GENOTOXICITY AND HISTOPATHOLOGICAL STUDIES ON THE LIVER AND KIDNEY OF MALE RATS FED ON DIET CONTAINING WASTE FAT RELEASED FROM CHICKEN DURING GRILLING PROCESS

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Food is essential to provide sustenance but may also be an important factor in the genesis of human diseases. An association between the intake of grilled or broiled meat and development of cancer was recorded by Thomson *et al*. (1996).

Grilling meat, fish or other foods with intense heat over a direct flame result in fat dripping on the hot fire and yielding flames containing a number of polycyclic hydrocarbons (PAHs), these chemicals adhere to the surface of the food. The more intense the heat, the more PAHs are present (Larsson, 1986). PAHs are produced from organic compounds by condensation of smaller units at high temperatures forming stable polynuclear aromatic compounds. At high temperature, organic compounds are easily fragmented into smaller compounds, mostly free radicals, which may then recombine to form a number of relatively stable PAHs (Lijinsky, 1991). Lin *et al*. (1996) showed that, mice fed high fat diet developed more serve disease and had a shorter life span.

Weisburger *et al*. (1994) found that the use of grilled meats in diet, where compounds formed on the surface of meat may be associated with increased risk of genotoxicity and cancer. They also showed that, normal intestinal bacteria can convert one of these compounds, 2-amino-3-methyl-3H-imidazo [4, 5-f] quinoline (IQ) to the 7-hydroxy metabolite, 2-amino-3,6-dihydro-3-methyl-7H-imidazolo[4, 5-f] quinolin-7-one(7-OHIQ),a direct- acting mutagen.

The surface of fried and grilled meat or fish contain powerful mutagens, Sugimura *et al.* (1977) isolated these mutagens and identified them as heterocyclic aromatic compounds with an exocyclic amino group and often an O-methyl group.

The heterocyclic amines formed by partial pyrolysis of amino acids, sugar or creatinine at high temperatures has been shown to be carcinogenic and mutagenic in mice and rats (Ohgaki *et al.*, 1991).

Food mutagens cause different types of DNA damage, nucleotide altera-

tions and gross chromosomal aberrations. Most mutagens begin their action at the DNA level by forming carcinogen-DNA adducts, which result from the covalent binding of a carcinogen or part of a carcinogen to a nucleotide. However, the effect of food mutagens in carcinogenesis can be modified by heritable traits, namely, low penetrant genes that affect mutagen exposure of DNA through metabolic activation and detoxification or cellular responses to DNA damage through DNA repair mechanisms or cell death (Goldman and Shields, 2003)

The single cell gel electrophoresis (SCGE) assay, also known as the comet assay, is a rapid, simple, visual and sensitive technique for detecting and analyzing DNA strand breakage in a variety of organs and various mammalian cells (Olive *et al*., 1990). The advantage of the comet assay is that it allows any viable eukaryote cells to be analyzed. For these reasons, the comet assay is now widely used in researches of biomonitoring and DNA damage processes to routine assessments of genotoxicity. Quantitative analysis for DNA damage has yielded several parameters, including tailed nuclei, tail length, DNA % in the tail, and tail moment in the comet assay (Tice *et al*., 2000).

Singh *et al.* (1988) made microgels slides and electrophoresing under alkaline conditions and removed the DNA supercoiling and denaturated the DS DNA to SS DNA .With this modification they obtained a dose response curve with respect to length of DNA migration. The resulting images were subsequently named ‘Comet ‘because of their appearance and their total length was considered directly related to the DNA damage. From that moment a range of applications of the Comet assay have been used in investigations of the physiochemical behavior of DNA, through studies of cellular responses of DNA damage, to biomonitoring of human population.

When the Comet assay technique is used to detect *in vivo* genotoxicity, the most important advantage is that DNA lesions can be measured in cells not engaged in mitotic activity, making it possible to assay many organs (Fairbairn *et al.*, 1995).

The grilled chicken fat was used by low income people in Egypt and used to prepare Howawshi. Thus the aim of this study was to evaluate the genotoxic and histopathologic effects on liver and kidney of rats fed on diet contained fats of grilled chicken.

MATERIALS AND METHODS

Preparation of grilled chicken fat

About fifteen kilograms from liquids released as waste from chicken during grilling process were collected from different restaurants in Fayoum province, Egypt. The liquid was held at low temperature (7± 2°C) for 3 hours for solidification of fat fraction that was then separated from other liquids, the fat portion was rendered by heating and filtered by using cheese cloth for separation of any

foreign matters, then the obtained fat was directly analyzed.

Animals and diets

Fifteen Swiss male albino rats weighing approximately 90±10 g were used. The rats were obtained from the Animal House of the Faculty of Science, Fayoum University, Egypt. Food and water were provided *ad-libitum*. After feeding on the basal diet for one week as adaptation period, the animals were divided into three groups with five rats in each group. The first group was fed on control diet (containing corn oil); while the second and the third groups were fed diets containing wasted fat as substitution for the diet fat at levels of 50 and 100%, respectively. The experimental diets that contained the grilled fat chicken and corn oil according to (AOAC, 1990). are presented in Table (1).

Genomic DNA isolation from liver and kidney

The liver and kidney were surgically removed from each rat and instantly immersed in liquid nitrogen and grounded using an autoclaved ceramic pestle. About 17 mg of the grounded tissue was added to 600 µl of Nuclei Lysis Solution, according to Surzycki (2000). Genomic DNA was fractioned on agarose gel electrophoresis (1.2%) and ethidium bromide staining.

Detection of DNA damage by the comet assay

The portions of the liver and kidney were minced and suspended in chilled homogenizing buffer (pH 7.5) 0.075 M Nacl and 0.024 M Na_2 EDTA, and then homogenised gently using homogeniser in ice. The cells suspension was centrifuged at 4°C, 700 X g for 10 min. The cells were resuspended in the cold buffer (Sasaki *et al.* 1997). Lymphocytes were isolated by the standard method according to Brulles and Wells (1977).

The comet assay was carried out under alkaline conditions, basically as described by Singh *et al*. (1988). In control and treated rats, digital images were scanned into an image analyzer (Comet analyzer software IV) to determine the length of DNA migration (Comet tail length) due to genotoxicity. Tail length, DNA % in the tail and tail moment were obtained by observing 25 nuclei for each rat. The tail length is the distance from the comet head to the last visible signal in the tail. The percentage of DNA in the tail is calculated from the fraction of DNA in the tail divided by the amount of DNA in the nucleus multiplied by 100. The tail moment is the product of the amount of DNA in the tail and mean distance of migration in the tail (Olive *et al.*, 1990).

Histopathological examination

After eight weeks the animals were scarified. The liver and kidneys were excised and fixed in neutral buffered formalin 10% the organs were routinely processed and sectioned at 4 to 5 μm thickness. The obtained tissue sections were collected on glass slides, deparaffinized and stained with Hematoxylin and eosin stain, bromophenol blue, fulgen technique

and PAS technique (Bancroft and Gamble, 2002). The sections are then observed under microscope at 400X magnification.

Statistical analysis of data

Comet sassy data were analysed for homogeneity of variance using the General linear Model procedure of statistical analysis system (SPSS, 1999). Variable means for treatments indicating significant differences in the ANOVA were compared and the significances were indicated using Duncan multiple range tests (Duncan, 1955).

RESULTS AND DISCUSSIONS

Electrophoretic pattern of liver and kidney DNA.

A comparison of the gel electrophoretic DNA band patterns in liver and kidney of control and treated rats are shown in Fig. (1) as revealed from the figure, the genomic DNA of control group showed definite bands, while the bands of treated groups gave damaged and smear bands. Moreover, the damage was clear in the liver of treated rats. This may be due to ingest of genotoxic compounds from grilled –processed chicken. Such compounds cause different DNA damage, most of genotoxic compounds begin their action at the DNA level by forming DNA-adducts, which bind mutagen to a nucleotide (Goldman and Shields, 2003). An increase in DNA damage was indicated by an increase in migration length of the stained DNA.

Comet assay

The head length, tail length , head intensity % , tail intensity % , tail migration and tail moment in the treated rats and the control , obtained using automated image analysis software are described in Table (2).

The results in Table (2) and Figs. (2, 3 and 4) showed that grilled chicken fat induced a significant concentration-dependent increase in the tail length, tail intensity%, tail migration and tail moments in the liver, kidneys and lymphocytes respectively. These results suggested that DNA damage observed was due to genotoxicity and comet assay might be used to detect the DNA damage induced by grilled fat in rat liver, kidneys and lymphocytes. The tail moment was chose to present the results because it facilitates comparison among the tested agents. In the control group, the tail moment was 0.50±0.24, 0.15±0.10 and 0.22±0.17 µm for the liver, kidney and lymphocytes respectively, where in the treated groups the tail moment increased in a dose-dependent fashion. When the grilled fat in rats diet was 5% tail moment of 3.50±0.29, 3.08±0.42 and 2.54±0.42 µm in the liver, kidney and lymphocytes respectively were observed. These results suggest that the *in vivo* comet assay might be used to detect the DNA damage induced by mutagen compounds released during grilled processed foods and the presence of several PAHs in the grilled fat is a measure of the potential for cellular injury through hydroxyl radicals genera-

tion. The changes that occurred in liver and kidney DNA may be due to the presence of the PAHs in the grilled fat which increase the free radicals in liver and kidney and this execs of free radicals combined with some ions can cause DNA damage (Guidarelli, 1997).

The amount of DNA breakage in a cell in the comet assay was estimated from the migration extent (tail length) of the genetic material in the direction of anode (Singh *et al.* 1988). Furthermore, the percentage of DNA in the tail (tail intensity) has been shown to be proportional to the frequency of DNA strand breaks (Olive *et al.*, 1990). Tail moment is a simple descriptor calculated by the computerized image analysis system considering both the migration tail length as well as the fraction of DNA migrated in the tail (Villarini *et al.*, 1998).

Histopathological findings

The liver

The hepatic parenchyma of the control rats consisted of several hepatic lobules separated from each other by very delicate connective tissue septa housing the portal triad. Each hepatic lobule contained a thin walled central vein surrounded by hepatic cords radiating towards the periphery. The hepatic cords were formed of hepatocytes arranged in cord mannar of one or two rows of cells. The hepatic cords were separated from each other by the hepatic sinusoids. The latter appeared wide irregular blood spaces lined by endothelial cells and Von Kupffer cells (Fig. 5). Similar findings were mentioned by Sharma and Iqbal (2005) and Sharma *et al.* (2008). The hepatocytes appeared polyhedral or hexagonal in shape showing intensive reaction to bromophenol blue indicating high contents of total protein (Fig. 6) as stated by Mazia *et al*. (1953) and Klastskin and Oconn (1993). The hepatocytes also showed intense magenta colour when treated with Feulgen technique as shown in Fig. (7) indicating high amount of DNA material in the nucleus and sometimes the cytoplasm as presented by Feulgen and Rossenbeck (1924). The normal hepatocytes exhibited strong PAS reaction indicating the normal and intense localization of mucopolysaccharides and/or glycogen (Fig. 8) as interpreted by Rawat *et al*. (2002).

The rats of the second group showed generalized diatation and congestion involving the hepatic arteries, lymph vessels, central veins and hepatic sinusoids. The hepatocytes appeared vacuolated ballooned and some of them showed degenerative changes while the nuclei appeared pyknotic and densely stained (Fig. 9). The hepatocytes lost some of their total protein which was indicated by the moderate bluish stainability (Fig. 10). Also, the DNA content of the hepatocytes especially in the nuclei became greatly decreased as indicated by the moderate magenta colour of Feulgen reaction (Fig. 11). Moreover the general polysaccharides in the hepatocytes of this group became reduced to a moderate amount as observed in Fig. (12).

In the third group, further degenerative changes in the liver became advanced and progressive resulting in fatty changes and accumulation of variable amount of fat in the hepatocytes while most of the nuclei appeared pyknotic and karyolytic. Also the hepatic sinusoids were markedly degenerated and destroyed (Fig. 13). The hepatocytes lost most of their total protein as indicated by faint blue stainabilty (Fig. 14). Furthermore, a marked depletion and great reduction of both DNA material and general polysaccharides to their minimal amount was an expected result in this group (Figs. 15&16). These results are in accordance with Aly (1997), El-Shamy *et al*. (1999), Kaboglu and Aktac (2002) as well as Hussein *et al*. (2007). The hepatocellular damage is augmented by the elevated liver function tests (GOT & GPT). Klastskin and Oconn (1993) attributed the dilatation and congestion of hepatic vessels and sinusoids to the direct toxic effect of the toxins leading to hepatocellular damage. The authors also reported that lymphocytic infiltration was seen near the central veins. The marked decrease in total protein, DNA material and general polysaccharides is attributed to the hepatocellular damage.

The kidney

The kidney of the control rats showed the normal histological structure of the renal corpuscles and renal tubules. The renal corpuscle consisted of tuft of blood capillaries surrounded by the Bowmann's capsule. The latter has a parietal layer lined by squamous cells and a visceral layer lined by podocytes. The renal tubules included proximal convoluted tubules lined by large pyramidal cells with brush border, distal convoluted tubules lined by cuboidal cells, loop of Henle and collecting tubules (Fig. 17). Similar findings were recorded by Ford *et al.* (1980) and Selim (2005). All the cellular components of the renal cortex of normal rats showed the normal contents of the total protein, and DNA material (Figs. 18 &19) as explained by Feulgen and Rossenbeck (1924) and Mazia *et al*. (1953). The general polysaccharides are concentrated in the glomerular tuft, brush borders and basement membranes of the renal tubule (Fig. 20) as reported by Selim (2005).

The kidney of the second group showed degeneration of the glomerular tuft with infiltration of lymphocytes. The renal tubules became vacuolated and lost their brush borders (Fig. 21). The cells of both renal corpuscle and renal tubules lost a considerable amount of total protein and DNA material as indicated by the moderate reaction for both bromophenol blue and Feulgen technique (Figs. 22 &23). In this group the general polysaccharides became greatly decreased in the glomerular tuft and basement membranes while the degenerated brush borders showed negative reaction (Fig. 24).

In the third group a progressive degeneration of the glomerular tuft and renal tubules took place (Fig. 28). Also focal areas of hyalinization were a marked

sign in the group together with loss of cellular architecture (Fig. 25). Moreover, the affected cells lost the majority of their cytoplasmic protein as indicated by the faint blue color (Fig. 26). Also the DNA content was reduced to a minimum degree as explained by weak magenta color (Fig. 27). These results are in accordance with Ford *et al.* (1980), Oslen *et al*. (1986) and Attia *et al*. (2005). The degenerative changes recorded in the kidney of the treated rats are augmented by the elevated kidney function tests as creatinine and urea. The loss of cellular protein may be due to disorganization of the rough endoplasmic reticulum that lacks the full complement of ribosomes (Early *et al.*, 1992). Tsuda *et al*. (2001) and Sasaki *et al. (2002*) stated that the food additives as well as fatty diet induced DNA damage. The depletion of polysaccharide is attributed to the damage of the renal tissue and brush borders (Klastskin and Oconn, 1993).

The degenerative changes observed in the present study would indicate the local action of the grilled fat on both liver and kidney as they considered the main sites of detoxification and excretion of toxic materials of administered substances (Hussein *et al.*, 2007).

SUMMARY

Fat released from chicken during grilling process, as waste, is used by some low income people in Egypt in cooking as dietary fat because it is a cheap fat source compared to other fats. The present study aimed to elucidate the genotoxic and histopatholgic effects of grilled chicken fat on the liver, kidney and lymphocytes of male albino rats. Fifteen two months old male rats were divided to three groups, the first group was fed on control diet, the second and third groups were fed diets containing wasted fat at substitution levels of 50 and 100 % from the fat of diet, respectively. The animals were fed *ad-libtium* for eight weeks. The single cell gel electrophoresis (Comet) assay is a simple and effective method for detecting DNA damage in control and treated rat cells, and the results showed significant increase in tailed nuclei (DNA damage), tail moment, DNA % in the tail and tail length in liver, kidney and lymphocytes of treated groups compared to the control. In addition, both liver and kidney of treated rats showed a marked degenerative changes congestion of blood vessels in addition to loss of protein, polysaccharides and DNA content.

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Table (1): Composition of experimental diets (g/100 g).

Ingredients	Control diet	Diet 1	Diet 2
Casein	11.26	11.26	11.26
Corn oil	5.00	2.50	0.00
Salt mix	4.00	4.00	4.00
Vitamin mix	1.00	1.00	1.00
Wheat bran (fiber source)	5.00	5.00	5.00
Grilled chicken fat	0.00	2.50	5.00
Starch	73.74	73.74	73.74
Total	100.00	100.00	100.00

Table (2): Comet assay parameters obtained by image analysis in cells isolated from liver, kidney and lymphocytes in rats after administration with diet contained grilled fat.

Treatments		Head length (microns)	Tail length (microns)	Head Intensity %	Tail Intensity %	Tail migration (microns)	Tail moment (microns)
Control	Liver	12.98± 0.37^{b}	7.62 ± 0.89^{C}	90.98± 2.76^{A}	7.22± 3.16^{B}	1.36± 0.61^{C}	0.50± 0.24^{C}
Diet with 2.5% grilled fat		19.04± 1.88^{a}	13.86± 1.91^{B}	81.97± 5.00^{A}	18.02± 5.00^{B}	4.34± 1.30^{B}	1.68± 0.51^{B}
Diet with 5% grilled fat		16.89± 0.95^{a}	18.35± 0.99^{A}	67.89± 2.23^{B}	32.11± 2.23^{A}	9.91± 0.64^{A}	3.50± 0.29^{A}
Control	Kidney	14.74± 2.44^{a}	6.44± 0.56^{C}	96.29± 2.43^{A}	3.71± 2.43^{C}	0.24± 0.15^{C}	0.15± 0.10^{C}
Diet with 2.5% grilled fat		13.96± 0.78^{a}	10.25± 1.18^{B}	80.37± 5.37^{B}	19.63± 5.37^{B}	3.27.± 1.06^{B}	1.44± 0.42^{B}
Diet with 5% grilled fat		14.34± 0.37^{a}	15.72± 0.92^{A}	66.84± 4.53^{C}	33.16± 4.53^{A}	8.54± 0.86^{A}	3.08± 0.42^{A}
Control	Lymphocytes	13.37± 0.36^{a}	6.74± 0.28^{B}	96.30± 2.86^{A}	3.70± 2.86^{B}	0.39± 0.25^{B}	0.22± 0.17^{B}
Diet with 2.5% grilled fat		17.08± 1.86^{a}	16.00± 0.93^{A}	80.98± 3.35^{B}	20.28± 2.86^{A}	7.45± 1.12^{A}	2.12± 0.20^{A}
Diet with 5% grilled fat		13.76± 0.79^{a}	14.54± 0.88^{A}	74.56± 3.22^{B}	25.44± 3.22^{A}	7.65± 1.16^{A}	2.54± 0.42^{A}

Values in the same column within the same item followed by different letters are significantly different ($P < 0.05$ for a to c; $P < 0.01$ for A to C)

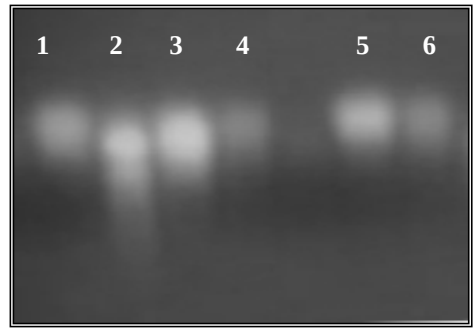

Fig. (1): Agarose gel electrophoretic pattern: Liver DNA (1:control); 2: (2.5% grilled fat); 3: (5% grilled fat); kidney DNA (4: control); 5: (2.5% grilled fat) and 6: (5% grilled fat).

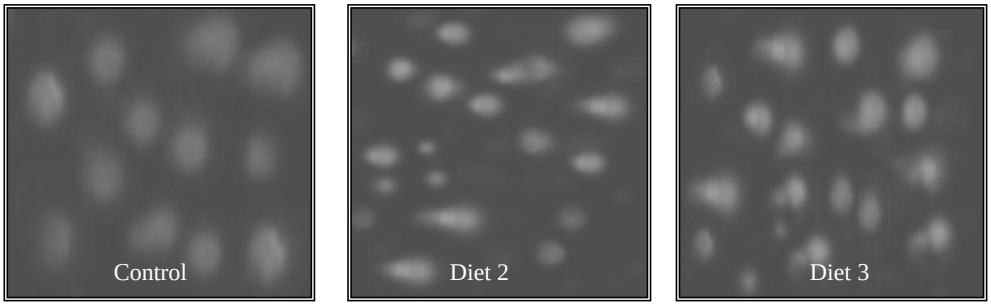

Fig. (2): Photomicrographs representative DNA damage (comet assay) in liver of rats fed on control diet and diet containing different concentrations of grilled chicken fat.

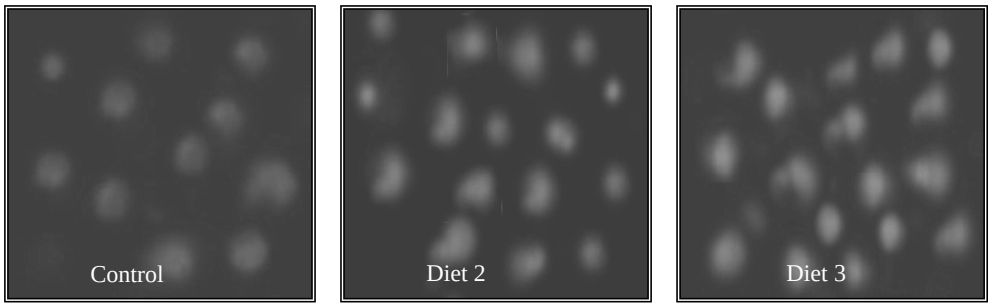

Fig. (3): Photomicrographs representative DNA damage (comet assay) in kidney of rats fed on control diet and diet containing different concentrations of grilled chicken fat.

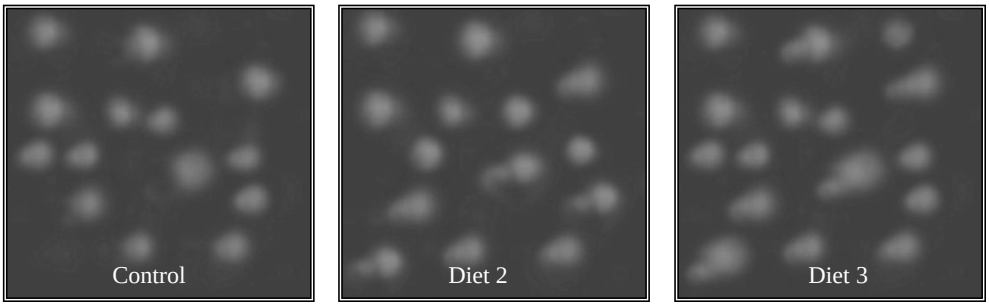

Fig. (4)**:** Photomicrographs representative DNA damage (come assay) in lymphocytes, of rats fed on control diet and diet containing different concentrations of grilled chicken fat**.**

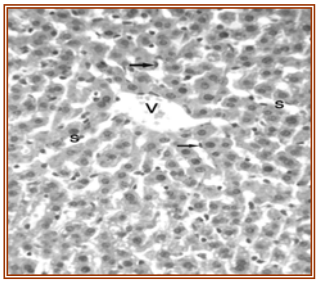

Fig. (5): A photomicrograph of the liver of control rats stained with H&E showing normal hepatic lobule has a thin walled central vein (V), hepatic cords radiating towards the periphery alternating with hepatic sinusoids(S) lined by Kupfer cells and endothelial cells (arrow). X, 400

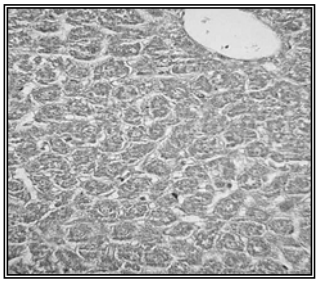

Fig. (6): A photomicrograph of the liver of control rats stained with bromophenol showing normal content of total protein in the hepatocytes as indicated by the intense blue stainability. X, 400

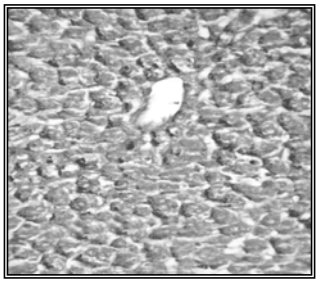

Fig. (7): A photomicrograph of the liver of control rats stained with Feulgen technique showing normal content of DNA in the hepatocytes as indicated by the intense magenta colour.X, 400

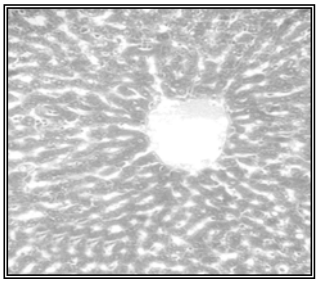

Fig. (8): A photomicrograph of the liver of control rats stained with PAS technique showing normal content of general polysaccharides as indicated by the intense red colour. X, 400.

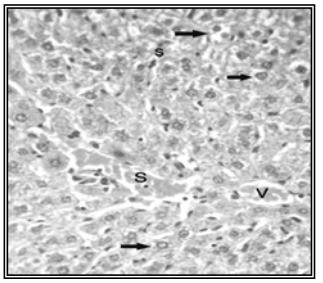

Fig. (9): A photomicrograph of the liver of rats feeding with 2.5% grilled fat stained with H&E showing dilated and congested central vein (V) and hepatic sinusoids(S). The hepatocytes (arrow) appeared vacuolated and some of them are degenerated. X, 400.

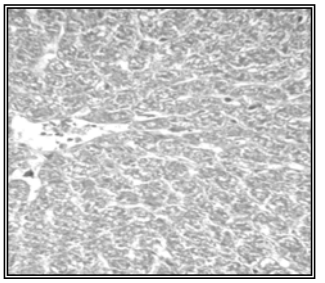

Fig. (10): A photomicrograph of the liver of rats feeding with 2.5% grilled fat stained with bromophenol showing moderate protein contents as indicated by moderate blue stainability. X, 400.

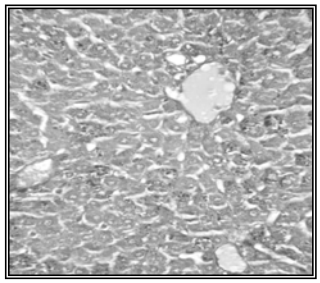

Fig. (11): A photomicrograph of the liver of rats feeding with 2.5% grilled fat stained with Feulgen technique showing reduced content of DNA in the hepatocytes as indicated by the moderate magenta colour.X, 400

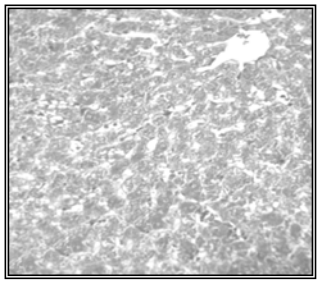

Fig. (12): A photomicrograph of the liver of rats feeding with 2.5% grilled fat stained with PAS technique showing moderate content of general polysaccharides as indicated by the moderate red colour. X, 400

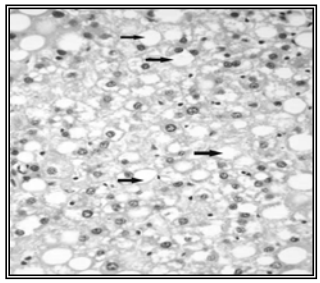

Fig. (13): A photomicrograph of the liver of rats feeding with 5% grilled fat stained with H&E showing that most of hepatocytes are degenerates with marked fatty changes (arrow). Most of the hepatic sinusoids are destroyed. X, 400

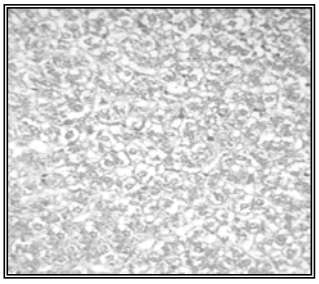

Fig. (14): A photomicrograph of the liver of rats feeding with 5% grilled fat stained with bromophenol showing marked depletion of protein contents as indicated by the weak blue stainability .X, 400

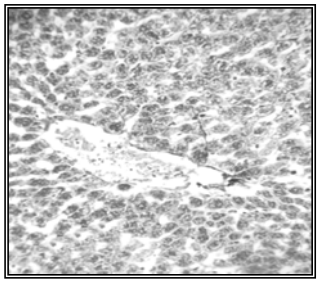

Fig. (15): A photomicrograph of the liver of rats feeding with 5% grilled fat stained with Feulgen technique showing a marked depletion of DNA in the hepatocytes as indicated by the weak magenta colour.X, 400

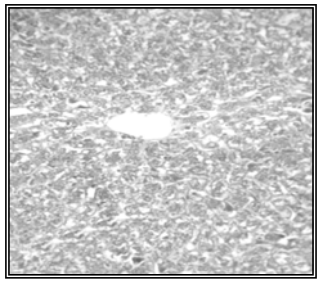

Fig. (16): A photomicrograph of the liver of rats feeding with 5% grilled fat stained with PAS technique showing a marked reduction of general polysaccharides as indicated by the weak to moderate red colour. X, 400

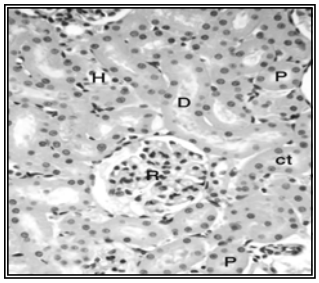

Fig. (17): A photomicrograph of the kidney of control rats stained with H&E showing normal histological structure of renal; corpuscle (R), proximal convoluted tubule (P), distal convoluted tubule (D), henles loop (H), and collecting tubule (CT). X, 400

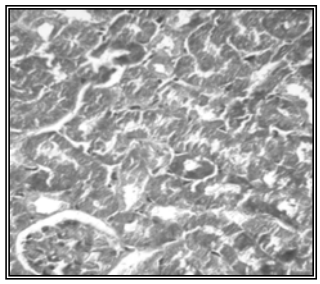

Fig. (18): A photomicrograph of the kidney of control rats stained with bromophenol showing normal protein content in the uriniferous tubules as indicated by intense blue stainability. X, 400

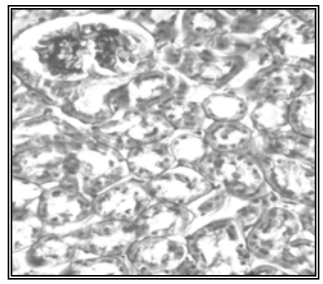

Fig. (19): A photomicrograph of the kidney of control rats stained with Feulgen technique showing normal DNA content in the uriniferous tubules as indicated by strong magenta colour.X, 400

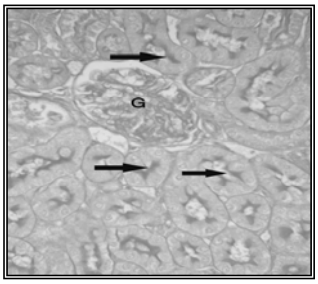

Fig. (20): A photomicrograph of the kidney of control rats stained with PAS technique showing strong reaction in the glomeruli (G), brush borders (arrow) and basement membrane (arrow head). X, 400.

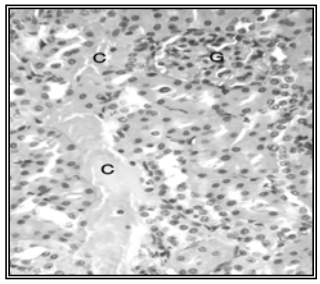

Fig. (21): A photomicrograph of the kidney of rats feeding with 2.5% grilled fat stained with H&E showing degeneration of glomerular tuft (G) and brush borders of renal tubules with accumulation of hyaline cast (C) beside lymphocyteic infiltration. X, 400.

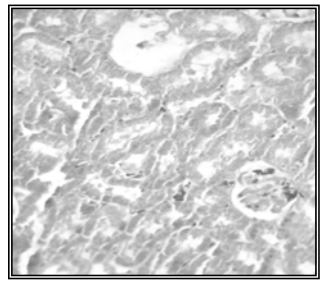

Fig. (22): A photomicrograph of the kidney of rats feeding with 2.5% grilled fat stained with bromophenol showing moderate protein content in the uriniferous tubules as indicated by moderate blue stainability. X, 400

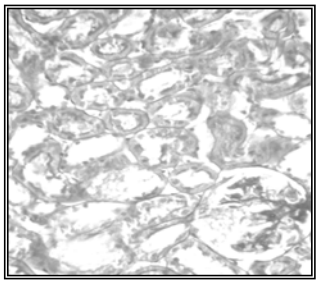

Fig. (23): A photomicrograph of the kidney of rats feeding with 2.5% grilled fat stained with Feulgen technique showing moderate DNA content in the uriniferous tubules as indicated by moderate magenta colour.X, 400.

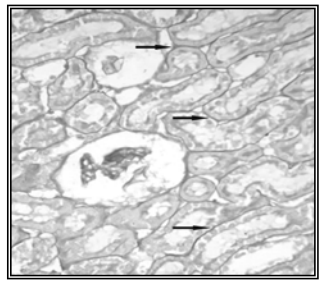

Fig. (24): A photomicrograph of the kidney of rats feeding with 2.5% grilled fat stained with PAS technique showing strong reaction in the basement membrane (arrow) and absent in the brush borders. X, 400.

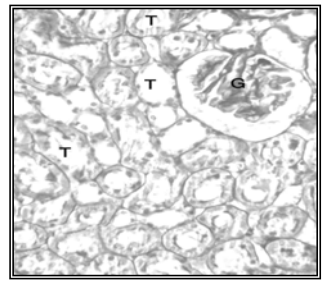

Fig. (25): A photomicrograph of the kidney of rats feeding with 5% grilled fat stained with H&E showing degeneration and hyalinization of glomerular tuft (G) and marked degenerationof the renal tubules (T) .X, 400.

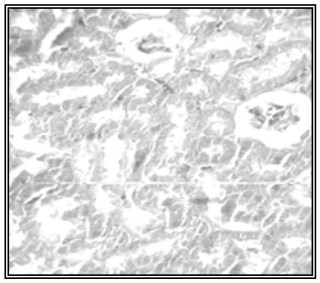

Fig. (26): A photomicrograph of the kidney of rats feeding with 5% grilled fat stained with bromophenol showing a marked reduction of protein content in the uriniferous tubules as indicated by faint blue stainability. X, 400.

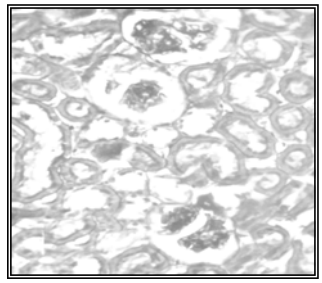

Fig. (27): A photomicrograph of the kidney of rats feeding with 5% grilled fat stained with Feulgen technique showing minimal DNA content in the uriniferous tubules as indicated by faint magenta colour.X, 400.

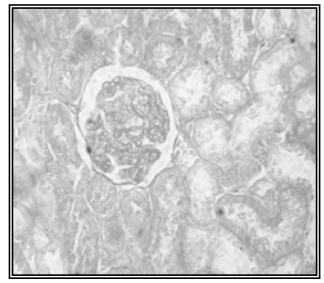

Fig. (28): A photomicrograph of the kidney of rats feeding with 5% grilled fat stained with PAS technique showing strong reaction in the basement membrane and absent in the brush borders. X, 400.