A prospective study of Green Tea and panax Ginseng against mutagens & carcinogens produced during Thermolyzed Meat and Fish

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Abstract: Effect of thermolyzed (ultra heated) Meat and fish on the nutritional, biochemical, histological, and hematological parameters as well as aberrations and sperm abnormalities was investigated in male albino rats. Animals were divided into two main groups: Meat group and Fish group. Each group was divided into six subgroups: G-I was fed the basal diet containing more than 12% dry frozen meat, G-II & G-III were fed the basal diet of G-I plus 5% of Green Tea and Panax Ginseng respectively. G-IV was fed the basal diet containing more than 12% Thermolyzed Meat, while, G-V & G-VI were fed the basal diet of G-IV plus 5% of Green Tea and Panax ginseng respectively. The same classification was done for the fish groups. It was found that the terminal body weight was increased non-significantly in all rat groups until the fifth week, and then decreased non-significantly until the end of the experimental periods (8 weeks). No effect on serum cholesterol, triglycerides, total lipids and glucose levels was found. On the other hand, significant values of both hepatic and renal function parameters were obtained among ultra heated meat and fish treated animals. Numerous histological alterations, particularly in the liver, were also observed. Moreover, anemia, leukocytosis, neutophilia, lymphocytosis and monocytosis were seen. Furthermore, chromosomes and sperms were adversely affected in such animals fed diet contained ultra heated meat and fish. The protective role of green tea and panax ginseng were observed in all biochemical, histological, and hematological parameters. In addition, improvement in chromosomal aberration and sperm abnormalities was also noticed.

Keywords: Ultra heated meat and fish, Green Tea, Panax ginseng, Albino rats.

1. Introduction:

   New types of mutagens have been isolated from proteinaceous foods prepared by using ultra heat. These mutagens are heterocyclic compounds with a free aromatic amino group and therefore are referred to heterocyclic amines (1).

   Stimulated meat flavors are frequently added to many processed foods as flavor enhancers. These flavors are normally produced by exposing different kinds of meat (beef, fish, chicken, etc.) to prolonged heating at degrees higher than cooking temperatures. Several studies reported that such ultra heated meats are mutagenic food (2 & 3). Also, some epidemiological data by Gerharsson and his team (4) suggested that people who ingested relatively large amounts of well cooked meat run a significantly higher risk of developing colorectal cancer, is available. The mutagenecity / carcinogenicity of such ultra heated protein rich foods may arise during the processes of prolonged treatment of temperature by forming a series of heterocyclic aromatic amines (HAAS) that have been found to be mutagens / carcinogens (5, 6, & 7).

   Differences in metabolic fate of different HAAS could account for the variable cancer-producing potential in different species. Because HAAS are found in a variety of cooked meats and fish that constitute a major part of our diet, they represent a potential risk factor for public health in the etiology of human cancer. Recent epidemiological studies have shown a positive association between intake of well done meat and increased risk of lung cancer, breast cancer, and colorectal cancer (8).

   The mutagenic and possibly carcinogenic products of the HAAS are metabolized and activated by enzymes of cytochrome systems-mediated N-hydroxylation to a number of hydroxylated metabolites which react with DNA to induced mutations (9). Hydroxyl free radicals are generated due to the cardio toxic effect of HAAS and subsequent single and double strand scission of DNA are produced (10). Also, N²-guanine adduct was found in various organs of mice (11), and rats (12) fed HAAS diets.

   The disturbance of DNA replication leads to abnormalities in the chromosomes (13). Although, humans usually use ultra-heated meat as a major part of their diet, there are no data regarding the possible cytogenic and sperm effects of feeding with well-
cooked meats. Therefore, the present study was planned to evaluate the chromosome aberrations and sperm abnormalities in rats fed diets containing meat and fish exposed to high heat treatment greater than cooking temperature.

The root of panax ginseng has been used as analeptic, stomachic tranquilizer, anti-psychotic, anti-fatigue agent, enhancement for sexual behavior, metabolism accelerator, protector from stress, increase gastro-intestinal tract motility, and erythropoietic agent in Asian countries and now used as a commercial medical drug all over the world (14). Many reports have demonstrated that panax ginseng suppressed the growth (in vitro) of cultivated tumor cells. Furthermore, it suppressed the growth of B16 melanoma transplanted into mice and showed a stimulative effect on the antitumor activity of mitomycin cultured tumor cells (15).

The green tea has been used successfully as an antioxidant, anticarcinogenic and antimitogenic agent (16). Moreover, some extracts of the green tea such as phenolic acids, catechins and β-carotene were found to be antimitogenic agents against mutagenicity of 2-amino-2-methyl imidazo [4,5-f] quinoline IQ (one of the major HAAS compounds). Furthermore, Sugamuma (17) and his team found that consumption of green tea is a practical and effective cancer preventive both before cancer onset and after cancer treatment. So, the purpose of the present study is to investigate the adverse effects of ultra-heated meat and fish administered to male albino rats on the nutritional, hematological, histological and biochemical parameters as well as sperm abnormalities and chromosomal aberrations. Also, to investigate the possible protective effects of green tea and panax ginseng against such hazards caused by diet containing these ultra-heated meat and fish.

2.Materials and Methods:

**Experimental Animals:**

Random bred male albino rats weighing approximately 101.61 ± 2.71 were obtained from Animal House Laboratory, National Research Center, Giza, Egypt. The rats were evaluated prior to initiation of the study to ensure a health condition and acclimation to the study environment. Clinically accepted animals were randomly assigned into 12 groups (10 animals / each).

**Environmental conditions:** A total of 120 rats were housed in stainless steel wire mesh cages on a bedding of wood chips (five animals / cage). They were kept in an ambient temperature of 25 ± 3°C, on a light / dark cycle of 12 / 12 hours and supplied rat chow (the diet) and fresh water ad libitum.

**Diet:** A basal diet (table 1) was formulated to meet threats nutrient’s requirements as recorded by Osfor (2003).

**Experimental Design:** This study was performed in two experiments. The first one was divided into 6 equal groups as follow:

**Group 1:** Fed the basal diet only, which contains 12% dry frozen (raw) meat and served as a negative control.

**Group 2:** Fed the basal diet plus 5 % green tea.

**Group 3:** Fed the basal diet plus 5 % panax ginseng powder.

**Both group 2 and 3 served as positive control groups.**

**Group 4:** Fed the basal diet which contains 12 % ultra-heated (fried) meat.

**Group 5:** Fed the same diet of group 4, plus 5 % green tea.

**Group 6:** Fed the diet of group 4, plus 5 % panax ginseng powder.

**Both group 5 and 6 served as positive control groups.**

The second experiment was also divided into 6 equal groups.

**Group 1:** Fed the basal diet only, which contains 12 % dry frozen (raw) fish and served as a negative control.

**Group 2:** Fed the basal diet plus 5 % green tea.

**Group 3:** Fed the basal diet plus 5 % panax ginseng powder.

**Both group 2 and 3 served as positive control groups.**

**Group 4:** Fed the basal diet which contains 12 % ultra-heated (fried) fish.

**Group 5:** Fed the same diet of group 4, plus 5 % green tea.

**Group 6:** Fed the diet of group 4, plus 5 % panax ginseng powder.

**Both group 5 and 6 served as positive control groups.**

**Vitamins and Minerals Mixture (g)**

- Copper sulphate (0.05), Ferric citrate (0.59), Zinc carbonate (0.053), Calcium carbonate (7.25), Calciumhydrogen phosphate (11.3), Di-sodium hydrogen phosphate (6.0), Potassium Iodide (0.003), Magnesium chloride (2.3), and Manganese sulphate (0.154).
- Thiamine (0.3), Riboflavin (1.0), pyridoxine (0.2), Calcium carbonate (6.0), Nicotinincacid (20.0), Cyanocobalamine (0.005), Folic acid (0.2), Biotin (20.0), Inositol (60.0), Choline chloride (60.0), vitamin A (4000 IU), vitamin D (1000 IU), Vitamin E (30 IU) and Vitamin K (50 IU).

**Test materials:** Green tea was purchased from the local market as dry green leaves of tea. It was present in boxes of one kilogram. It was given mixed with the diet after grinding in a dose of 5% of the diet for 8 weeks.
### Table (1): Composition of the basal Diet:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorghum</td>
<td>39</td>
</tr>
<tr>
<td>Corn yellow</td>
<td>31.6</td>
</tr>
<tr>
<td>Barley</td>
<td>8</td>
</tr>
<tr>
<td>Meat meal</td>
<td>8</td>
</tr>
<tr>
<td>Corn cobs</td>
<td>7.3</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>4</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.3</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.4</td>
</tr>
<tr>
<td>Di-calcium Phosphate</td>
<td>0.2</td>
</tr>
<tr>
<td>Lime stone</td>
<td>0.4</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.3</td>
</tr>
<tr>
<td>Vitamins and Minerals Mixture*</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Calculated Nutrient Composition</strong></td>
<td></td>
</tr>
<tr>
<td>Crude protein</td>
<td>11.99</td>
</tr>
<tr>
<td>Energy</td>
<td>3404.2</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>4.46</td>
</tr>
<tr>
<td>Ether extract</td>
<td>7.51</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.71</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.61</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.45</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Panax ginseng was purchased from Arab Drug Company, Cairo, Egypt. It is present in boxes of 20 capsules (2 strips, each strip contains 10 soft gelatin capsules, each capsules contains 850 mg of panax ginseng powder). It was given mixed with the diet in a dose of 5% of the diet for the same time interval. This dosing regimen is similar to that prescribed for human use.

**Clinical Observation:**

The rats were observed daily throughout the experimental period. Complete physical examination was conducted weekly. Body weights were measured prior to offering the diet and recorded individually every week. Food consumption was calculated every other day to the nearest gram for each group of rats as the difference between the amounts of offered and residual food.

**Clinical Pathology:** Blood samples for hematochemical and clinical chemistry determinations were obtained from the retro-orbital plexus of veins of all rats on the day before they were scheduled for euthanasia.

**Clinical chemistry:** Clotted blood samples were centrifuged and the serum was removed by aspiration for subsequent determination of the following.

**Liver function tests:**
- Alanine aminotransferase (ALT) “Unit / dl”
- Aspartate transaminase (AST) “Unit / dl” (18)
- Alkaline phosphatase “Unit / L” (19)
- Albumin “mg / dl” (20)
- Bilirubin “mg / dl” (21)
- Glucose “mg / dl” (22)
- Total proteins “mg / dl” (23)

**Renal function tests:**
- Urea “mg / dl” (35)
- Creatinine “mg / dl” (24)

**Lipid metabolism:** Cholesterol, triglycerides and total lipids “mg / dl” (25).

**Hematology:** Blood sample containing EDTA as anticoagulant was used for the determination of hemoglobin contents, erythrocyte and leucocyte counts (total and differential).

**Histopathological studies:**

Immediately after sacrifice of animals, samples of the liver and kidney tissues were fixed in 10% formal saline, dehydrated, cleared, embedded in paraffin wax, and were sectioned at 7 um. Paraffin sections were stained with Hematoxylin and Eosin stain (26).

**Chromosome analysis:**

Femur bones of each rat were collected and the bone marrow was pooled in a sterile tube containing 5 – 6 ml of sigma medium. 0.2 ml of 0.05 colchicine was added to each tube (27). Tubes were then incubated at 37°C for 1.5 – 2 hours. Then, tubes were centrifuged for 10 minutes at 1000 rpm and the supernatant was discarded while sedimented cells were incubated with 75 mM KCl for 20 minutes at 37°C and then centrifuged again. The cells were then fixed several times with methanol : acetic acid (3 : 1). Cells were then dropped gently on dry clean slides and stained with Giemsa stain. Chromosomal aberrations were recorded in at least 50 metaphase spreads for each animal (28).

**Sperm analysis:**

For sperm-shape analysis, the epididymis was excised and minced in about 10 ml physiological saline, dispersed and filtered to exclude large tissue fragments. Smears were prepared after staining the sperms with aqueous Eosin Y. at least 4000 sperms per subgroup were assessed for morphological abnormalities. Epididymal sperm count was also determined by hemocytometer (29 & 30).

**Statistical analysis:**

The obtained data were statistically analyzed after (31). Wilcoxon’s Signed-rank sum test was used for the statistical analysis of the sperm results (32).

**3. Results and Discussion:**

The aim of frying meat or fish is to obtain a golden brown, crispy crust with good flavors, which is induced by the Millard reaction or non-enzymatic browning and also to make the protein coagulable. A high temperature in the outer layer of the meat in combination with decreased moisture content causes chemical and physical changes that form the crust. Food mutagenic heterocyclic aromatic amines (HAAs), isolated from the crust of boiled and fried meat or fish, are speculated to be among the most plausible environmental carcinogens relevant to neoplasia in humans (33). More than 20 HAAs have been isolated and their structures have been fully elucidated. Heterocyclic amines are carcinogenic in various organs in mice, rats, and monkeys. They have been shown to induce tumors in the liver, oral cavity,
stomach, lung, skin, small intestine, hematopoietic system, blood vessels, urinary bladder, and lymphoid tissues of the rodents (34). In addition, HAAs causes severe atrophy in the salivary glands and pancreas of rats (35). Furthermore, human colon DNA adduct levels are approximately 10 times greater than in rodents at the same dose and time point following exposure (36). For that we used the rat as an experimental model in our study.

In the present study, there was a non-significant increase in the body weight of rats in group 4, 5, and 6 of both experiments when compared with the negative control group, till the fourth week of the experimental period. Furthermore, there was a non-significant decrease in the body weight of the same groups from the fifth week until the end of the experimental period. No information is available on the effect of HAA on the body weight and food consumption. Our explanation is that the UHM or UHF act as appetizer so, the animal ate more food and the food consumption was increased, and that led to increase the body weight. When the adverse effects of HAAs begin to appear from the fifth week of the experimental period, the consumption begins to decline and the body weight gradually decreased. This result is in agreement with that obtained by (37), who recorded that the average body weight of 300 and 100 ppm IQ (2-amino-3 methyl imidazo [4,5-f] quinoline), one of the most important HAAs, treated mice were significantly lower than the control values by 18.9 and 6.4 %, respectively. They claimed that this might be due to presence of aberrant crypt foci in the colons of mice that lead to decrease in food consumption.

There were non significant increases in the mean values of serum glucose, total lipids, cholesterol, and triglycerides in all treated rats which fed diets containing UHM or UHF. This increase in blood glucose level in our study was in accordance with what obtained by Salem et al., (38) who claimed that linogliride which is one of the important HAAs has been shown to possess significant hyperglycemic activity in rats. Furthermore, the hypercholesterolemic, hyperglycemic and hyperlipidemic results in this study were in agreement with the results obtained by other authors (39 & 40), who have reported that Tetrazole urea (one of the most important HAAs) at doses of 3 mg / kg body weight , elevated plasma total cholesterol 67 % in fed rat model of hypercholesterolemie and 47% in fed dogs. Also, it increased triglycerides 52 % and total lipids 13 % in rats with pre-established hypercholesterolemia.

Addition of green tea and panax ginseng to the diet contains UHM and UHF, improved the levels of glucose, total lipids, cholesterol, and triglycerides, through improving he metabolism of carbohydrates and lipids, and the physiological functions of the liver. These results are in accordance with the results obtained by Oguri et al., (41) who recorded that green tea catechins (0.2 mmol) in diet containing HAAs was forum to clearly suppress the formation of both MeIQX and PhIP (the strongest and vary famous HAAs) being 32.75 %. These phenolic antioxidants also reduced the total mutagenicity of the same heterocyclic amines (MeIQX and PhIP) which are a good substrate found in several human foods.

The present study demonstrated a significant decrease in serum total proteins, albumin, globulin and Albumin / globulin ratio in all treated rat groups. On the other hand, total bilirubin, liver enzymes ALT (Alanine Transferase). AST (Aspartate Transfase) and alkaline phosphatase were all significantly elevated in the serum of these treated rats, when compared with the negative control group. These biochemical results were confirmed by histopathological examination of the liver of UHM / UHF treated animals, which showed numerous histological alteration including congestion, multiple haemorrhages, mononuclear infiltration and cellular degeneration in the form vacuolation of the cytoplasm and pyknosis of the nuclei. The liver is the most frequent target site for most HAAs especially in rats, mice and monkeys (42).

The protective role of either green tea or panax ginseng on the liver was evidenced by the partial improvement in both the biochemical parameters and histological alterations. All the biochemical parameters still showed significant differences with the control groups. Moreover, histological examination still revealed haemorrhages, cellular infiltration and cellular degeneration, but to less extent than those present in UHM / UHF treated rats. A new class of carcinogens (HAAs) formed during the boiling or fying of creatinine containing food including fish and meat, can be inhibited by green tea that is a rich source or micronutrients with antioxidants properties. Green tea is a major source of epigalo-catechin gallate and theaflavin and the associated thearubigins,which are polyphenolic compounds,act as antioxidant (14). Tea constituents epigalo-catechin (EGC) and epigalo-catechin-3-gallate (EGCG), inhibit the enzymes (functional O-acetyl tranferase), which directly scavenge the reactive electrophile, whereas the complexing agent chlorophylline complexe with heterocyclic amines and facilitate the degradation of active metabolism (43).

Similar to the live, the kidneys of the animals treated with UHM or UHF showed a significant increase in serum levels of urea and creatinine. These results were confirmed by the histological examination of the kidneys f such rats which revealed congestion, multiple interstitial hemorrhage and
nearly mononuclear cellular infiltration. De-Stefani (44) demonstrated that red meat, barbecued meat, protein and heterocyclic amines intakes were associated with a significant increase in the risk of renal cell carcinoma. Furthermore, gadolinium DTPA (one of the major heterocyclic aromatic amines) induced a significant increase in serum creatinine, during its elimination through the kidney. Moreover, gadolinium is nephrotoxic and leads to reversible renal failure (45). However, the addition of either green tea or panax ginseng to the UHM / UHF in our study resulted in complete protection of the kidneys. This was evidenced by complete reversibility of both serum urea and creatinine, so that they were returned back nearly to the initial control values. Furthermore, histopathological examination of the kidney revealed figures which were more or less similar to the control figures.

There was a significant decrease in the R.B.Cs count, hemoglobin concentration and packed cell volume in all animals that received ultra-heated meat and fish. However such rats showed a significant increase in the W.B.Cs count, neutrophils, lymphocytes and monocytes, while eosinophiles and basophils revealed no significant differences. In accordance with our results, Kaleagasioglu (46) and his team reported that 3.7- bis-(3-trifluoromethylphenyl)-1,5,3,7dioxadiaocane, at a dose of 0.12 mmol / kg body weight / day for three consecutive days in male Wister rats, caused haematolytic anemia, neutrophilia, lymphocytosis, enlargement of the spleen and enhanced production of granulocytes / macrophages. This means that, heterocyclic aromatic amines are haematotoxic on the red blood cells and induce lymphocytosis (47). Furthermore, PhIP, which is one of the main HAAs is carcinogenic in rats and mice causing lymphomas (enlargement of the lymph nodes and spleen), with lymphocytosis (48). PhIP is activated to a form that will bind to albumin, haemoglobin ad W.B.Cs count in the peripheral blood of five human volunteers that were administered a dietary-relevant dose of PhIP. Moreover, PhIP is bioavailability to the colon, with levels in normal tissue in range 42 – 122 pg PhIP / g tissue (49).

In our study, the addition of ginseng extract to the UHM or UHF resulted in complete reversibility of the above mentioned haematological parameters. On the other hand, the addition of green tea significantly improved only the white blood cells parameters, while the red blood cells parameters still showed a significant difference when compared with the control groups.

It was clear that rats that were fed diets containing UHM or UHF had more frequent chromosome aberrations (especially the structural ones) compared with those which were fed diets containing frozen meat or frozen fish. The group of animals which had diet containing UHF had the highest percentage of numerical aberrations. These results indicate that such foods have mutagenic effect on chromosomes Induction of chromosome aberrations may be due to formation of HAAs in UHM and UHF. Consequently these mutagens (HAAs and their metabolites) can interact with cellular DNA to form mutations (8) leading to anomalies in the chromosomes as a result of disturbance of DNA replication (21). In similar studies, Breneman (6) showed a significant response of sister chromatid exchange (SCE) and micronuclei in mice fed MelQx (2-amino-3,4-dimethylimidazo [4,5-f] quinoline) (one of the major kinds of HAAs) diet, and suggested that the increase of micronuclei and SCE confirms that MelQx and / or its metabolites reached peripheral lymphocytes in quantities sufficient to induce chromosome aberrations. Also, Director (50) reported that the induction of SCE in mice fed PhIP (2-amino-1-methyl – 6 phenylimidazo [4,5-b] pyridine (another kind of HAAs). In a previous study, Ohgaki (51) suggested that the induction of tumor in the liver, lung and intestine in female mice after receiving MelQx, might be an indicator for observable chromosome damages.

In the present study, a significant increase in the number of morphologically abnormal sperms and a significant decrease in sperm count have occurred in animals fed diets containing UHM or UHF. The consistence high incidence of chromosome aberrations as a result of potential formation of HAAs or their metabolites may be indicative of a general susceptibility of these animals to chromosome de-arrangements of gonadal cells causing abnormalities in sperm shape and reduction in sperm count (52). Evidence that sperm shape abnormalities induced by selected mutagens and carcinogens have been reported (53). The increase in abnormally shaped sperm and the decrease in sperm count may lead to infertility (54).

In our study, animals fed diets containing UHM or UHF plus green tea or panax ginseng showed a decrease in chromosome aberrations and morphological sperm abnormalities with an increase of sperm count. These results indicate that the green tea and ginseng have a protective role in body cells against the observed mutagenic effect of UHM or UHF diets. Evidence that some components of green tea such as catechins and ascorbic acid have anti-mutagenic agents against the mutagenic effect of IQ (2-amino-3-methylindazo [4,5-f] quinoline) in salmonella typhimurium TA 100 were observed (26).
### Table (2): Body weight of rats fed diet containing thermolyzed meat and fish (mean ± S. D., n=10)

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Fish</th>
<th>Mean</th>
<th>Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-III</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>G-IV</td>
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<td></td>
<td></td>
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<tr>
<td>G-V</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-VI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Means (%):** 3.341±0.13; 4.88±0.14; 4.01±0.17; 3.91±0.12; 4.00±0.10. **Gaps:** 4.11±0.10; 4.22±0.11; 2.73±0.07; 4.01±0.12; 4.00±0.10. **Significant at < 0.05**

### Table (3): Biochemical parameters of rats fed diet containing thermolyzed meat and fish (mean ± S. D., n=10).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>G-I</th>
<th>G-II</th>
<th>G-III</th>
<th>G-IV</th>
<th>G-V</th>
<th>G-VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alk. Pho. (U / L)</td>
<td>4.53±0.168</td>
<td>0.015±0.01</td>
<td>0.035±0.17</td>
<td>1.68±0.067</td>
<td>2.26±0.22</td>
<td>3.02±0.01</td>
</tr>
<tr>
<td>AST (U / L)</td>
<td>66.75±3.21</td>
<td>7.334±0.13</td>
<td>171.4±5.1</td>
<td>224.5±9.34</td>
<td>166.2±5.8</td>
<td>189.2±11.8</td>
</tr>
<tr>
<td>ALT (U / L)</td>
<td>4.53±0.168</td>
<td>0.015±0.01</td>
<td>0.035±0.17</td>
<td>1.68±0.067</td>
<td>2.26±0.22</td>
<td>3.02±0.01</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>1.0±0.41</td>
<td>1.25±0.48</td>
<td>1.00±0.40</td>
<td>1.25±0.48</td>
<td>1.00±0.40</td>
<td>1.25±0.48</td>
</tr>
</tbody>
</table>

**Means (%):** 8.7±0.3; 16.2±0.3; 23.4±0.3; 30.6±0.3; 37.9±0.3; 45.1±0.3. **Significant at < 0.05**

### Table (4): Hematological parameters of rats fed diet containing thermolyzed meat and fish (mean ± S. D., n=10):

<table>
<thead>
<tr>
<th>Parameter</th>
<th>G-I</th>
<th>G-II</th>
<th>G-III</th>
<th>G-IV</th>
<th>G-V</th>
<th>G-VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.B.Cs. (×10³)</td>
<td>9.01±0.29</td>
<td>8.01±0.29</td>
<td>7.01±0.29</td>
<td>6.01±0.29</td>
<td>5.01±0.29</td>
<td>4.01±0.29</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>12.79±0.19</td>
<td>11.79±0.19</td>
<td>10.79±0.19</td>
<td>9.79±0.19</td>
<td>8.79±0.19</td>
<td>7.79±0.19</td>
</tr>
<tr>
<td>P.C.V. (%)</td>
<td>43.6±1.39</td>
<td>42.6±1.39</td>
<td>41.6±1.39</td>
<td>40.6±1.39</td>
<td>39.6±1.39</td>
<td>38.6±1.39</td>
</tr>
<tr>
<td>W.B.Cs. (×10³)</td>
<td>6.28±0.27</td>
<td>6.28±0.27</td>
<td>6.28±0.27</td>
<td>6.28±0.27</td>
<td>6.28±0.27</td>
<td>6.28±0.27</td>
</tr>
</tbody>
</table>

**Means (%):** 9.2±0.2; 10.2±0.2; 11.2±0.2; 12.2±0.2; 13.2±0.2; 14.2±0.2. **Significant at < 0.05**

### Table (5): Chromosomal Aberrations of rats fed diet containing thermolyzed Meat (mean ± S. E., Chi-square, n=10):

<table>
<thead>
<tr>
<th>Groups</th>
<th>Chromatic Gaps</th>
<th>Chromatic Breaks</th>
<th>Chromatic Aberration</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-I</td>
<td>1.00±0.41</td>
<td>1.25±0.48</td>
<td>1.00±0.40</td>
</tr>
<tr>
<td>G-II</td>
<td>1.00±0.41</td>
<td>1.25±0.48</td>
<td>1.00±0.40</td>
</tr>
<tr>
<td>G-III</td>
<td>1.00±0.41</td>
<td>1.25±0.48</td>
<td>1.00±0.40</td>
</tr>
<tr>
<td>G-IV</td>
<td>1.00±0.41</td>
<td>1.25±0.48</td>
<td>1.00±0.40</td>
</tr>
<tr>
<td>G-V</td>
<td>1.00±0.41</td>
<td>1.25±0.48</td>
<td>1.00±0.40</td>
</tr>
</tbody>
</table>

**Means (%):** 0.00±0.00; 0.00±0.00; 0.00±0.00; 0.00±0.00; 0.00±0.00; 0.00±0.00. **Significant at < 0.05**

### Table (6): Chromosomal Aberrations of rats fed diet containing thermolyzed Fish (mean ± S. E., Chi-square, n=10):

<table>
<thead>
<tr>
<th>Groups</th>
<th>Chromatic Gaps</th>
<th>Chromatic Breaks</th>
<th>Chromatic Aberration</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-I</td>
<td>0.00±0.41</td>
<td>0.75±0.48</td>
<td>0.00±0.40</td>
</tr>
<tr>
<td>G-II</td>
<td>0.00±0.41</td>
<td>0.75±0.48</td>
<td>0.00±0.40</td>
</tr>
<tr>
<td>G-III</td>
<td>0.00±0.41</td>
<td>0.75±0.48</td>
<td>0.00±0.40</td>
</tr>
<tr>
<td>G-IV</td>
<td>0.00±0.41</td>
<td>0.75±0.48</td>
<td>0.00±0.40</td>
</tr>
<tr>
<td>G-V</td>
<td>0.00±0.41</td>
<td>0.75±0.48</td>
<td>0.00±0.40</td>
</tr>
</tbody>
</table>

**Means (%):** 0.00±0.00; 0.00±0.00; 0.00±0.00; 0.00±0.00; 0.00±0.00; 0.00±0.00. **Significant at < 0.05**

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Plate I

Fig. 1: A photomicrograph of liver section in Group-I showing the normal architecture of the liver (H & E stain X 150).

Fig. 2: A photomicrograph of liver section in Group-IV showing marked congestion (C) and haemorrhage (H), (H & E stain X 150).

Fig. 3: A photomicrograph of liver section in Group-IV showing congestion (C), moderate mononuclear infiltration (I) and marked degeneration of the most liver cells (D), (H & E stain X 150).

Plate II

Fig. 4: A photomicrograph of liver section in Group-V showing necroinflammatory cellular infiltration (I), and moderate degeneration of the most liver cells (H & E stain X 150).

Fig. 5: A photomicrograph of liver section in Group-VI showing congestion (C), mild mononuclear infiltration (I) and mild haemorrhage (H), and degeneration of some liver cells (D), (H & E stain X 150).
Fig. 6. : A photomicrograph of kidney section in Group-I showing the normal architecture of the kidney (H & E stain X 150).

Fig. 7. : A photomicrograph of kidney section in Group-IV showing interstitial haemorrhage (H & E stain X 150).

Fig. 8. : A photomicrograph of kidney section in Group-IV showing congestion and mononuclear infiltration (I). (H & E stain X 150).

Plate IV

9. : A photomicrograph of Kidney section in Group-V showing mild haemorrhage (H). (H & E stain X 150).
Table (7): Sperm Abnormalities of rats fed diet containing thermolyzed Fish (mean ± S. E., n=10):

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sperm Number</th>
<th>Tail Abnormality</th>
<th>Types of Head Abnormalities</th>
<th>Total</th>
<th>Sperm count (×10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>Big</td>
<td>Small</td>
</tr>
<tr>
<td>Meat</td>
<td>G-I</td>
<td>4000</td>
<td>45</td>
<td>1.125</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>G-II</td>
<td>4000</td>
<td>40</td>
<td>1.00</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>G-III</td>
<td>4000</td>
<td>38</td>
<td>0.95</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>G-IV</td>
<td>4000</td>
<td>230</td>
<td>5.75**</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>G-V</td>
<td>4000</td>
<td>84</td>
<td>2.1*</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>G-VI</td>
<td>4000</td>
<td>72</td>
<td>1.8</td>
<td>10</td>
</tr>
<tr>
<td>Fish</td>
<td>G-I</td>
<td>4000</td>
<td>39</td>
<td>0.975</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>G-II</td>
<td>4000</td>
<td>47</td>
<td>1.175</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>G-III</td>
<td>4000</td>
<td>44</td>
<td>1.100</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>G-IV</td>
<td>4000</td>
<td>213</td>
<td>5.35**</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>G-V</td>
<td>4000</td>
<td>74</td>
<td>1.85*</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>G-VI</td>
<td>4000</td>
<td>47</td>
<td>1.175</td>
<td>12</td>
</tr>
</tbody>
</table>

*Significant at < 0.05; ** Significant at < 0.01
Furthermore, they reported that the inhibitory effects of green tea may be due to binding with the mutagens or the inhibition of enzymes activities with consequent reduction of DNA mutations and chromosome aberrations. In another study, Ito (55) reported that green tea has suppressed the chromosome aberrations induced by aflatoxin B1 in rat bone marrow cells in vivo. Considering ginseng, the mode of action of this substance against mutagenic foods was not clear. However, several studies demonstrated that ginseng is well known to have protective role against many toxicants by preventing or decreasing such toxicants or one of their metabolites to reach inside the cells and scavenging the free radicals (54). The protective action of ginseng against chromosome aberrations was observed in study of Ramadan et al. (55), who found that the chromosome aberrations had lowered in rats injected with ginseng plus ochratoxin, compared with those injected with ochratoxin alone.

4. Conclusions and Recommendations:

Heterocyclic aromatic amines (HAAs) resulting from ultra heating of proteinaceous materials are toxicants and mutagenic. They act directly on the body tissues or indirectly through their action on body enzymes. The results of the present study proved that both green tea and panax ginseng extract possessed a good protective action against the toxic and mutagenic effects of HAAs.

More concrete measures should be taken to reduce the hazards resulting from exposure to the HAAs. Otherwise, nutritional education with some social effort services should be done through women, wives and mothers, because they are still the principal decision makers concerning the family food. Further studies might be needed on long-term feeding on ultra heated proteinaceous materials and their effects on different enzymes and hormones. Furthermore, more than one dose of green tea and panax ginseng could be used in such studies to achieve the best results.

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