Assess the Effect of Cucrumen in Histopathological Changes on the Cerebral Cortex of Offspring Rats Toxicity Induced By Fried Foods

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Abstract: Acrylamide is an industrial chemical formed in food containing starch like chips, crisps and bread during heat processing and preparation of food. Acrylamide used in preparing polyacrylamide which is used in water purification from organic substance, paper, oil field industries and preparation of poly acrylamide gels using in laboratory searching. The present investigation shows the effect of dietary fried food on the histoarchitecture of the cerebral cortex, female Sprague-Dawley rats were feeding with fried potato chips or fried bread alone or combined at dose 15%, 30% supplemented basil diet before 6 weeks of gestation and 6 weeks during the gestation and lactation, the offspring's reaching 3 weeks old. Microscopic examination of sections of the cerebral cortex of offspring's maternally fed on fried potato (G2) showing some cells appeared with distorted deeply stain ,malformed in pia mater with loss of density in plexus nerve fibers and dilatation blood capillary in piamater with many necrotic cells, Cerebral cortex of (G ,3,4) showed rupture piamater layer, with infiltrated inflammatory cells in molecular layer, The most prominent alterations in external granular such as most nuclei in external granular cell shrunken surrounded by spaces and dilated congested blood capillary in piamater and molecular layer with loss of density in plexus in molecular layer(G ,5,6 &7). The using of curcumen showing ameliorative cerebral tissue, represented in low doses of acrylamide (fried potato 15%, fried bread 15% mixed fried bread & potato 15%),) groups: indicated the external granular cell appeared nearly normal, with moderate ameliorative. On the other side, the groups feds on high dose of acrylamide (fried potato 30%, fried bread 30% mixed fried bread & potato 30%), showed remarkable changes, represented necrotic cells& dilated blood capillary, inflammatory tricked in pia matter and loss of plexus, necrotic cells & dilated blood capillary.

Key words: Cucrumen; Histopathological; Cerebral Cortex; Offspring Rat; Toxicity; Fried Foods

1. Introduction

Recently high levels.... were unexpectedly detected in widely consumed food items notably French fries, potato crisps, bread (WHO, 2002). French fries and potato chips are common parts of to children's menus fast in fast food restaurant, over the past35 years ; these familiar food contain high levels of toxic and carcinogenic by products not found in the uncooked foods (Mitka, 2002, Madbouly,2006). Acrylamide can be generated during the heating of specific foodstuffs as a result of Maillared reaction between amino acids and sugar (Stadler,2002, Mottram,2002). The 64th Joint FAO/WHO Expert Committee on Food Additives concluded that an intake of 1 g/kg body weight/day of ACR could be taken to represent the average for the general population (FHO/IPCS,2006). However ,infants and small children may be more highly exposed to ACR due to their lower body-weights and high consumption of snacks, so that their relative ACR intake is estimated to b 2 to 3-fold higher than in adults (FHO/IPCS,2006).The potential for exposure to ACR through human milk has been demonstrated in a study conducted with two women (Soergel et al., 2002).

Other early studies demonstrated that ACR induces cumulative neurotoxicity linked to nerve terminal damage in the CNS and PNS where distal axon swelling and subsequent to be the hallmark degeneration were considered morphological features of this toxic axonopathy (Miller and Spencer , 1985 and Lehning et al., 1998). At the molecular level, it has been postulated by Carlson and Weaver, 1985 that covalent binding of ACR to CNS proteins may play an important role in ACR toxicity, resulting in inhibition of a number of enzymes and essential compounds.

Many previous studies on ACR induced toxicity during the developmental stages were conducted with the focus on behavioral endpoints ,but detailed assessment of changes in target organs tissues has been scarce. In the present study ,we thereby performed a histopathological assessment of the frontal cortex of brain of rat offspring maternally fed on fried potato chips and fried bread during the gestation and lactation period.
In the present study, to investigated the protective effects of the well-known antioxidant curcumin on ACR induced histological changes in brain.

Curcumin (diferuloylmethane) is a natural compound present in turmeric, a rhizome of the plant Curcuma longa Lin. Plant material in the human diet contains a large number of natural compounds, which may be of benefit. In protecting the body, one of the plants with constituents reputed to possess antioxidant properties was curcumin. It is extensively used as a dietary spice and pigment in Asian cooking and also as an herbal medicine for inflammatory diseases. Curcumin is the principal curcuminoid found in turmeric and is generally considered as its most active constituent (Duvoix et al., 2005; Sharma et al., 2005). Turmeric exhibits anti-tumor, anti-inflammatory and anti-infectious activities with low toxicity (Cohly et al., 1998, Mathuria and Verma, 2008). Furthermore, the present study aims to evaluate the histological effects in the cerebral cortex of offspring's maternally females rats fed on diet containing fried potato and bread and investigate the possible protective effect of curcumin.

In the previous literature regarding detailed histological change in acrylamide induced neurotoxicity is lacking. Thus the present study was aimed to investigate the histological changes in the cerebral cortex of off springs.

2. Materials and Methods

Experimental Animals

The present study was carried out on 80 mature virgin females and 26 fertile males of Wistar albino rats weighing 120-150 g. were obtained from Hellwan Animal Breeding Farm, Ministry of Health, Cairo, Egypt and used for experimentation. Rats were housed in individual cages and maintained in a room with good ventilation at 23 °C. The housing room was maintained on a 12:12 h light: dark cycle Standard diet composed of 20% casein, 15% corn oil, 55% corn starch, 5% salt mixture and 5% vitaminized starch (Egyptian Company of Oils and Soap Kafr-Elzayat Egypt). Daily examination of virgin smear of each virgin female was carried out to determine the estrous cycle. Mating was induced by housing proestrous females with untreated male at the ratio of 3 females with 1 male for 12 hour between 8 P.M till 8 A.M. In the next morning, the presence of sperm in vaginal smear determined the zero day of gestation. During the experiment, the animal groups were fed with diets and water ad libitum.

The doses

Female rats fed with varied concentrations of potatoes chips, fried bread alone, accompanied at doses (15%, 30%) supplemented basil diet 6 weeks before gestation and 6 weeks during the gestation and lactation periods, offspring until reaching 3 weeks old from parturition. This doses is low if compared to the doses used by (Hassan et al., 2011), which was 50%.

Curcumin supplementation:

Powder curcumin was obtained from the market and mixed with basil diet at concentration of 2% and supplied for feeding during experimental period (Young et al., 2004). Animals were fed on diet containing 0.2g/100g curcumin powder.

The offspring rats were arranged into 14 groups each was composed of 5 pregnant rats as follows:

- Control offspring rats fed basil diet(G1).
- Fried potato chips 15% for 3 months. (G2)
- Fried bread 15% for 3 months. (G3)
- Mixture of fried potato and fried bread (7.5% + 7.5%) for 3 months(G4).
- Fried potato chips 30% for 3 months(G5).
- Fried bread 30% for 3 months.
- Fried potato and fried bread mixed at (15% + 15%) at 3 months.

The same previous groups were repeated with 2% curcumin.

The offspring rats aging 3 weeks, experimental groups were sacrificed, and brain of offspring was 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.

3. Result:

Microscopic examination of sections of the cerebral cortex of the offspring of control rats showed the layer of the cerebral cortex, the molecular layer covered with piamater, the external granular, external pyramidal, internal granular, internal pyramidal and polymorphic layer (Fig.1). The molecular layer was thick and contained dense plexus of nerve fibers with few cells. Whereas, the external granular & external pyramidal contained numerous granular cells and pyramidal cells.

Several histopathological alterations were seen in the sections of cerebral cortex of the offspring of each group. Examination of sections of frontal cortex of offspring's maternally fed on fried potato (G1) showing some cells appeared with distorted deeply stain, malformed in pia mater with loss of density in plexus nerve fibers and dilatation blood capillary in piamater with many necrotic cells fig (2). Cerebral cortex of group (3) showed rupture pia mater layer, with infiltrated inflammatory cells in molecular layer (Fig.3)

Inspected Cerebral cortex sections obtained from group(4) revealed dilated congestion blood vessels with fibrosis epithelial layer in pia- matter and . Loss of
density in plexus nerve fibers resulted to reduced nerve
cells (Fig.4).
Different alterations in the architecture of
cerebral cortex sections for group (5). The most
prominent alterations in external granular such as
most nuclei in external granular cell shrunken
surrounded by spaces and dilated congested blood
capillary in piamater and molecular layer with loss of
density in plexus in molecular layer. Cerebral cortex
sections of group (6) revealed necrosis in external
granular neurocytes and some appeared shrunken
pyknotic nuclei, these cells are surrounded by
unstained areas (Fig.6). Group (7) sections of cerebral
cortex showed that completely degenerative areas of
cerebral tissue and enlargement of blood capillary
and some necrosis are seen. Figure (8) related to
the same group exhibited cells in external granular
showing necrosis of cells, pyknotic nuclei, and
unstained area surrounded some cells. The using of
curcumen showing amolirative cerebral tissue,
represented in fried potato (15%) fried bread (15%)
groups: indicated the external granular cell appeared
nearly normal, with moderat amolirative in group fed
with mixed fried bread & potato (15%).
On the other side, the groups feds on fried
potato (30%), fried bread (30%) Mixed fried bread &
potato (30%), showed remarkable changes,
represented necrotic cells & dilated blood capillary
(12), inflammatory thicked in pia matter and loss of
plexus (13), necrotic cells & dilated blood
The average number and the percentage of
embryos of different groups are shown in Table (1),
the treated groups with low doses recorded
decreasing in their percentage in G2,G3 and G4
with respect to the control group. However the
treated groups with high doses recorded decreasing
in their percentage in G5,G6 and G7 with respect
to the control group and groups with low doses.
Curcumin improved decreasing in the percentage
especially group G11.

Table (1): Showing the average number and the percentage of embryos of different groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Average ±SD</th>
<th>Percentage</th>
<th>Group</th>
<th>Average ±SD</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>8.8 ± 0.8</td>
<td>-</td>
<td>G8</td>
<td>9.2 ± 0.8</td>
<td>-5</td>
</tr>
<tr>
<td>G2</td>
<td>6.4 ± 0.5</td>
<td>-27</td>
<td>G9</td>
<td>6.2 ± 0.8</td>
<td>-30</td>
</tr>
<tr>
<td>G3</td>
<td>6.2 ±0.4</td>
<td>-30</td>
<td>G10</td>
<td>6.2 ± 0.8</td>
<td>-30</td>
</tr>
<tr>
<td>G4</td>
<td>6 ± 0.7</td>
<td>-32</td>
<td>G11</td>
<td>6.4 ± 1.1</td>
<td>-27</td>
</tr>
<tr>
<td>G5</td>
<td>4.6 ± 0.5</td>
<td>-48</td>
<td>G12</td>
<td>4.6 ±0.5</td>
<td>-48</td>
</tr>
<tr>
<td>G6</td>
<td>4 ± 0.7</td>
<td>-55</td>
<td>G13</td>
<td>4.8 ± 1.1</td>
<td>45</td>
</tr>
<tr>
<td>G7</td>
<td>4.4 ± 0.5</td>
<td>-50</td>
<td>G14</td>
<td>4.6 ± 1.1</td>
<td>-48</td>
</tr>
</tbody>
</table>

4. Discussion:
The literature contain little information concerning
the possible histopathological effects of feeding of fried
potato chips and fried bread on brain tissue of both
pregnant rats and their offspring’s ,that is why it was
particularly chosen in the current work. The present
investigation showed that the alterations in neurological
cells were defined by very characteristic changes in the
cerebral tissue of offsprings maternally feed with fried
potato chips and fried bread including necrosis of
several sings of constituent nerve cell. Variable degree
of degeneration were noticed in the cell undergoing
pyknotis and necrosis. Also , there were various vascular
changes which including marked dilated, congested blood capilla ry with fibrosis epithelial layer.
The present findings revealed that the brain is sensitive
to the cytotoxicity of feeding on fried potatoes chips
and fried bread during both gestation and laction
periods. Cell necrosis may be due to inhibition of the
synthesis of DNA needed for growth and maturation of
the cell (Scott,1971). Moreover, animals administration
acrylamide orally or fed a diet high in fried foods
(Tarek ,2000) had higher levels of hemoglobin DAN
adduct compared to unexposed animals. It has been
demonstrated that reproductive toxicity is not only
induced by ACR, but also by its metabolite glycidamide
(GA) ( Adler, 2000, Doerge ,2007).
In addition, acryl amide binds to dietary proteins
under intestinal conditions. Although acryl amide
monomers readily diffuse through Caco-2 minelayers,
acryl amide uptake from food in the human intestine
may differ from these experimental conditions, acryl
amide contains a reactive terminal double bond, which
may interact with food ingredients, mainly proteins,
DNA, and RNA . we consider that acryl amide is most
likely bound covalently to glutathione via Micheal
addition of cysteine residues to yhe reactive terminal
double bond. However also a binding to the primary
amino group cannot be excluded. (Godin ,et al., 2002).
This suggests that acrylamide may occur during
heating of these foods at high temperatures. Many
studies observation of acrylamide formation in starchy
foods as a result of heating at higher than 120 0C
revealed the results that temperatures higher than 100
0C is enough for acrylamide formation, (Stadler,2002,
The studies performed in order to understand how acrylamide is formed in fried or roasted foods showed it occurs as a result of Maillard Reaction between carbonyl group of reduced sugars (glucose, fructose) during treatment with heat and amino group of amino acids especially asparagine amino acid (Stadler, Mottram, 2002, Mottram ,2002). ACR occurs at medium level (5-50 µg/kg ) as a result of cooking foods rich in protein and at higher levels (150-40000 µg/kg) as a result of cooking foods rich in carbohydrate such as potatoes and cereals, (Mendel, 2003.). Asparagines constitutes 14% of total free amino acid in wheat flour, 18% of total free amino acid in ray, and 40% of total amino acid content of potatoes used in production of potato chips is asparagine (Martin and Ames, 2001.).

The observation recorded in the present work that damage in cerebral cortex of brain rats , mightly reflect and support the toxic of acrylamide formed during cooking of vegetable foods such as potatoes and cereals on nervous tissues.

The obtained result may be attributed to the increased level of ACR in the blood of treated mother as well as in the umbilical cord blood of neonates as a result of its higher affinity of forming N-terminal haemoglobin adducts. In view of the shorter life span of neonatal erthyrocytes and the lower body weight of newborn infants , the relative internal dose of ACR adducts in neonates (in microgram's /Kg body weight ) must be assumed to be at least equal to that of the mother. Because of the high cell-replication rates during fetus development, trans-placental exposure of neonates to ACR might raise concerns (Schettgen et al., 2004). It has been suggested that ACR manifested toxicity principally toward growth and development (Garey et al., 2005, Wang et al., 2010). FHO/IPCS, (2006) reported that toxicity studies of in ACR have hitherto mainly been performed using adult animals to mimic occupational exposure , the data for effects during fetal, infantile and pubertal periods are rather limited. Therefore , it is important for risk assessment of ACR exposure in human to evaluate toxicity taking into account physiological differences between adults and fetuses infants that might influence sensitiviti. It is a evident that ACR is a developmental toxicant in rodents, because suppression of offspring body weight results from maternal exposure (Garey et al., 2005).

Soergel et al. (2002) found that from 10 to 50% of dietary ACR in pregnant women was transferred via blood through the placenta to the fetus. Breast milk was found to contain up to 18.8/mg/L of ACR. Because water soluble ACR can pass both placental and blood brain barriers, the authors suggested that to protect fetuses’ pregnant women should not consume high-ACR food.


The obtained results are in agreement with many investigators who studied the neurotoxicity of ACR. Edwards et al.,(1991) reported that the manifestations of ACR intoxication was closely similar in human and experimental animals and were in the form of swollen axons and or decrease in numbers of large axons diameter, Takahashi et al. (2008) obtained similar results after studying the effect of ACR on rat offspring. In vivo and in vitro chronic treatment of rats with ACR was found to produce a substrate-dependent, toxicologically specific inhibition of brain mitochondrial respiration. This inhibition of mitochondrial energy production might play a role in the neurotoxin mechanisms of action for ACR (Medrano and LoPachin, 1989). Following administration ACR exposure was found to be linked to nerve terminal damage in the central nervous system and peripheral nervous system (Lehning et al., 2003 and LoPachin et al., 2003).

It was demonstrated that ACR can produce neurological toxicity in the absence of axonopathy; i.e. whereas equivalent neurotoxicity can be induced by intoxication over a wide range of daily dose-rates, axon degeneration in PNS and CNS occurred only during long-term exposure to lower ACR dose-rates (LoPachin et al., 2002; Lehning et al., 2003).

Recently, Allam et al. (2011) reported that prenatal and perinatal ACR disrupts the biochemical machinery, cause oxidative stress and induce structural
changes in the developing rat cerebellum. Hassan et al. (2011) reported that the feeding of fried potato chips during gestation caused histopathological changes on the development of retina in albino rat. Saker et al. (2011) found the same results by using acrylamid, which supported the current study. This changes may be due the presence of acrylamide or its metabolite.

Recently, it was also reported that antioxidants could exert their beneficial effects by abstracting reactive free electrons from free radical intermediate postulated to be formed in the Maillard reaction (Friedman and Levin, 2008). Curcumin which is a cheap available natural plant could a meliorate changes in brain. Antioxidants are the frontline of defense against free radicals (Osawa and Kato, 2005). The antioxidant mechanism of curcumin is due to its specific conjugated structure of two methoxylated phenols and an enol form of diketone.

This structure is responsible for free radical trapping ability as a chain breaking antioxidant (Masuda et al., 2001). The ability of curcumin to chelate the toxic metals was shown by Daniel et al. (2004). They found that curcumin significantly protects against lipid peroxidation induced by heavy metals, lead and cadmium in the rat brain homogenate, as well as reduces lead-induced structural damage in the hippocampus. Curcumin prevents free radical generation by competing with peroxidant metals for cell binding sites, which decrease the possibility of free radical formation or by maintaining the activities of antioxidant enzymes like SOD and catalase (Reddy and Lokesh, 1992). Huang et al. (2008) reported that a single dose of curcumin (20 or 40 mg/kg, intraperitoneally) was effective in inhibiting the increase in glutamate level in the hypothalamus during lipopolysaccharide-induced systemic inflammation in rabbits.

Oxidative stress has been proven to be involved in mutation, chromosome aberration, tumor promotion, and cancer development and repeatedly addressed as an important mechanism of indirect genotoxicity (Spit et al., 2002).

In principle, chemicals that give rise to excess ROS production and lipid peroxidation will cause different types of toxicity, including genotoxicity and cell death. These adverse effects can be suppressed by antioxidants, which can eliminate ROS (Weisburger, 2001). Curcumin, a polyphenol derived from the herbal remedy and dietary spice, turmeric, is well known for its antioxidant properties, because it is able to suppress the generation of ROS and decrease lipid peroxidation (Chan, 2005). The present study revealed that slightly improvement in cerebral cortex alteration caused by acrylamide, this is may be its attributed to its antioxidant and free radical scavenging activities.

In addition, Chen et al. (2010) reported that a single intraperitoneal injection of curcumin (20 mg/kg) attenuated airway hyperreactivity induced by ischemia-reperfusion of the pancreas in rats.

Conclusion
Curcumin could prevent neurotoxicity of ARC in the rat’s brain. If Hyperhomocysteinemia is one of the pathological reasons for neurodegenerative disorders such as sporadic AD or Parkinson’s disease, curcumin may be an effective prophylactic agent in the prevention of oxidative stress by ACR. Furthermore, data of this study suggest that curcumin’s mechanism for protection of the brain against the toxicity of ACR may be inhibition of brain lipid peroxidation and improvement of learning and memory deficits in rats. Further studies are required to reveal the exact mechanism of ACR in cell death process (apoptosis or necrosis) and the neuroprotective properties of curcumin must be studied in more detail. Curcumin which is a cheap available natural plant could a meliorate changes in brain.

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