HISTOLOGICAL ALTERATIONS IN THE LIVER OF MOTHER RATS AND ITS WEANLINGS FED ON FRIED BREAD AND THE PROTECTIVE EFFECT OF CURCUMIN

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ABSTRACT

This study dealt with the evaluation of the histological alteration due to feeding on fried bread and the protective role of curcumin on the liver of pregnant rats and their offspring. The results showed that feeding on fried bread caused different histopathological lesions in the liver of female rats and their offspring closely similar to those induced by feeding on a diet containing acrylamide.

These lesions included vacuolated cytoplasm, dilated blood vessels, congested blood vessels, haemorrhage, pyknosis, necrosis and complete degenerated areas. The emerged data showed that fried bread induced histological alterations in the liver of rats similar to those caused due to acrylamide in food. The present findings suggested that curcumin has slight protective effect against the degenerative effects of fried bread.

Key Words: Histological Alteration - Curcumin – Bread — Acrylamide.

INTRODUCTION

Discovery of the presence of a “probable carcinogen” in a range of fried and oven-cooked foods by Swedish scientists caused worldwide concern. Carbohydrate rich foods were posed as a cancer threat in the headlines, and food manufacture, consumers and health professionals quickly sought additional information guidance and reassurance (Ahn et al., 2003). The mechanism behind the formation of acrylamide when food is cooked has been published as Maillard reaction, which occurs between amino acids and reducing sugars that can generate acrylamide when food is heated above 100 °C. The higher the temperature used to prepare a starchy food and the longer it is cooked, the more likely that the three dimensional chemical structure of its enzymes will denature and become inactive, ineffective or worse yet, a toxic, cancer-causing mutagen like acrylamide (Mottram et al., 2002).

Marlowe et al. (1986) indicated that acrylamide is efficiently absorbed from the stomach and eliminated by the liver, kidney, and pancreas. A previously unrecognized affinity of acrylamide or a metabolic product was demonstrated for fetal skin in late gestation and for adult epithelia of oral cavity, esophagus, stomach, and bronchi. Also, acrylamide or its metabolite appears to bind to spermatids at specific stage near maturation.

Tareke et al. (2000) showing that a strong increase in the level of acrylamide induced Hb adduct in rats fed on fried diet, compared to control rats fed on un-fried diet. Also Tareke et al. (2002) observed a reaction products (adducts) of acrylamide with N termini of hemoglobin. They added that moderate levels of acrylamide (5-50 microgram/Kg) were measured in heated protein-rich foods and higher contents (150-4000 µg/Kg) in carbohydrate-rich foods, such as potato products and crisp bread. Acrylamide could not be detected in unheated control or boiled foods (<5 µg/Kg). Histopathological alterations in liver, kidney, brain and erythrocyte due to acrylamide exposure were documented (Dixit et al., 1984; Segerback et al., 1995 and Gamboa et al., 2003).

Recently turmeric is gaining a lot of attention for its potential medicinal properties. Curcumin is the major yellow coloring matter isolated from turmeric plant (Curcuma longa). Curcumin (diferulylmethane), a phenolic compound, has been shown to possess antioxidant, free radical scavenging, anti-inflammatory, antieancer, anti-thrombotic, hypoglycemic and hypocholesterolemic effects (Ruby et al., 1995; Chuang et al. 2000 and Devasena et al., 2002). Moreover, curcumin is not toxic for human up to 800 mg/kg day (Cheng et al., 2001).

The present study aims to evaluate the histological alterations in the liver of female rats and their progeny fed
on diet containing fried bread and also to investigate the possible protective effect of curcumin.

MATERIALS AND METHODS

Forty two adult virgin female rats weighing (180-200 g) were obtained from the Experimental Breading Farm at Helwan, Egypt

Fried breads were prepared at home, mixed with basil diet at concentration of 15% and 30% supplied for feeding female rats for 6 weeks before and after pregnancy. Powder curcumin (turmeric) was obtained from the market and mixed with basil diet at concentration of 2% and supplied for feeding during experimental period (Gamboa et al., 2003).

Control and all experimental groups were placed in cages overnight with untreated males (in a ratio of 1 male to 3 females). Then, in each morning vaginal smears were prepared and examined under the microscope. The presence of sperms in the vaginal smear during estrus denoted day zero of gestation.

Female rats were arranged into 7 groups each was composed of 6 pregnant rats as follows:

1- Control pregnant rats fed basil diet.
2- Acrylamide treated pregnant rats (daily oral doses of 25 μg/kg for 3 months).
3- Acrylamide + 2% curcumin treated pregnant rats.
4- Rats fed on Fried bread (15%) for 3 months.
5- Rats fed on Fried bread (15% + 2% curcumin) for 3 months.
6- Rats fed on Fried bread (30% + 2% curcumin) for 3 months.
7- Rats fed on Fried bread (30%) for 3 months.

The mother rats of both control and experimental groups were sacrificed after 12 weeks of beginning of experiment and weanlings rats aging 3 weeks.

Livers of female and their weanlings were removed, fixed in 10% formal saline, dehydrated, cleared and embedded in paraffin wax. Sections were cut at 5-7μ thickness and stained with haematoxylin and eosin.

RESULTS

Control liver

The normal structure of the liver of control female rat is shown in Figure (1). The hepatic lobule contains a central vein from which radiate, anatomicizing and branching cords of hepatic cells. The spaces lying between these plates constitute the hepatic sinusoids surrounded by a discontinuous layer of flattened endothelial cells with a little amount of cytoplasm and flattened darkly stained nuclei. Kupffer cells are distinctly large and possess prominent nuclei. They appear frequently bulging into the sinusoidal lumens. The hepatocytes are polyhedral in shape and enclosing a homogeneously fine granulated acidophilic cytoplasm and embodying a centrally placed large spherical nucleus, a prominent nucleolus and distinct chromatin particles. The liver structure of weanlings is similar to that of mother rats.

Experimental groups

The liver of female rats fed on diet containing acrylamide (25 μg/kg) exhibited different histopathological lesions. Signs of cell degeneration, pyknosis, necrosis vacuolation, congestion and bleeding were perceptible in most parts of the hepatic tissue (Fig. 2). The addition of curcumin in the diet as a protective agent against the side effects of acrylamide did not prevent these effects in female rat liver. Inspected liver sections of rats of this group showed mostly the same degenerative changes recorded in the liver of rats fed on diet containing acrylamide. Figure (3) shows dilated blood sinusoids, oedematous blood vessels and some karyolysis in the nuclei of the hepatocytes of this group.

Inspected liver sections obtained from female rats fed on diet containing 15% fried bread for three months showed different alterations in comparison with control. These alterations were manifested in vacuolation in the hepatocytes cytoplasm, karyolysis in hepatocyte nuclei, dilatation in blood vessels, and some of these blood vessels were engorged with blood. Moreover increase of Kupffer cells and some necrotic cells were noticed (Fig. 4). The addition of curcumin simultaneously with diet containing 15% fried bread ameliorated the hepatic pattern in rats, whereas different histological lesions were still found. Dilated blood vessels and pyknosis were encountered in different parts of the liver tissue of this group (Fig. 5).

Severe histopathological lesions were noticed in the liver tissue of female rats fed on diet containing 30% fried bread for three months, where, lymphocytic infiltration, vacuolation, pyknosis, and focal necrosis were delineated in different parts of the tissue (Fig. 6). A slight protective effect for curcumin against the adverse effect of fried bread could be detected in the liver of female rats after three months of feeding. Investigated liver sections of this group revealed congested blood vessels, pyknotic hepatocytes nuclei, and vacuolated hepatocytes cytoplasm (Fig. 7).

Feeding on diet containing acrylamide induced severe histological changes in the liver of weanlings similar to those observed in the liver of mothers. Inspected liver sections obtained from this group revealed dilated blood vessels, vacuolated hepatocytes cytoplasm, pyknotic nuclei and necrotic cells were greatly encountered. Investigated liver sections from this group showed oedematous blood vessels, inflammatory cells, necrotic cells and complete degenerated area (Fig. 8). These lesions denoting that curcumin has no protective effect against the adverse effect of acrylamide.

The feeding on diet containing 15% of fried bread for three months resulted in some degenerative changes in the
Figure 1: Liver section of control female rat showing central vein (C.V.), hepatic cords (H.C.) and hepatocytes (H.). (H/E X200).

Figure 2: Liver section of female rat fed on diet containing acrylamide showing necrosis (N.), pyknosis (P.), vacuolation (V.), and degeneration (D.). (H/E X 400).

Figure 3: Liver section of female rat fed on diet containing acrylamide and curcumin showing oedematous blood vessel (O.), hyperthrophied Kupffer cells (K.), increase of endothelial cells and dilated blood sinusoids (D.S.). (H/E X 400).

Figure 4: Liver section of female rat fed on diet containing 15% fried bread showing vacuolation (V.), karyolysis (Kr), increase of Kupffer cells (K) and necrotic cells (N.). (H/E X 400).

Figure 5: Liver section of female rat fed on diet containing 15% fried bread and curcumin showing dilated blood vessel (D.B.V.) and pyknotic nuclei (P.). (H/E X 400).

Figure 6: Liver section of female rat fed on diet containing 30% fried bread showing pyknosis (P.), vacuolation (V.) and necrotic area (N.). (H/E X 400).
Figure 7: Liver section of female rat fed on diet containing 30% fried bread and curcumin showing congested blood sinusoids (C.B.V.) and vacuolated hepatocyte cytoplasm (V.). (H/E X 200).

Figure 8: Liver section of weanling rat fed on diet containing acrylamide and curcumin showing necrotic cells (N.) and degenerated areas (D.). (H/E X400).

Figure 9: Liver section of weanling rat fed on diet containing 15% fried bread showing haemorrhage (H.), lymphocytic aggregation (L.), degeneration (D.) and vacuolation (V.). (H/E X400).

Figure 10: Liver section of weanling rat fed on diet containing 15% fried bread and curcumin showing haemorrhage (H.), oedema (O.) and pyknosis (P.). (H/E X200).

Figure 11: Liver section of weanling rat fed on diet containing 30% fried bread showing vacuolation (V.), haemorrhage (H.) and necrosis (N.). (H/E X 200).

Figure 12: Liver section of weanling rat fed on diet containing 30% fried bread and curcumin showing increase of Kupffer (K.) and endothelial cells (E.) and pyknotic nuclei (P.). (H/E X 400).
liver of weanling rats. Liver sections obtained from this group revealed necrotic changes in the hepatocytes, vacuolation in the cytoplasm, congestion in the blood vessels, haemorrhage, proliferation of lymphocytes and some degenerated areas (Fig. 9). Investigated liver sections obtained from weanlings maternally fed with 15% fried bread in combination with curcumin for three months exhibited incomplete protective effect against the side effects of fried bread. Pyknosis, oedematous and congested blood vessels, vacuolated cytoplasm and haemorrhage were still perceived (Fig. 10).

The results showed that fried bread feeding (30% of the diet) caused drastic effects in the hepatic tissue of weanling rats similar to those obtained in the case of mothers. Figure (11) shows congestion in blood vessels, increase of inflammatory cells, haemorrhages, focal necrosis and vacuolation in the hepatocytes cytoplasm. The role of curcumin as protective agent against the side effect of fried bread feeding was well defined. The liver of this group exhibited an increase in both endothelial and Kupffer cells, while most of the hepatocyte nuclei were mostly karyolitic or pyknotic (Fig. 12).

**DISCUSSION**

The levels of acrylamide was analyzed in over 100 Swedish food samples including bread, pasta, rice, fish, sausages, beef, pork, biscuits, breakfast cereals, beer, ready meals, pizzas, and popcorn (Tareke et al., 2002 and Ahn et al., 2003).

The present study clarified close relationships between feeding on diet containing fried bread and histopathological abnormalities in the liver of female albino rats during 3 months (6 weeks before gestation and 6 weeks after gestation). The histological defects of the hepatic tissue were characterized by increased fatty changes and dilatation of the central and portal veins and sinusoids in the hepatic cells of female rats of the experimental groups fed on diet containing fried bread. Similar histopathological alterations in livers of weanling rats maternally the females of experimental groups with highest intensity of lesions in those maternally fed on fried diet. These histological effects were closely similar to those obtained after exposure to acrylamide.

The histopathological defects in maternal tissues reflected the abnormal histological pattern in weanling offspring. These may be attributed to the transplacental passage of acrylamide and its metabolites to weanling tissues interfering with cell differentiation and exerting those drastic effects on their histological pattern (Ikeda et al., 1985).

From the present findings, not only acrylamide exerted intoxication but also swallowing of fried bread. Diembeck et al., 1998 reported that acrylamide may be readily absorbed from skin during handling of slices of fried diet during eating. Once acrylamide absorbed, it was conjugated by glutathione- S- transferase to N- acetyl- S- (3- amino-3 oxopropyl) cysteine or reacted with cytochrome P_{450} to produce glycidamide (Miller et al., 1982; Calleman et al., 1990; Bergmark et al., 1991 and Sumner et al., 1999). Glycidamide formation occurred as a result of marked inhibition of glutathione- S- transferase by acrylamide (Mukhtar et al., 1981 and Dixit et al., 1982).

Furthermore glutathione- S- transferase is a detoxifying enzyme in the liver and takes many isofoms and plays a great role in maintaining cell function. Conjugation of acrylamide or glycidamide with this enzyme may interfere with cell function and promote cell death. Bergmark et al. (1991) and Barber et al. (2001), established that the deleterious effects of acrylamide or its metabolite glycidamide seemed to be the formation of adduct with sulfhydryl groups on hemoglobin and other proteins. These may reduce the hemoglobin surface of carrying oxygen to tissues causing cell degeneration.

Segerbach et al. (1995) identified that injection of acrylamide tend to caused formation of DNA adducts. This formation of DNA adduct may interfere with cell replication causing cell damage. Increased DNA adducts in liver of adult rats may explain the systemic effect of the acrylamide and its metabolites and may illustrate the induced cell damage (Gamboa et al., 2003). Autoradiographic study carried out by Marlowe et al. (1986) showed that extensive distribution of acrylamide or its metabolite in the fetal tissues, showed increased rates of binding activity in RNA, DNA and hemoglobin.

Curcumin improved survival and minimized oxidative stress, hepatocellular injury and hepatic necroinflammation (Shapiro et al., 2006). Although the use of curcumin as a protective agent against different diseases have been reported (Jain and De Filipps, 1991 and Devasena et al., 2002), a protective effect of curcumin against the adverse effects of fried bread feeding could not be detected in the present findings, whereas slight ameliorative effect could be detected in the liver of female rats and their progeny fed on diet containing fried bread simultaneously with curcumin. However, besides natural curcumin, several analogue of curcumin have been synthesized and tested (Dinkova-Kostova and Talalay, 1999 and Ishida et al., 2002). Tetrahydrocurcumin, an antioxidative substance that is derived from curcumin by hydrogenation, has been shown to have a protective effect on oxidative stress in cholesterol-fed rabbits (Naito et al., 1991 and Ishida et al., 2002). 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Studies have also been performed to examine the synergy of curcumin with other antiproliferative agents. Deeb et al. (2003) investigated whether curcumin and TNF-related apoptosis inducing ligand (TRAIL) cooperatively interact to promote death of tumor cells. The concentrations at which neither of the two agents alone produced significant cytotoxicity in tumor cells, cell death was markedly enhanced (two- to three-fold) if tumor cells were treated with curcumin and TRAIL together. Depending on the available results, more studies will be needed to evaluate the protective effect of curcumin and its derivatives against cytotoxicity induced by different chemicals.

REFERENCES


