The Effect of Caffeine on Liver During Foetal Development: An Experimental Study in Albino Rats

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Caffeine when given in various doses has been shown to produce significant changes in the histology of liver in developing foetuses.

Key Words: Albino rats, effect of caffeine, Foetal development

Caffeine is widely used by human beings as a component of various drugs or beverages. Although it was identified as a potential teratogen in early 1960, it was not considered serious as malformations due to caffeine had not been reported in human beings till then. In 1970 Food and Drug Administration of the USA cautioned pregnant women not to take caffeine or to take it sparingly.

Caffeine has been shown to be a teratogen in mice when given in doses of 50-200mg/kg for two weeks[1]. It was shown to reduce the reproductive capacity, reduce the foetal size and weight in mice receiving caffeine during pregnancy[2]. After a study of the effect of caffeine on four generations of mice it was found to reduce fertility, increase incidence of premature deliveries, reduce litter size and increase the incidence of digital defects[3]. Both brewed and instant coffee given to two generations of rats lowered the birth weight, delayed ossification and enlarged liver and kidney[4,5].Similarly studies on the effect of caffeine on developing chick embryo revealed that it produces gross morphological defects in them[7,8,9]. Although, caffeine has been shown to interfere with various biochemical processes in the cell[10], little information is available on the effects of caffeine on the histology of various tissues in developing embryo. The present study was, therefore, designed to investigate the effect of caffeine on the histology and morphology of liver in developing rat embryos.

Material and methods

For this research work 60female 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.For15 days animals were kept without treatment to allow for acclimatization and were provided with chick feed No.3 and tap water ad libitum.

Mating was started after 2 weeks by placing 56 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 intramuscular injection of caffeine during first, second and third week of pregnancy respectively.

The experimental groups were further divided into 3 sub-groups B_1, B_2, B_3, C_1, C_2, C_3 and D_1, D_2 and D_3,comprising of; six animals each. Sub-groups B_1, kC_1 and D_1 were given 100mg/kg of caffeine daily during 1ast,2nd and 3rd weeks of pregnancy, respectively. Sub groups B_2, C_2 and D_2 were given 150mg/kg of caffeine daily during 1st,. 2nd and 3rd weeks, respectively. Were as sub-groups B_3, C_3 and D_3 were given 200mg/kg of caffeine daily during 1st, 2nd and 3rd week, 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, behavioural 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 foetuses was removed and fixed in bouin's solution for forty-eight hours. The foetuses 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 5-6μm thick and stained with Ehrlich's Haematoxylin, Eosin and PAS.

Miscorscopic observation

After staining, the liver tissues were observed under a light microscope at various magnifications. Comparison between untreated liver cells and treated cells was done by observing various categories of the cells. The size, shape and position of cells was noted. Variation in number of cells was noted by counting the number of cells of different groups under a microscopic field.

Observations

Macroscopic appearance: Mean weight of foetal liver was 244.16mg. After fixation the foetal liver was dull brown in colour, with well developed right and left lobes.

***Microscopic appearance*:** The liver was surrounded by a connective capsule with no lobulation. Two types of cells were seen.

1. Hepatocytes
2. Blood cells

Hepatocytes appeared as rounded cells with homogenous cytoplasm and a rounded vesicular nucleus situated in the centre.

Blood cells-both red and white blood cells were present. Mature red cells were identified as pale staining cells with a lighter centre, normoblast were present in between mature red blood cells and were identified because of their eccentric nucleus. White blood cells were present in various stages of maturation, few megakaryocytes were also seen, these were large cells with homogenous irregular cytoplasm and contained 3-4 nuclei. The larger blood vessels had a well defined endothelial lining, whereas in the developing blood vessels, present in between the cells, a well defined endothelial lining was absent. Between the cells there were clear spaced, probably developing bile ducts.

Sub-group B_1, C_1, D_1:

150 mg/kg of caffeine given during 1st, 2nd and 3rd week of pregnancy respectively. Mean liver weight was 223, 171,181mgrespectively.There was no change in the gross appearance, the number of cells was considerably less with large intercellular spaces, while in C_2 and D_2 there was no formed blood vessel and number of developing blood vessels was also decreased. (Fig. 3).

Sub-group B_3, C_3, D_3:

200mg/kg of caffeine given during 1st, 2nd and 3rd week of pregnancy respectively. Mean liver weight was190, 154 and 140mg respectively, showing a slight decrease in B_3 also with marked reduction in C_3 and D_3.

Microscopically the changes already identified in previous subgroups were more enhanced with complete disappearance of developing blood vessels (Fig.4).

Discussion

A number of studies have reported gross morphological changes in developing embryos[2,5]. Little information is available on the effect of caffeine at the cellular level. Major changes caused by the administration of caffeine include a significant reduction in the mean liver weight in embryos receiving 100mg/kg and 150mg/kg given during1stand 2nd week of pregnancy and 200mg/kg of caffeine given during 1st,2nd or 3rd week of pregnancy.

However, the gross morphology of the liver was not affected by the treatment and all the characteristic lobes were distinctly present.

Although various; types of cells were present, there were significant changes in the number and structure of cells.

In rats receiving100mg/kg of caffeine the overall number of cells decreased and loose connective tissue appeared in intercellular spaces. This is an indication of inhibition in cell division. The increase in connective tissue may be due to a change in normal protein synthesis in liver.

When 150mg/kg of caffeine was given in addition to changes mentioned above decrease in the number of blood vessels was also observed. Similarly, when 200mg/kg was administered these changes were further increased and the development of blood vessels was almost completely inhibited. These changes may be due to defects in the cells which inhibits the passage of certain inducing substances or reduced supply of oxygen due to the damages.

The changes observed in liver cells are suggestive of changes in their metabolism. Such
metabolic changes have been observed previously.

Caffeine is known to increase 3' 5' cyclic monophosphate in cells and may; interfere withcellgrowth[1].Caffeine is structurally similar to adanine and guanine and therefore, may act directly on nucleic acids to cause chromosomal aberrations[10].Foetal hypoxia caused by caffeine can interfere in nucleotide metabolism and ultimately inhibit DNA synthesis and cell division.

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