The Effect of Formaldehyde on the Histological Structure of Pancreas in Rabbits

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Abstract

Formaldehyde is an important chemical agent which is widely used in medical fields and industries; but it has been associated with excess risk of cancers.The present experimental study was designed to know the effect of formaldehyde effect on the pancreas. Therefore 24 male rabbits were randomly subdivided into 3 groups (I,II,III) and exposed to vapour of 10% formaldehyde (12 ppm) for the following periods: 2, 4 and 6 months respectively; beside 8 rabbits were exposed to vapour of distilled water as a control group. The physical observations of the exposed rabbits showed general signs of loose of appetite and aggressive behaviour. Light and electron microscopical observation of the exposed groups revealed no changes in the sections which had been examined and they have the same structures of those sections which obtained from the control group. From these findings, it can be concluded that formaldehyde has no effect on the pancreas.

Key words: formaldehyde; Pancreas; rabbits.

Introduction

Formaldehyde was introduced in 1893 by Blum as a fixative and embalming fluid. It is well known as a preservative, a sterilizer and embalming fluids; and approximately 2.1 million workers are exposed to formaldehyde [1,2].Domestic exposures occur mainly from consumer products that include textiles , insulation , paper, cosmetics and wood-products [3] .

Formaldehyde exposure has toxic effects on respiratory system, gastrointestinal tract, heamopoitic tissue and nervous system [4,5,6,7].

Several epidemiological studies have analyzed the potential association between formaldehyde and cancer risk in humans. These have been reviewed in three consecutive monographs of the International Agency for Research on Cancer (IARC) which classified formaldehyde as 'carcinogenic to humans' (Group 1) [8] .

Most reviews on the carcinogenicity of formaldehyde have focused on cancers of the respiratory tract. Two previous epidemiological studies [9,10] have suggested that exposure to formaldehyde may increase the risk for pancreatic cancer, while the most recent study [11] concluded that the null findings among industrial workers and the lack of biological plausibility would argue against formaldehyde as a cause of pancreatic cancer.

The objective of the present investigation was to know the histopathological effects of formaldehyde vapor on the pancreas of rabbits in relation to duration of exposure since there is no available literature concerning the duration needed for ca. pancreas development in rabbits after exposure to formaldehyde; so this study is regarded as a first experimental study in this subject.

Materials & methods

Animals and design of the work:-

Thirty two adult male rabbits (local breed) were collected; their weight and age range between 871-1618 grams and 22-41 weeks respectively, all were kept under the same environmental conditions.

The animals kept in stainless steel mesh cages. The temperature was maintained at 22- 32 C^o, a dry, absorbent, bedding material, like wood particles was provided in all cages. All animals were allowed free access to food and water.

All the animals were observed for 10 days before the beginning of the experiment to exclude any possibility of abnormal behavior and disease.

The animals were randomly assigned irrespective of age and weight into the following groups:

1-Control group.
2-Exposed group I.
3-Exposed group II.
4-Exposed group III.

Each group includes of 8 animals and all the rabbits of each group were weighted before and after exposure.

The 3 exposed groups were exposed to the vapor of 10% formaldehyde for the following periods:

Group I for 2 months.
Group II for 4 months.
Group III for 6 months.

The 10% formaldehyde solution was placed in 400- steel containers which is covered by nylon mesh and filled periodically. All the animals were exposed to the same constant surface area of 10% formalin solution.

Analytic procedure [12] was applied several times during the exposed period of this work to get more accurate and precise determination of formaldehyde (ppm) in the atmosphere of the exposed cages. From this analytic measurement the concentration

of formaldehyde in the exposed cages was (12 ppm).

While the rabbits of the control group were exposed to a vapor of distilled water, under the same condition of housing, feeding and duration of the exposure.

Dissection & Macroscopic examination:

The experimental and control animals were killed at the end of periods: 2, 4, and 6 months respectively.

Each rabbit was anaesthetized with diethyl ether soaked cotton, in an air–tighten jar.

The animal lay down on a dissecting board then the abdominal cavity opened and the pancreas removed and examined to detect any lesion

Fixation and preparation of paraffin section:

Biopsy of 5.mn thickness pieces from different parts of the pancreas were taken and fixed by 10% formalin solution. Then procedure of preparing the paraffin section slides and staining by Harris, Haematoxylin and Eosin (H&E)was performed to prepare a stained histological sections for light microscopic examination [13].

Tissue preparations for electron microscopy were done according to Hayat [14].

Statistical analysis

The following statistical methods were used for the analysis of data:

1-Standard statistical methods were used to determine the mean and standard error.

2-Paired t- test was used to identify the statistical difference between before and after treatment values.

3-Unpaired t- test was used to identify the statistical difference between after treatment values and control values of the same duration.

4-Statistical results were considered significant at $p \leq 0.05$ [15].

Results

The exposed animals showed lower appetite for food and liquid intake as well as a significant decrease in body weight particularly among exposed group III (**Table-1).** This decrease of weight in the treated groups differs from one group to another and from that of control animals according to the duration of the exposure; as well as among the same group before and after exposure (group I, $p = 0.003$, for group II and III, $p < 0.001$).

The rabbits also showed aggressive behaviour and movement at the beginning of the exposure time.

Table (1): The weight of the animals (before and after experiment) in grams.

Animal NO.	Control group		Group I		Group II		Group III	
	Before	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**
1	1138	1404	1033	1154	991	913	891	953
2	873	1311	890	979	1264	1183	960	991
3	1311	1530	1368	1260	1309	1139	1311	1442
4	1171	1380	871	958	973	978	1243	1171
5	878	1212	1168	968	1618	1547	979	1221
6	1201	1409	924	1031	1128	975	929	1033
7	961	1937	1054	1250	960	951	1226	1237
8	1154	1309	878	968	1372	1346	1153	1084
Mean	1085.8	1343.5	1023.2	1071	1202	1129	1086.5	1141.5
SD	157.5	194.1	401.1	402.1	228.5	223.5	77	71

Light microscopical observations

Group 1 (control rabbits)

The architecture of the pancreas was preserved, it was composed of many lobules, and in between those very thin septa were observed. These lobules contained many acini that regarded as the exocrine portion of the pancreas. Scattered among these acini were the islet of Langerhans which represented the endocrine part of the gland.

All the sections of the pancreas that were taken from the control group were similar to each other. The gland showed no definite fibrous capsule but there was a thin areolar tissue from which thin septa extend into the gland to divide it into many lobules (**fig. 1**). The exocrine portion was composed of secretory acini. The acinar cells were pyramidal in shape and closely packed around central lumen forming acini which were of serous type. The peripheral part of each acinus was occupied by deeply basophilic stained and rounded nuclei while the inner part contained dense eosinophilic zymogen granules (**fig. 2**).

The extraglandular elements include the excretory ducts, blood vessels and connective tissues (**fig. 3**). Centroacinar cells were observed,

these cells were pale with large nuclei and contained no zymogen granules (**fig. 2**).

Intercalated ducts were seen in between the acini, each duct was lined by flattened cuboidal epithelium (**fig. 4**), larger interlobular ducts were observed in between the lobules and lined by low columnar epithelium (fig. 3).

Spreading in the exocrine pancreas were the islets of Langerhans, which were spheroidal masses of pale staining cells. The cells of the islets were polygonal, and arranged in cords (**fig. 5**).

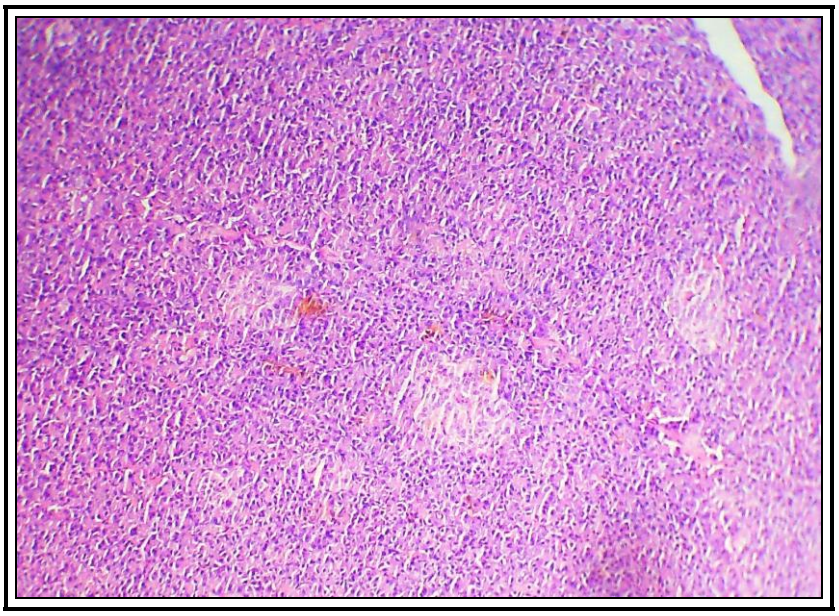


Figure (1): Light micrograph of the rabbit pancreas showing the exocrine and endocrine tissues. (H & E X 100).

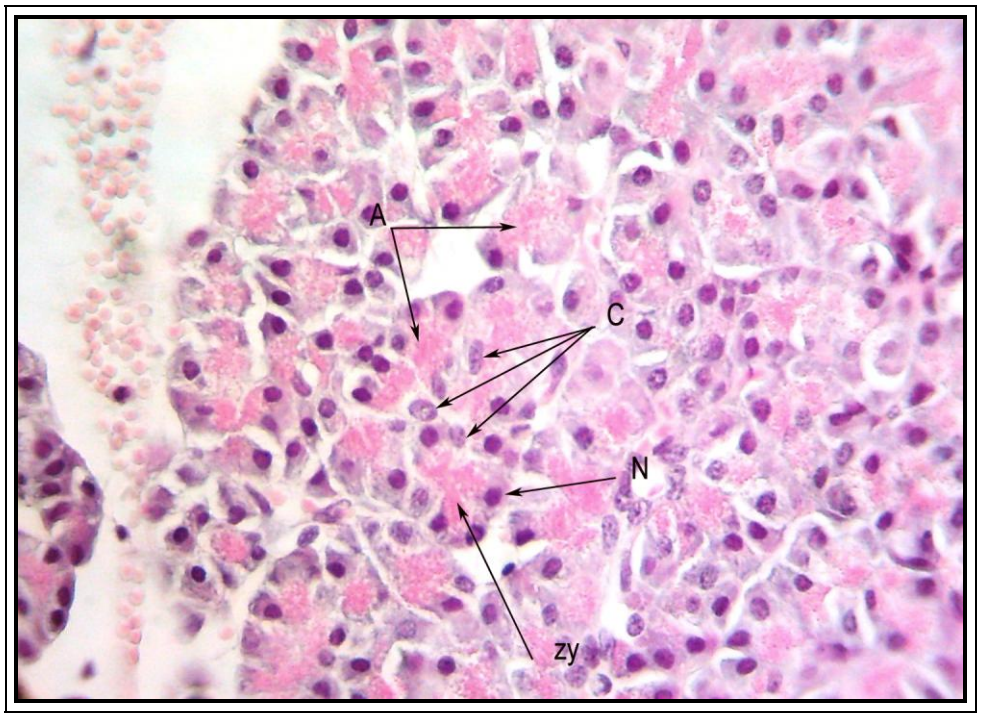


Figure (2): Light micrograph of the rabbit acinar cells of pancreas (A) with their nuclei (N), zymogen granules (zy) and centroacinar cells (C). (H & E X 600).



Figure (3): Light micrograph of the extraglandular tissue of the rabbit pancreas showing interlobular duct, blood vessel (Bv), connective tissue (CT) and acinar cells (A). (H & E X 400).

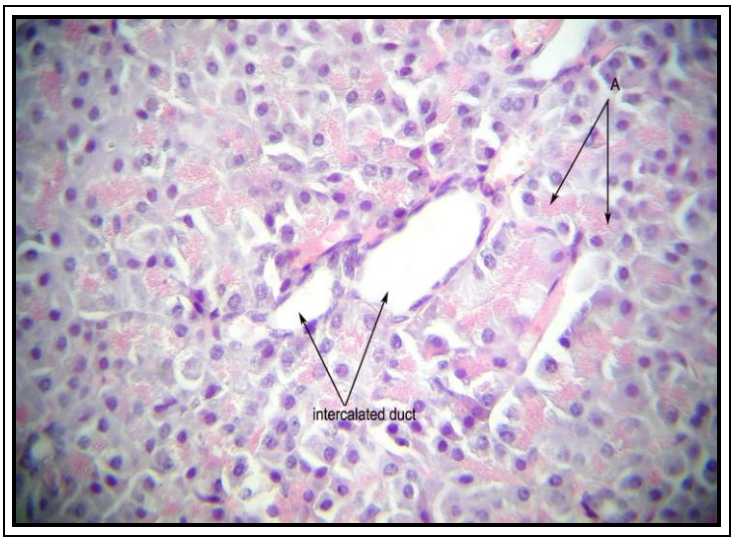


Figure (4): Light micrograph of the rabbit exocrine pancreas showing intercalated duct and acinar cells (A). (H & E X 400).

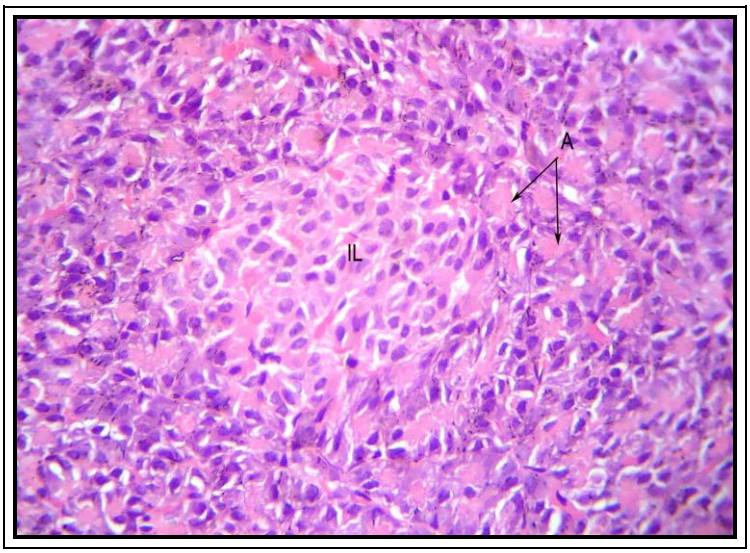


Figure (5): Light micrograph of the rabbit pancreas showing islet of Langerhans (IL) and acinar cells (A). (H & E X 400).

Electron microscopical observations
Group 1 (control rabbits)

The exocrine part of the pancreas was formed of serous cells which were pyramidal in shape and they were clustered in a roughly spherical fashion around a central lumen forming an acinus. Each pancreatic acinar cell was enveloped by a plasma membrane which showed distinct regional variation. The lateral and basal plasma membrane was straight and unfolded, while the luminal part carry a number of microvilli projecting into the acinar lumen (**fig. 6 and 7**).

The nuclei were almost circular in shape, basally located and surrounded by nuclear membranes. Each nucleus contained single or multiple nucleoli (fig. 6 and 7). The characteristic feature of pancreatic acinar cells was the presence of abundant well developed rough endoplasmic reticulum which was chiefly distributed basally and laterally to the nucleus. The rough endoplasmic reticulum consisted of closely packed parallel cisternae together with many ribosomes attached to the outer surface of the membranes. In addition to that many free ribosomes were present within the cytoplasm (**fig. 8**).

Elongated than those of A cells, the granules were numerous and the zymogen granules were mostly circular in profile and mainly occupied the apical cytoplasm. They consisted of electron dense material surrounded by a limiting membrane (fig. 6 and 7). Centroacinar cells were frequently seen in association with the acinar cells, forming part of the acinus and they were easily recognized by their large irregular nuclei, an absence of zymogen granules and by their low electron density (fig. 6).

The endocrine portion of the pancreas was composed of islets of Langerhans, which were formed of cells of irregular polygonal shape, central spherical nuclei, and small rod like mitochondria. Numerous cell types were present in the islets according to their secretory granules.

These cells include:

A cells: They were located at the periphery of the islet. Granules were numerous and uniform in size and membrane- bound with a very dense core and a less dense outer layer (**fig. 9**).

B cells: They were centrally located in the islet and forming the majority of the cell types, their mitochondria were more spherical and less bounding membrane was loose with a dense crystalline core in a matrix of low electron density (**fig. 10).**

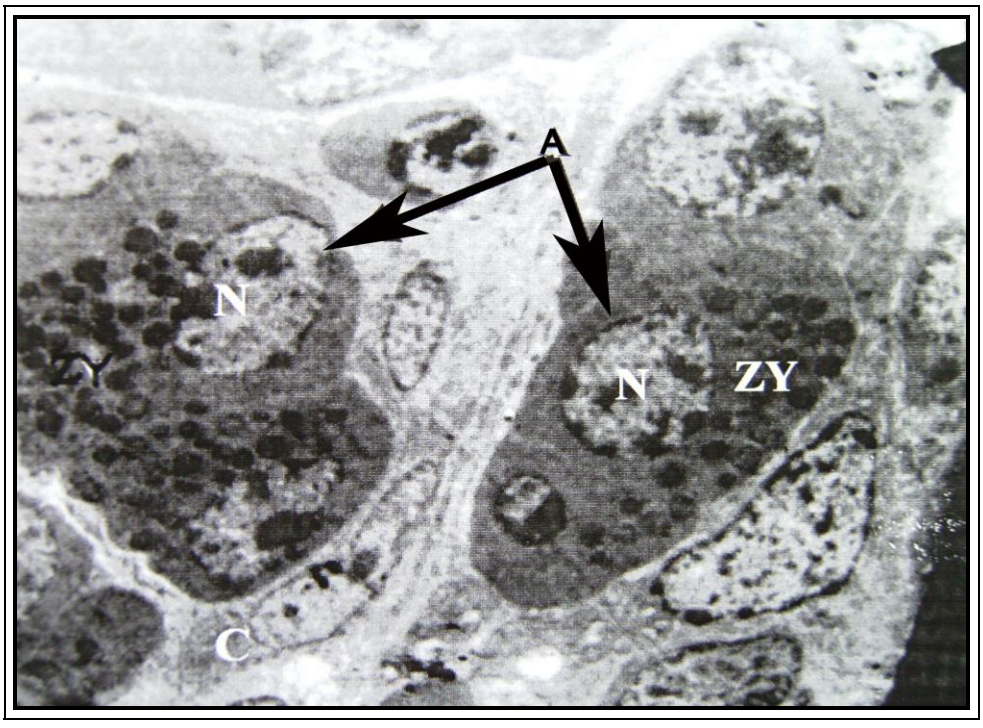


Figure (6): Electron micrograph of the rabbit exocrine pancreas showing the acinar cells (A) with their nuclei (N), zymogen granules (ZY) and centroacinar cells (C). (X 2600).

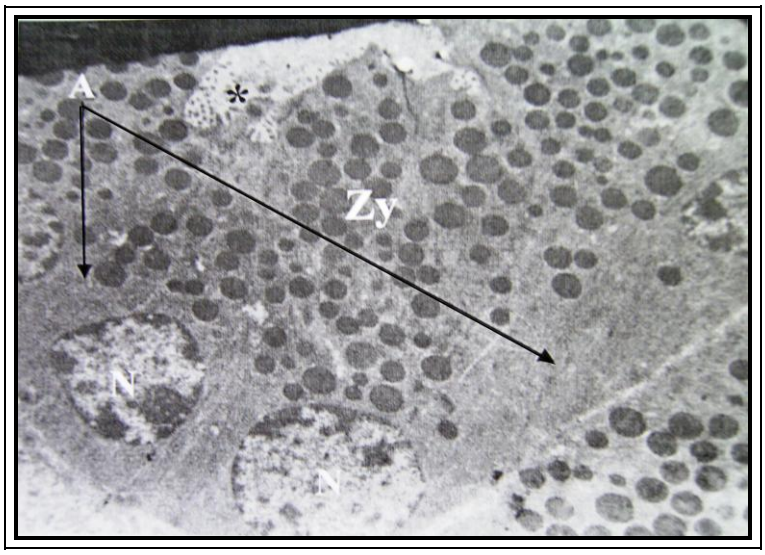


Figure (7): Electron micrograph of the rabbit exocrine pancreas showing the acinar cells (A) with their nuclei (N), zymogen granules (Zy) and microvilli (*). (X 2600).

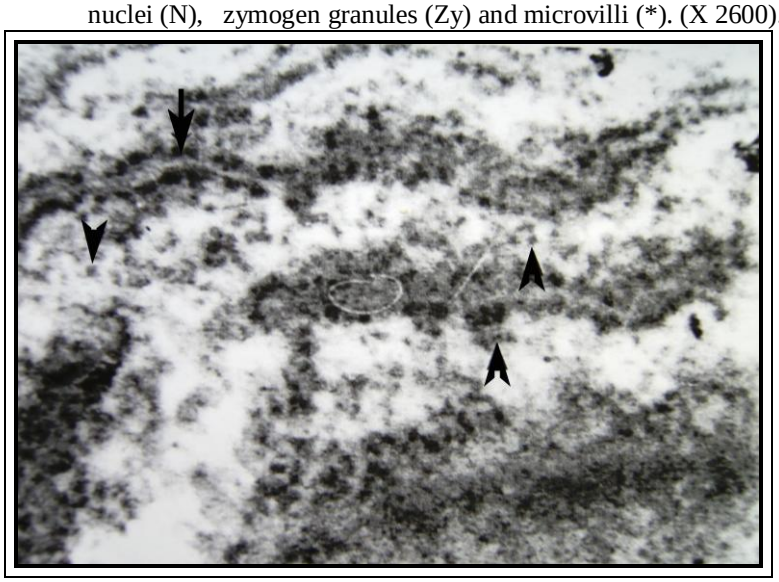


Figure (8): Electron micrograph of the rabbit exocrine pancreas showing well developed rough endoplasmic reticulum (→) and ribosomes (arrow head). (X 16000).

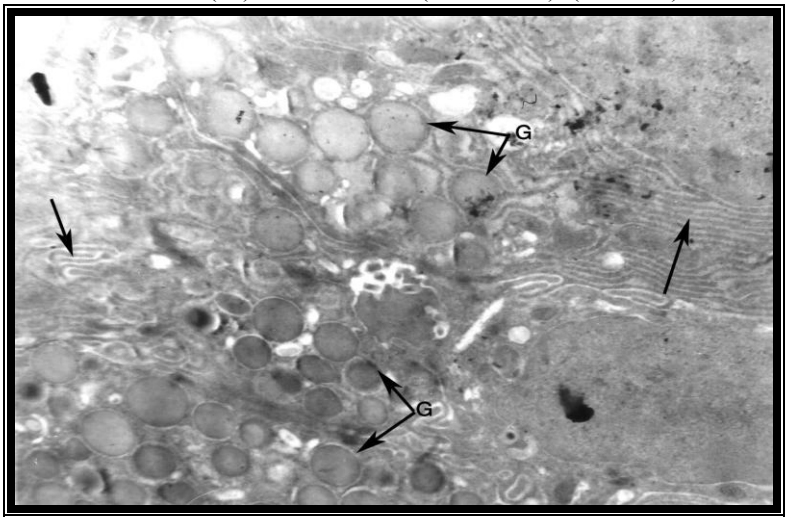


Figure (9): Electron micrograph of the rabbit A cell showing glucagon granules (G), rough endoplasmic reticulum (→). (X 8700).

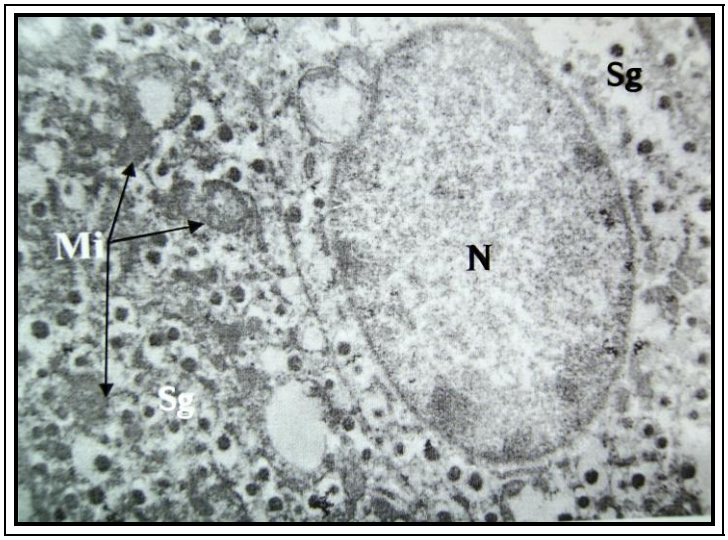


Figure (10): Electron micrograph of the rabbit B cell showing insulin granules (sg), mitochondria (Mi) and nucleus (N). (X 8700).

Exposed groups:

The architecture of the pancreas was preserved, it was composed of many lobules, and in between those very thin septa were observed. These lobules contained many acini that regarded as the exocrine portion of the pancreas. Scattered among these acini were the islet of Langerhans which represented the endocrine part of the gland.

There were no histological changes detected in both exocrine & endocrine portions and all three exposed groups have the same histological structure of the control group .

Exposed groups (EM)

The exposed groups revealed no changes in the sections which had been examined under the electron microscope, and they (exocrine and endocrine portions) have the same structures of those sections which obtained from the control group.

Discussion and Conclusion

The present work noticed lower food and liquid intake with a significant decrease in the body weight of the FD- exposed animals. This could be due to the stress and irritation of the animals as a result of FD-expos specially the mucous membranes of the nasal cavities and eyes [16].

The rabbits showed aggressive behaviour and movement at the beginning of the exposure time, this may be due to its effect on the central nervous system. There is a neuropsychological effect such as loss of concentration and mood alterations in man and in animals exposed to FD [17,18].

The present study showed no relationship between formaldehyde exposure and the pancreatic cancer.However, six cohort studies failed to report observed or expected deaths from pancreatic cancer among formaldehyde workers [19,20,21,22,23,24].

This suggests no relationship between pancreatic cancer and formaldehyde exposure. While two studies evaluated pancreatic cancer risk and increasing formaldehyde exposure especially among embalmers, pathologists, and anatomists [9,10].

The case control study of Kernan et al. [10] also reports high rates of pancreatic cancer among workers involved in medical and other health-related services.

A study found [11] a small increase in pancreatic cancer risk that appears to be limited to embalmers, pathologists and anatomists.

The small increased risk of pancreatic cancer among medical professionals and embalmers could result from a diagnostic bias since these groups would likely be subjected to better diagnostic procedures than the general

Population given their professions and socioeconomic status.

The occupational or lifestyle characteristics of pathologists, anatomists and embalmers, rather than formaldehyde, are likely to be the underlying factors associated with the excess risk of these neoplasms among these professionals [25].

In rats and monkeys exposed to formaldehyde by inhalation, approximately 40% of formaldehyde undergoes rapid biotrans - formation immediately after absorption to formate, which is partially incorporated via

normal metabolic pathways into one - carbon pool of the body or farther oxidised to carbon dioxide. The remainder 40% was incorporated into macromolecules, while the other 20% of formaldehyde was excreted in the urine. The macromolecules are covalently bound as DNA-protein cross – links in the tissues [26, 27, 28].

Some studies have shown that formaldehyde is almost completely absorbed at the site of contact and is rapidly metabolized by enzymes, formaldehyde induced lesions in the distal organs have not been observed among experimental animals [29].

In rhesus monkeys exposed to formaldehyde, the DNA-protein cross-links were found in highest concentration in the nasopharynx, less in the larynx

To major proximal bronchi and none at the lung parenchyma [30].

Observations in humans and experimental animals show no increase in background formaldehyde levels in the blood of formaldehyde exposed rats and humans [31,32].

Thus, formaldehyde is likely to be rapidly metabolized and detoxified upon contact with the respiratory tract and is unlikely to reach the pancreas in concentrations sufficient to cause damage.

The findings of this study did not suggest that formaldehyde exposure is a major contributor to the etiology of pancreatic cancer. This finding is consistent with a recent review and meta-analysis studies; and the evidence supporting a causal relation between formaldehyde exposure and pancreas cancer remains weak. Further experimental study may be needed to confirm the positive association between formaldehyde exposure and pancreatic cancer.

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