

Chronic Effect of Artificial Butter Flavoring on the Liver and Kidney of Male Albino Rats. (A Histopathological Study)

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Abstract: Diacetyl is used to give taste and smell of common foodstuffs such as margarine, snacks, candy, baked goods and pet food and other products. 2,3 - diacetyl butanedione is a yellowish liquid is usually mixed with other ingredients to produce the flavor of butter or other flavors in a variety of food products. Associated with inhalation of vapors butter flavor first with bronchiolitis among workers in the aerobic production of microwave popcorn and the study of inhaled diacetyl with decomposition and deformation in the epithelial tissue examination showed hypertrophy of the cells of the liver lobule, and necrosis in the pipe near the kidneys. In this study was used 36 male of rats ranging weights from 90 to 110 grams, were divided into 3 groups. Control group consisting of 12 rats were given distilled water and also two treatment each consisting a total of 6 Gzazn. Was given a dose of Melgr M / kg of body weight per day for two weeks in a row. The samples were taken from the liver and kidney of control and treatment rats to study the histopathological changes and fixed in formalin solution and then prepared to stage pieces and paint a Eocene Alheimatoxlin dye Histological study. This study showed many of the pathological changes (kidney) shows severe necrosis and hemorrhage in vitro sparse - necrosis sharp with pool in the tubular cavity cells. Nkerzh pipeline leak lymphocytes - Tklso necrosis pipeline and the accumulation of acidic substances in in vitro bundled extended pipeline with Nkerzh pipe show combines acidic substances in vitro twisted. Hyper cells Almiznchimih and leakage of lymphocytes and shows necrosis cell phone and cell death and shows severe bleeding and shows decomposition Alanabibi and lose summit Interior cilia cells that help the nomination (Liver) injury and hepatic cells Taatdkhmha apoptosis and clotting Nkrvsat granulomas hepatic cirrhosis of the plasma cells and lymphoid aggregates necrosis and fibrosis squint pockets bleeding blood vessels dilate sinusoidal.

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Key words: Histological structure, Liver, Kidney, Male albino rats.

Introduction

Diacetyl is a yellow to green liquid or powder that is found in a variety of foods and beverages. It contributes to the beer and wine, and it has the form of an emulsion at low levels and flavor candy at higher levels. It is a natural by-product of the conversion of glucose to ethanol by yeast during fermentation in beer, and there is also naturally in low concentrations in coffee, vinegar, dairy products, honey, and fruit. Diacetyl is used primarily on food additives artificial in flour, Alchoko of Ath, and cooking oils, candy, chips, and more than that it is responsible for the smell of butter. Diacetyl is the flavor of the main composite dairy products and devoted conduct extensive research on this topic and diacetyl are produced by some species of the genera of lactic acid bacteria (LAB) including family Streptococcus, Leuconostoc, Lactobacillus, Pediococcus and Oenococcus (Ford *et al.*, 1980). It is noteworthy aldehydes and ketones, which are characterized by the presence of the carbonyl group, and also for carbonyl compounds. And Keaton binary (CH₃-CO-CO-CH₃), 2,3 - butanedione or diacetyl (sometimes referred to as biacetyl), perhaps best known for being the compound

responsible for the characteristic odor and flavor of butter (Van Neil *et al.*, 1929). As well as being the main flavor of butter compound, diacetyl is present in products wine (made from grapes), brandy, roasted coffee, and many other fermented foods. It is also found in beer, but in these drinks is considered to be the characteristic damage (Selim, 2005). In wine, Diacetyl first nominated for the National Toxicology Program (NTP) in 1994 by the National Cancer Institute's automated, metabolism, carcinogenicity studies through the mouth. Because of this chemical was not palatable in water dosed and has been very volatile in dosed feed, take oral feeding tube is only possible exposure. Conducted NTP studies dispose of chemical oral in rats have shown that near the metabolism full of diacetyl to CO₂ based on the limitations in exposure route, characteristics of irritant strong this chemical, and metabolism full close of CO₂, it was believed that it is unlikely that the effects of other ulceration in the stomach will be disclosed in the tube - feeding studies in the short term. Based on this information, were not recommended toxicity studies and was formally withdrawing the nomination in 1999. In 2007, the butter flavor was nominated

Industrial (ABF) and two of the major volatile components, diacetyl and acetoin, by the management of the United Food and Commercial Workers Union for testing a long-term inhalation of respiratory toxicity, and general toxicity and carcinogenicity. Because ingestion is the most common route of human exposure for the ABF, has been given little attention to the potential toxicity of inhaled vapors. Inhalation exposure to high concentrations of vapors ABF occurs primarily in the food industry, and until recently there were no reports of any adverse health effects (Kalstskin and Oconn, 1993). The toxicity of inhaled ABF first under the microscope in 2000 after a high proportion unusually young workers in a factory microwave popcorn have been diagnosed with bronchiolitis obliterans (OB), a disease that is irreversible airway obstruction that is often fatal (Morgan 2006).

The objective of this study and research knowledge of the pathological effects on the liver and kidney in rats and to learn the toxic effects of diacetyl in rats and mice.

2. Materials and Methods

2.1. Method Diacetyl Structure

buttery taste (Diacetyl), A model strawberry flavor consisting of ethyl butanoate, ethyl 3-methylbutanoate, (Z)-hex-3-enol, 2-methylbutanoic acid, 5-hexylhydro-2(3H)-furanone

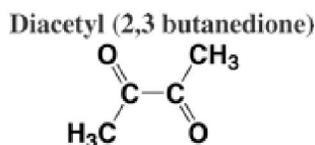


Figure 1. 2-Dimensional representation of diacetyl
[<http://www.thewinemerchantinc.com/educational/WineAcid.html>].

2.2. Animals

The experimental animals used in this study total of 36 were male albino rats, *Rattus rattus* (90 – 110 g). They were supplied with food and water ad libitum under standard conditions of light, humidity and temperature (22-25°C).

The study covered four groups of animals, group A served as a control group and composed of 12 rats and two experimental groups B, and C each group consisted of 12 rats. The insecticide was used. Diacetyl Structure as commercial formulated form of a concentrated suspension produced by Astrachem. K S A, and dissolved in distilled water. dose of Diacetyl (10, 25, 100, 200, 400 mg/kg b.w.t orally (Patterson *et*

al., 2007) administered orally via stomach tube to the treated group B, and C daily for 2 weeks respectively. Rat serving as controls group (A) animals were sacrificed and the liver and kidney were removed then fixed in 10% neutral buffered formalin, embedded in paraffin, sections of 1-3 μ were stained with haematoxylin and eosin for histological examination (Bancroft and Stevens, 1996).

3. Results.

Morphological changes:

Most of the animals showed groups of (B-C), tremors, bloody tears. Mice treated here anorexic occurred two weeks after the partial period experimental. The mice usually most affected by shortness of breath and often suffering from diarrhea. About 30% of the mice in the group have led to her death when increasing the duration of the transaction B, C. Body weight:

On the first day of the experiment body weight of animals ranging from 155-170 g and during the first weeks of the dose decreased by 30%. During the following weeks of the experiment observed a slow increase in body weight.

Histological of liver

Liver gland retina organized cells in the tapes intersect with each other made up the network. And organized these tapes in groups. Each group will be Vsissaqaibdia located canaliculi bile between the cells and collects each group of them to be the course of biliary small falls with Aaúaan bloody artery and vein. Hesmaan is a space portal, and the Baabi find Addinn the spreads between the hepatic lobules and Eugdorad in the middle is called hepatic lobule central vein; and Eugdfra from the portal vein in the portal space and the second branch of the hepatic portal vein and branches within the lobule into a network of capillaries located between the tapes between the liver cells and collects the central veins of different lobules to be two intravenous the alqaibdian Hepatic lobules Tapes contain glandular hepatic cells and central vein in lobule Center and then the capillaries and blood sinuses between the tapes The Baabi spreads. Each of which contains a branch of the portal vein and a branch of the hepatic artery and biliary tract cubic a padded Btalaúah small, and join with each other connective tissue extends between the lobules.

Histological changes: A (Control group) of liver

Histological examination of rats liver in group A (control group) showed normal picture during all the experimental intervals.

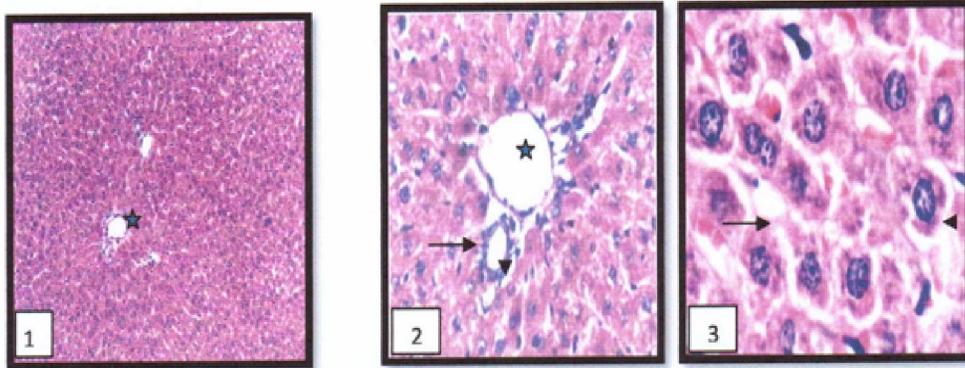


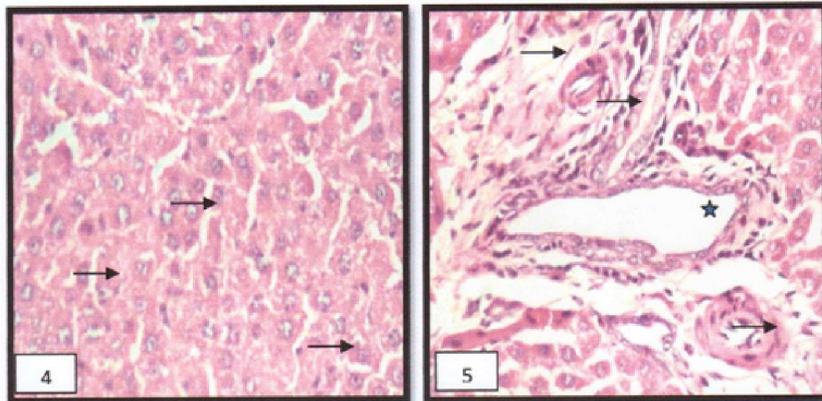
Fig.1- 3 photomicrographs of liver sections stained with hematoxylin–eosin stain (H&E).

1 - Section taken from the liver of a control rat shows normal liver histology central vein (★) (H&E) x100

2 - Cross section of the liver showing a Branch of portal vein, Biliary duct, Branch of hepatic artery and bile stream channel (arrow). (H&E) 100

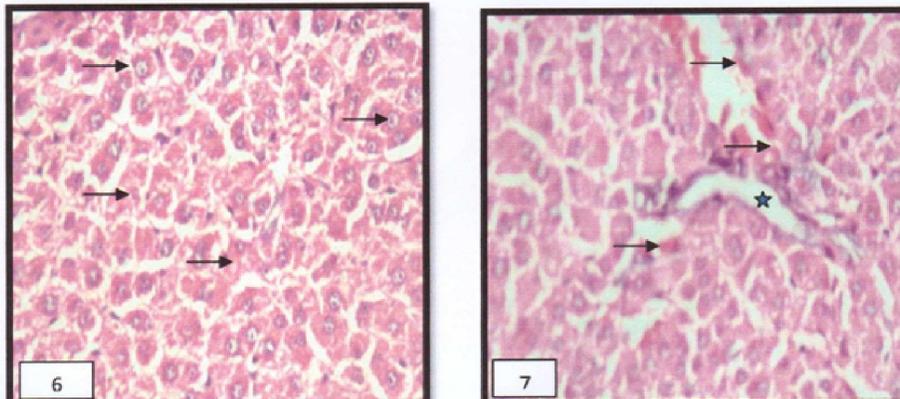
3 - Sections taken from the liver of rats showing, Hepato cytes, Sinusoid (arrow). (H&E) x 400

B (treated group 1 weeks)



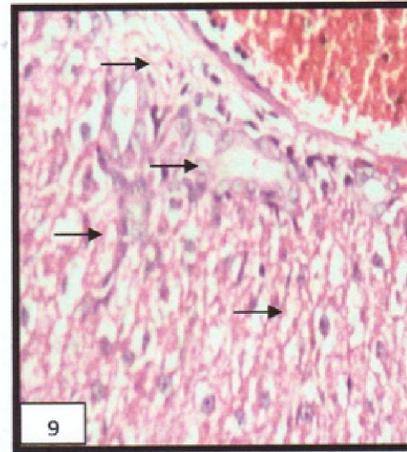
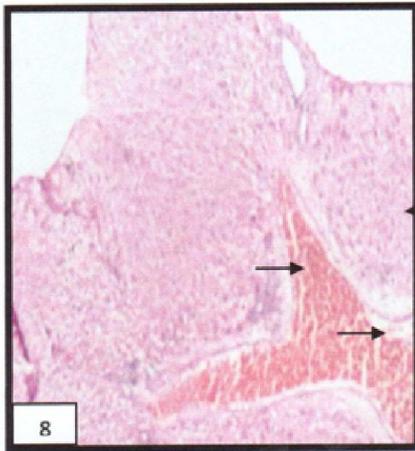
4 - Sections taken from the liver of rats treatment (one week) and shows the Necrotic cell (arrow) and cellular fragmentation, Inflammation of Hepatic sinusoids (arrow). (H&E) x

5 - Change from the liver of rats treated with carvedilol show few fibrosis in the portal area central and portal veins associated with few inflammatory cell infiltration and breadth of hepatic sinusoids (arrow). (H&E) x 100.



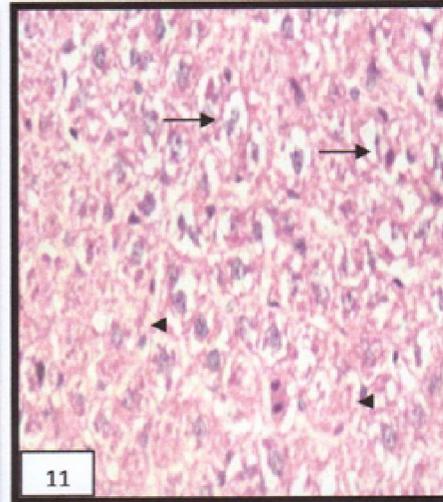
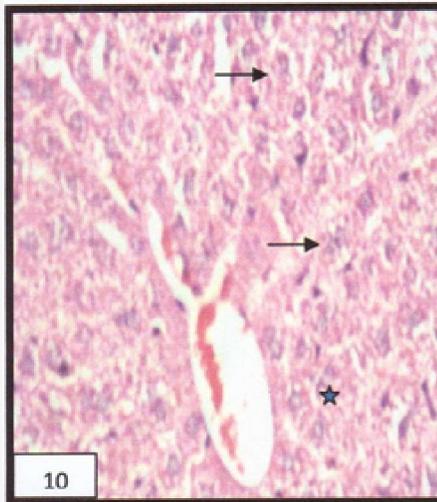
6 - Shows cell lysis and Pyknotic cell. Karyarr hexis of nuclei and the fragmentation of Hepato cytes and breadth of Hepatic sinusoids (arrow). (H&E) x100

7 - There is a serious inflammation of the Hepatic sinusoids, Karyolysis, changes in some hepatocytes (arrow) as well as few fibroblastic cells proliferation in between at the surrounding area. The portal area showed also dense fibrosis (arrow). (H&E) x100

C(treated group 2 weeks)

8- Sections taken from the liver of rats treatment (two week) and shows the show inflammatory cell infiltration with edema and extravasated red blood cells were detected in the portal area associated with fatty changes in some hepatocytes (arrow).(H&E)100

9 - Acute inflammation of the sinus hepatic () and was nominated recession hematoma, Necrotic cell, Kary alysis cell death in abundance, analyzes and clear abnormalities in Portal tract (arrow).(H&E) x1001



10- Show inflammatory cell infiltration with edema and extravagated red blood cells changes in some hepatocytes and mild congestion in the central and portal veins associated with few inflammatory cell infiltration (arrow).(H&E) x100

11 - Hepatic cell death () and fragmentation in the cells, Pyknotic cell, Necrotic and analyze the entire liver cells to the disintegration of the connective tissue (arrow).(H&E) x100

Histological of kidney.

kidney important to get rid of nitrogenous material composed of thin Foreign portfolio. Followed crust petition granular stretch inside then spinal form of a pyramid extending within the college basin. Containing the urinary units and each unit consisting malpighian capsule round and tubules the Boulez curled long characterized into three. parts first Relatively broad, thick-walled and wraps around

himself in the crust layer next Malpighi an capsule and wrapped called Proximal convoluted tubules.

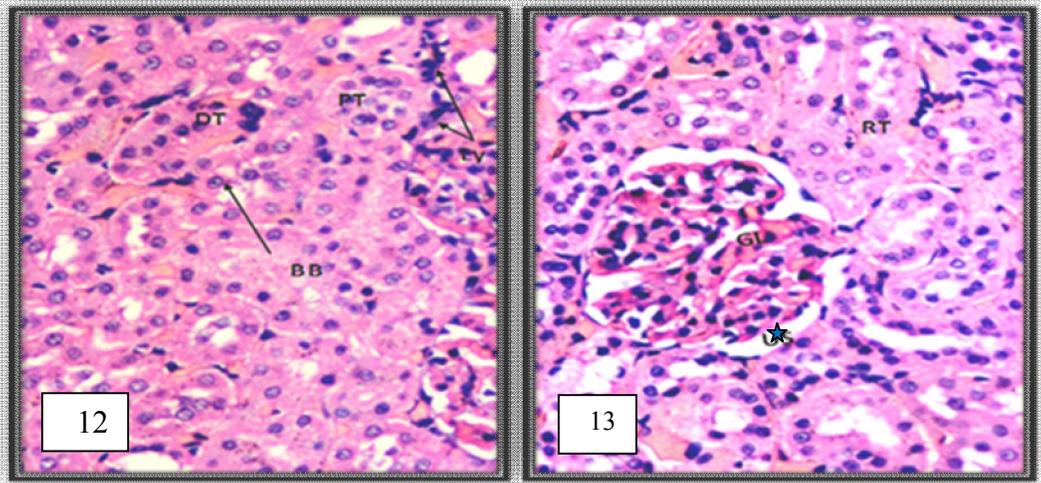
The second part extends into the spinal bend again to the crust and the so called loop of Henle and then stretched to be coiled pipe remote 1/portfolio composed of fibrous connective tissue 2/crust contains Kiryat Mlbeja and coiled tubes far and near 3/ Bowman's capsule double-walled surround Blood capillaries of capillaries are kippah and tubes wrapped nearby Tjoifaa tight and walls thick

composite of cells cubic Aohermeh and have the edge Faragonyh and pipes remote Tjoifaa wider and walls are thinner and cells cubed small and does not have the edge Faragonyh and appears in the section of a large number of nuclei more than

pipe nearby shows fabric Dhaamadam to meet between connective tissue tubules and blood capillaries.

A (Control group) of kidney

Histological examination of Rats kidney in group A (control group) showed normal picture during all the experimental intervals.

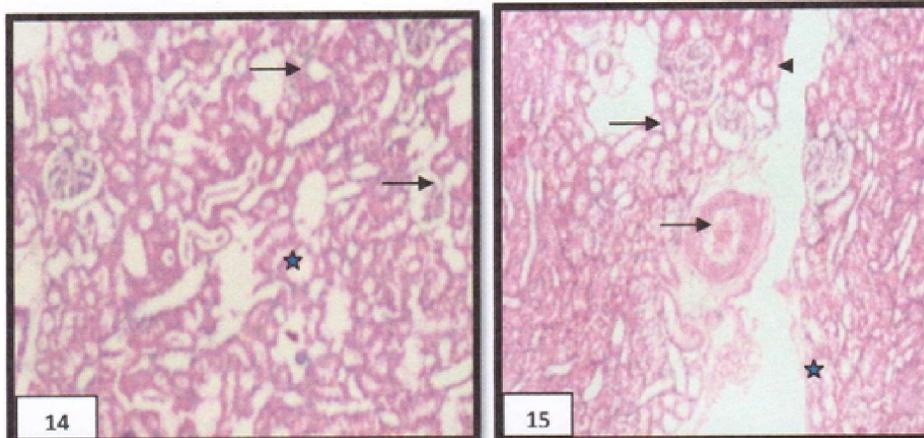


Photomicrographs of kidney sections stained with hematoxylin–eosin stain

12- Section taken from the kidney of a control rat shows normal kidney histology Distal convoluted tubule (DT) Proximal convoluted tubules (PT)thereare brush border(BB).(H&E) x100

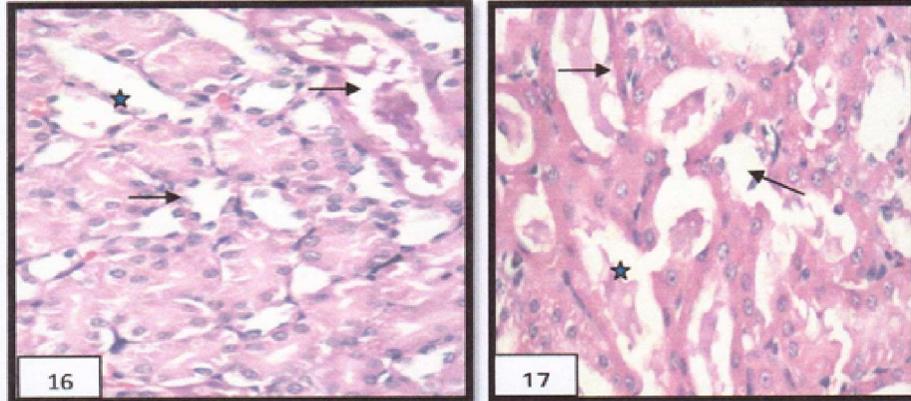
13- Cross section of the kidney showing normal Bowmans capsule Glomerulus (GL). (H&E) x100

B (treated group 1 week)



14 - Cross-sections in college treatment for (one week) and shows where severe damage in (Bowman's capsule) And squamous cell laceration and visceral layer (★) and lined by podocytes with infiltration of lymphocytes (arrow).(H&E) x100

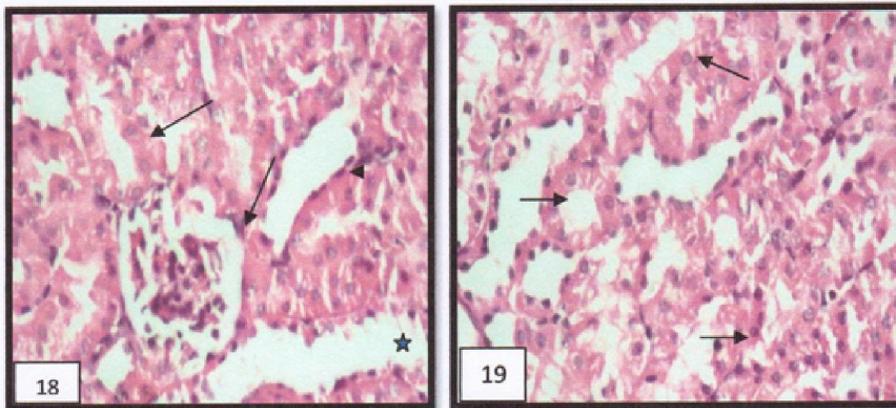
15 - Acute inflammation of connective tissue and occurrence of bloody nominated and intravascular coagulation medicines(arrow).(H&E) x100



16 - Acute inflammation and blood accumulation within the renal tubules and cell death and decomposition of Necrotic cell (arrow).(H&E) x400

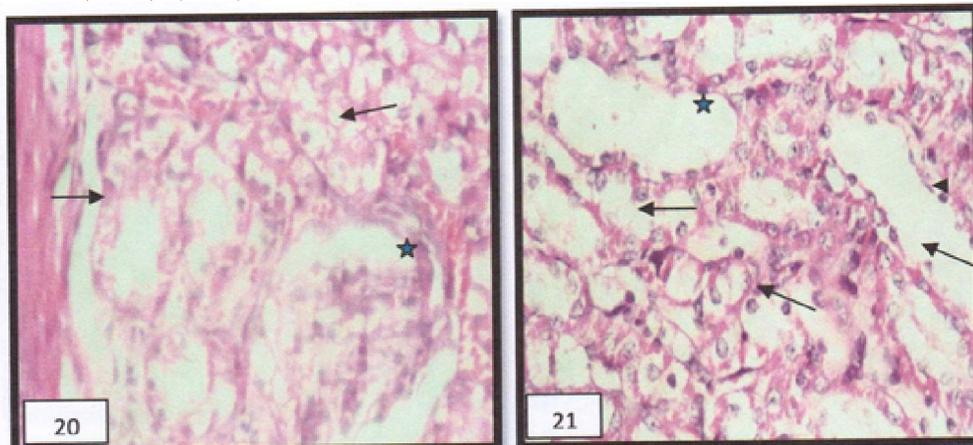
17 - Fragmentation of nuclei and Karyarr hexis, Necrotic cell and squamous cell carcinoma. (arrow).(H&E) x400

C(treated group 2 weeks)



18 - Cross section of kidney treatment for (two weeks) and shows decomposition glomerulus and lack of brush Border Necrotic cell Kary alysis of Proximal convoluted tubules (arrow).(H&E)x400

19 - Cross section of kidney treatment and deformation analyzes to relative intact tubule, pyknotic nuclei, Proximal convoluted tubules (arrow). (H&E) x400



20 – Tubule with widened lumen (), vacuolized, damaged blood vessel for squamous layer of the renal tubules. Nominated bloody sharp between proximal convoluted tubules (arrow). (H&E) x400

21-- Necrotic cell and disorganized tubule. Nuclei fragmentation and lack of squamous epithelial thickness and increase the widening cavities pipeline and lack of brush border proximal convoluted tubules (arrow). (H&E) x400

Conclusions

Thousands of workers exposed in the flavor of the food industry chemicals diacetyl and others that make up the ABF.

Breathing apparatus should be used and additional ventilation to reduce worker exposure to these chemical. It must be available for setting exposure standards in the workplace or exposure limits in the short term for artificial butter flavor, diacetyl, or acetoin inadequate inhalation toxicity data. The proposed studies provide data on drug and dose - response for all three vehicles, and in particular with regard to damage to the respiratory system, liver, kidneys, and also help in determining the toxic component (s) of the ABF. This will be the pathological abnormalities and toxicity to determine safe levels of exposure for ABF, diacetyl and acetoin, and to develop guidance to protect the health of workers in occupations involving the use of these chemicals.

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