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Biological Effects of Some Synthetic Flavoring Agents on Experimental Animals

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ABSTRACT

The introduction of synthetic food flavorings has been widely increased in Egypt. These materials are used in both industrial and household processing scale. Many of these materials, which commonly used in the household preparations, are anonymous. The use of not controlled food flavorings (unknown) might exposure the consumers health to various health risks. These unknown flavoring materials used in food processing were subjected to chemically and biologically evaluation. Heavy metals contents and FT-IR analysis were determined. A nutrition experiment on rats was conducted to determine growth parameters, liver and kidney functions. Also, histological examination on brain and testes was done. Results indicated that studied heavy metals content of flavoring agents did not exceed the permissible limits of each that stated by international organizations. FT-IR spectra indicated that the studied unknown flavoring agents contained many functional groups such as hydroxyl, vinyl, carbonyl, amide, alcohol and aliphatic amine groups. As for growth parameters, body weight of all studied groups significantly decreased via increasing dosages of synthetic flavoring agents (10, 40, 70 and 100 mg/kg/day) compared with those of control group. Flavoring agents had a negative effect on liver and kidney functions where liver enzymes activity (ALT and AST), creatinine and urea concentrations of treated groups were significantly increased compared with those of control group. While, SOD activity and albumin content were significantly decreased. With increasing the administrated concentration of synthetic flavors the adverse effects of biological parameters had been significantly increased. As well as, brain histological sections proved to show congestion and edema. Testes showed irregular shape of seminiferous tubules, atrophy of leydig cells and disturbance in spermatogenesis. Results indicated that the used doses of the synthetic food flavorings were mostly attributable to hepatocellular damage, renal failure and decrease in spermatogenesis process. In conclusion, synthetic food flavorings had negatively affected the biological performance of Albino male rats. More studies could be required to re-evaluate the health effects of used food flavors in the local markets. Government must take conclusive decisions and put restrictions to reduce these hazard components import, handling and using.

Keywords: Flavoring agents, liver functions, ALT, AST, SOD, albumin, creatinine, urea and histological examinations.

INTRODUCTION

Food additive is any substance not classified as food, which is added to, or used in or on, food at any stage of food processing. These materials are commonly used to keep the food quality, texture, consistency, taste, colour and alkalinity or acidity, or to serve any other technological function in relation to food (Anon., 1980 and Anon., 1984). However, there are about 2500 chemically defined flavoring substances used in EU and USA. Most of these substances (60%) were evaluated by Flavor and Extract Manufacturers' Association of the United States experts (FEMA) and were recognized by the US Food and Drug Administration (FDA) to be Generally Recognized As Safe (GRAS) substances, as they are considered safe for their intended use. Most of flavoring substances are volatile organic chemicals, have simple, well characterized structures with a single functional group and low molecular weight. More than 52% of these substances added to food in USA are simple aliphatic acyclic and alicyclic alcohols, aldehydes, ketones, carboxylic acids and related esters, lactones, ketals and acetals (Munro *et al*., 1999). FAO (1997) and WHO (2011) adopted infrared technique as one of identification methods of food additives including flavoring agents where FAO used this method to characterize more than 250 certain flavoring agents. By great increasing of food additives utilization, there also has emerged considerable scientific data linking food additive intolerance with various physical and mental disorders, particularly with childhood hyperactivity (Feingold, 1973 and Smith, 1991). Monosodium glutamate (MSG) is added to some processed foods to provide a strong meaty taste. MSG at examined doses may cause an adverse effect on the hepatic and renal functions which might be due to oxidative stress induced by MSG on the liver and renal tissue (Tawfik and Al-Badr, 2012).

Many unknown artificial flavors enhancers are highly handled in the local markets in Egypt. These chemicals are added to different snacks such as popcorn, chips and pasta without any regulations or maximum limits. Accordingly, this current study was carried out to chemically and biologically evaluate these flavoring agents. A feeding experiment was conducted to measure the effects including growth parameters, liver and kidney functions. Also, histological examination was carried out on brain and testes tissues.

MATERIALS AND METHODS

Materials:

Four different synthetic food flavorings (enhancers) namely chicken, ketchup, cheese and kabab, were purchased from the local market, Mansoura city, Egypt.

Eighty five male Albino rats (120-150 ± 2g, 10 weeks age) were obtained from National Organization Drug Control and Research (NODCAR), Giza, Egypt.

Heavy metals determination:

Heavy metals (Mn, Cd, Pb, Cu, Zn and Ni) were determined in the Central Laboratory Unit, Faculty of Science, Mansoura University, Egypt. Samples (triplicate 0.2 g) were digested using 5mL nitric acid, 2 mL perchloric acid. Samples were heated at 90°C until all the materials were dissolved. After digestion, samples were diluted with deionized water, filtered. Volume made up to 10 mL using deionized water. thereafter, samples were determined, using flame atomic absorption spectrophotometer (Perkin Elmer, Model 2380) (UNEP/FAO/IAEA/IOC, 1984).

Metal pollution index (MPI) calculated by multiplication of studied heavy metal concentrations (ppm), then 6th root of the result using the formula reported by Usero *et al*. (1997).

$$MPI = (Mn \times Cd \times Pb \times Cu \times Zn \times Ni)^{I/6}$$

Fourier Transformer Infra-Red spectroscopy (FT-IR):

A standard procedure with 1mg of investigated flavoring agents grinded with 200 mg of KBr was used for FT-IR analysis at Spectral Analyses Unit, Chemistry Department, Faculty of Science, Mansoura University, Egypt. The spectra were recorded at room temperature as average of 32 sample scans using a ThermoFisher Nicolete FT-IR, model IS10, USA. A Nernst rod was the excitation source and a DTGS detector with spectral range: 400-4000 cm^{-1}. The resolution was 4 Cm^{-1} and optical velocity was 0.6329.

Biological assay:

Animals and experimental design:

The animals were housed in separate stainless steel cages under controlled conditions at constant temperature (24°C) and given free access of food and water throughout the experimental period. After adaptation period (7 days), rats were divided into seventeen groups (n=5), all groups (G1, G2, G3, G4 and G5) were fed on basal diet only (10% casein, 10% corn oil, 5% cellulose, 1% vitamin mixture, 4% salt mixture, 70% corn starch (Lana Peter and Pearson, 1971). All animals were fed on basal diet for 90 days. The rats were fed according to the following scheme:

G1: Rats fed on basal diet only (negative control).
G2: Rats fed on basal diet + chicken flavor.
G3: Rats fed on basal diet + catchup flavor.
G4: Rats fed on basal diet + cheese flavor.
G5: Rats fed on basal diet + kabab flavor.

Rats were daily treated with 1mL of flavoring agents solution using oral stomach tube containing the following ratios: 0, 10, 40, 70 and 100 mg/Kg of body weight per day.

Rats were observed daily for the appearance of any symptoms of discomfort that might be related to study treatments. Body weight (BW) of the rats was recorded at the beginning of the experiment and at the end of experiment period (90 days), the percentage of weight gain was calculated according to the following equation:

(Final weight - Beginning weight) ÷ Beginning weight × 100.

Treatments and animals maintenance were in accordance with the guidelines of Pharmacy Faculty Labs. animals care and committee, which follows the international animal care and use guidelines(ILAR, 1996).

Blood sampling:

At the end of experimental period, animals were fasted (overnight), anesthetized with diethyl ether and sacrificed. Blood samples were collected from the eye plexuses of rats by a fine capillary glass tube and immediately placed on ice. Serum samples were collected into dry clean centrifuge tubes, the serum was separated after centrifugation for 10 min at 3000 rpm (1500xg) and kept at -20°C until analysis.

Biochemical analysis:

The following biochemical parameters were estimated by test reagent kits (Bio-diagnostics, Egypt) mentioned below. Liver functions: alanine aminotransferase (ALT,U/L) and aspartate aminotransferase (AST, U/L) (Bergmeyer and Harder, 1986), albumin (g/dL) Gustafsson (1976) and super oxide dismutase (SOD, U/ml) (Marklund and Marklund, 1974). Kidney functions: creatinine (g/dL) (Faulkner and King, 1976) and Urea (g/dL), (Patton and Crouch, 1977).

Histological examination:

Autopsy samples were taken from brain and testes of rats in different studied groups and fixed in 10% formal saline for 24h. Samples were washed using tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56ºC in hot air oven for 24h. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by sledge microtome.

The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stain for routine examination through the light electric microscope (Banchroft *et al*., 1996).

Statistical analysis:

Data (heavy metals and biochemical analysis data) were presented as mean ± standard error (n=3). Data were analyzed using analysis of variance (two ways ANOVA), while comparisons were made using Duncan's test at $P<0.05$ level of significance using SPSS (2008) version 17 program for windows.

RESULTS AND DISCUSSION

Heavy metals compositions:

Results (Table 1) indicate that manganese content recorded about 0.035 ppm in all samples with little significant differences, meanwhile copper content ranged from 0.017 ppm in kabab flavor to 0.127 ppm in catchup flavor with high significant differences between the four samples. Zinc content was around 0.015 ppm with low significant differences with F value of 8.93. Although Mn, Cu and Zn were considered as heavy metals, they also were microelements for human nutrition. Thus there were no recommended maximum or permissible limits for them except in case of Zn, where USDA (2014) reported 1.00 ppm Zn as maximum level in food additives.

Concerning cadmium, lead and nickel contents (ppm), it was illustrated that Ni content was the highest mineral found in the selected samples (Table 1), it varied from 0.188 to 0.306 ppm in catchup and cheese flavors, respectively. There were high significant differences between all samples and there was no any maximum limits for Ni in food additives. On the other hand, Cadmium content ranged from 0.129 to 0.144 ppm in catchup and cheese flavors, respectively. There were highly significant differences between all samples with F value of 840. Finally, Lead content recorded 0.130 ppm in chicken flavor and 0.155 ppm in cheese and kabab flavors. But Cd and Pb content in all studied

samples did not exceed the permissible limits stated by FAO (2002), EOSQ (2010) and USDA (2014).

Metal pollution index (MPI) was calculated to compare the overall metal contents of flavoring agents investigated in this study. MPI ranged from 0.059 to 0.077 in kabab and catchup flavors, respectively. This index could help to expect the highest polluted flavoring agent. It could be predicted that catchup flavor may have the strongest effect on albino rats followed by chicken flavor depending on its content of heavy metals.

Table (1): Heavy metals content (ppm) of the studied synthetic food flavorings:

Flavoring agents / Heavy metals	Chicken	Catchup	Cheese	Kabab	Maximum Limited level (ppm)
Manganese (Mn)	0.037±0.0005[a]	0.034±0.0003[b]	0.034±0.0006[b]	0.036±0.0005[a]	-
Cadmium (Cd)	0.138±0.0003[c]	0.129±0.0002[d]	0.144±0.0002[a]	0.142±0.0001[b]	No limits[1] ,1.00[2,3]
Lead (Pb)	0.130±0.0002[c]	0.137±0.0005[b]	0.155±0.0002[a]	0.155±0.0002[a]	1.00[1,2,3]
Copper (Cu)	0.057±0.0008[b]	0.127±0.0004[a]	0.047±0.0001[c]	0.017±0.0003[d]	-
Zinc (Zn)	0.017±0.0006[a]	0.014±0.0002[b]	0.013±0.0007[b]	0.013±0.0005[b]	1.00[3]
Nickel (Ni)	0.262±0.0012[b]	0.188±0.0018[d]	0.306±0.0018[a]	0.247±0.0016[c]	-
MPI*	0.074	0.077	0.072	0.059	

Mean values ± standard error (n=3). Means of samples having the same letter(s) within a row are not significantly different (P<0.05).
[1]FAO (2002),[2]EOSQ (2010), [3]USDA (2014).
*** MPI means Metal Pollution Index.**

FT-IR spectroscopy:

Data in Figure (1) show FT-IR of studied flavoring agents (A- chicken, B- catchup, C- cheese and D- kabab) in the spectral range of 400-4000 Cm^{-1}. Illustrated results indicated that these unknown flavoring agents were complexes of more than component. The identification of each functional group was done by infrared tables of common absorption frequencies. All studied unknown flavoring agents contained many of functional groups. The highest absorptions in the FT-IR spectrum of chicken flavor (Fig. 1-A) are noticed at 3442, 2926, 1636, 1023 and 576 Cm^{-1} that are due to hydroxyl group, vinyl group, amide group, aliphatic amine group and bromoalkene group (C-X), respectively.

In catchup spectrum (Fig. 1-B), the peaks obtained at 3412, 2927, 1636, 1411, 1053 and 604 Cm^{-1} indicated the presence of hydroxyl, vinyl, amide, alkyl, aliphatic amine and vinyl cis-distributed alkene groups, respectively. Whereas, cheese spectrum (Fig. 1-C) showed some peaks at 3432, 2926, 1744, 1658, 1156, 1084 and 576 Cm^{-1} represents hydroxyl, vinyl, carbonyl, amide, alcohol, aliphatic amine and bromoalkene groups (C-X), respectively. Finally, the strongest absorptions in the FT-IR spectrum of kabab flavor (Fig. 1-D) are observed at 3431, 2926, 1743, 1654, 1413, 1158 and 1084 Cm^{-1} which are due to hydroxyl group (O-H) with high concentration of alcohol and phenols, vinyl group ($C=CH_2$), carbonyl group (C=O) esters and lactones influenced by conjugation and ring size, amides, alkyl (methylene), tertiary alcohol group (C-O) and aliphatic amines (CN), respectively. From previous results, it could be easily seen that chicken and cheese flavors contained more functional group than those in catchup and kabab flavors.

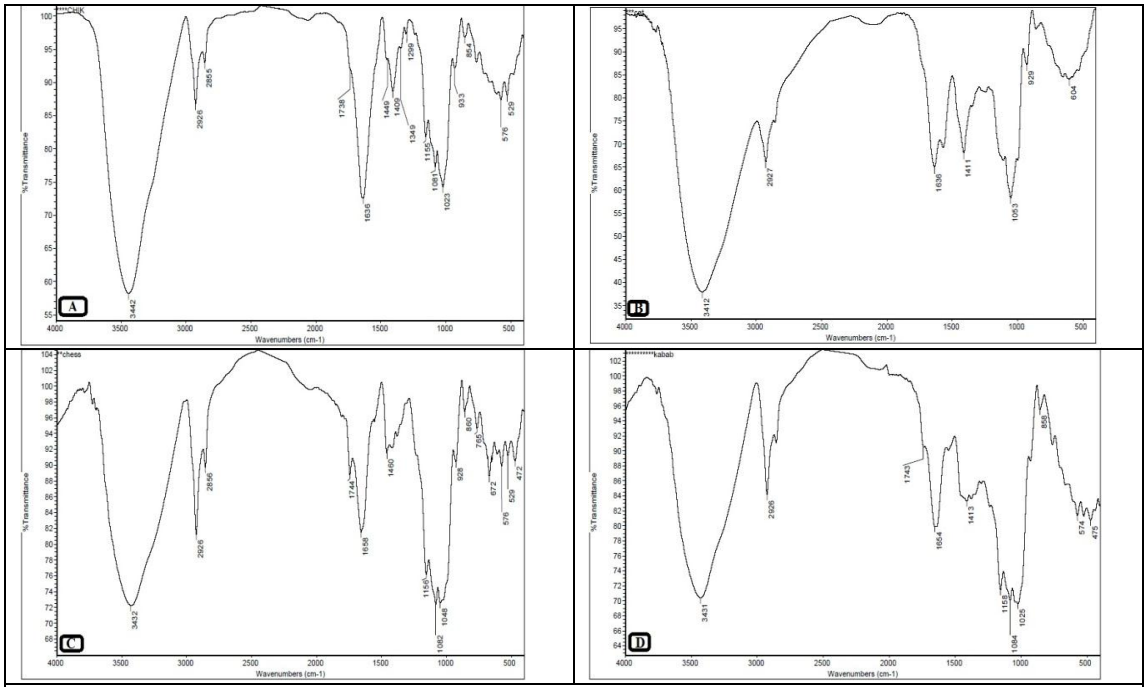

Figure (1): The FT-IR spectra of studied flavoring agents (A- chicken, B- catchup, C- cheese and D- kabab) in the spectral range of 400-4000 cm^{-1}.

Biological assay:

Growth parameters:

The increase (%) in body weight was presented in Table (2). At day 0, there were no significant differences in body weight between different groups. After experimental period, body weight of all tested groups was significantly decreased by increasing dosages of synthetic food flavorings (0, 10, 40, 70 and 100 mg/kg/day) compared with the control group. Among the tested groups, cheese and catchup groups were the highly effective groups, while the other two groups showed slight decrease in body weight compared with the control group.

Table (2): Effect of studied synthetic flavoring agents on growth parameters.

Groups	Parameters	Initial weight (g)	Final weight (g)	Weight gain %	Weight gain (g)/day
G1 (control)		148.00±1.53^{a}	297.00±7.21^{a}	100.63±3.37^{ab}	1.66±0.07^{a}
G2 (chicken flavoring agent)	10	139.67±3.71^{ab}	287.00±10.02^{ab}	105.40±2.01^{a}	1.64±0.07^{ab}
	40	135.67±6.98^{abcd}	267.00±20.53^{abc}	96.27±5.24^{abc}	1.46±0.15^{abcd}
	70	131.00±4.00^{bcd}	261.50±13.50^{abc}	99.49±4.21^{ab}	1.45±0.11^{abcd}
	100	122.50±2.50^{d}	220.50±20.50^{c}	79.74±13.06^{cd}	1.09±0.20^{d}
G3 (catchup flavoring agent)	10	137.67±3.38^{ab}	264.67±12.12^{abc}	92.08±4.84^{abcd}	1.41±0.10^{abcd}
	40	136.67±4.67^{ab}	263.00±19.66^{abc}	91.91±7.92^{abcd}	1.40±0.17^{abcd}
	70	129.00±2.65^{bcd}	241.00±9.61^{bc}	86.68±3.83^{abcd}	1.24±0.08^{bcd}
	100	132.67±1.45^{bcd}	237.00±7.51^{c}	78.74±6.61^{cd}	1.16±0.09^{d}
G4 (cheese flavoring agent)	10	143.00±3.79^{ab}	287.00±19.40^{ab}	100.27±8.24^{ab}	1.60±0.17^{abc}
	40	134.33±6.36^{abcd}	248.33±12.14^{bc}	84.85±0.97^{bcd}	1.27±0.06^{abcd}
	70	135.67±1.20^{abcd}	238.67±8.69^{c}	75.97±6.90^{d}	1.14±0.10^{d}
	100	123.00±3.00^{cd}	232.00±5.00^{c}	88.64±0.54^{abcd}	1.21±0.02^{cd}
G5 (kabab flavoring agent)	10	142.00±1.53^{ab}	268.33±2.60^{abc}	88.98±1.26^{abcd}	1.40±0.02^{abcd}
	40	135.33±2.91^{abcd}	264.33±8.69^{abc}	95.23±2.40^{abcd}	1.43±0.07^{abcd}
	70	140.00±3.00^{ab}	262.00±19.00^{abc}	86.94±9.57^{abcd}	1.36±0.18^{abcd}
	100	130.50±8.50^{bcd}	248.50±24.50^{bc}	90.00±6.40^{abcd}	1.31±0.18^{abcd}
Sig. value		0.015	0.028	0.031	0.031

Mean values ± standard error (n=3). Means of samples having the same letter(s) within a column are not significantly different (P<0.05).

Liver functions:

The effect of selected synthetic flavoring agents on liver functions (ALT, AST, albumin and SOD activity) was determined (Table 3). Results showed a significant increase in liver function enzymes with increasing the doses of synthetic flavoring agents, compared with control group. The highest activity values in ALT and AST were observed in chicken and catchup flavor groups after feeding on 100 mg/kg/day (140.50, 111.50 U/L for chicken and 164.67, 106.33 U/L for catchup, respectively).

In general, there were a significant differences between the control and treated groups. The highly adverse affects were observed in treat of rats with chicken and catchup followed by cheese and kabab, flavoring agents.

The albumin concentration was 3.95 g/dL in the control group. No significant changes were observed in the treated groups compared with control groups (Table 3), except some treatments such as chicken, cheese and kabab (70, 100 mg/kg/day). In these groups the albumin concentration was decreased by ratios ranged from 12.15% to 17.72%.

On the other hand, SOD activity value of control group was 29.80 U/mL. SOD values were significantly decreased in all groups compared with those of control. No significant changes were found with the treated groups (Table 3).

The serum ALT and AST shows functional activity of liver. Any increases in the activities of these enzymes indicates negative effects due to the doses used.

The increase in the ALT and AST activity may be an indicator of liver damage. These used flavoring agents may contain monosodium glutamate could easily release free glutamate. The diminution of GLU produces ammonium ion (NH_4^+) that could be toxic unless detoxified in the liver by the reactions of the urea cycle. Thus, the excess of ammonium overload that may occur as a result of an increased level of glutamate following MSG intake could damage the liver, consequently releasing the ALT enzyme that may lead to its observed elevation. This increase could also be explained by free radical production which reacts with polyunsaturated fatty acids of cell membrane leading to impairment of mitochondrial and plasma membranes resulting in enzyme leakage (Poli *et al*., 1990).

Albumin content decreased in the serum of MSG dosed rats (Tawfik and Al-Badr, 2012). Studies performed in MSG-treated rats showed a significant decrease in serum albumin levels compared with control group. The synthetic function of liver was altered by MSG, so albumin level decreased. On the other hand, Mean serum urea of the control animals was 35.2 mg/dL and that of the MSG treated rats were 31.4 and 24.1 mg/dL of two MSG doses. Urea is the major nitrogen-containing metabolic product of protein catabolism. The significant reduction in serum urea concentration throughout the experimental period may be attributed to impairment of the urea cycle leading to reduced production of the metabolic product. Serum creatinine of the treated rats was observed to be higher than that in control group (Tawfik and Al-Badr, 2012).

Table (3): Effect of studied flavoring agents (mg/kg/day) on liver functions.

Groups	Parameters	ALT (U/L)	AST (U/L)	SOD (U/mL)	Albumin (g/dL)
G1 (control)		34.00±3.79^{a}	39.00±4.51^{a}	29.80±0.35^{a}	3.95±0.09^{a}
G2 (chicken flavoring agent)	10	86.00±20.65^{bcde}	75.00±11.15^{bcd}	23.07±1.54^{bc}	3.68±0.06^{abc}
	40	116.33±2.96^{det}	98.33±2.19^{de}	20.37±1.18^{de}	3.63±0.04^{abc}
	70	125.50±26.50^{etg}	106.50±17.50^{e}	16.80±1.10^{tg}	3.45±0.11^{bc}
	100	140.5±25.50^{tg}	111.50±31.50^{e}	16.60±0.20^{tg}	3.25±0.22^{c}
G3 (catchup flavoring agent)	10	102.00±5.57^{bcdet}	84.67±15.07^{cde}	29.17±0.15^{a}	3.78±0.15^{ab}
	40	104.33±32.37^{cdet}	93.67±3.71^{de}	27.87±0.09^{a}	3.63±0.03^{abc}
	70	125.00±5.57^{etg}	100.00±1.73^{de}	25.13±0.69^{b}	3.61±0.05^{abc}
	100	164.67±18.22^{g}	106.33±6.69^{e}	21.50±0.57^{cd}	3.54±0.04^{abc}
G4 (cheese flavoring agent)	10	64.67±2.03^{abc}	46.67±0.88^{ab}	27.57±0.41^{a}	3.58±0.13^{abc}
	40	65.67±8.76^{abc}	48.67±0.88^{ab}	23.90±0.66^{bc}	3.60±0.21^{abc}
	70	77.33±5.24^{abcde}	50.33±2.91^{ab}	20.47±0.58^{de}	3.45±0.10^{bc}
	100	92.50±26.50^{bcdef}	57.50±17.50^{abc}	17.40±0.60^{f}	3.47±0.06^{bc}
G5 (kabab flavoring agent)	10	53.33±6.89^{ab}	40.67±3.53^{a}	23.23±1.10^{bc}	3.72±0.18^{ab}
	40	69.67±1.67^{abcd}	46.33±1.20^{ab}	18.77±0.82^{ef}	3.63±0.22^{abc}
	70	77.50±9.50^{abcde}	48.50±0.50^{ab}	14.70±0.80^{g}	3.47±0.07^{bc}
	100	77.50±2.50^{abcde}	62.50±9.50^{abc}	14.55±0.45^{g}	3.43±0.05^{bc}
Sig. value		0.000	0.000	0.184	0.000

Mean values ± standard error (n=3). Means of samples having the same letter(s) within a column are not significantly different (P<0.05).

Kidney functions:

The concentration of creatinine and urea (g/dL) in both control and active groups are shown in (Table 4). The results showed significant differences between the control and treated groups. There are also significant changes among the active groups, depending on the type and the concentration of the flavoring agents used. The increase in creatinine and urea concentrations with highly correlated with the increasing of used flavoring agent doses. The highest concentration of creatinine (0.88 g/dL) was found in rats treated with Kabab flavoring agents (100mg/kg/day), while cheese flavor (100mg/kg/day) had the highest urea concentration (45.95 g/dL).

Table (4): Effect of studied flavoring agents (mg/kg/day) on kidney functions.

Groups	Parameters	Creatinine (g/dL)	Urea (g/dL)
G1 (control)		0.47±0.05^{a}	18.67±1.25^{abc}
G2 (chicken flavoring agent)	10	0.55±0.02^{abc}	22.30±0.21^{abc}
	40	0.63±0.07^{bc}	23.43±1.71^{abcd}
	70	0.68±0.07^{bcd}	26.05±2.85^{abcd}
	100	0.68±0.08^{cd}	28.30±1.80^{bcde}
G3 (catchup flavoring agent)	10	0.52±0.04^{ab}	20.60±0.32^{ab}
	40	0.53±0.01^{abc}	21.37±0.37^{abc}
	70	0.62±0.01^{bc}	22.57±0.61^{abc}
	100	0.67±0.06^{bcd}	26.50±2.42^{abcde}
G4 (cheese flavoring agent)	10	0.54±0.01^{abc}	23.00±1.62^{abc}
	40	0.64±0.01^{bc}	33.43±2.93^{def}
	70	0.65±0.02^{bcd}	37.97±5.02^{f}
	100	0.79±0.16^{de}	45.95±7.45^{e}
G5 (kabab flavoring agent)	10	0.60±0.03^{abc}	28.80±4.08^{bcde}
	40	0.62±0.02^{bc}	29.47±0.88^{bcde}
	70	0.67±0.02^{bcd}	29.90±0.60^{cdef}
	100	0.88±0.01^{d}	35.00±2.30^{ef}
Sig. value		0.000	0.000

Mean values ± standard error (n=3). Means of samples having the same letter(s) within a column are not significantly different (P<0.05).

The obtained data (creatinine, ALT and AST) are in good agreement with those of El-Malky *et al*. (2014) and Sarhan *et al*. (2014), who reported that artificial colorants used in foods caused rises in urea, creatinine, ALT and AST, which were strictly associated to the injury of renal and liver functions.

The serum urea nitrogen is a measure of renal function. Normally, the serum urea nitrogen level rises in heart failure, dehydration, or a high protein diet and low urea nitrogen level can be observed in liver and renal damage or in liver diseases (Johnson *et al*., 1972).

Histological examination:

Concerning to histological examinations, Figure (2) showed histological changes in male rats brain as affected by different commercial flavoring agents (chicken, catchup, cheese and kabab) with daily intake of 10, 40, 70 and 100 mg/kg body weight for 90 days as compared with control group.

At first, the control group (Fig. 2-A) showed the granular cell layer (G) of the dentate gyrus of the hippocampus formed mainly of three layers: polymorphic layer (O), granular cell layer (G) and molecular layer (M).The granular cell layer (G) is formed mainly of small granule and pyramidal cells that are densely packed and have large vesicular nuclei; their processes are well apparent in the molecular layer. The large dispersed basket cells and interneurons in both molecular and polymorphic layers could be seen.

Chicken flavoring agent treated groups (Fig. 2-B,C,D,E) showed apparent decrease in the number and density of granular pyramidal cells in rats treated with 70 mg/kg/day. The granular cells appear loosely packed. Few shrunken cells (SC) with a dark acidophilic cytoplasm, pyknotic nuclei and an increase in the white space around them were seen. An increased blood vessels could be noted. A decrease in the thickness of the granular cell layer (G) compared with the control group was observed in 100mg/kg/day group (Fig. 2-E). Granular cells were few in number and appeared loosely packed. Some degenerated granule neurocyte (DG) with

dark cytoplasm and pyknotic irregular nuclei were seen. Halo areas around the cells can be seen. Few cells hade vesicular nuclei. It could be noted also the presence of molecular layer (M) and the polymorphic layer (O).

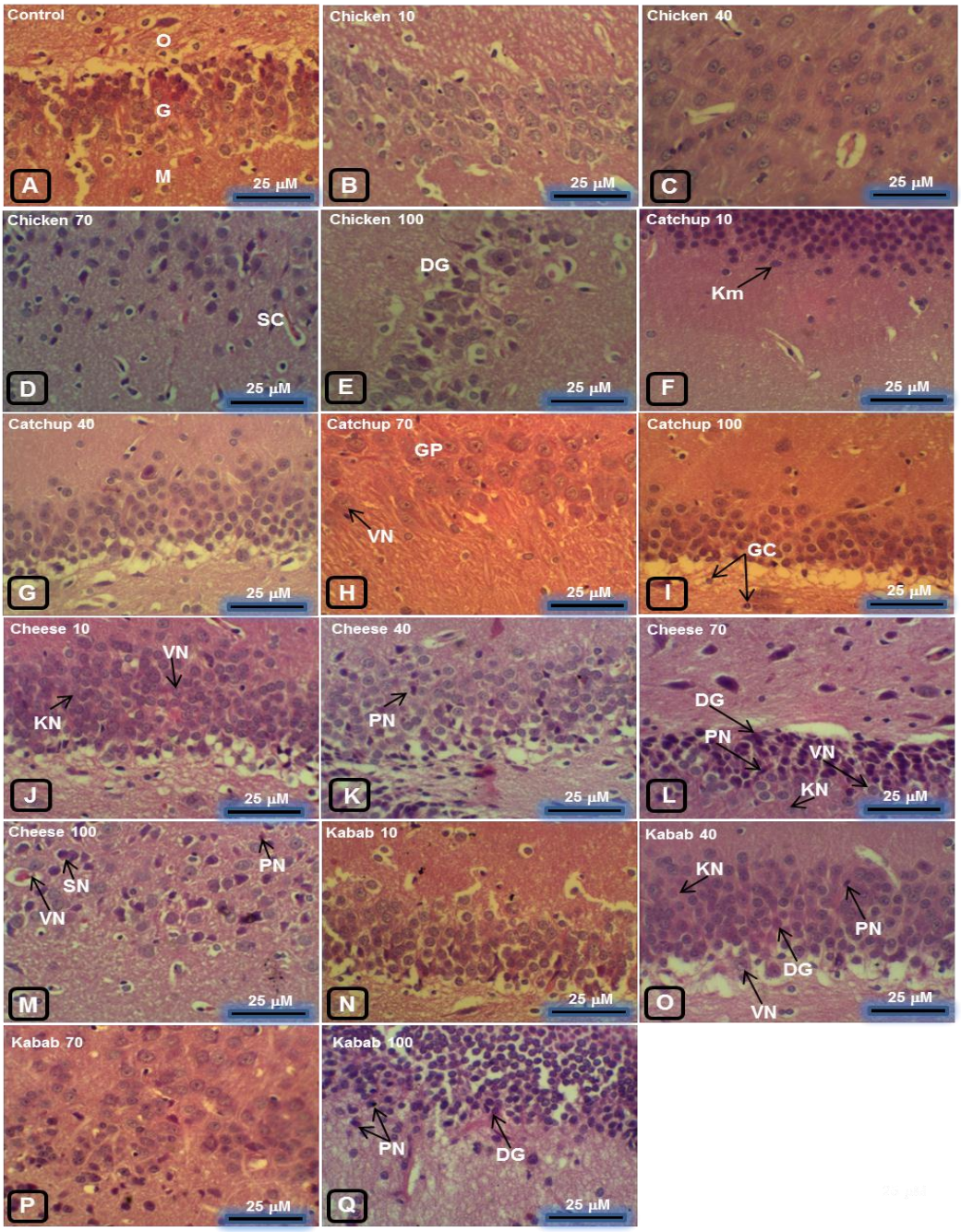

Figure (2): Histological changes in male rats brain as affected by different commercial flavoring agents (chicken, catchup, cheese and kabab) with daily intake of 10,40,70 and 100 mg/kg body weight for 90 days.

Figure (2-F) (catchup 10mg/kg/day) showed that the granular pyramidal cells were densely packed and havelarge nuclei "Karyomegaly" (Km), their processes were well apparent in themolecular layer and few glial cells were evident.Apparent decrease in the number and density of granular pyramidal cells (GP) in the catchup 70mg/kg/day group (Fig. 2-H) were noticed. Many shrunken cells with a dark acidophilic cytoplasm, pyknotic nucleiand an increase in the white space around them were seen. Some karyolitic nuclei could be seen. There was apparent decrease in thickness of the pyramidal cell layer. Few cells had vesicular nuclei (VN). The group treated with catchup flavor (100mg/kg/day) (Fig. 2-I) showed that the granular pyramidal cells appeared loosely packed. Many shrunken cells with a dark acidophilic cytoplasm, pyknotic nuclei and an increase in the white space around them were observed. Few glial cells (GC)were evident. The presence of molecular layer (M) and the polymorphic layer (O) was seen. a decrease in the thickness of the pyramidal cell layer compared with the control group was noted.

Meanwhile, cheese flavor treated group showed many granular cells of the dentate gyrus of the 10 mg/kg/day group (Fig, 2-J) with vesicular nuclei (VN). Few karyolitic (KN) and few cells are degenerated with a dark cytoplasm andpyknotic nuclei could be seen. But some degenerated granule cells with dark cytoplasm and pyknotic irregular nuclei (PN) were seen in 40 mg/kg/day group (Fig. 2-K). Halo areas around the cells and some karyolytic nuclei could be seen. The same figure indicated a decrease in the thickness of the granular cell layer compared with the control group. Few cells had vesicular nuclei could be observed. Figure (2-L) (70 mg/kg/day) showed many degenerated granular cells (DG) with dark cytoplasm and pyknotic irregular nuclei (PN) and halos around them. Some karyolytic nuclei (KN)could be seen. The same figure indicated an apparent decrease in the thickness of the granular cell layer compared with the control group. Few cells had vesicular nuclei (VN). The group treated with cheese flavor 100 mg/kg/day (Fig. 2-M) showed apparent decrease in the number and density of granular cells The granular cells appeared dispersed and loosely packed. Many shrunken neurons (SN) with a dark acidophilic cytoplasm, pyknotic nuclei (PN) or vesiculated nuclei (VN), beside an increase in the white space around them and an increased in blood vessels were seen.

Finally, kabab flavor treated group showed many granular cells of the dentate gyrus of the 40 mg/kg/day level with vesicular nuclei (VN). Areas devoid of granular cells "neurons of the granular region". Some karyolytic nuclei (KN) could be seen. Few degenerated cells (DG) with a dark cytoplasm and pyknotic nuclei (PN)could be noted. While, 100 mg/kg/day treated group showed many degenerated granular cells (DG) with dark cytoplasm and pyknotic irregular nuclei (PN) and halos around them.

Figure (3) showed histological changes in male rats testes as affected by different commercial flavoring agents (chicken, catchup, cheese and kabab) with daily intake of 10, 40, 70 and 100 mg/kg body weight for 90 days as compared with control group. Control group (Fig. 3-A) showed a normal section from rat testes with closely packed well-organized seminiferous tubules (ST) with normal epithelial stratification (ES) and surrounded by interstitial connective tissue (IC) that contains clusters of Leydig's cells (LC). Seminiferous tubules lined by several layers of spermatogenic cells (SC)were seen resting on the basement membrane (BM). Their lumen (L)was reduced and contains large numbers of active and fully developed spermatozoa (Sp).

The same figure showed shrinkage of the seminiferous tubules (SS) with irregularity of their basement membrane (IB) in chicken flavor treated group with 10mg/kg/day (Fig. 3-B). Degenerated tubules lined with germ cells having pyknotic nuclei (PN) and vacuolated cytoplasm widening of the intercellular spaces. While the group treated with 40 mg/kg/day showed disorganized spermatogenic cells with haphazard chromatin content. Degenerated tubules are lined with germ cells having pyknotic nuclei (PN) and vacuolated cytoplasm. A multinucleated giant cell was seen inthe lumen of the seminiferous tubule. There was a moderate hyperplasia of the interstitial tissue cells, and interstitial acidophilic exudates (AE).In addition, most of the seminiferous tubules were lined by severallayers of spermatogenic epithelium. The interstitial connective tissue (IC) was between the tubules in 70 mg/kg/day group (Fig. 3-D). As for 100 mg/kg/day group, there were degenerated tubules lined with germ cells having pyknotic nuclei and vacuolated cytoplasm. A multinucleated giant cell (GC) was seen in the lumen of the seminiferous tubule. Apparent hyperplasia of the interstitial tissue cells and interstitial acidophilic exudates (AE) were observed (Fig. 3-E).

Catchup flavor treated group with 10 mg/kg/day showed many markedly distorted seminiferous tubules with irregular outlines and disorganized epithelium. Germinal epithelium was formed of a few spermatogenic cells with darkly stained nuclei and wide lumina (WL). Some tubules had no sperms and a reduction in the thickness of their epithelial lining. Some tubules had a relatively narrow lumina with a few sperms. Many areas of interstitium were wide (Fig. 3-F). The second level of catchup flavor (40 mg/kg/day) showed distorted seminiferous tubules with irregular outlines. Most of the tubules had normal epithelial stratification and sperms were observed inside their lumina. A few tubules had disorganized germinal epithelium with a few sperms in their lumina (Fig. 3-G). Many distorted seminiferous tubules (DS) were observed in 70 mg/kg/day group (Fig. 3-H). Most of the seminiferous tubules are packed together with regular outlines and narrow interstitium (NI). Acidophilic hyaline material (AH)was observed between some tubules. Most of the tubules had normal epithelial stratification and sperms were observed inside their lumina. Some seminiferous tubules are markedly distorted with very wide lumina, no sperms, and a reduction in the thickness of their epithelial lining with a few sperms. Figure (3-I) showed the changes occurred in male rat testes treated with 100 mg/kg/day catchup flavor for 90 days. The figure showed many distorted seminiferous tubules with irregular outlines (IO),disorganized epithelium and wide lumina (WL). Seminiferous tubule epithelium was formed of a few separated spermatogenic cells with darkly stained nuclei.

Cheese flavor treated group (10 mg/kg/day – Fig. 3-J) showed dilated seminiferous tubules. Some germinal epithelial cells appeared apoptotic, The germinal epithelium was disorganized with sloughedor vacuolated cells. Area of acidophilic exudate (AE) was seen in-between the seminiferous tubules. Some seminiferous tubules were devoid/empty from sperms in their lumina. While the group treated by 70 mg/kg/day (Fig. 3- L) referred to irregular seminiferous tubules (IS) and diminution in the number of lining spermatogenic cells and mature sperms. Many lining cells exhibit vacuolated cytoplasm and deeply stained nuclei. Focal loss of spermatogenic cells leaving empty spaces could be noted. Moderate dilatation of some interstitial spaces filled with eosinophilic excaudate

(EE) was seen. Widely separated degenerated tubules (DG) lined with germ cells having pyknotic nuclei and vacuolated cytoplasm separated by dilated interstitial spaces could be observed in 100 mg/kg/day group (Fig. 3-M). Diminution in the number of lining spermatogenic cells and weakly developed spermatozoa were seen.

Finally, as for kabab flavor treated group (Fig. 3-N,O,P,Q), some of the seminiferous tubules were widely separated from each other with regular outlines and narrow interstitium. Acidophilic hyaline (AH) material (Fig. 3-O,P)was still observed between some tubules. Some tubules had normal epithelial stratification except for some areas of separation in their epithelial lining. Sperms were observed inside their lumina. A few tubules had disorganized germinal epithelium with a few sperms in their lumina.

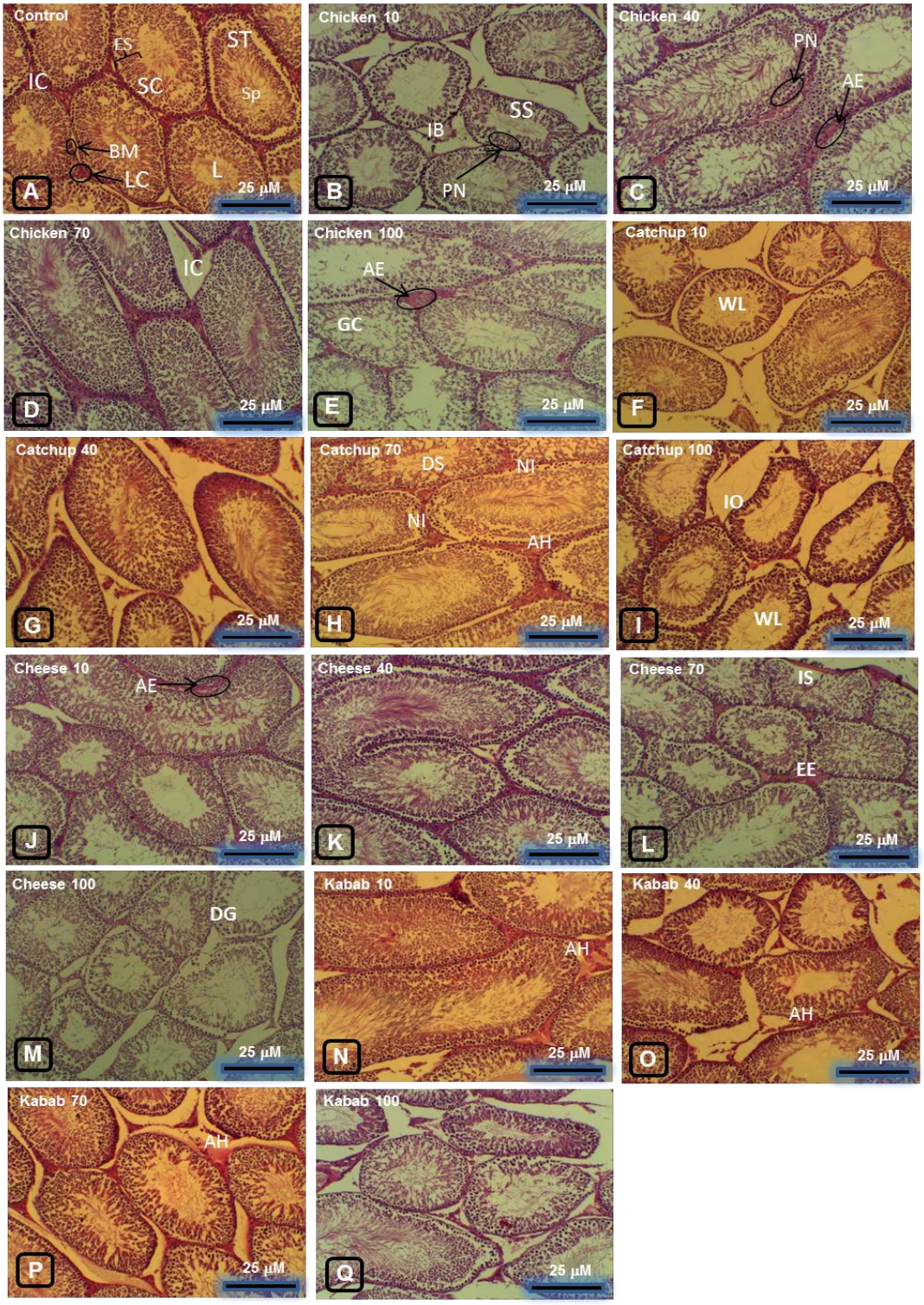

Figure (3): Histological changes in male rats testes as affected by different commercial flavoring agents (chicken, catchup, cheese and kabab) with daily intake of 10,40,70 and 100 mg/kg body weight for 90 days.

From all previous histological results, it could be ensured that these used flavoring agents with various dosages caused harmful effects for brain tissues such as shrunken cells, pyknotic nuclei, degenerated granule neurocyte, Karyomegaly and karyolytic nuclei. In addition, some negative changes were occurred in rat testes such as pyknotic nuclei, interstitial acidophilic exudates, narrow interstitiumand disappearing of sperms.

These presented histological results were in compatible with those of Khiralla *et al*. (2015), who stated that there were several negative changes in histological architecture of brain caused by treated with the synthetic colorants.

CONCLUSION

In conclusion, the chemically unknown artificial flavoring agents had toxic effects on liver and kidney. So, they were considered chemical hazards from food safety view where human health will be affected by overuse of such synthetic compounds. Further studies must be carried out to explore their chemical structure, especially aroma compounds present in these unknown materials. Then a toxicity study should be done to determine permissible limits of such materials.

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التأثير البيولوجي لبعض مكسبات الطعم الصناعية علي فئران التجارب

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في الآونة الأخيرة زاد استخدام المنكهات الغذائية الصناعية على نطاق واسع في مصر. حيث تستخدم هذه المواد في تجهيز المواد الغذائية في كلاً من النطاق الصناعي و المنزلي. ولكن العديد من هذه المواد، التي يكثر استخدامها في تحضير المواد الغذائية مجهولة المصدر. استخدام هذه المواد (المنكهات الغير معروفة) قد يعرض المستهلك لمخاطر صحية مختلفة. لذا فقد تم تقييم هذه المواد المكسبة للطعم غير معروفة التركيب و المستخدمة في تصنيع الغذاء كيميائياً و بيولوجياً. فقد تم تقدير المعادن الثقيلة و إمتصاص طيف الأشعة تحت الحمراء. كذلك تم تصميم تجربة تغذية لتقدير مقاييس النمو ووظائف الكبد و الكلى. و أيضاً تم عمل إختبارات هستولوجية على كلاً من المخ و الخصي. أظهرت النتائج أن محتوى مكسبات الطعم تحت الدراسة من المعادن الثقيلة كان في الحدود المسموح بها دولياً. كما أظهر إمتصاص الأشعة تحت الحمراء إحتواء هذه المواد المكسبة للطعم على العديد من المجموعات الفعالة مثل الهيدروكسيل ، الفينيل ، الكربونيل ، الأميد ، الكحولات و الأمينات الأليفاتية. كما أن وزن الجسم قد إنخفض إنخفاضاً معنوياً في جميع المعاملات مع زيادة الجرعات المستخدمة من مكسبات الطعم الصناعية (١٠ ، ٤٠ ، ٧٠ و ١٠٠ مجم/كجم/يوم) و ذلك مقارنة بالمجموعة الضابطة. كما أظهرت النتائج التأثير السلبي لمكسبات الطعم المستخدمة على وظائف الكبد و الكلى حيث إزدادت فاعلية إنزيمات الكبد (ALT, AST) و كذلك تركيزات كلاً من الكرياتينين و اليوريا مقارنةً بالمجموعة الضابطة. بينما إنخفضت فاعلية إنزيم SOD و كمية الألبيومين. كما لوحظ زيادة هذا التأثير العكسي لهذه المقاييس الحيوية مع زيادة تركيزات مكسبات الطعم المستخدمة. وقد أظهرت النتائج الهستولوجية إلى أن الجرعات المستخدمة من هذه المكسبات الصناعية كان مصحوباً بتلف في خلايا المخ وانخفاض في تكوين الحيوانات المنوية. بصفه عامة يمكن القول بأن هذه المواد المكسبة للطعم الصناعية كان لها تأثير سلبي على النشاط البيولوجي لذكور الفئران البيضاء. لذلك يمكن أن نوصي بأن هناك حاجة ماسة لمزيد من الدراسات لإعادة تقييم الآثار الصحية و إختبارات التسمم للمنكهات الصناعية التي تستخدم في الأسواق المحلية. كما يجب على الجهات المعنية إتخاذ القرارات الرادعة و وضع محاذير للتقليل من إستيراد و تداول و إستخدام هذه المركبات الخطرة على صحة الإنسان.

الكلمات الدالة: مكسبات الطعم – وظائف الكبد - SOD – ALT – AST - الألبيومين – الكرياتينين – اليوريا – الاختبارات الهستولوجية.