14mg (Table A).

- The kidneys were enclosed in a connective tissue capsule and divided intocortex and medulla. The cortex contained a number of glomeruli, with Bowman's capsule. The mean diameter of glomeruli was 15μm and the mean capsular space was 1μm. Also present in the cortex were numcrous tubules, sectioned obliquely and transversely, the transversely sectioned tubules were of two types, one with smaller lumen and larger cells (proximal convoluted tubules)

and the other with larger lumen (distal convoluted tubules .

- The medulla had connective tissue stroma. Two types of tubules with mean diameter of 2 and 5μm were present.
- The thickness of cortex and medulla was approximately equal (100 and100μm respectively), with both containing numerous scattered capillaries.

Subgroups B1,C1, D1

- 100mg/kg caffeine given during 1st, 2nd and 3rd week of pregnancy respectively.
- Mean weight 67, 5, 51 and 56.5mg (Table A).
- Microscopically no change was observed in B1, C1 and D1, the capsular space was increased to 2μm and 1.5μm respectively, and an increased connective tissue stroma with fewer normal tubules was seen in medulla.

Subgroups B2, C2, D2

- 150mg/kg caffeine given during 1st, 2nd and 3rd week of pregnancy respectively.
- Mean weight 55.9, 42.7 and 41.5mg (Table A).
- Microscopic findings were similar to the previous subgroups in B2. In C2 and D2 the capsular space was further increased to 3μm with appearance of some degenerated glomeruli. The medulla had abundant connective tissue stroma with very few tubules.

Subgroups B3,C3,D3

- 200mg/kg of caffeine given during 1st, 2nd and 3rd week of pregnancy respectively.
- Mean weight 50, 35, 9, 89, 3 (Table A).
- Majority of the glomeruli appeared as clumps of dead cells with large capsular spaces (4-5μm). Medulla had very few tubules and an abundant connective tissue stroma the thickness of medulla was decreased as compared to that of the cortex (70μm and 100μm respectively).

Table A: Comparative table of mean foetal kidney weight.

Groups	Mean Wt. of fetal kidney	Standard deviation	Comparison of experimental groups with control
A	64.14	4.04	
B1	67.5	4.6	P<0.05 insignificant
B2	55.9	4	P<0.05 insignificant
B3	50	7.2	P<0.05 insignificant
C1	51	7.7	P<0.01 significant
C2			P<0.01 significant
C3	35.9	7.05	P<0.01 significant
D1	56.5	7.8	P<0.05 insignificant
D2	41.5	8.96	P<0.01 significant
D3	89.3	18	P<0.01 significant

Discussion

When caffeine is administered at the rate of 150mg/kg during the first week of pregnancy, it does not produce a significant change. However, when givend uring the second and third week it produces and increase in the capsular space and some degeneration in the glumeruli, an increase in the connective tissue stroma of medulla and reduced number of tubules. These effects are further accentulated in the embryos of rats receiving 200mg/kg. THe observations show that caffeine when administered to pregnant female rats adversely affects the development of kidney in the developing embryos and that the effect is both time and dose dependent.

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