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Hazardous Effects Induced by Inhalation of Isopropanol.

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ABSTRACT

The purpose of this study was to explore the effects induced by inhalation of isopropanol vapor for 2 and 4 weeks on the trachea, lung, liver and kidney. Thirty-two healthy Wister rats divided equally into four groups. Group 1 (G1) control rats were allowed to inhale fresh air, G2 were allowed to inhale IPA vapor at concentration of 240 ppm for two weeks, G3 and G4 were exposed to IPA as in G2 for four weeks. In addition, rats of G4 left without exposure for further two weeks as a recovery period. At the end of each experimental period, rats dissected; their tracheas, lungs, livers and kidneys extracted and processed for light microscopy. Tracheal mucosa showed focal destruction, desquamation, and the lamina propria showed oedema. Lungs showed emphysema in some air alveoli and bronchiolar hyperplasia with polyp formation. The hepatic parenchyma showed degenerated hepatocytes with some pyknotic nuclei. Degenerated kupffer cells, with dilatation and congestion in the portal veins. Renal glomeruli showed congestion, increase in their cellularity. Our results suggest that inhalation of the IPA vapor at low concentrations could have cytotoxic effects on the structure and function of these organs.

Keywords: Isopropanol inhalation, rats, trachea, lung, liver, kidney.

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INTRODUCTION

Isopropanol (also known as Isopropyl alcohol, 2-propanol, and propan-2-ol) is an aliphatic alcohol hydrocarbon that becomes volatile and colorless by rubbing compound [1] liquid and highly flammable [2]. Isopropanol is found in many household cleaning agents in aftershave lotions, toiletries, disinfectants, antifreeze, paint thinners [3] and used as a sterilizing agent and 'rubbing' alcohol [4]. This solvent is reported as innocuous in various test systems [5]; popular for pharmaceutical applications; presumably due to the low toxicity of any residues. Also, it is used as a gasoline additive [6].

Isopropanol can enter the environment as emissions from its manufacture and use as a solvent. It naturally occurs as a volatile plant and released during the microbial degradation of animal wastes [7]. It has a bitter taste and emits an odor resembling that of ethyl alcohol and acetone. It is relatively cheap to purchase and is 2 to 3 times more potent than ethanol.

It is readily available and most toxic ingestions are in children after accidental intake. In adults, most ingestions are in alcoholics, as a substitute for ethyl alcohol, or in attempted suicides [8]. Isopropanol can cause intoxication by ingestion, inhalation, or absorption through the skin or the mucus membranes [3]. Unlike ethanol, this alcohol is absorbed rapidly and completely via ingestion, inhalation and transdermally, producing greater CNS effects that are more depressant than ethanol at comparable concentrations.

Clinical effects of isopropanol are similar to those of ethanol and include vomiting, dizziness, nausea, abdominal pain, confusion, stupor, hypotension, and hypoglycemia [9]. IPA has an anesthetic effect but irritates the respiratory system if inhaled [10, 11]. In addition, a higher number of sinus cancers and laryngeal cancers have been found among workers involved in IPA production [11]. Human data on toxic inhalation of IPA show cerebral edema and CNS toxicity [12]. Other features following ingestion include hemorrhagic gastritis [13] renal tubular necrosis and hemolytic anemia [4]. Furthermore, human studies show that women may be more susceptible to the toxic effects of inhaled IPA [14].

The toxicity of IPA inhalation, especially at low concentration for short experimental periods, was not fully described before. Thus, the present study aimed to study the potential effects of IPA vapor at low dose and the period of time it required depended on the biological responses of Wister rats as a mammalian model. We studied the histological alterations in the tracheas, lungs, livers and kidneys so as to obtain information for understanding the actual toxicity of IPA.

MATERIALS AND METHODS

Chemical reagents

Isopropyl alcohol: CAS registry no. 67-63-0 was purchased from Sigma (Nasr City, Cairo, Egypt). All other chemicals were purchased from local standard companies.

Experimental animals:

Thirty two healthy Wister rats (weighing1100±10g) were fed on pellets of standard rodent laboratory diet and had access to water ad libitum throughout the experimental period. All rats were acclimatized for two weeks before the experiment in an air-conditioned room at temperature of 20-24 ºC, a relative humidity of 50±5%, and a 12h light/12h dark cycle. This study was carried out in accordance with the protocol approved by the Institutional Animal Care and Use Committee (IACUC), of the University of Umm Al-Qura (Permit Number: 4320656). Also, all Procedures were carried out in accordance with the international guidelines for care and use of laboratory animals. All efforts were made to minimize suffering.

Experimental Protocol

Animal grouping

Rats were randomly and equally divided into four main groups, each was further subdivided into four subgroups, two rats for each in hand-made wire cages that can be distributed equally in four glass exposure chambers designed for inhalation. G1 non-exposed rats are considered as the control group, whereas the remaining groups were exposed for 10 minutes at concentration of 240 ppm IPA for 2, 4 and 6 weeks, respectively.

Groups	Experimental periods		
	dose/weeks	Recovery	Total
G1	non-exposed	---	---
G2	Exposed*/2weeks	---	2weeks
G3	Exposed*/4weeks	---	4weeks
G4	Exposed*/4weeks	2 weeks	6weeks
*Exposure dose: 240 ppm for 10 minutes/day			

Inhalation Chambers

Each inhalation chamber (IC) can accommodate four wire cages distributed side by side and provided with generator, auto-sampler and analyzer for controlling IPA vapor. Before inhalation, each chamber was disinfected using ethyl alcohol that sprayed continuously every 24hrs. Waste trays formed of paper were fixed daily underneath the wire cages as sterile substrates.

Inhalation of Isopropanol (IPA)

According to [7] who recommended that the IPA inhalation level was 400 ppm for 3 to 5 min. Rats of the present work were allowed to inhale IPA vapor sent from the generator at concentration of 240 ppm in the inhalation chamber for 10 minutes/day for 2, 4 and 6 weeks, respectively.

At first, rats of all groups were transferred into disinfected ICs. G1 rats were allowed to inhale normal air in the first IC, whereas other chambers of the remaining groups were allowed to inhale IPA vapor sent from the generator at concentration of 240 ppm supplied through the central roof into the chamber from above and aspirated from below. The inhalation period for G2 rats was 10 minutes/day for two weeks, whereas the inhalation periods for G3 and G4 were 10 minutes/day for four weeks. G4 rats were left without exposure for further two weeks as a recovery period.

Preparation of specimens

At the end of the experimental period for each group, rats were fastened overnight and then sacrificed by cervical dislocation. Their abdomen were incision and small pieces of tested organs were extracted, washed in saline solution and immediately immersed in cold buffered formalin 10% for 48h.

Histopathological investigations

The fixed samples from trachea, lung, liver and kidney of different groups were washed, treated with conventional grade of alcohol, cleared using xylene, embedded in paraffin and sectioned at five microns thickness [15]. The sections were stained with hematoxylin and eosin and then examined with light microscope for histopathological evaluation.

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RESULTS

Clinical signs (Animal symptoms, food consumption, general activity) and mortality

No gross effects or significant differences in body weight were observed during the study period in any of the rats exposed to IPA when compared with the control group (data not shown). However, the treated rats consumed water and rodent food as usual except at exposure and post-exposure time, which extends for about one hour. During the first period of exposure, rats were hyperactive and then suffered from short breathing. At the end of the experiment, they were sprawled on the glebe of their cages. No mortality observed in any of the animals in G1 and G2, whereas 12.5% (only one rat in G3 and G4) died at the end of 4^{th} week.

Ophthalmoscopy

Ophthalmologic examinations did not show any treatment-related ocular lesions in any of the animals (data not shown).

Histopathological findings

The treated Wister rats showed distinct histopathological changes in the trachea, lung, liver and kidney on microscopic observation when compared with the Control Group, indicating highly affected cells.

Trachea:

Light microscopic study of trachea of group of Wister rats were kept as a control group and showed normal architecture. Figure 1 showed the layers of the tracheal wall, the respiratory epithelium including pseudostratified columnar cells and goblet cells. The lamina propria consists of highly loose, vascular supporting tissue, hyaline cartilage, which is a specialized connective tissue. The surfaces of cartilage are covered with a thin fibrous layer or membrane, the perichondrium and a loose connective tissue around the trachea the adventitia.

The tracheal mucosal epithelium of G2 showed hyperplasia with deciliated region and hyperplasia of goblet cells, some inflammatory cell infiltrate the underling lamina propria and congestion in the blood vessel (fig. 2). In Group 3, the trachea of rats showed focal destruction and desquamation of lining mucosal epithelium, deformation of seromucous glands, congestion in blood vessels in the lamina propria with severe inflammatory cell infiltration (figs. 3&4).

Microscopic examination of trachea of G4 revealed deciliation, degeneration in the mucosal epithelium, accumulation of cytoplasm in the upper portion of the mucosal epithelial cells, and goblet cells hyperplasia with inflammatory cell infiltration in lamina propria (figs. 5&6) .

Lung:

Figure 7elucidated normal histological structure of a rat's lung in Group 1. The respiratory bronchioles are further divided into several alveolar ducts which have numerous alveoli, and pulmonary vessel. The bronchiole wall consists of ciliated cuboidal epithelium and a layer of smooth muscle disposed in a spiral manner (fig. 8).

Light microscopic study of lung of G2 showed bronchiolar hyperplasia with polyp formation and peribronchiolar muscular hypertrophy; severe congestion and dilatation in blood vessel, peribronchiolar lymphoid hyperplasia with infiltration of mononuclear cells (fig. 9) , emphysema in some alveoli, peribronchiolar, perivascular and perialveolar inflammatory cell infiltration (fig. 10) . In the lungs of G3 rats, inflammatory changes happened including mature lymphoid follicles with inflammatory cell infiltration, bronchiolar hyperplasia with peribronchiolar muscular hypertrophy, interstitial and peribronchiolar edema, sheet of polymorph leukocytes fills the alveolar duct with severe congestion and dilatation in blood vessel (figs. 11&12) . The alveolar spaces were

collapsed, their walls appeared thicker than normal (collapse in air alveoli) and the perialveolar capillaries were greatly dilated; polymorphs are also present in the alveolar walls (fig. 12).

The significant histopathological changes were observed in the lungs of rats in Group 4 showed severe pneumoniain pulmonary tissue, focal lymphoid hyperplasia with cellular infiltration, acute dilatation with hemolysis in the blood vessels and severe pulmonary oedema (fig. 13). Figure 14 showed emphysema in lung section with narrowing in alveolar spaces due to thickening in their walls, dilated capillaries and interstitial oedema.

Liver:

Light microscopic study of liver of G1 rats, considered as the Control Group, showed no histopathological alteration. Figure 15 showed normal liver architecture with the central vein and surrounding polygonal hepatocytes arranged in radial branched cords and separated by blood sinusoids containing some Kupffer cells. The liver sections of G2 showed dilation in the portal vein, appeared longitudinally oriented. Some hepatic lobules showed focal degeneration in the hepatocytes (fig. 16).

In figure 17, the portal vein showed severe dilatation and congestion and the hepatocytes became degenerated in the hepatic parenchyma with some pyknotic nuclei. The liver of G3 treated rats showed infiltration of few inflammatory cells around the portal vein, which seemed congested and severely dilated. The bile duct showed deformation and some hepatocytes appeared to be degenerated (fig. 18) . Figure 19 of G3 showed degenerated hepatocytes (necrosis) with some pyknotic nuclei, karyomegaly (enlargement of hepatocyte cytoplasm and nucleus) and cytoplasmic vacuolations, and Kupffer cells in some sinusoids seemed degenerated.

Microscopic examinations revealed severe histopathological changes in the livers of G4 rats, rigorous cytoplasmic vacuolations in degenerated hepatocytes, apoptotic changes and numerous degenerated hepatic cells. The blood sinusoids were highly dilated and congested with Kupffer cells proliferation. Some acini were lined by malignant ductal epithelial cells containing bile –stained debris (fig. 20). Figure 21of G4 rats showed some angular hepatocytes with condensed nuclear material within the hypereosinophilic cytoplasm. Other hepatocytes exhibited severe cytoplasmic vacuolations and degeneration with faded pyknotic nuclei or apoptotic nuclei.

Kidney:

Figure 22 clarified normal histological structure of glomeruli and renal tubules of the kidneys of rats in the Control Group (G1). Renal tissues of rats in G2 showed congestion in the glomeruli, dilatation and congestion in blood vessels; hemorrhage in the renal space and between the convoluted tubules (figs. 23&24). Some renal tubules showed degenerated epithelium (fig. 25).

Light microscopic study of kidneys in G3 rats showed congestion in the glomerular tuft, an increase in the cellularity of the glomerulus and renal corpuscle were partially or completely occluded. Focal necrosis in some renal tubules, the lumen of some collecting tubules and glomerulus were blocked by dense casts (fig. 26). While figure27 showed severe congestion and dilatation in the blood vessels and degenerated epithelium in some renal tubules and focal necrosis in other tubules, the renal spaces were significantly reduced and showed an inflammatory cellular infiltration.

The significant histopathological changes were observed in the kidneys of G4 rats showed partially or completely occluded renal corpuscles that appear to be more congested. The stroma contains densely eosinophilic (colloid) casts; hemorrhage was observed between the renal tubules, some renal tubules showed degenerated epithelium, others were dilated and contained colloid, whereas other tubules lost the lumen (fig. 28).

In figure 29 the Kidney of G4 rats showed that the center of glomerulus was shrunken and the epithelial of Bowman's capsule were degenerated. Severe dilatation in some blood vessels and the tubular damage epithelium appeared to be degenerated. A branching papillary structure appeared with delicate cords of connective tissue

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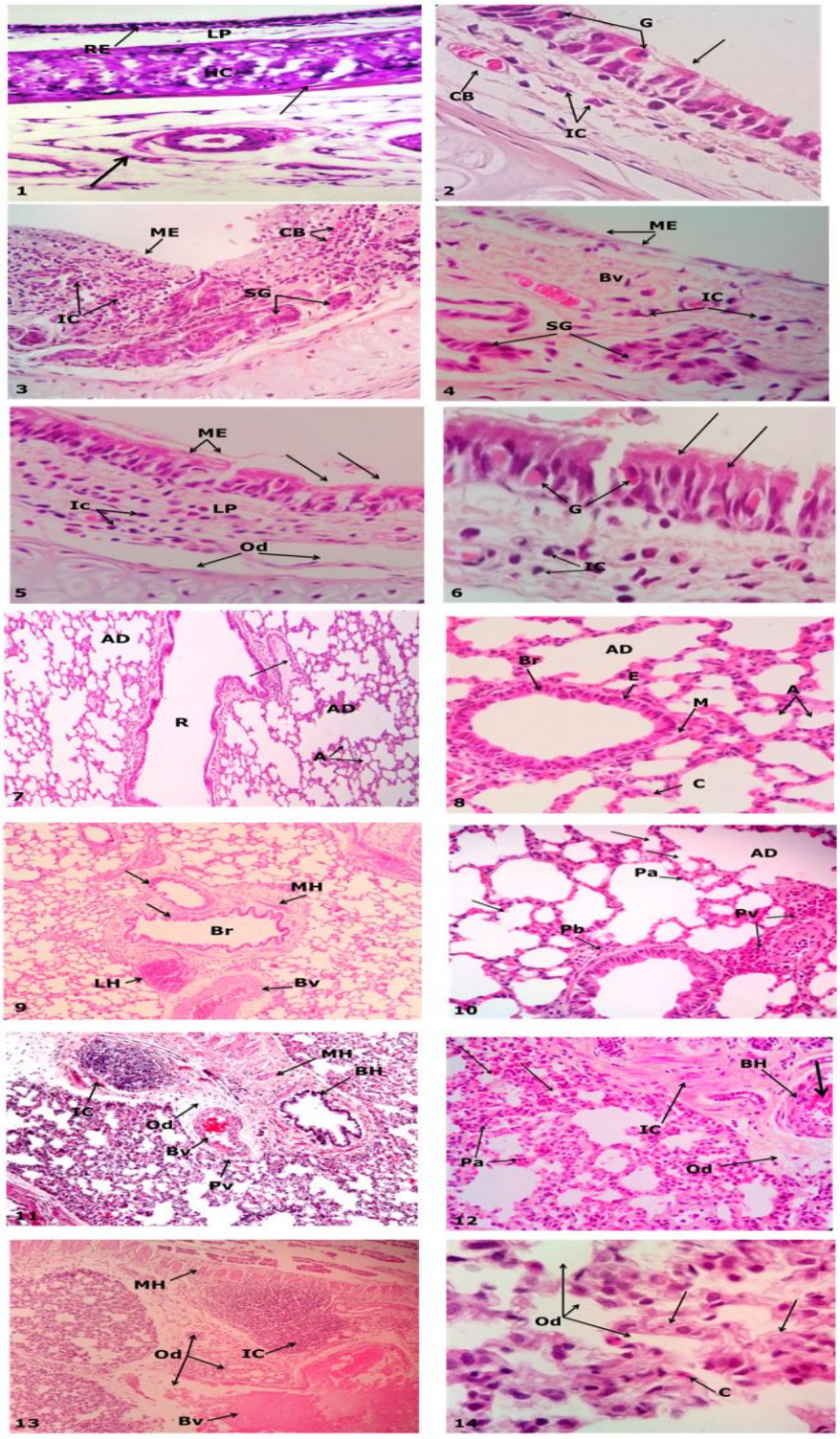
covered with cuboidal epithelial cells which have small round nuclei with no mitotic (may be tumor cells). Figure 30 showed severe degeneration in the epithelium of renal tubules. Hemorrhage diffused between renal tubules and the epithelium of some other tubules was ragged from undergoing necrosis with acute tubular necrosis.

Histopathological alterations induced by inhalation of Isopropanol



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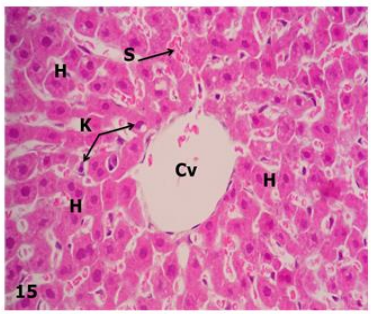

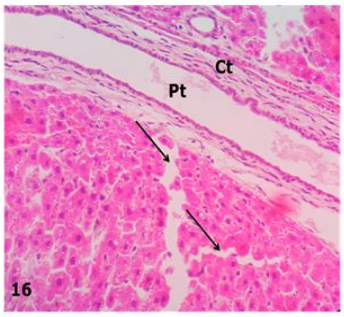

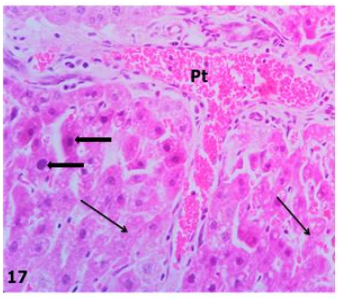

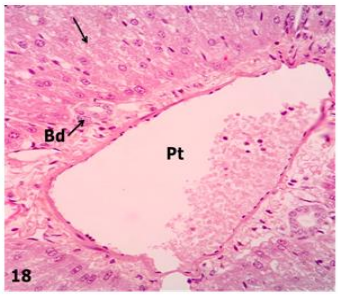

Fig. 1 Trachea of rat of control group (G1) showed the layers of the tracheal wall, the respiratory epithelium (RE), the lamina propria (LP), hyaline cartilage (HC), the perichondrium (thin arrow) and a loose, connective tissue around the trachea adventitia (thick arrow). H&E X 40.

Fig. 2 Trachea of rat in G2 showed hypoplasia in the mucosal epithelium with deciliated region (arrow), with inflammatory cell infiltration(IC) in the underling lamina propria and congested blood vessel (CB) goblet cells (G) hyperplasia. H&E X 100.

Fig. 3 Trachea of rats in G3 showed focal destruction and desquamation of lining mucosal epithelium (ME), deformation of seromucous glands (SG) congested blood vessels (CB) in the lamina propria with sever inflammatory cell infiltration (IC). H&E X 40.

Fig. 4 Magnified micrograph of trachea of rats in G3 showed deformation and destruction in mucosal epithelium (ME), congestion and dilatation of the blood vessels (Bv) in the lamina propria with numerous inflammatory cell infiltration (IC) destruction of seromucous glands (SG). H&E X 100.

Fig. 5 Trachea of rats in G4 showed deciliation (arrows), hyperplasia and degeneration in the mucosal epithelium (ME) and oedema (Od) in the lamina propria (LP) with inflammatory cell infiltration (IC). H&E X 100.

Fig. 6 Magnified micrograph of trachea of rats in G4 showed accumulation of cytoplasm in the upper portion of the mucosal epithelial cells (thin arrows) goblet cells (G) hyperplasia and little inflammatory cell infiltration (IC). H&E. X 400.

Fig. 7 Lungs of rats in G1 showed normal histological structure of the respiratory bronchioles (R), alveolar ducts (AD) which have numerous alveoli (A), and pulmonary vessel (arrow). H&E X 40.

Fig. 8 Lungs of rats in G1 clarified the normal histological structure of bronchiole (Br), smooth muscle (M), alveolar ducts (AD) alveoli (A), epithelial cells (E) and capillaries (C). H&E X100.

Fig. 9 Lungs of rats in G 2 showed bronchiolar (Br) hyperplasia with polyp formation (thin arrows) and peribronchiolar muscular hypertrophy (MH), dilated and congested blood vessel (Bv), peribronchiolar lymphoid hyperplasia (LH) with infiltration of mononuclear cells. H&E X40.

Fig. 10 Lungs of rats in G2 showed alveolar ducts (AD), emphysema in some alveoli (arrows); peribronchiolar (Pb), perivascular (Pv) and perialveolar (Pa) inflammatory cell infiltration. H&E X100.

Fig. 11 Lungs of rats in G3 appeared infiltrated by lymphocytes and plasma cells, bronchiolar hyperplasia (BH), peribronchiolar muscular hypertrophy (MH), interstitial and peribronchiolar oedema (Od), focal lymphoid hyperplasia with inflammatory cell infiltration (IC) and congestion, perivascular lymphoid hyperplasia (Pv) with congested blood vessel (Bv). H&E X 40.

Fig. 12 Lungs of rats in G3 showed congestion in the bronchiole (thick arrow), bronchiolar hyperplasia (BH) peribronchiolar oedema (Od), focal lymphoid hyperplasia with inflammatory cell infiltration (IC), collapse in air alveoli(thin arrows),perialveolar (Pa) inflammatory cell infiltration and the alveolar capillaries are greatly dilated. H&E X100.

Fig. 13 Lungs of rats in G4 showed sever damage in pulmonary tissue (pneumonia), focal lymphoid hyperplasia with inflammatory cell infiltration (IC) acute dilatation and congestion with hemolysis in the blood vessels (Bv), muscular hypertrophy (MH) and severe pulmonary oedema (Od). H&E X 40.

Fig. 14 Lungs of rats in G4 showed emphysema and narrowing in alveoli due to thickening in their walls (arrows), dilated capillaries(C) and interstitial oedema (Od). H&E X 400.

Fig. 15 Livers of rats in G1 showed normal histological structure of the central vein (Cv),and surrounding polygonal hepatocytes (H) arranged in radial branched cords and separated by blood sinusoids (S) containing some Kupffer cells (K). H&E X100.

Fig. 16 Livers of rats in G2 showed dilated portal vein (Pt) oriented longitudinally surrounded by connective tissue (Ct), the hepatic lobule showed focal degeneration (arrows) in the hepatocytes. H&E X100.

Fig. 17 Livers of rats in G2 showed sever dilatation and congestion of the portal vein (Pt), degenerated hepatocytes in the hepatic parenchyma (thin arrows) some pyknotic nuclei (thick arrows). H&E X100.

Fig. 18 Livers of rats in G3 showed few inflammatory cell infiltration surround the portal vein (Pt), which is congested and severely dilated, deformation of bile duct (Bd) and hepatocytes appeared degenerated(arrow). H&E X100.

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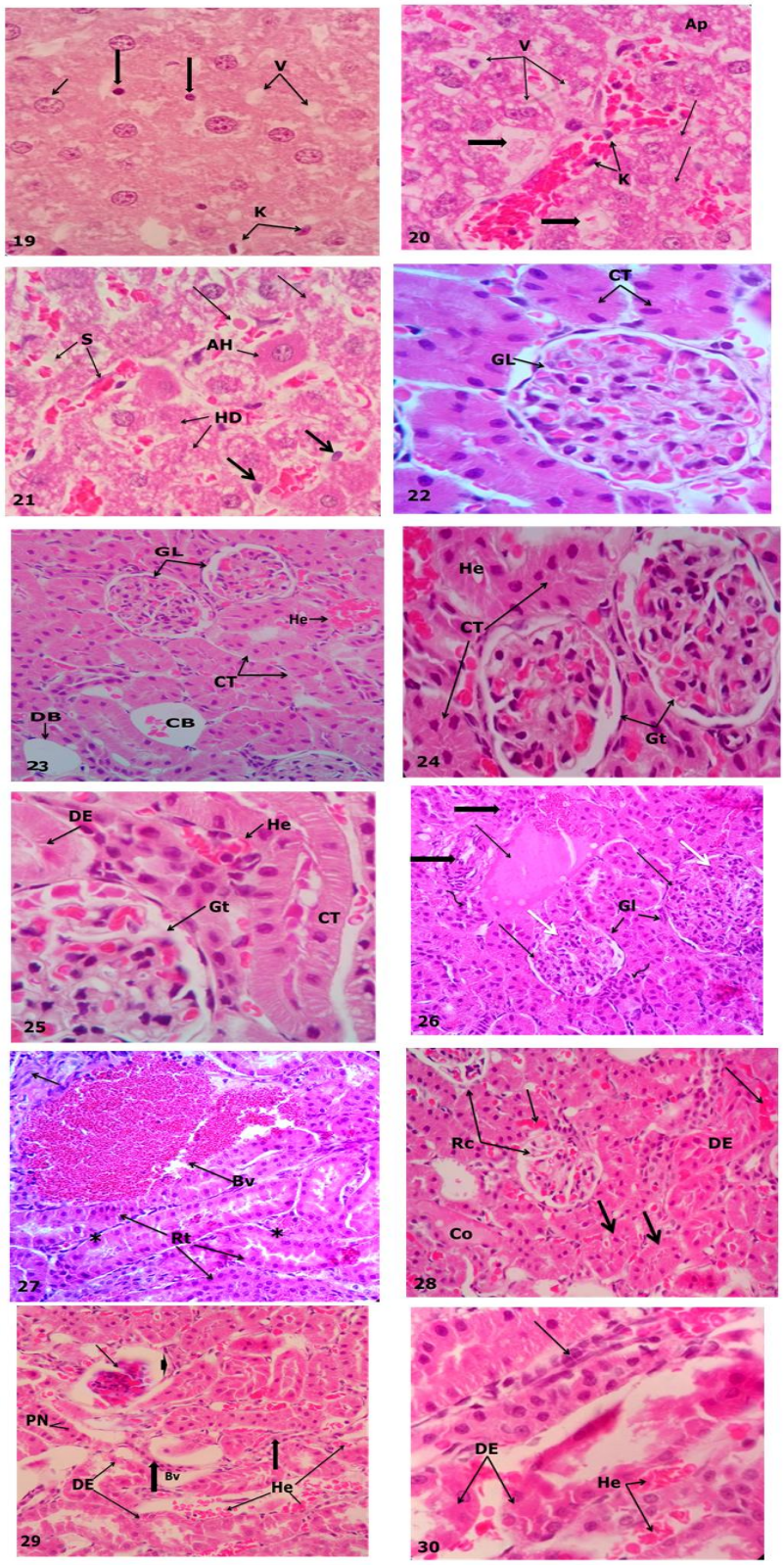

Fig. 19 Livers of rats in G3 showed degenerated hepatocytes (necrosis); some pyknotic nuclei (thick arrows), cytoplasmic vacuolations (V), thin arrow indicates enlargement of hepatocyte cytoplasm and nucleus (karyomegaly) and few degenerated Kupffer cells in some sinusoids (K). H&E X400.

Fig. 20 Livers of rats in G4 exhibited severe cytoplasmic vacuolations (V) in degenerated hepatocytes, apoptotic (Ap) changes and numerous degenerated hepatocytes (thin arrows). The blood sinusoids are highly dilated, and congested with Kupffer cells (K) proliferation, some acini are lined by malignant ductal epithelial cells contain bile –stained debris (thick arrows). H&E X400.

Fig. 21 Livers of rats in G4 showed some angular hepatocytes (AH) with condensed nuclear material within the hypereosinophilic cytoplasm, other hepatocytes exhibited sever degeneration (HD) with faded or pyknotic nuclei(thick arrows), apoptotic nuclei (thin arrows),sever cytoplasmic vacuolations , highly dilated and congested blood sinusoids(S). H&E X400.

Fig. 22 Kidneys of rats of the Control Group (G1) showed normal architecture of glomeruli (Gl), and convoluted tubules (CT). H&E X1000.

Fig. 23 Kidneys of rats of G2 showed congestion in the glomeruli (GL), dilated (DB) and congested blood (CB) vessels, hemorrhage (He) in the renal space and between the convoluted tubules (CT). H&E X400.

Fig. 24 Magnified micrograph from figure 23 showed congestion in the glomerular tuft (Gt), hemorrhage in the renal space (He) convoluted tubules (CT). H&E X1000.

Fig. 25 Kidneys of rats in G2 showed congestion in the glomerular tuft (Gt), hemorrhage (He) in the renal space (arrow), and between the convoluted tubules (CT). Note that some tubules showed degenerated epithelium (DE). H&E X1000.

Fig. 26 kidneys of rats in G3 showed congestion in the glomerular tuft with, increase in the cellularity of the glomerulus (Gl), partially or completely occluded renal corpuscle (white arrows), inflammatory infiltrate (thick arrows) in the stroma, focal necrosis in some renal tubules (curved arrows) ,the lumen of some collecting tubules is blocked by dense casts (thin arrows) . H&E X400.

Fig. 27 Kidneys of rats in G3 showed e congestion and dilatation in the blood vessels (Bv) and degenerated epithelium in some renal tubules(Rt) and focal necrosis in other, the renal spaces were significantly reduced (stars) and showed inflammatory infiltrate (arrow). H&E X400.

Fig. 28 Kidneys of rats in G4 showed partially or completely occluded renal corpuscles (Rc) that appear more congested, the stroma contains densely eosiphinic (colloid) casts (thin arrows), diffuse hemorrhage is observed between the renal tubules , some renal tubules showed degenerated epithelium(DE) others are dilated and contain colloid (Co) ,other tubules have no lumen (thick arrows) H&E X400.

Fig. 29 Kidneys of rats in G4 showed glomerulus (center) is shrunken (thin arrow) and the epithelial of Bowman's capsule are degenerated (arrow head), severe dilatation in some blood vessels (Bv), diffuse hemorrhage (He) in between the renal tubules and sever tubular damagedegenerated epithelium (DE), delicate cords of connective tissue are covered with single layer of cuboidal epithelial cells, thick arrows). There are many pyknotic nuclei (PN). H&E X400.

Fig. 30 Kidneys of rats in G4 showed sever degenerated epithelium (DE) of renal tubules, diffuse hemorrhage (He) is observed between the renal tubules, the epithelium of the tubules seen here is ragged from undergoing necrosis with acute tubular necrosis (thin arrow) H&E X1000.

Abbreviations

A	**Alveoli**	**DE**	**Degenerated epithelium**	**MH**	**Muscular hypertrophy**
AD	**Alveolar ducts**	**E**	**Epithelial cells**	**ME**	**Mucosal epithelium**
AH	**Angular hepatocytes**	**G**	**Goblet cells**	**Od**	**Oedema**
Ap	**Apoptotic**	**Gl**	**Glomeruli**	**Pa**	**Perialveolar**
Bd	**Bile duct**	**Gt**	**Glomerular tuft**	**Pb**	**Peribronchiolar**
BH	**Bronchiolar hyperplasia**	**H**	**Hepatocytes**	**PN**	**Pyknotic nuclei**
Br	**Bronchiole**	**HC**	**Hyaline cartilage**	**Pt**	**Portal vein**
Bv	**Blood vessel**	**HD**	**Hepatocytes degeneration**	**Pv**	**Perivascular**
C	**Capillaries**	**He**	**Hemorrhage**	**R**	**Respiratory bronchioles**
CB	**Congested blood vessel**	**IC**	**Inflammatory cell infiltration**	**Rc**	**Renal corpuscles**
Co	**Colloid**	IPA	**Isopropanol**	**RE**	**Respiratory epithelium**
Ct	**Connective tissue**	**K**	**Kupffer cell(s)**	**Rt**	**Renal tubules**
CT	**Convoluted tubules**	**LH**	**lymphoid hyperplasia**	**S**	**Blood sinusoids**
Cv	**Central vein**	**LP**	**lamina propria**	**SG**	**Seromucous glands**
DB	**Dilated blood vessel**	**M**	**Smooth muscle**	**V**	**Cytoplasmic vacuolations**

DISCUSSION

Isopropyl alcohol (IPA) is a chemical commonly used in a variety of commercial applications [16]. In 1994, 1.5 million tons of isopropyl alcohol was produced in the United States, Europe, and Japan [6]. This compound is primarily produced by combining water and propane in a hydration reaction. It is also produced by hydrogenating acetone [6, 17].

The majority of isopropanol exposures occur in children less than 6 years of age, although isopropanol poisoning appears to be a reasonably common occurrence, deaths are rare. Isopropanol is rapidly absorbed

following ingestion with peak plasma concentrations occurring within 30 min. It can also be absorbed following inhalation or dermal exposure [18].

Toxicity in patients exposed to harmful levels of IPA via inhalation can be distinguished by the presence of a fruity odor on the breath of an IPA poisoned patient; this is a result of the production of acetone as the isopropanol is metabolized in the liver [9]. Acute IPA intoxication is thought to occur secondary to either direct toxicity from IPA itself or its metabolite acetone, but it is controversial with regard to the substances that could be the primary agent causing toxicity [19].

The present study confirmed that low exposure inhalation dose for different periods cause IPA toxicity on respiratory and some vital organs. IPA toxicity is dose and time-dependent. The allowable IPA level recommended by [10] in the USA is 400 ppm. Exposure to 400 ppm IPA can affect the mucosa of the nose and trachea [20].

Our data clearly showed that (240ppm) of IPA causes severe pathological degeneration of the respiratory mucosa, liver and kidney. Clinical data on the effects of acute IPA toxicity in humans are well described [16]. Also, inhalation studies at higher levels showed severe intoxication including gastrointestinal symptoms, nausea and vomiting, coma, cardiovascular toxicity, altered neurological functions, respiratory failure, and hemodynamic instability [8, 16, 21-23]. However, the effects of IPA on the nervous system are similar to those of ethanol or butanol ingestion, but are probably more severe and more persistent [4, 24]. Moreover, IPA administration by the oral route has identified developmental and reproductive effects [25]. Other features following ingestion include hemorrhagic gastritis [13] haematemesis, hypotension [26], hypothermia, sinus tachycardia and frequent premature ventricular beats [27].

Our study suggests that the target organs for IPA include respiratory tracts, livers and kidneys of rats. The present study only considered these four organs and not to be all-illusive. This study is consistent with the published literature that shows that IPA is an irritant of the skin, mucous membranes, and the upper respiratory tract [9]. Also, the inhalation of IPA leads to respiratory lung infiltrates and failure [28].

In vivo studies proved that IPA is rapidly absorbed through the respiratory epithelia especially through the lung parenchyma into systemic circulation with an absorption efficiency of 85–99% [14, 29, 30]. Presentation of acute IPA inhalation injury was reported to be similar to the ingestion injury, likely secondary to the rapid/efficient absorption of IPA through the lung parenchyma [12] and respiratory depression [26] which are the major symptoms following substantial exposure.

Histological alteration observed in the tracheal mucosa showed deciliation, hyperplasia, deformation, destruction and desquamation. The lamina propria showed congested blood vessels, deformation of seromucous glands, congestion in blood vessels, and severe inflammatory cell infiltration. In the same context, an acute effect of IPA toxicity on the mucociliary system in the tracheal mucosa appeared. The loss of ciliated cells of trachea has been reported by [31] while [20] demonstrated that the IPA vapor caused deterioration of the ciliary activity of the respiratory mucosa that markedly declined**.** They added that his deteriorating effect can be recovered within 2 weeks. In contrast, the present observation proved that this period is not sufficient for complete recovery.

Additionally, they observed hyperplasia of goblet cells, deformation of seromucous glands and severe inflammatory cell infiltration in lamina propria. Common tracheal features appeared after hydrocarbon inhalation hyperemic and dilated vasculature with intraluminal hemorrhage and inflammatory cell infiltration into the underlying connective tissue. Tracheal glands taking place in the lamina propria seemed to increase following several hydrocarbon exposure [32]. The degree of cellular infiltration and deformation of seromucous glands is gradually increased with the period of inhalation. This result was also demonstrated by [20] who exposed Guinea-pigs to IPA, [33] who used conditioned gases with the dogs, [34] who used O-Chlorobenzylidene Malononitrile (CS) with the rats and by [35] who used Gasoline with Guinea pigs.

The present work proved that lungs of the exposed rats showed severe pneumoniain pulmonary tissue, pulmonary oedema and emphysema in the lung section with narrowing in alveoli due to thickening in their walls.

The histological evaluation after acute exposure to isopropyl alcohol (IPA) showed extensive pneumonitis and lung oedema in animals [16]. In addition, we declared that the exposed lung showed focal lymphoid bronchiolar and peribronchiolar hyperplasia with cellular infiltration and acute dilatation with hemolysis in the blood vessels. This is in agreement with [16] who stated that the examination of lung after acute exposure to IPA showed obliteration and remodeling of smaller bronchioles with associated prominent peribronchiolar metaplasia and chronic inflammation.

Several studies were carried out to characterize the acute pulmonary inflammatory responses to aliphatic hydrocarbon inhalation in rats [36-38]. This study proved that the inhalation of IPA for 4 weeks at low dose caused severe damage in the tissues of the trachea and lung in spite of the recovery period, which lasted for two weeks.

The hepatic parenchyma indicated variable damages after IPA inhalation, Karyomegaly, focal degeneration, some hepatocytes with some pyknotic nuclei and cytoplasmic vacuolations. Other hepatocytes had condensed nuclear material within the hypereosinophilic cytoplasm. The observed blood sinusoids were highly dilated and congested with Kupffer cell proliferation. The portal areas showed bile duct deformation, severe dilation and congestion in the portal vein with infiltration of few inflammatory cells.

Similar results were reported by [39] when rats exposed to1, 3-Dichloro2-propanol inhalation, and by [40] when a human was exposed to small quantities of aerosols (volatile hydrocarbons). Also, [29] described foamy vacuolization of liver cells after acute exposure to ISP, whereas [41] concluded that the fatty liver was a result of acute IPA administration.

Isopropyl ingestion is usually showing variable damages in renal function [30, 42] and chronic renal toxicity [43]. Our microscopic examination revealed renal tubular necrosis and this was also reported by [4, 44, 45]. Moreover, the present work suggests congestion and dilatation in the blood vessels hemorrhage diffused in the renal space and between the convoluted tubules with inflammatory infiltrate cells. Also, the lumen of some glomerulus were blocked by dense casts, and tubules are filled with small to moderate amounts of eosinophilic smooth to follicular material (proteinosis). This was also reported by [21] who stated that hyaline droplets appeared within the kidneys of all male rats exposed to IPA.

Moreover, renal damage was observed, including hypertrophied nucleoli, thickening of the basement membrane in the glomerular tufts, and reduction of Bowman's space. Some renal corpuscles were partially or completely occluded. Despite these findings, some studies revealed that minimal to mild effects to the kidney including renal tubular proteinosis and tubular dilation were observed following exposure, and there was no corresponding evidence of alterations to the glomeruli [43].

Inhalation of IPA at low concentration for a short period of time has left sever impact in liver and kidney tissues difficult to be cured during the suggested recovery period in this research. We concluded that the present results proved the general toxic effects and target organ toxicity of IPA via repeated inhalation, which can aid in the process of risk assessment.

Ethical approval: "All procedures performed in studies involving animals were in accordance with the ethical standards of the Institutional Animal Care and Use Committee (IACUC), of the University of Umm Al-Qura (Permit Number: 4320656) at which the studies were conducted."

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