THE EFFECT OF FORMALDEHYDE ON THE LIVER OF ADULT MALE ALBINO RATS AND POSSIBLE PROTECTIVE ROLE OF VITAMIN C

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ABSTRACT

Background: Formaldehyde is found in different kinds of medicine and industrial products, cigarette smoke, and even numerous vegetables, fruits and seafood that have been illegally preserved with formalin. The water soluble vitamin C is a strong antioxidant that scavenges free radicals and other reactive nitrogen and oxygen species. Even in small amounts, vitamin C can defend nucleic acids, lipids and proteins against oxidative damage. ***Objectives:*** the aim of this study is to elucidate the possible changes that take place in the liver of adult male albino rats after intraperitoneal injection of formaldehyde and the possibility of oral vitamin C's hepatoprotectivity against it. ***Design:*** forty-five adult male albino rats were utilized in this work. These animals were allocated randomly into three main groups. **Group I** (control groups) included 3 subgroups each contained 9 rats : –ve control received no treatment , +ve control which were injected intraperitoneally by 1 ml of distilled water for 10 days and vit.C +ve control group which received a daily dose of ascorbic acid (100mg/kg bw) dissolved in distilled water by gavage for 10 days. **Group II:** contained 9 rats, which were injected intraperitoneally with a daily dose of formaldehyde (10mg/kg BW) for 10 days**. Group III:** contained 9 rats that were injected intraperitoneally with a daily dose of formaldehyde (10mg/kg BW) concomitantly with daily dose of ascorbic acid (100mg/kg BW) by gavage for 10 days. By the end of the experiment, blood samples were collected for biochemical study of ALT (Alanine aminotransferase), AST (Aspartate aminotransferase) and albumin and all animals were anaesthetized by ether inhalation. Liver specimens were dissected out and weighed then subjected to histopathological, immunohistochemistry, and morphometrical examination. ***Results:*** Administration of formaldehyde at a dose of 10 mg/kg caused increase in serum activities of ALT and AST ,but regarding albumin ,it remained unaffected .It caused increase in the liver weights and induced several histopathological changes in the liver of adult male albino rats as congested dilated central veins, portal veins and blood sinusoids with increase in the thickness of the wall of the portal vein. Meanwhile, some hepatic lobules showed multiple necrotic foci around central and portal veins. On the other hand, vit.C partially improved the state of oxidative stress as evidenced by iNOS (inducible nitric oxide synthase) immunohistochemistry. It also reduced the degree of hepatic fibrosis as evidenced by Mallory trichrome histochemical staining. ***Conclusion:*** Exposure to formaldehyde led to pronounced hepatic damage which is partially limited by vit.C coadministration. ***Recommendations:*** Many special precautions should be taken to limit the occupational and environmental exposure and the level of food and water contamination with formaldehyde and use vit.C as a supplement to limit the toxic effects on the liver.

Keywords: formaldehyde, liver, vit.C

INTRODUCTION

Formaldehyde (FA) is a colorless and highly water-soluble aldehyde. Its intake occurs through the topical, oral, injection and mostly via the respiratory system. It can be inhaled in smoke due to the combustion of burning fossil fuels and in the fumes of paints and in cigarette smoke***(Nazaroff & Singer, 2004)*** . It can be ingested in fresh water , food and drugs .In food , it can occur naturally or through contamination as it can be added as a preservative or disinfectant agent .It can result also from cooking or smoking of foods ***(Yang et al., 2007)***. Even in infancy, children are exposed by injection to formaldehyde present in diphtheria, polio and tetanus vaccine preparations as a result of the manufacturing

process ***(Metz et al., 2004; Thaysen-Andersen et al., 2007)***. Several malignancy treating drugs are formulated with formaldehyde which is required for drug activation **(*Evison et al., 2008*)** or release formaldehyde ***(Levovich et al., 2008)***. Some cosmotics especially hair smoothing products containing FA or methylene glycol which require the use of heat that leads to volatilization of both FA gas as well as methylene glycol vapors . This increases the potential for hair stylist and consumer exposure to FA from methylene glycol formulated in keratin products **(Golden and Valentini, 2014).**

The International Agency for Research on Cancer (IARC) has classified FA in group 1 (human carcinogen). It was based on inhalation causing squamous cell carcinoma in rats and naso-pharyngeal cancer in humans ***(IARC 2006)***. Recently, the classification has been expanded with FA causing leukaemia and limited evidence of sinonasal cancer in humans ***(IARC 2012)***.

(Shimizu et al., 2003) postulated that formaldehyde's hepatotoxic effect can occur in many species, including humans, following injection, ingestion, or inhalation. FA induces oxidative stress which has been reported to be the mechanism of FA toxicity in multiple tissues in the exposed rats and mice, in liver, lymphocytes, heart, brain, lung, testes and ovaries ***(NTP, 2010; Lino-dos-Santos-Franco et al., 2011; Zhou et al., 2011 and Wang et al., 2012)***.

Nitric oxide (NO) and the oxidative metabolite, peroxynitrite (ONOO-) are referred to as reactive nitrogen species (RNS). Nitric oxide (NO) is one of the essential regulators of several biological processes, it is produced by the activity of nitric oxide synthase (NOS) from arginine **(Kaplan et al., 2012)**.There are three major isoforms of NOS : (1) endothelial NOS (eNOS), (2) neuronal NOS (nNOS), which are expressed constitutively and both are termed constitutive nitric oxide synthase (cNOS); and (3) induced NOS (iNOS) by endotoxin and inflammatory mediators **(Wen et al., 2011)**.

Vitamin C is a water soluble vitamin and a potent reducing agent, so that its two major functions are as an antioxidant and as an enzyme cofactor ***(Combs, 2012)***. Vit.C is a strong antioxidant that scavenges free radicals and other reactive nitrogen and oxygen species. Thus, even in small amounts; it can defend indispensable molecules in the body, such as proteins, lipids and nucleic acids against oxidative damage. These reflect its hepatoprotective effect ***(Padayatty et al., 2003)* .**

The aim of this work: the aim of this study is to elucidate the possible changes that take place in the liver of adult male albino rats after intraperitoneal injection of FA and the possibility of oral vitamin C hepatoprotective effect against it using biochemical, histopathological, immuno-histochemistry and morphometrical examination.

MATERIAL AND METHODS

- **MATERIAL :**
- **Chemicals:**

1- **Formaldehyde (FA):** it was obtained in the form of formalin liquid 37% from El Gomhoria Company for Chemical and Medical Trading, Zagazig, Egypt.

2- **Vitamin C:** it was obtained in the form of powder, (L-ascorbic acid) from El Gomhoria Company for Chemical and Medical Trading, Zagazig, Egypt. At the time of each administration, it was freshly prepared by dissolving in distilled water. **Kits for immunohistochemistry:** were supplied by (DAKO life trade Egypt).

- **Animals :**

Forty-five healthy adult male albino rats weighing 310 -320 gm were obtained from the Laboratory Animals' Unit at the Faculty of Medicine, Zagazig University were used in the present study. All animals were kept under hygienic conditions. Standard food and water ad-libitium were allowed. All rats were handled in accordance to the standard guide for the care and use of laboratory animals ***(Institute of laboratory animal resources,1996)***.

Adult albino rats were chosen in this work as they could be housed, bred and handled without difficulties. Also, they have long life span and they are relatively disease free ***(David et al., 2000).*** Moreover, the cellular organization of the rat liver is quite similar to that of other mammalian species, confirming that the rat represents a useful animal model for studies of liver structure and function ***(Baratta et al., 2009)***.

Male albino rats were preferred because they have constant hormone levels in comparison to females who have variable ones. This variability should not be ignored as hormones can play a role in many inflammatory responses.Recent experiments showed significant differences in mitochondrial injury, nuclear condensation, ER (Endoplasmic Reticulum) status, and plasma membrane permeability between sexes presenting female cells as being more sensitive, at certain exposure times ***(Beery & Zucker, 2011)*** .

They were divided into three groups:

Group I: (Control): It was divided into three subgroups. Each subgroup included 9 rats:

- Subgroup **(1a):** negative control which received no treatment.
- Subgroup (**1b):** positive control, which received daily dose (1 ml) of distilled water by intraperitoneal injection for 10 days.
- Subgroup (**1c):** vitamin C positive control, received a daily dose of L-ascorbic acid (100mg/kg BW) dissolved in distilled water by gavage for 10 days ***(Uboh, et al., 2012).***

Group II: (FA treated group): It included 9 rats and they received a daily dose of formaldehyde (10mg/kg BW) by intraperitoneal injection for 10 days ***(Ucmakli et al., 2013).***

Group III (FA& vit.C treated group): This group included 9 rats .They received daily dose of intraperitoneal injection of formaldehyde (10mg/kg BW) concomittaantlly with gavage of vitamin C (100mg/kg BW) for 10 days ***(Uboh, et al., 2012).***

By the end of the experiment (10 days), all animals were anesthetized by ether inhalation and venous blood samples were collected by means of micro-capillary glass tubes from the retro-orbital plexus ***(Johnson,2007)*** for assessment of liver functions (Alanine transferase (ALT), Aspartate transferase (AST) and albumin) and then animals were sacrificed. Liver specimens were dissected out and weighed then subjected to histopathological, immunohistochemistry and morphometrical examination.

- **Methods**

1- Determination of plasma ALT, AST and albumin:

Collected blood samples from the rats at the end of the experiment were centrifuged at 5000 rpm, 4 ◦C, for 15min; the plasma samples were stored at −30 ◦C. AST, ALT and albumin were analyzed by the semiautomated clinical chemistry analyzer Microlab 300. AST and ALT were assayed according to the method of ***Reitman and Frankel (1957),*** *while albumin was calculated* according to the method described by ***Doumas et al.,(1971)***.

2-light microscopic examination

A-Histopathological and histochemical study:

Samples from liver were rapidly fixed in 10% formol saline for 48 hours, dehydrated through graded alcohols and embedded in paraffin. Transverse sections of 5µm thickness were obtained from all specimens stained with hematoxylin and eosin (Hx&E) and other sections stained with Mallory trichrome stains ***(Bancroft and Gamble, 2008).***

B-Immunohistochemical study for iNOS:

Sections of 5 µm in thickness were prepared from paraffin embedded tissues. After deparaffization, endogenous peroxidase was quenched with 3% H2O2 in deionized water for 5–10 min. Nonspecific binding sites were blocked by incubating the sections in 10% normal rabbit serum for 10–15 min. The sections were then incubated with polyclonal rabbit anti-iNOS (dilution 1:25) overnight at 4 °C, followed by incubation with biotinylated goat-antirabbit IgG at room temperature for 10–15 min. After phosphate buffred saline (PBS)

rinses of 3 × 3 min, the horseradish-peroxidase-conjugated streptavidin solution was added and incubated at room temperature for 10–15 min. The antibody binding sites were visualized by incubation with a diaminobenzidine–H2O2 solution. The sections incubated with PBS instead of the primary antibody were used as negative controls. Brown-yellow granules in cytoplasm or nuclei were recognized as positive staining for iNOS. Activity of iNOS tissues was shown by commercial iNOS immunohistochemical stain kits (DAKO life trade Egypt). Streptavidin–biotin complex was used. Cytoplasmic staining with iNOS was revealed to be positive.

3-Morphometric study:

The morphometric study was done using Image analyzer software (Leica Qwin 500 Image Analyzer, England). The total images per animal were 15 images. According to this method, we used an optical magnification of 400 for counting the number of hepatocytes with positive immune reaction using the interactive measure menu.

4-Statistical analysis

All the grouped data were statistically evaluated with SPSS, version **18.0** software ***(Lee & Lim, 2011)***. Testing methods included one-way analysis of variance (ANOVA) for comparisons between more than two groups in normally distributed data and Kruskal Wallis test was used for comparison between more than two groups in not normally distributed data followed by least significant difference (LSD) test for comparison between two groups. *P*-values of ≤ 0.05 were considered to indicate statistical significance, while *P*-values of <0.01 indicate highly significant results. All the results were expressed as mean ± S.D**.**

RESULTS

1- Biochemical results :

- **ALT, AST and Albumin**

The results of the present study showed a highly significant increase in serum activities of ALT and AST (P<0.001) in FA treated group when compared with that of the control groups and FA& vit.C group. While, there was a non-significant statistical difference between the control groups and FA&vit.C group. However, regarding albumin (ALB), the study showed a non-significant statistical difference among the different groups**(Tables 1&2).**

2- Liver weight and statistical results:

Statistical analysis of the liver weight revealed a highly significant statistical increase (p>0.001) in the FA treated group when compared with that of the control groups and FA& vit.C group. There was a non-significant statistical difference between the control and FA&vit.C groups **(Tables 3&4).**

3- Light microscopic examination :

A. **Histopathological results :**

Examination of Hx&E stained sections of liver of control adult male albino rats revealed that the (-ve)control group **(Fig.1a)** and vit. C treated (+ve) control **(Fig.1b)** stained sections had the same histological structure of the liver, thus in the following stains and groups the (-ve) control slides represented the control groups.

Examination of Hx&E liver stained sections of these groups revealed that each hepatic lobule was formed of tightly packed cords of hepatocytes radiating from the central vein. The hepatic cords were composed of polygonal hepatocytes with rounded vesicular nuclei and acidophilic cytoplasm. Blood sinusoids with their endothelial lining were noticed between hepatic cords **(Fig.1c)**. Portal triad is composed of a portal vein, which has large lumen and thin vessel wall, hepatic artery, which has small lumen and a wall of smooth muscle and bile ductile which is lined by single cuboidal cells with dark rounded nuclei **(Fig.1d).**

In the FA treated group, examination of Hx&E stained sections showed a marked loss of normal liver architecture with variable hepatocellular changes representing different degrees of lobular affection **(Fig.2a)**. Hepatic lobules revealed some hepatocytes with ill-defined borders and condensed nuclei, while others had also vacuolated cytoplasm.

Congested dilated central veins and blood sinusoids with mononuclear cellular infiltration around the central vein were also observed **(Fig.2b)**.

The portal area showed congested portal vein, marked cellular infiltration and bile duct proliferation **(Fig.2c and Fig.2h)**. Some portal areas showed an apparent increase in the thickness of the wall of the portal vein **(Fig.2g)**.

Meanwhile, some hepatic lobules showed multiple necrotic foci around central and portal veins that appeared to be formed of a collection of cell debris and infiltration of lymphocytic cells **(Fig. 2d, Fig.2e and Fig.2f)**.

On the other hand, examination of liver sections of the FA & vit.C treated group showed variable degrees of improvement according to the severity of changes observed in FA treated group. Some hepatocytes showed vacuolated cytoplasm and condensed nuclei. While, other lobules revealed normal lobular architecture**(Fig.3a)**, as hepatocytes had vesicular nuclei and acidophilic cytoplasm radiating from central vein. Many cells were binucleated. Slightly dilated blood sinusoids and central vein were still present **(Fig.3b)**.

B. **Histochemical results :**

The control group stained sections revealed few blue-stained collagen fibers around portal area **(Fig.4a)** and the central vein **(Fig.4b)**.

While, in the FA stained sections, examination showed marked increase in the amount of the blue-stained collagen fibers in the portal areas **(Fig.4c)** and around the central veins **(Fig.4d)**. Meanwhile, stained sections of the FA&vit.C treated group revealed few collagen fibers around the portal areas **(Fig.4e)** and central veins (**Fig.4f)**.

4- Morphometrical study

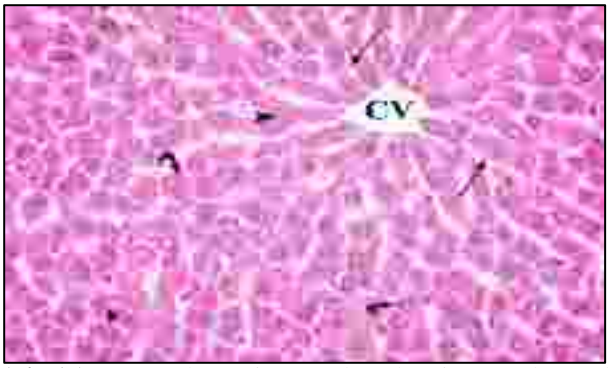
The results of the present study showed a highly significant increase ($P<0.001$) in the number of hepatocytes with positive immune reaction for iNOS in the FA treated group when compared with that of the control group and the FA& vit.C treated group. Also, there was a significant statistical difference ($p<0.05$) between the control and FA&vit.C treated groups **(Tables 5 &6)**.

(Fig.1a):A photomicrograph of a section in the liver of the control (-ve) (1a) group showing part of a hepatic lobule with tightly packed cords of hepatocytes radiating from central vein **(CV)**.

(Hx&E X 100)

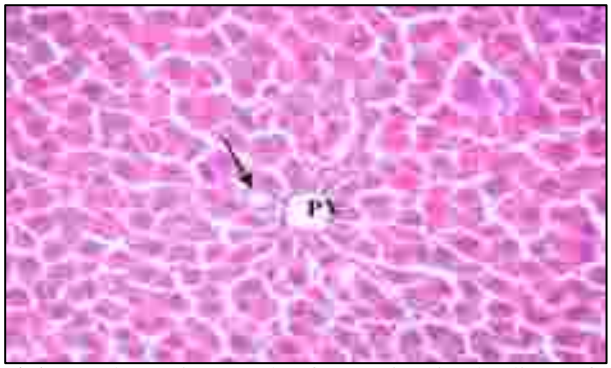
(Fig.1b):A photomicrograph of a section in the liver of the vit.C control (+ve) group (1c) showing a part of hepatic lobule with tightly packed cords of hepatocytes radiating from central vein (CV).

(Hx&E X 100)



(Fig.1c): A photomicrograph showing polygonal hepatocytes (curved arrow) radiating from central vein (CV) with rounded vesicular nuclei and acidophilic cytoplasm. Narrow radiating blood sinusoids (thin arrow) in between liver cords and their lining endothelium are seen. Binucleated cell are also seen (arrow heads).

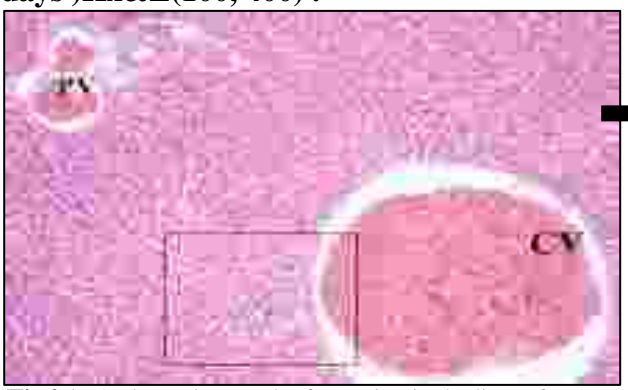
(Hx&E X400)



(Fig.1d): A photomicrograph of a section in the liver of the control group showing portal area containing portal vein (PV) and bile duct (arrow) .

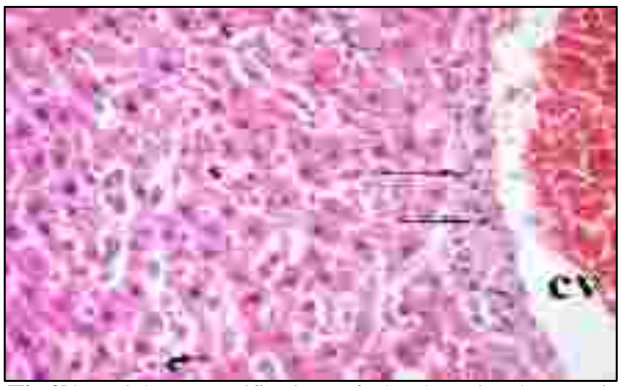
(Hx&E X400)

Figure (1): photomicrographs of the liver of (group I) control groups (group Ia (-ve) control and group Ic ,vit.C (+ve) control treated by a daily gavage of L-ascorbic acid (100mg/kg BW) for 10 days)Hx&E(100, 400) .



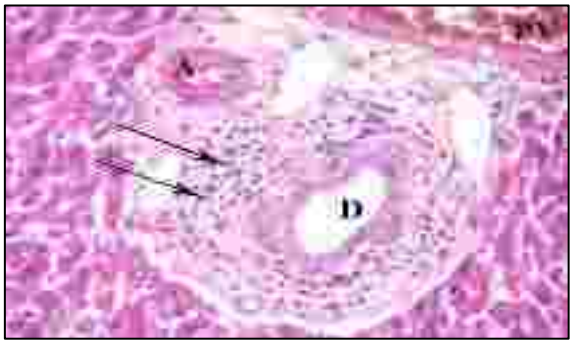
(Fig.2a): A photomicrograph of a section in the liver of FA treated group showing loss of the normal arrangement of hepatocytes and dilated congested central (CV) and portal (PV) veins.

(Hx&E X 100).



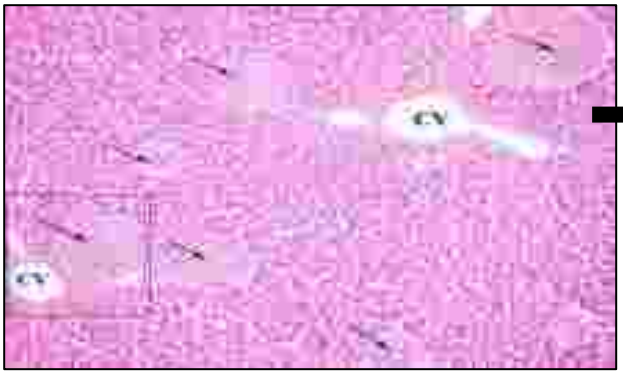
(Fig.2b): Higher magnification of the box in the previous photomicrograph showing mononuclear cellular infitration (thin arrow) around dilated congestd central vein (CV) . Irregularly dilated congested blood sinusoids (S) and hepatocytes with darkly-stained nuclei (curved arrow) are also seen .

(Hx&E X 400).



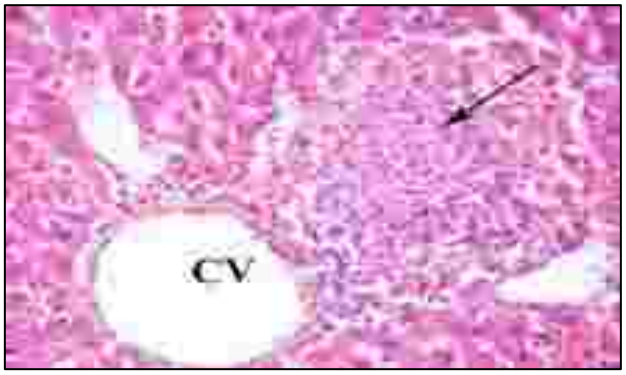
(Fig.2c): A photomicrograph showing mononuclear cellular infiltration (arrows).around part of dilated congested portal vein (PV) ,proliferation of bile ducts (D) and thick walled hepatic artery (A) are also seen.

(Hx&E X 400).



(Fig.2d): A photomicrograph of a section in the liver of FA treated group showing loss of normal arrangement of hepatocytes and multiple necrotic foci (thin arrows).

(Hx&E X 400).

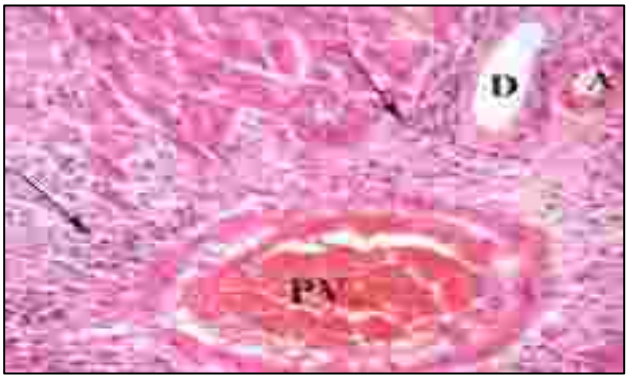


(Fig.2e): Higher magnification of the box in the previous photomicrograph showing necrotic foci formed of a collection of cell debris and infiltration of lymphoid cells (arrows).

(Hx&E X 400).

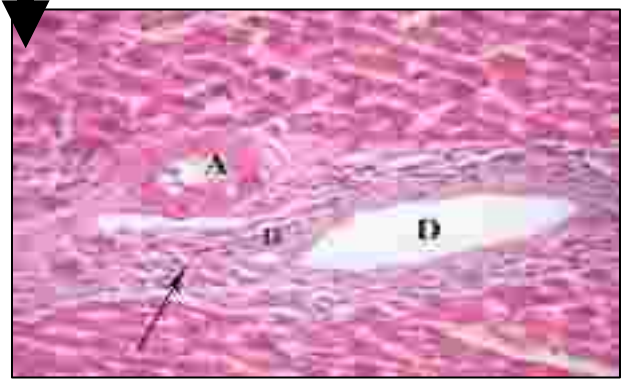
(Fig.2f): A photomicrograph of a section in the liver of the FA treated group showing loss of the normal arrangement of hepatocytes and dilated congested portal vein(PV) . Necrotic focus is also seen (thin arrow).

(Hx&E X 100)



(Fig.2g): Higher magnification of the A box in the previous photomicrograph showing mononuclear cellular infiltration (arrows) and thick walled dilated congested portal vein (PV).

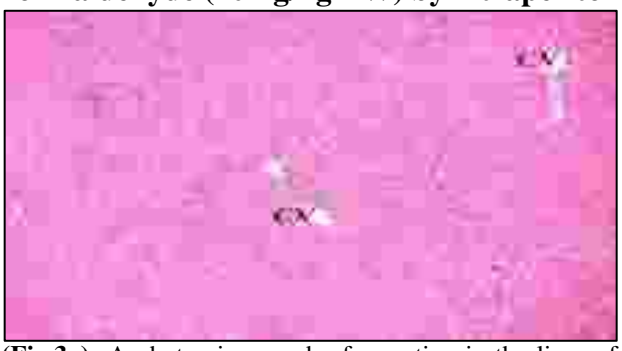
(Hx&E X 400)



(Fig.2h): Higher magnification of the B box in the previous photomicrograph showing bile duct proliferation (D) and one lined by multiple layers . Mononuclear cellular infiltration (arrows) and thick walled hepatic artery (A) are also seen .

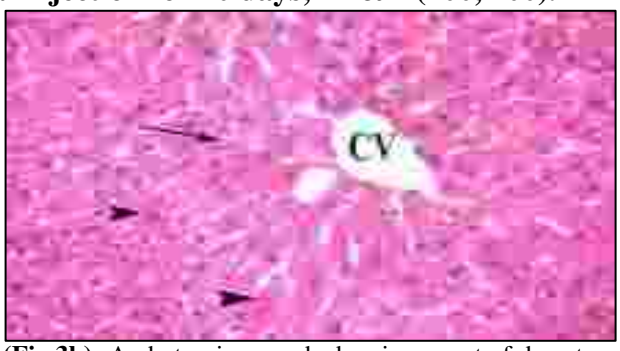
(Hx&E X 400)

Figure (2): photomicrographs of liver of FA treated group (group II) that received a daily dose of formaldehyde (10mg/kg BW) by intraperitoneal injection for 10 days, Hx&E (100, 400).



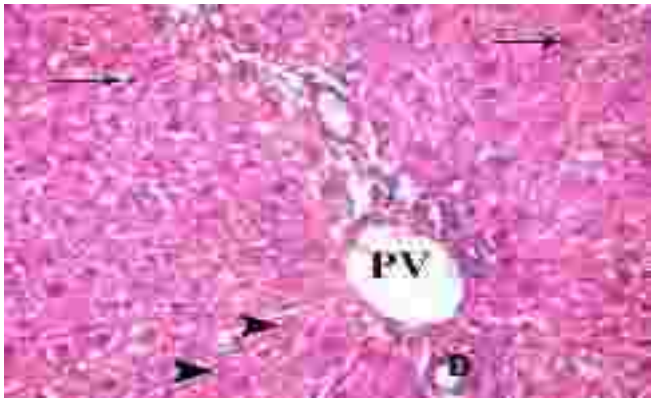
(Fig.3a): A photomicrograph of a section in the liver of FA& vit.C treated group showing slightly preserved liver architecture with hepatocytes arranged around central vein (CV) .

(Hx&E X 100)



(Fig.3b): A photomicrograph showing most of hepatocytes with vesicular nuclei and acidophilic cytoplasm (arrow heads) radiating from slightly dilated central vein (CV). Binucleated cells (arrows) are also seen .

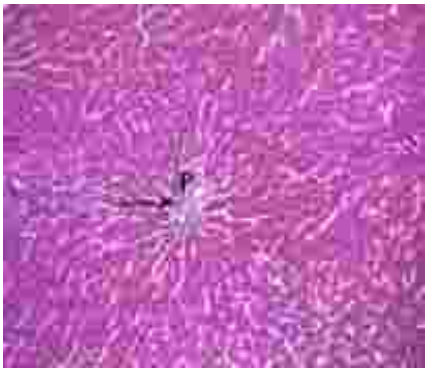
(Hx&E X 400)



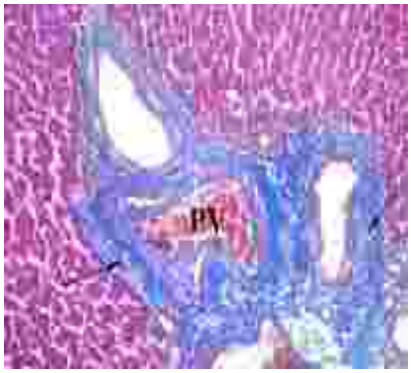
(Fig.3c): A photomicrograph of a section in the liver of FA& vit.C treated group showing portal area with portal vein (PV) and bile duct (D) .Some hepatocytes with acidophilic cytoplasm and vesicular nuleus (arrow head) and others with darkly stained nuclei (arrows)are seen

(Hx&E X 400).

Figure (3): photomicrographs of the liver of FA &vit.C treated group (group III) which received daily dose of intraperitoneal injection of FA (10mg/kg BW) concomitantlly with gavage of vitamin C (100mg/kg BW) for 10 days Hx&E (100, 400).



(Fig.4a): A photomicrograph of a section in the liver of the control group showing few blue stained collagen fibers (arrow) in the portal area (P). **(Mallory's trichrome x 200)**.

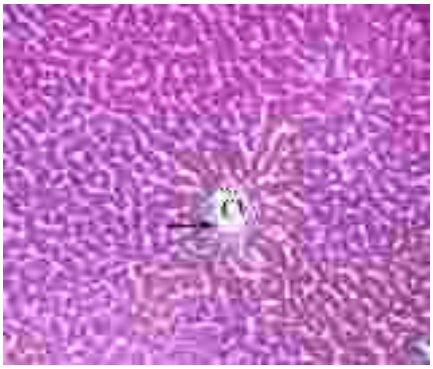


(Fig.4c): A photomicrograph of a section in the liver of FA treated group showing abundant blue stained collagen fibers (arrow) in portal area surrounding portal vein (PV).

(Mallory's trichrome x 200)

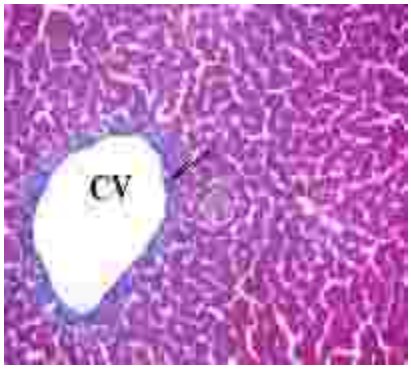
(Fig.4e): A photomicrograph of a section in the liver of FA& vit.C treated group showing blue stained collagen fibers (arrow) around the portal area.

(Mallory's trichrome X 200)



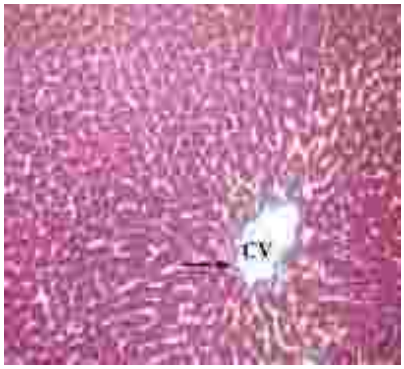
(Fig.4b): A photomicrograph of a section in the liver of the control group showing few blue stained collagen fibers (arrow) around the central vein (CV).

(Mallory's trichrome x 200)



(Fig.4d): A photomicrograph of a section in the liver of FA treated group showing abundant blue stained collagen fibers (arrow) around the central vein (CV)**.**

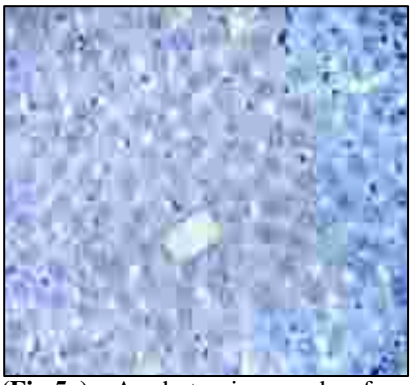
(Mallory's trichrome x 200)



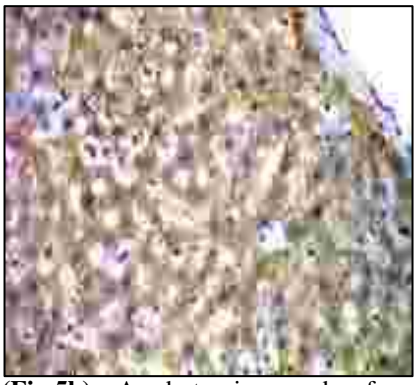
(Fig.4f): A photomicrograph of a section in the liver of FA& vit.C treated group showing few blue stained collagen fibers (arrow) around the central vein (CV).

(Mallory's trichrome X 200)

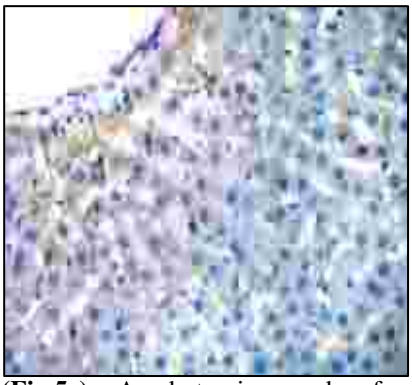
Figure (4): photomicrographs of the liver of control, FA and FA& vit.C treated groups Mallory trichrome (200).



(Fig.5a): A photomicrograph of a section in the liver of a control adult albino rat showing negative immune reaction for iNOS in the cytoplasm hepatocytes. (**Immunoperoxidase technique for iNOS X 400**).



(Fig.5b): A photomicrograph of a section in the liver of FA teated adult albino rat showing severe positive immune reaction for iNOS in the cytoplasm of hepatocytes. **(Immuno-peroxidase technique for iNOS X 400).**



(Fig.5c): A photomicrograph of a section in the liver of FA&vit.C treated adult albino rat showing mild immune reaction for iNOS in the cytoplasm hepatocytes **(Immunoperoxidase technique for iNOS X 400)** .

Figure (5): photomicrographs of the liver of control, FA and FA& vit.C treated groups) (iNOS immunohistochemical X 400)

Table (1): Statistical analysis of liver function tests of Alanine transferase (ALT), Aspartate transferase (AST) and albumin (ALB) in the different studied group using ANOVA (analysis of variance) test.

	(-ve) control	Vit.C (+ve) control	FA treated	FA&vit.C treated	F	p
ALT:						
Mean ± SD	**33.2 ± 1.30**	**37 ± 2.83**	**78.8 ± 3.35**	**35.8 ± 9.18**	**90.36**	**<0.001****
Range(U/L)	**32 – 35**	**34 – 40**	**75 – 83**	**22 – 46**		
AST:						
Mean ± SD	**117.6 ± 3.13**	**127.4 ± 2.41**	**269.4 ± 68.43**	**129.4 ± 16.29**	**21.17**	**<0.001****
Range (U/L)	**115 – 121**	**0125 – 130**	**201 – 371**	**113 – 154**		
ALB:						
Mean ± SD	**3.58 ± 0.08**	**3.63 ± 0.15**	**3.59 ± 0.20**	**3.75 ± 0.13**	**1.3**	**0.31**
Range(g/dl)	**3.5 – 3.7**	**3.52 – 3.8**	**3.32 – 3.82**	**3.60 – 3.87**		**NS**

FA:formaldehyde NS: non significant **: highly significant (p<0.001) .
SD:standard deviation

Table (2): Least significant difference test (LSD) for comparison of the changes of the mean values of liver function tests of Alanine transferase (ALT), Aspartate transferase (AST) and albumin (ALB)ALT, AST and ALB in-between groups.

	ALT	AST	ALB
–ve Control vs. +ve control	**0.26 NS**	**0.66 NS**	**0.59 NS**
–ve Control vs. FA treated	**<0.001****	**<0.001****	**0.92 NS**
–ve Control vs. FA&vit.C	**0.34 NS**	**0.6 NS**	**0.09 NS**
+ve Control vs. FA treated	**<0.001****	**<0.001****	**0.66 NS**
+ve Control vs. FA&vit.C	**0.72 NS**	**0.93 NS**	**0.24 NS**
FA treated vs. FA&vit.C	**<0.001****	**<0.001****	**0.12 NS**

NS: non-significant

(:** highly significant **(p<0.001).**

Table (3): Statistical analysis of (Mean ± SD) of liver weight in the different studied group using ANOVA (analysis of variance) test

	(-ve) control	Vit.C (+ve) control	FA treated	FA& vit.C	F	p
Liver Weight:						
Mean(gm) ± SD	**7.96 ± 0.05**	**7.62 ± 0.16**	**10.99 ± 0.71**	**7.91 ± 0.72**	**48.11**	**<0.001****
Range(gm)	**7.9 – 8**	**7.5 – 7.8**	**10.4 – 11.88**	**6.82 – 8.6**		

SD:standard deviation FA:formaldehyde

Table (4): Least significant difference test (LSD) for comparison of the changes of the mean values of Liver weight in-between groups.

	Liver weight
–ve Control vs. +ve control	0.31 NS
–ve Control vs. FA treated	**<0.001****
–ve Control vs. FA&vit.C	0.87 NS
+ve Control vs. FA treated	**<0.001****
+ve Control vs. FA&vit.C	0.39 NS
FA treated vs. FA&vit.C	**<0.001****

NS: non-significant (**: highly significant **(p<0.001).**

Table (5): statistical analysis of morphometry in the different studied group using Kruskal Wallis test.

	control	FA treated	FA&vit.C treated	k	p
Morphometry:					
Mean ± SD	**1.8 ± 0.83**	**73.8 ± 5.22**	**9.2 ± 5.76**	**15.5**	**0.001****
Range(+ve cells)	**1 – 3**	**65 – 79**	**3 – 14**		

SD:standard deviation

Table (6): Least significant difference test (LSD) for comparison of the changes of the mean values of Morphometry in-between groups.

	iNOS Morphometry
–ve Control vs. FA treated	**<0.001****
FA treated vs. FA&vit.C	**<0.001****
–ve Control vs. FA&vit.C	**0.008***

*: Significant (p<0.05)

**: highly significant (p<0.001)

DISCUSSION

Humans are exposed to FA every day due to its presence in different kinds of medicine and industrial products such as building materials, cosmetics, cigarette smoke, and photochemical smog also even various fruits, vegetables and seafood that may be illegally preserved with formalin. FA has been documented to be potentially carcinogenic, making it a subject of major environmental and search concern ***(Heck et al., 1990;Metz et al., 2004 and Tang et al., 2009).***

In the current study, there were statistically significant increases in AST and ALT levels in the FA treated group when compared with that of the control groups. This result is in accordance with ***(Gulec et al., 2006)*** who gave the same dose intraperitoneally and said that this provided evidence of liver cell damage . ***(Khatun et al., 2015)*** added that, ALT and AST are useful serum markers for FA hepatotoxicity and postulated that their elevation may be due to FA's metabolite, a free radical that binds to lipoprotein and leads to peroxidation of lipids of ER.

On the other hand, these changes were statistically leveled similar to the control groups in FA & vit.C treated group. This result is in harmony with ***(Uboh et al., 2012)*** who stated

that vit. C has the ability to normalize levels of AST and ALT. Also these results are in agreement with ***(Bashandy & AlWasel, 2011)*** who also reported that vitamin C normalized levels of AST & ALT, in addition to alkaline phosphatase, blood hydroperoxide and MDA in the liver of carbon tetrachloride intoxicated rats ***(Bakand et al., 2005)*** reported that, liver is affected following FA exposure as it is involved in its metabolism. The major metabolic enzymes involved in the metabolism of FA, particularly GSH-dependent formaldehyde dehydrogenase and NAD-dependent aldehyde dehydrogenase, have been detected in human liver and red blood cells and in a number of animal tissues, such as respiratory and olfactory epithelium in the rat.

FA disturbs the oxidant-antioxidant balance in various tissues and cause oxidative stress in parallel with tissue damage.Its administration caused significant increases in MDA (Malondialdehyde) (especially in lung, liver and testis) and PC (Protein carbonyl), which are products of lipid and protein oxidation, respectively. It also results in a decrease in the activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) ***(Songur et al., 2010)*** . Moreover, ***Ye et al., (2013)*** in their work following FA inhalation revealed that the greatest increase in ROS occurred in bone marrow and liver.

In the present study, the liver weight was statistically increased in the FA treated group when compared to the control groups. Also, there was a non-significant statistical difference between the control groups and FA & vit.C treated group. This result is in accordance with ***Liu et al., (2014)***, who gave a 20mg/kg intragastric daily dose of FA for only 5 days and reported compensatory hypertrophy in the liver organs .

Kum et al., (2007) reported that, FA inhalation alone has no statistical effect on the liver weight of the adult rats but combination of FA with xylene caused increase in the weight of the liver. The previous authors added that, the increase in liver weight might has been partly attributable to passive congestion, hepatic inflammation, hyperemia and edema.

In the present work, Hx&E stained sections in the treated group with a 10 mg/kg intraperitoneal dose of FA revealed disrubted normal liver architecture with cellular infiltration in the portal area and around the central vein. Furthermore, hepatocytes showed variable degrees of affection. Some hepatocytes showed hyperchromatic nuclei which is a degenerative change denoting apoptosis as proved by ***Taylor et al., (2008)***, while others had cytoplasmic vacuolizations. Mononuclear cellular infiltration around central vein , between liver cords and in the portal area was noticed . These results were in agreement with those of ***Pekmez et al., (2008)*** and ***Bakar et al., (2014)*** who gave the same dose intraperitoneally .

Treesh et al., (2014) who gave 30 mg/kg of FA intraperitoneally for 5 days, detected the same results in addition to some signs of fatty degeneration. Also, ***Cikmaz et al., (2010)*** reported the same results after exposure of rats to subacute and subchronic FA inhalation.

In the present work, in the FA treated group multiple necrotic foci were noticed in some hepatic lobules formed of a collection of debris cell and infiltration of lymphocytic cells. ***Edinger & Thompson, (2004)*** referred the presence of necrotic foci to lysis of cells and formation of cell debris , which initiate phagocytic infiltration .They were mostly located around blood vessels, that is in the liver around the central vein , these results are in concordant with those of ***Soni et al., (2013)*** who exposed rats to FA orally . Also necrotic foci were noticed beside the portal area and this result is in agreement with that of ***Uppal et al., (2012),*** who exposed rabbits to formalin 40% by inhalation .

Soni et al., (2013) explained that cell necrosis in liver of mice is based on the PARP-1 (Poly ADP-ribose polymerase-1) protein fragment which is responsible for DNA repair process. However, if the DNA damage is too severe, then the cell will not be able to repair

such damage and this case can lead to hyper activation of PARP-1 protein resulting in depletion of cellular NAD+ and ATP that direct cell death through the mechanism of necrosis ***(Moubarak et al., 2007 and Chaitanya et al., 2010)***

This group also showed dilated and congested central vein and blood sinusoids. ***Hu et al., (2013)*** confined these finding to portal hypertension. On the other hand, ***Rockey (2001)*** and ***Puche et al., (2013)*** attributed sinusoidal dilatation to activation of perisinusoidal cells that had contractile properties.

Bile duct proliferation was evident in the examination of the FA treated group. ***Alison et al., (2001), Roskams, (2003)*** and ***Richardson et al., (2007)*** reported that a ductular reaction is the regenerative proliferative response to many types of liver injuries in humans, which is seen as duct-like structures. Under the condition of severe and chronic liver injury caused by drugs, viruses, and toxins, hepatic stem cells proliferate and differentiate both into mature hepatocytes and into biliary epithelial cells. However ***Roskams et al., (2004)*** and ***Alvaro et al., (2007)*** reported that the cellular changes might be due to hepatic stem/ progenitor cell activation, proliferation of preexisting cholangiocytes, or ductular metaplasia of mature hepatocytes.

In the current study, in FA treated HX&E slides, the wall structure of some portal veins showed a tendency towards arterialization. ***Wen et al., (2015)*** attributed this result to smooth muscle cell (SMC) proliferation and hypertrophy in the portal vein that were observed using optical and electron microscopy. They indirectly proved that ET-1 (Endothelin-1) and NO (Nitric oxide) were closely related with portal venous vascular remodeling and that tumor necrosis factor and increased ET-1 synthesis will lead to SMC proliferation and hypertrophy, inducing vascular remodeling.

In the current work, Mallory's trichrome stained sections of FA treated group revealed a marked increase in collagen fibers in the portal tract and around central vein .These results are in concordant with those of **Treesh et al., (2014)** . **Galli et al., (2005)** stated that ROS are involved in the development of hepatic fibrosis as they induce hepatic stellate cells (Ito cells) proliferation and collagen synthesis.

Regarding morphometrical analysis of the number of hepatocytes with (iNOS) positive immunoreaction that were later confirmed statistically ,the FA treated group showed a highly significant statistical increase ($P<0.001$) as compared with that of the control group . This result is in accordance with ***Ucmakli et al., (2013)*** who gave the same dose intraperitoneally. On the other hand, ***Sögüt et al., (2004)*** who exposed rats to subacute FA inhalation, detected normal NO levels in the studied groups suggesting that overproduction of free radicals by NOS isoform had not been occurred .

Ucmakli et al., (2013) postulated that, the mechanism of the effect of FA on the expression of iNOS is not exactly clear. Assuming that this effect occurs by one of the two ways, the first is that FA or its metabolites act like a stimulator that induces iNOS protein synthesis directly. This is supported by ***Speit, (2006)*** who stated that as FA is a water-soluble molecule, it can easily diffuses into membranes and directly crossreacts with DNA-protein chains .However ***Ferrer et al.,(2010)*** pointed out that ROS mediates the induction of iNOS gene expression . The second way may be through cytokines and this theory is supported by ***Persoz et al., (2010)*** who reported that FA has a stimulating impact on cytokines, which affects iNOS metabolism . Several studies showed that cytokines such as TNF-α and IL-1β are effective on iNOS mRNA synthesis and iNOS activity or expression ***(Horie et al., 2009)***

Vitamin C (L-ascorbate) is a hydrophilic molecule, and, therefore, it is found mostly in body fluids. Ascorbate is critical for the biosynthesis of collagen and is able to scavenge superoxide anions, hydroxyl and peroxynitrite radicals ***(Birlouez-Aragon & Tessier, 2003 and Deruelle & Baron, 2008)*** .

In the current work, examination of the Hx&E liver stained sections of the rat group simultaneously treated with vit.C in accompany with FA showed variable degrees of improvement according to severity of changes observed in the FA treated group . These results are in agreement with that of ***Abdulqader & Mustafa , (2014)*** .

Moreover, binucleated hepatocytes were seen may be due to the regenerative attempt of the cells as reported earlier in the liver of carbaryl treated rats by ***Munglang et al., (2009).*** Also, in this group's Mallory stained sections , the liver contains few collagen fibers around central vein and in the portal area .

Concerning iNOS immune-histochemical assay, a highly statistical significant decrease (p. value<0. 001) in FA&vit.C treated group was observed when compared to that of FA and a significant statistical difference ($p<0.05$) when compared to the control groups. This coincides with the results of ***Zhuroms'kyi & Skliarov, (2011)*** who revealed that vit. C displayed a pronounced antioxidant action diminishing the activity of iNOS**.**

Adikwu & Deo, (2013) attributed the hepatoprotective effect of vitamin C to its antioxidant property. It potentiates the activities of free radical scavengers, SOD (Superoxide dismutase), CAT (Chloramphenicol acetyltransferase) and GSH-Px (Plasma glutathione peroxidase), thereby preventing microsomal lipid peroxidation, liver fibrosis, liver necrosis and hepatic inflammation . Vitamin C is an important free radical scavenger, thus it can protect biomembranes from peroxide damage. On the other hand, **Kuo, (2013)** reported that the only well-established antioxidant role of vitamin C is its reduction of oxidized vitamin E .

CONCLUSIONS

Intraperitoneal administration of formaldehyde at a dose of 10 mg/kg/day produced hepatotoxic effects in the adult male albino rat. These effects were evidenced by the serological, histopathological and immune-histochemical changes. Vit.C could provide partial protection against those toxic effects.

RECOMMONDATIONS

Much more attention should be paid for limiting the occupational and environmental exposure to formaldehyde. Special precautions must be taken to limit the level of the environmental, water and food contamination by formaldehyde and many alternatives should be developed to improve the safety profile of formaldehyde. Moreover, highly exposed individuals advised to take vit.C supplementation to limit the toxic effects of formaldehyde on the liver.

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تأثير الفورمالدهيد على الكبد فى ذكور الجرذان البيضاء البالغة و احتمالية الدور الوقائى لفيتامين سى

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يوجد الفورمالدهيد فى العديد من الادوية، المواد الصناعية والعديد من الخضروات ،الفاكهة والماكولات البحرية التى يتم حفظها فى الفورمالين . أما فيما يتعلق بفيتامين سى ،فهو أحد الفيتامينات القابلة للذوبان في الماء و له القدرة على التخلص من الشقائق الحرة و حماية الاحماض الامينية والبروتينات من الضرر التأكسدى .

كان الهدف من هذا البحث هو تحديد التغيرات التى قد تحدث فى الكبد نتيجة التعرض لمادة الفورمالدهيد وكذلك امكانية الوقاية من هذه الآثار الضارة بإستخدام فيتامين سى . أستخدم فى هذه الدراسة خمسة واربعون من ذكور الجرذان البيضاء البالغة التى تم تقسيمها الى ثلاث محموعات رئيسية .المجموعة الاولى تم اعتبارها كمجموعة ضابطة وتم تقسيمها الى ثلاثة مجموعات فرعية كل مجموعة منها تحتوى على ٩ فئران(واحدة مجموعة ضابطة سلبية لم تعطى اى علاج ، و اخرى مجموعة ضابطة موجبة تلقت (١ مل) من الماء المقطر يوميا عن طريق الحقن الى داخل الغشاء البريتونى لمدة ١٠ ايام و اما الاخيرة مجموعة ضابطة موجبة تم اعطائها فيتامين سى يوميا عن طريق الفم بجرعة مقدارها ١٠٠ مجم/ كجم لمدة ١٠ ايام) ،المجموعةالثانية التى احتوت على ٩ فئران تم إعطائهم ١٠ مجم /كجم من مادة الفورمالدهيد عن طريف الحقن الى داخل الغشاء البريتونى يوميا لمدة ١٠ ايام والمجموعة الثالثة و التى تألفت ايضا من ٩ فئران تم حقنهم يوميا ب١٠مجم/كجم من مادة الفورمالدهيد وبالتزامن اعطائهم فيتامين سى (١٠٠مجم/كجم)عن طريق الفم يوميا لمدة ١٠ ايام . فى اعقاب انتهاء العشرة ايام تم سحب عينات من دم الفئران لفحص مستوى الانزيمات الكبدية و الألبيومين .ثم تم تخدير الفئران و استخراج الكبد لوزنه . و تم أخذ عينات من الكبد واعدادها للفحص الهيستوباثولوجي ،الهيستوكيميائي المناعى والدراسة المورفومترية .

اظهرت الدراسة زيادة ملحوظة فى انزيمات الكبد و زيادة كبيرة فى اوزان اكباد الفئران فى المجموعة المعالجة بالفورمالدهيد و التى أوضحت نقص ملحوظ فى المجوعة المعالجة بفيتامين سى بالتزامن مع الفورمالدهيد جعلها تقريبا طبيعية مقارنة بالمجموعات الضابطة ، : كما اتضح من نتائج هذه الدراسة أن الفورمالدهيد يحدث تغيرات هستولوجية واضحة فى نسيج الكبد فى ذكور الجرذان البيضاء البالغة تمثلت فى وجود اتساع و احتقان فى الشعيرات الدموية و الاوردة المركزية وفى المنطقة البابية ،زيادة سمك الوريد فى المنطقة البابية ونخر فى انسجة الكبد بالاضافة الى زيادة فى الألياف الغروية حول الوريد المركزي وفى المنطقة البابية . و فيما يتعلق بالصبغة الهستوكيميائية المناعية للنيتريك اوكسيد سينسيز المحرض فقد أظهرت تفاعلا إيجابيا داخل سيتوبلازم الخلايا الكبدية فى المجموعة المعالجة بالفورمالدهيد . و على الجانب الاخر وجد أن إعطاء فيتامين سى بالتزامن مع الفورمالدهيد بإمكانه الحد من هذه التغيرات النسيجية الضارة على مستوى المجهر الضوئى.لذلك نوصى بإعطاء فيتامين سى فى حالات التعرض المتكرر للفورمالدهيد للحد من تاثيره الضار على الكبد.