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ALTERED MORPHOLOGY OF LIVER AND PANCREAS TISSUES OF OFFSPRINGS OF ALBINO RATS BY CHARRED MEAT

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Summary: The effects of food processing and or cooking practices in the variations in morphological changes in tissues were investigated. The relationship between consumption of charred meat, which is believed to be rich in nitrosamine by pregnant mothers and the adverse effects on the growth of their offsprings, alterations in morphology of tissues like liver and pancreas were studied. Meat was subjected to charcoal fire roasting without curing and was thereafter fed to pregnant rats. The results shopwed growth retardation of the offsprings, micromorphological changes in tissues such as liver (generalized apoptotic processes and hepatocellular necrosis) and pancreas (increased islet cells density and scattered acinar hyperplasia with solid cellular area) in the offsprings of the female albino rats that were fed on 60 and 80 percent of charred meat regimen during gestation and lactation periods when compared to control. These observations have shown that meat curred or uncured when subjected to charcoal fire roasting may cause alteration in the morphology of the foetal tissues.

Key Words: *Charred Meat; Growth Retardation; Tissue Morphology; Liver; Pancreas.*

Introduction

In the mid sixteenth century, Paracelsus asserted that all substances are poisons, and that there is none which is not a poison. The right dose differentiates a poison and remedy (Paracelsus 1493 – 1541). This assertion was true then, it is still true today, and will continue to be true in pharmacology and toxicology.

Our environment contains a great variety of naturally occuring carcinogenic factors which have been speculated to play a major role in cancer formation (Nobuyuki, 1998) and to cause serious pathological abnormalities in human and animals. Humans are exposed concurrently and sequentially to a large variety of environmental carcinogens, ranging from foods, food additives to food processors. (Rywotycki, 2000, Smith, 1991, Sugimura, 1985).

Several chemicals such as nitrates and nitrites used as food additives and for meat curing, are ubiquitous in plants, are found naturally in many foods, and are implicated in liver and kidney cancers when subjected to dry heating. Their property to be converted to nitrosamines (Scalan, 2003), which are potential carcinogens, poses a serious health hazard.

Food processing, and or cooking practices can cause large variations in the total mutagenic activity and in the amount of specific mutagens present in muscle-containing foods which can lead to different toxic effects (Felton and Knize 1990) Studies have shown that dry heating actually produces greater percentage of some types of carcinogens compared with aqueous heating (Commoner, 1998). Compounds like Dimethyl Nitrosamine (DMN) and Diethyl Nitrosamine (DEN) which are naturally present in meat in very low concentration (Rywotycki, 2000) are carcinogenic to liver, and kidney (Takayuki and Bjeldanes, 1993) and perhaps other organs when subjected to dry heating during roasting. A nitrosamine-rich diet of cured mutton has been shown to cause damage to pancreatic beta cells (Helgason *et al* 1982) in a study on mice. It is therefore, that when meat is charcoal-fire roasted and consumed frequently and in large quantity, the nitrosamine exposure will be high and hazardous to the exposed individual, especially, the foetus.

With the high consumption rate of popularly known roasted meat “soya meat” in Nigeria, for example in Calabar, Municipality, Nigeria especially by pregnant women, some of whom crave for such meat from the roadside; poised us to examine the extent of alterations in morphology of foetal liver and pancreas and the effect on their growth.

Materials and Methods

Animals: Twenty female albino rats (disease free) were obtained from the animal house of the Department of Pharmacology, College of

Medical Sciences, University of Calabar. They were caged in plastic cages with stainless steel mesh floor and well ventilated tops. The cages were equipped with stainless steel feed containers and plastic drinkers with stainless nozzles. Saw dust was used as beddings and was changed daily. The animals were housed under a hygenic, and well ventilated environment at room temperature of 29 ± 1^0c and 12 hours day light/darkness cycle. The feed, bought from a commercal stock (Agro Feed Nigeria Plc) in Calabar and the tap water were available ad libitum.

The Feed:

The fresh cow meat was purchased from Calabar abattoir. The meat was carefully prepared to remove fats, bones and other particles, washed using running tap water. It was sliced into small pieces, and in bits placed on a wire gauze without treatment with spices or cured. It was then roasted with charcoal fire on a tripod burner. With periodic turning, the meat reached edible state, and was ground using manual grinding device (Victoria Grain Mill) to a mash that was stored in a glass container for gradual use. The mash (ground meat) was mix with grower mash from Agro Feed Nigeria Plc in a percentile ratio.

Animals:

Twenty female non-pregnant albino rats with mean weight of 127.8 ± 3.79 were randomly selected, and were divided into four groups of five rats each. All the female rats were mated with adult male rats restrained from sex for seven days to increase their sexual urge for opposite female sex. The male rats were withdrawn after 10 days after the pregnancy had occurred prior to experimental feeding pattern. The female rat of each group was fed on normal feed and mixed with charred meat corresponding to dosages and feeding patterns described by Essien and Akpan (2006), throughout the gestation period and three weeks thereafter, except on the night preceeding the morning prior to sacrifice.

For growth determination, the weights of female rats were recorded on zero day, and on the day of sacrifice. While the weights of all the litters (offsprings) in each group were recorded on the day of delivery and on the day of sacrifice. (three weeks thereafter)

Histopathological Analysis:

Selected tissue specimens viz. liver and pancreas, for histopathological analysis were carefully dissected from the mother rats and their corresponding litters during the sacrifice. All the tissues were blotted dry using blotting paper, weighed and recorded. They were further subjected to normal routine histological procedures, stained with Hematoxylin-Eosin and examined using the light microscope. The main significant histopathological changes, especially morpholocellular changes were noted and recorded.

Results

The effect of charred meat intake by mother rats on the body weight of their corresponding offsprings is shown in Table 1, Fig.1 and Fig. 2A and 2B. The results showed a dose related significant ($P<0.05$) reduction in body weight of the offsprings compared with the control. The photograph in Fig.1 showed growth retardation among the offsprings of mothers that received high dosages of charred meat. However, there were no significant ($P<0.05$) weight changes in the mothers compared with the control. Histograph in Fig.3A shows section of the liver of the offsprings of pregnant rats fed on normal feed wihout charred meat (control), while data in Fig.3B, C and D show sections of the liver of the offsprings of pregnant rats fed on 40, 60, and 80 percent of charred meat respectively, showing changes in cell morphology. Cell morphology changes include generalized apoptotic and hepatocellular necrosis in the liver of the offsprings whose mothers received high dosages (60 and 80 percent) of charred meat. Fig.3C & D show hepatocellular dysplasia, enlarged and prominent nuclei, and loss of polarity of cells, and widespread of hepatocellular necrosis and apoptosis (Fig. 3D).

Table 1: Effect of charred meat intake on the body weight of mother rats and their offsprings.

Dose	Mother Rat Initial Weight	Final Weight	Change in Body Weight	Offspring Initial Weight	Final Weight	Change in Body Weight
Control	147.8 ± 4.38	196.3 ± 3.25	48.5 + 15.38	6.6 ± 0.11	26.3 ± 0.47	19.7 ± 0.44
40	111.0 ± 0.0	166.5 ± 4.95	55.5 ± 4.95	6.5 ± 0.14	24.3 ± 0.34	17.8 ± 0.23*
60	129.3 ± 1.64	146.3 ± 16.52	17.0 ± 14.89	6.3 ± 0.12	12.6 ± 0.25	6.3 ± 0.27**
80	130.0 ± 11.06	159.7 ± 14.64	29.7 ± 11.92	6.2 ± 0.09	10.0 ± 0.34	3.8 ± 0.29**

* - *P<0.05; ** - P<0.01*

Fig. 4B, C and D show sections of the pancreas of the offsprings of the pregnant rats that were fed on 40, 60 and 80 percent of charred meat compared with the control (Fig.4A). There were marked changes in cell morphology, ranging from increased islet cells density (Fig 4B) to scattered acinar hyperplasia with solid cellular areas (Fig 4C and D).

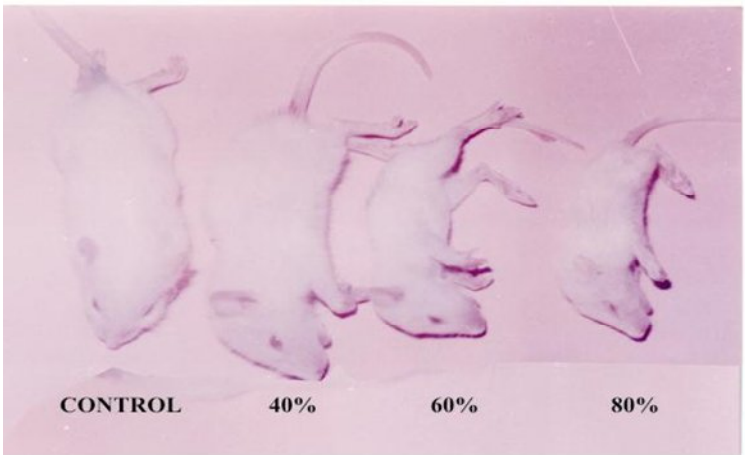

Fig 1: Photomicrograph of three weeks old litters showing differences in size corresponding to feeding mothers with percental mixture of charred meat as indicated.

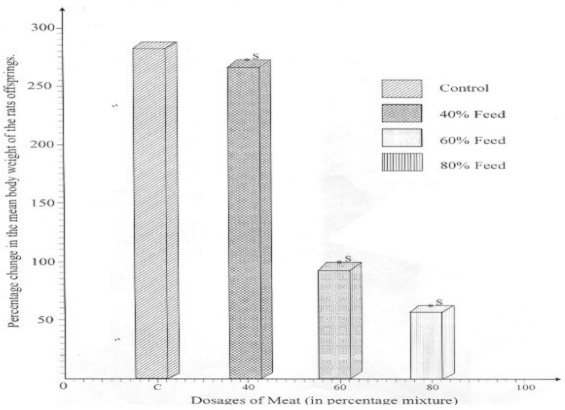

Fig. 2A: Mean percentage changes in body weight of litters of pregnant rats that received charred meat during gestations and three weeks compared with the control

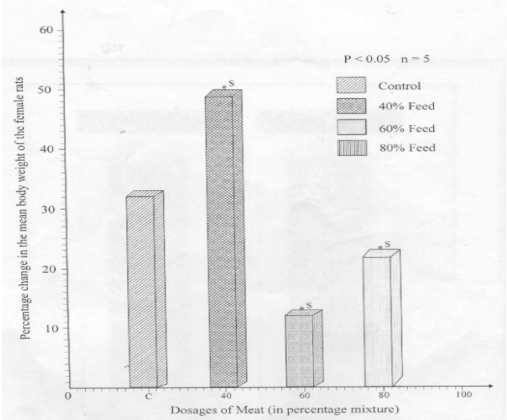

Fig.2B: Percentage change in mean body weight of mother rats showing no relation between weight change and feeding pattern.

Discussion

The results of this investigation show that ingestion of large quantity of charred meat by pregnant rats during gestation and lactation periods resulted in growth retardation among their offspring and alteration in morphology of liver and pancreas of their offfsprings. This is in line with the report that effect of charred meat depends, to a greater degree, on the details of processing (Felton and Knize, 1990 and Commoner, 1978). It is likely that the naturally occurring nitrosamine in meat, when subjected to charcoal fire roasting, could be raised to a concentration toxic enough to cause alteration in morphology of tissues of liver and pancreas and perhaps other organs.

Curred meat was not used for this study since nitrates and nitrites used in meat curing could be influenced by bacterial action in Gastro-Intestinal Tract and heat to form series of different N-nitroso compounds, like nitrosamines and nitrosamides (Rywotycki 2000). Intensity of charcoal fire and the uniformity in the distribution of heat were not of essence in the study since the aim was to simulate condition almost identical to that obtained locally in "soya meat" preparation. Refrigeration of the meat was avoided in order to prevent possible decomposition of some food components in the meat. It is worthy to note that DMN and DEN present in meat, when subjected to heat acted synergistically with other mutagenic agents such as polycyclic aromatic hydrocarbons and heterocyclic amines (Veith 2004 and Sugimara, 1985). These compounds have been found in vary traceable amounts in meat, especially, when subjected to smoking and roasting. This study is in accord with the view expressed by Scalan (2003) that most nitrosamine are mutagens and some are transplacental carcinogens, and hence cause alterations in tissue morphology. Takayuki and Bjeldanes (1993) reported that DMN added to diet resulted in a dose-related morphological changes in kidney, and the present study shows a dose-related morphological changes in liver and pancreas of the litters of the experimental pregnant rats fed on high dosages of charred meat.

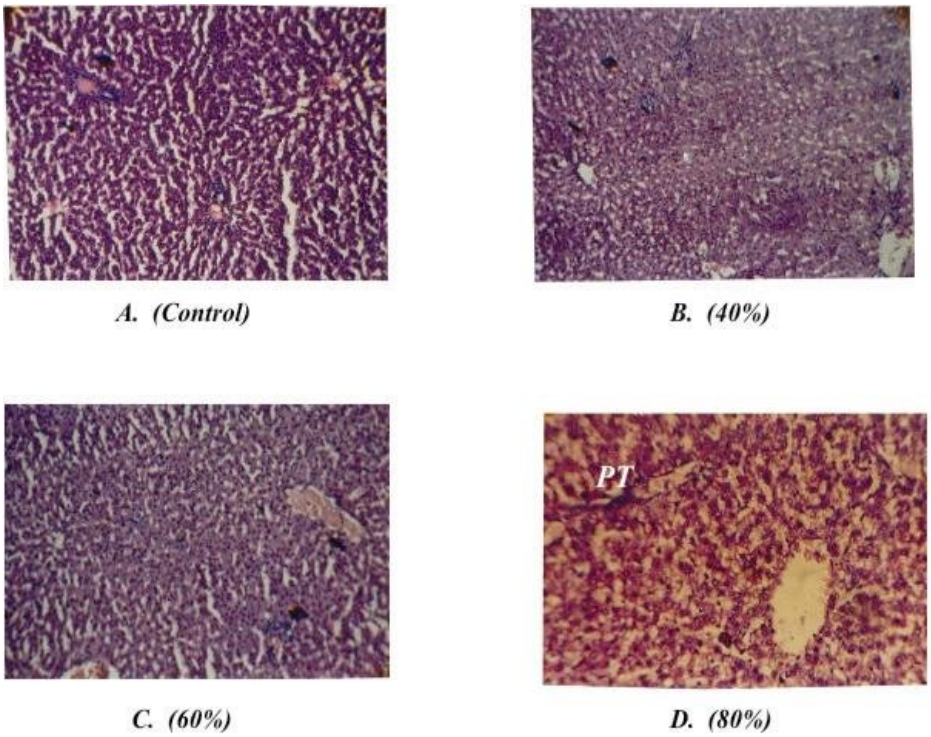

Fig.3: Section of the liver of litters of the pregnant rats fed on normal feed (A-control) and different doses of charred meeat (B-D) showing dose related changes in the cell morphology (H&E x300). B-shows mild apoptotic process of the hepatocytes; C-dyspatic hepatocytes have enlarged and prominent nuclei; D-Shows widespread hepatocellular necrosis and apoptosis PT (portal tract) - markedly inflamed.

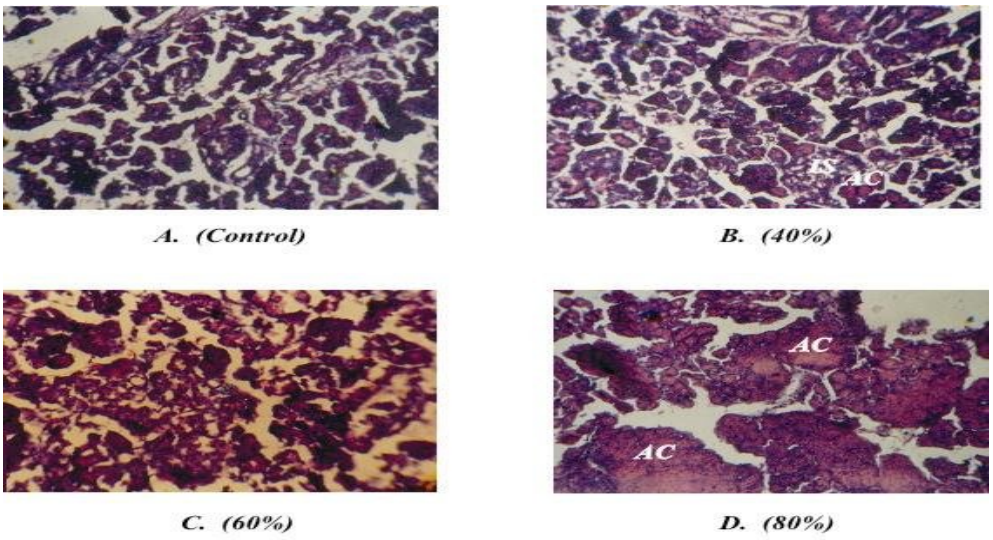

Fig.4: Section of the pancreas of the litters of the pregnant rats fed on normal feed (A-control); and various percentages of charred meat. (H&E x300). B –shows increased islet cell density. The islet cells (IS) and acinar (AC) appeared normal; C-Acinar are hyperplastic with solid cellular areas D widespread of markedly hyperplastic acinar with solid cellular areas are observed.

Conclusion

The basic aims and objectives of this study were to examine the role of food processing practices as a causative factor in inducing morphological changes in the offsprings of the exposed mothers to this feed. The hypothesis has been confirmed that pregnant rats fed on graded amounts of charcoal-fire roasted meat during gestation and lactation for three weeks resulted in dose-related weight retardation, morphological changes in liver and pancreas of offsprings whose mother received higher doses (60 and 80 percent) of charred meat. Hence, charred meat consumption regularly in large quantity during pregnancy alters the tissue morphology, leading to pathological changes in the affected organs, and growth retardation of their offsprings. Pregnant women craving for "soya" meat should be advised on the health hazards involved in consuming large quantity of roasted (soya) meat.

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