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Hematological and immunohistochemical changes in testes associated with exposure to the formaldehyde Vapor in male rats

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Abstract

Aims: the present study was performed to investigate the hematological and immunohistopathological changes in the testes of experimental rats after exposure to formaldehyde. **Method:** Twenty-four Albano Waster male rats weighing (150-170g) were divided into four groups (6 rats for each group). The control group was not exposed to the formaldehyde vapor. In group two the rats were exposed directly to formaldehyde vapor with a mean concentration of 1.5 ppm (1 hour daily) for 20 days in a special chamber. In group three the rats were exposed directly to formaldehyde vapor with a mean concentration of 1.5 ppm (2 hours daily) for 20 days in a special chamber. In group four the rats were exposed directly to formaldehyde vapor with a mean concentration of 1.5 ppm (3 hours daily) for 20 days in a special chamber. After the experiment ended, blood samples were collected from the rats for hematological parameters. Testes organs were immediately removed from experimental rats for immunohistochemical examination and KI67 immunoreactivity was performed using an Avidin-Biotin-Peroxidase (ABP) immunohistochemical method with KI67 monoclonal antibody (dilution 1:100; DAKO Japan Co, Tokyo, Japan). **Results**: Exposed groups showed increased testes weight, decreased red blood cell parameters and platelets, and increased white blood cells. Ki-67 immunoreactivity decreased with exposure duration, along with structural damage to seminiferous tubules and inflammatory cell accumulation in longer exposures.

Conclusion: Formaldehyde vapor exposure, even at low doses, can cause hematotoxicity and detrimental effects on testicular tissue structure and cell proliferation.

Keywords: Formaldehyde vapor, Hematological parameters, Ki-67-ir immunohistochemistry, Testes tissue.

Introduction

Formalin is an aqueous solution of 37-40% of formaldehyde. The extensive use of formalin in fruit, vegetables, fish, meat, and milk for long-term preservation creates a serious hazard to public health and nutrition at an alarming rate. In the manufacturing, clothing, furniture, plastics, medicinal, cosmetic, and pharmaceutical industries, formalin is frequently used and has a significant impact on regular users **(1,2)**.

Formaldehyde is an organic carbon compound commonly employed for embalming cadavers, preservation of biological specimens, and as a disinfectant. Apart from being an occupational hazard, it is also a common source found indoors from unvented fuel-burning appliances, kerosene heaters, baby shampoos, cosmetics, and pressed wood products **(3)**.

Formaldehyde induces cytotoxicity in air passages, skin, liver, and various other organs. The exposure to

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this compound through inhalation affects a wide range of populations including medical professionals, laboratory technicians, funeral home employees, students exposed to cadavers, cosmetologists, and industrial workers **(4).**

Formaldehyde toxicity has been reported in multiple tissues or organs (liver, heart, brain, lung, and gonads) and also can induce inflammation of the lining of the mouth, throat, and gastrointestinal tract, subsequent ulceration, and necrosis of the mucous lining of the gastrointestinal tract, causing parenchymatic organ lesions, cancer, and leukemia in the eye and upper respiratory tract **(5).**

Formaldehyde toxicity can cause various histopathological changes that indicate the damage of the liver tissue, focal hepatic necrosis, hepatic enlargement, decreased weight, and degeneration of hepatocellular fatty that is related to the length of the period of exposure, also leads to kidney disease, cancer, and respiratory disease **(6).**

Gnanadeepam *et al* **(7)** reported that the role of formaldehyde as a potential toxin exerts adverse outcomes, especially on the male reproductive system and the possible mechanism underlying the reproductive toxicity of formaldehyde is that the compound crosses the blood testes barrier and inflicts injury by increasing the reactive oxygen species thereby depleting the cellular glutathione levels and inducing oxidative stress.

Golalipour *et al* **(8)** showed that formaldehyde exposure can cause a severe decrease in germ cells, spermatogenesis arrest, and a thickening of the basement membrane of seminiferous tubules in the testis of rats. Intraperitoneal injection of formaldehyde can disrupt the Leydig cells and spermatogenesis arrest at dosages of 5, 10, and 15 mg/kg in rats **(9).**

Oxidative stress factors play a critical role in the pathogenesis of male reproductive toxicity and produce a high level of active free radicals and reactive oxygen species (ROS) and thus may lead to spermatogenesis dysfunction or even to sperm death **(10,11).**

The testicular tissue is highly susceptible to oxidative stress because testicular membranes are highly rich in poly-unsaturated fatty acid and the oxidative damage to this poly-unsaturated fatty acid of the cell membrane leads to impairment of membrane fluidity and permeability, presence of rich polyunsaturated fatty acids in the mitochondrial membrane of sperm and low amounts of antioxidants makes sperm more susceptible to lipid peroxidation **(12,13).**

The present study was performed to investigate the hematological and immunohistopathological changes in the testes of experimental rats after exposure to formaldehyde.

Materials and methods

The present study was carried out at the Environmental Toxicology Laboratory, Department of Environmental Studies, Institute of Graduate Studies and Research, Alexandria University, Alexandria, Egypt.

Experimental animals: A total number of twenty-four Albano Waster male rats weighing (150-170g) were obtained from the animal house of Faculty Medicine, Alexandria University. Animals were handled following the principles of laboratory animal care as contained in the NIH Guide for Laboratory Animal Welfare and the experimental protocol was approved by the Local Ethics Committee and Animals Research. The rats were housed in stainless steel bottomed wire cages after grouping into four groups (6 rats in each cage) and maintained at a temperature of 22 ± 2°C, relative humidity of 40-60%, with a 12 h/12 h light/dark cycle and allowed free access to food and water, after one week the rats assigned to four groups (six rats for each group) and treated as following experimental protocol:

- Group I (control): Control rats were fed a basal diet given tap water and not exposed to formaldehyde vapor.
- Group II (1-hour exposure): Rats were fed a basal diet given tap water and exposed directly to the vapor of 10% formaldehyde with a mean concentration of 1.5 ppm for a period (of 20

days) for (1 hour daily) in a special chamber made designed according to the Gnanadeepam *et al* (7) method and modified with openings for light and ventilation at different levels.

- Group III (2 hours exposure): Rats were fed a basal diet given tap water and exposed directly to the vapor of 10% formaldehyde with a mean concentration of 1.5 ppm for a period (20 days) for (2 hours daily) in a special chamber made designed according to the Gnanadeepam *et al* (7) method and modified with openings for light and ventilation at different levels.

- Group IV (3 hours exposure): Rats were fed a basal diet given tap water and exposed directly to the vapor of 10% formaldehyde with a mean concentration of 1.5 ppm for a period (20 days) for (3 hours daily) in a special chamber made designed according to the Gnanadeepam *et al* (7) method and modified with openings for light and ventilation at different levels.

After the end of the time of exposure, the rats of each group were transported to another cage and kept in the laboratory animal quarters far from the exposure rooms with normal ventilation and normal temperature.

At the end of 20 days of the experimental period, the rats were starved overnight, euthanized with the administration of a dose of chloroform and cessation of respiration then dissected, blood samples taken from inferior vena cava and collected in clean class tubes with EDTA anticoagulant. Complete blood pictures (CBC) were shown from collected blood samples by automatic method (Celltac X kx 021n automated hematology analyzer, Japan CARE Co, Ltd.), which included hemoglobin (Hb), Red blood cells (RBCs), White blood cells (WBCs), Platelet and Packed cell volume (PCV). Testes organs were immediately removed from experimental rats for immunohistochemical examination and put into 10% neutral buffer formalin as a fixative solution. Fixation time was limited to 24 hours and the fixed tissues were stored in 70% ethyl alcohol until they were processed. The fixed tissues were dehydrated through a graded series of ethanol and embedded in paraffin according to standard procedures **(14).**

KI67 immunoreactivity was performed according to Tousson *et al* **(15).** Kidney KI67 distribution of receptor subunits was examined in deparaffinized sections (5µm) using an Avidin-Biotin- Peroxidase (ABP) immunohistochemical method (Elite–ABC, Vector Laboratories, CA, USA) with KI67 monoclonal antibody (dilution 1:100; DAKO Japan Co, Tokyo, Japan).

Statistical Analysis:

Study results were expressed as mean ± standard error (SE). Statistical analyses were performed with one-way analysis of variance (ANOVA) using SPSS 17. Group mean compared for significance in($P<0.05$).

Results

The results observed a significant increase in the weight of the testes in all the groups that were exposed directly to the formaldehyde vapor with an increase in the time of exposure as compared with the control group in ($P< 0.05$), figs (1), grossly both right and left testes were seen massive swelling with congested and enlarged of the blood vessels and some of the patchy hemorrhage, fig (2).

The results of this study observed a significant decrease in Red blood cells (RBCs), Hemoglobin concentration(Hb), Hematocrit (HCT), Mean corpuscular volume (MCV)and Mean corpuscular hemoglobin concentration(MCHC), figs(3,4,5,6,7), also significant decrease in platelet accounts (Plt) in all groups after exposure to the formaldehyde vapor in different times as compared with control group, fig (8).

Formaldehyde vapor exposure observed a significant effect by increasing the total white blood cells (WBCs)most commonly in period times (2,3 hours) of exposure as compared with the control group, fig (9).

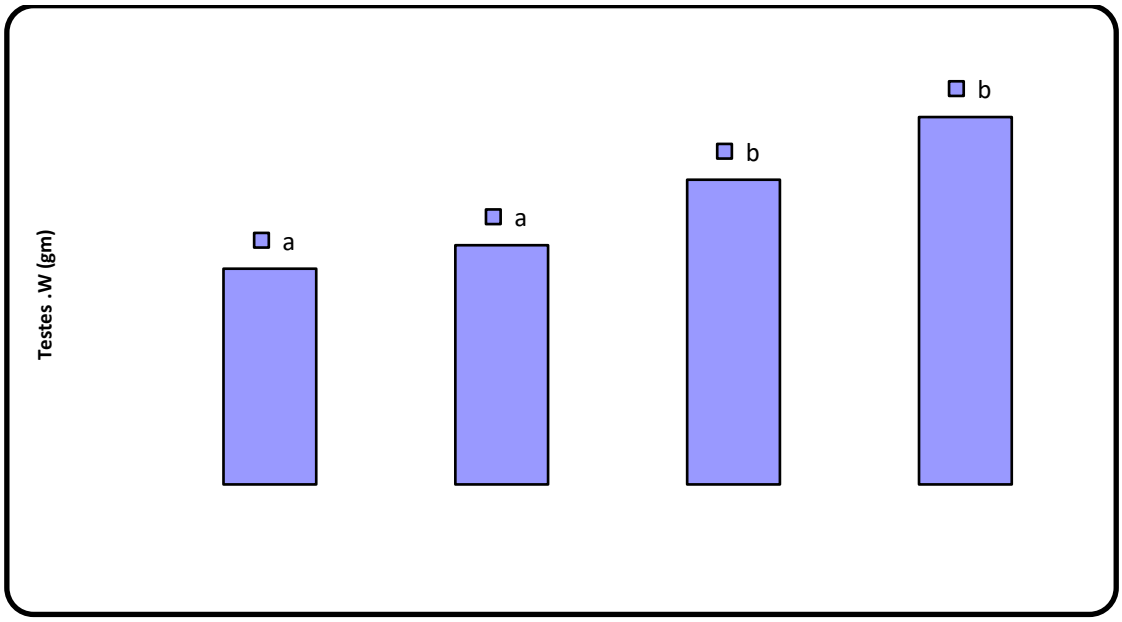

Fig (1): Changes in testis weights in male rats after exposure to formaldehyde vapor at different times. Values are expressed as means±SD of per group. Means with different letters are significantly different (P<0.05).

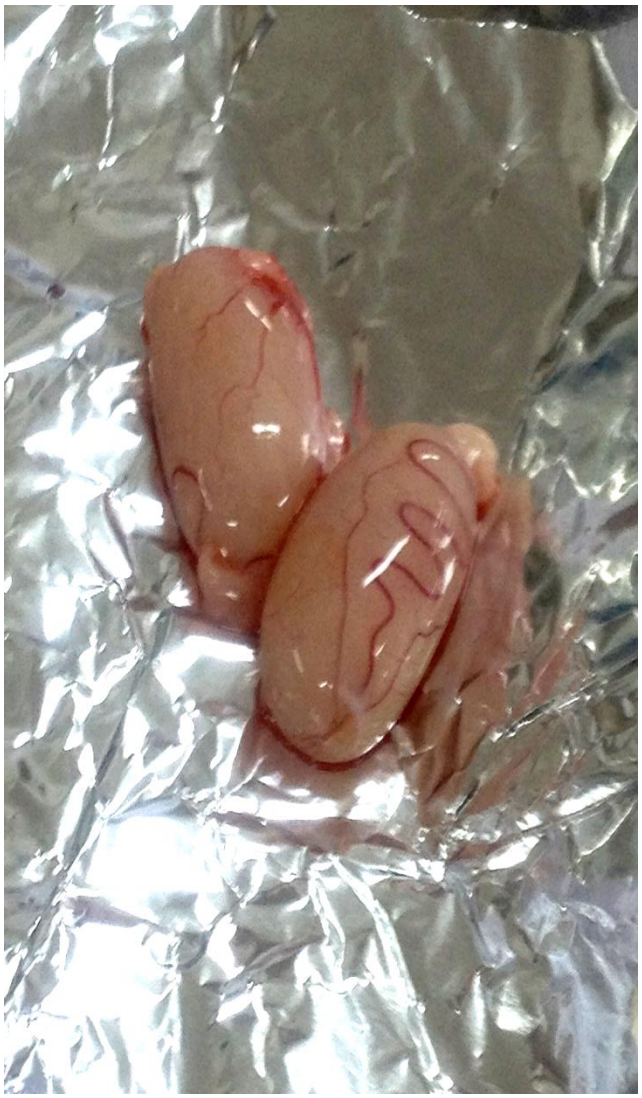

Fig (2): Showing cross-section of testes for the male rats after exposure to formaldehyde vapor at different times, observed massive swelling, congested with enlarged blood vessels.

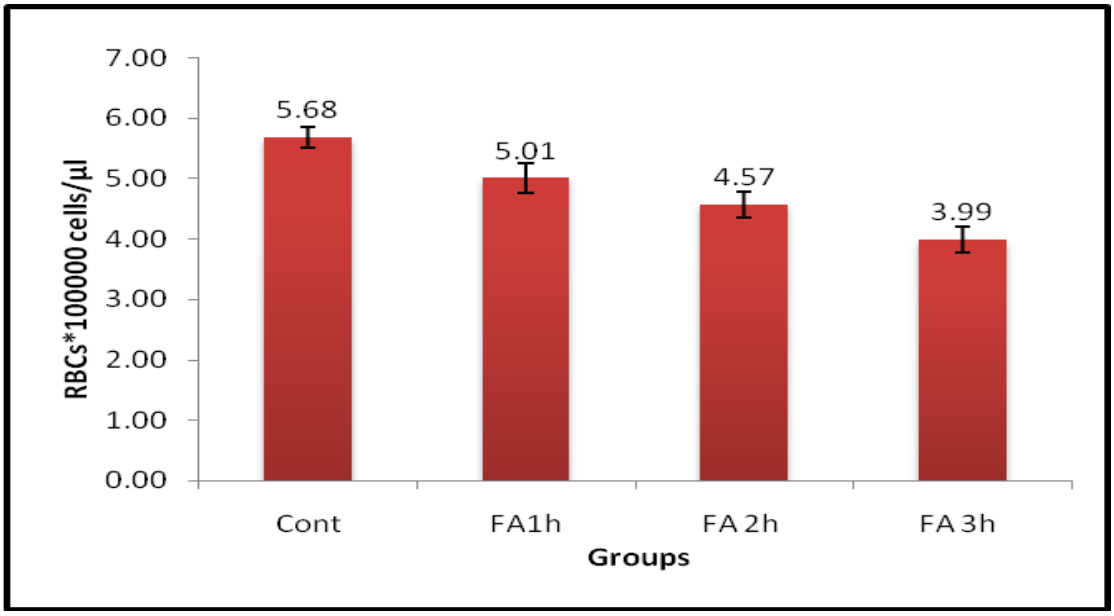

Fig (3): Changes in Hemoglobin concentration (Hb) in male rats after exposure to Formaldehyde Vapor at different times. Values are expressed as means±SD of per group. *FA: Formaldehyde

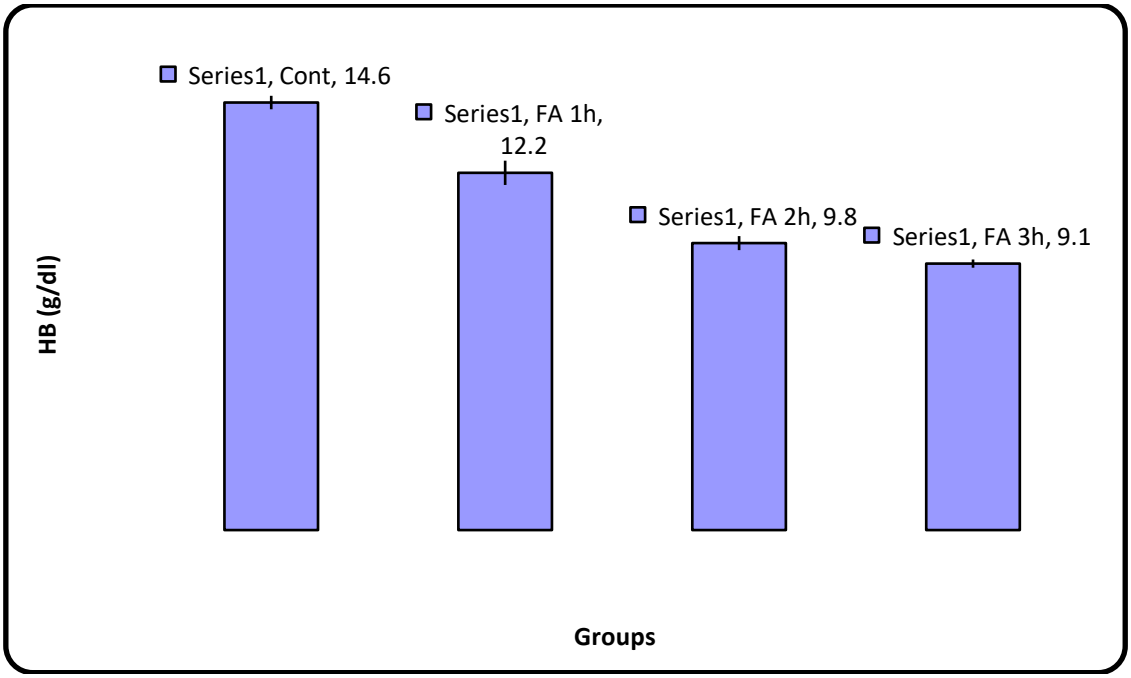

Fig (4): Changes in Hemoglobin concentration (Hb) in male rats after exposure to formaldehyde vapor at different times. Values are expressed as means±SD per group. *FA: Formaldehyde

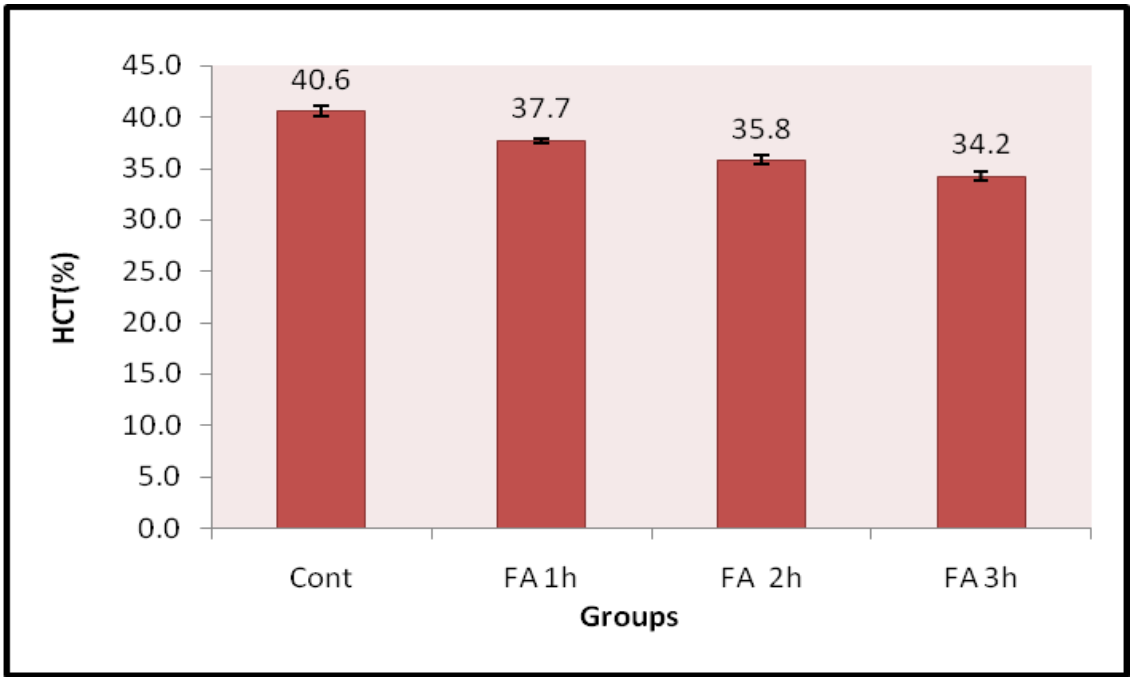

Fig (5): Changes in percentage of Packed cells volume (HCT) percentage in male rats after exposure to formaldehyde vapor at different times. Values are expressed as means±SD per group. *FA: Formaldehyde

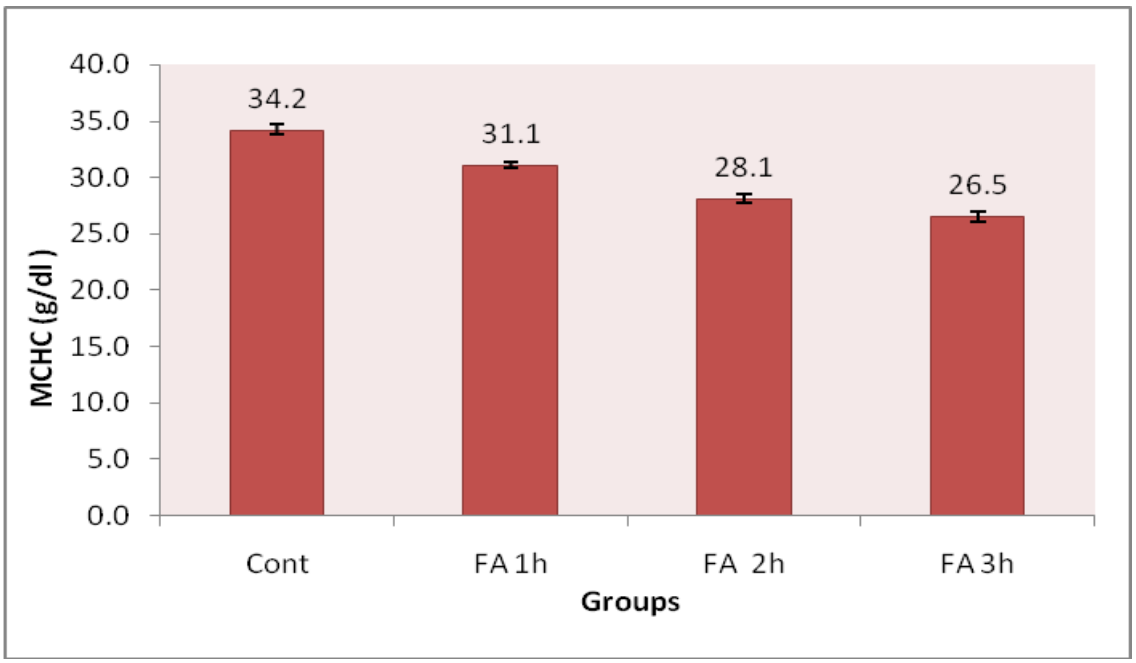

Fig (6): Changes in (MCHC) level in male rats after exposure to formaldehyde vapor at different times. Values are expressed as means±SD per group. *FA: Formaldehyde

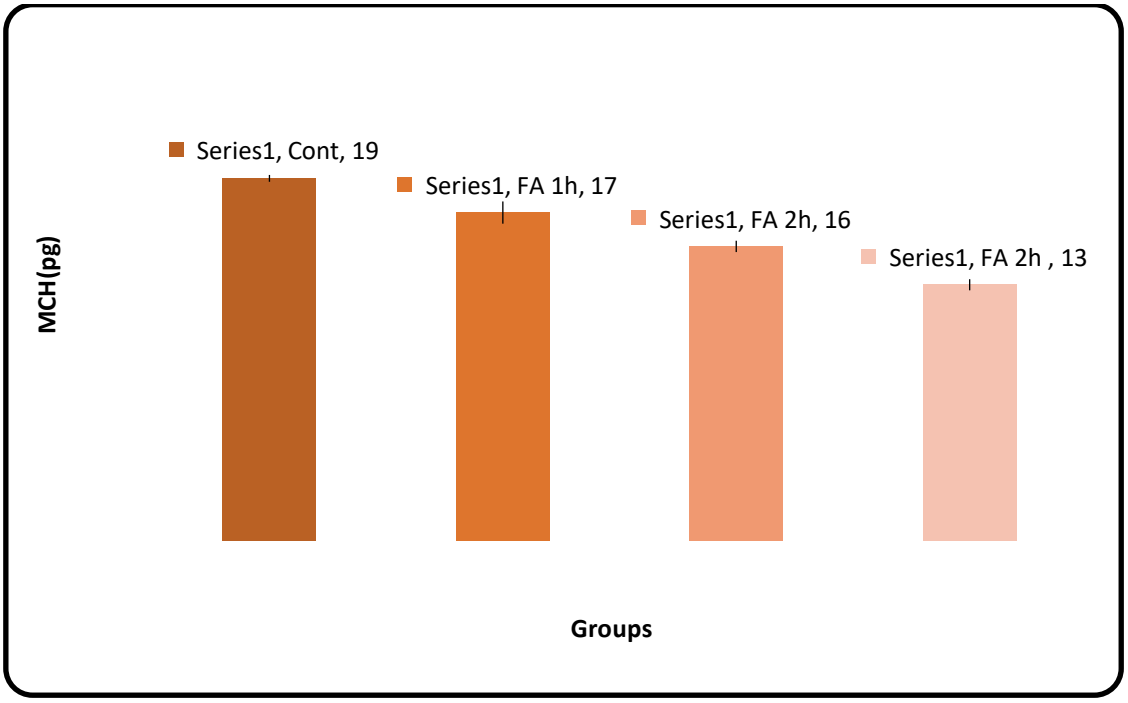

Fig (7): Changes in (MCH) level in male rats after exposure to formaldehyde vapor at different times. Values are expressed as means±SD per group. *FA: Formaldehyde

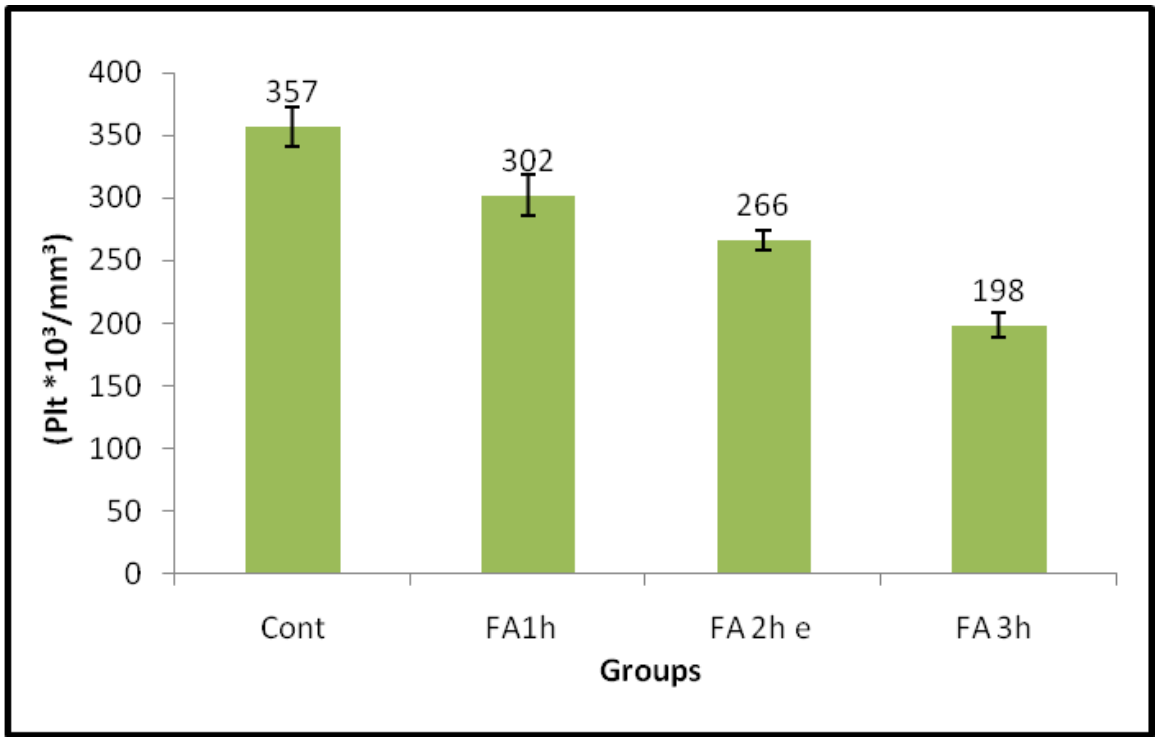

Fig (8): Changes in Platelet (plt) count in male rats after exposure to formaldehyde vapor at different times. Values are expressed as means±SD per group. *FA: Formaldehyde

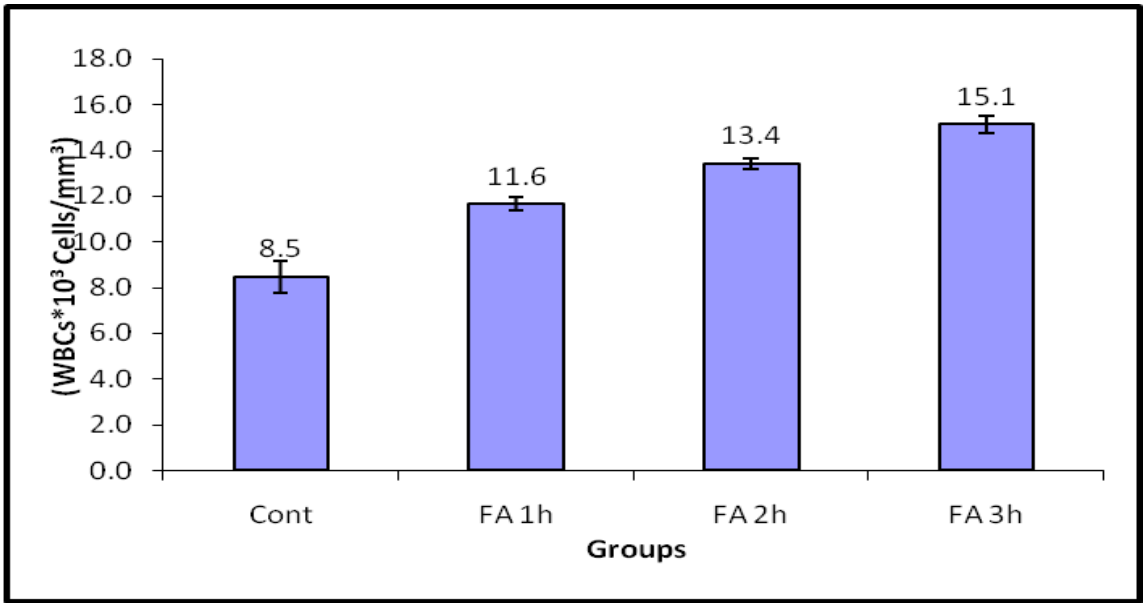

Fig (9): Changes in White blood cells (WBCs) level in male rats after exposure to formaldehyde vapor. Values are expressed as means±SD per group. *FA: Formaldehyde

The present study observed the effect of Ki-67 immunoreactivity (Ki-67-ir) in testes tissue sections in the different groups under study. The spermatogonia and Sertoli cells in the control group showed a strong positive reaction for Ki-67-ir stain, while the sperms showed a negative reaction, and there are normal and regular cycles of spermatogenesis with normal sertoil and lydig cells, (fig 10).

Detection of Ki-67 immunoreactivity (Ki-67-ir) in testes tissue sections for the rats' exposure to the formaldehyde vapor for 1 hour daily for 20 days showed mild to moderate positive reaction for Ki-67-ir in spermatogonia and Sertoli cells, with an increase the space between the germ cells in some somniferous tubules, also disruption in the association between Sertoli cells and germinal cells, and accumulation of lymphocytes in connective tissues around the somniferous tubules fig(11).

Photomicrograph of the testes tissue sections for the rats after exposure to the formaldehyde vapor for(2 and 3 hours) showed a faint positive reaction with (Ki-67-ir) in the spermatogonia and sertoil cells, while the lydig cells observed moderate to strong positive reaction with (Ki-67-ir) with increase the space between the germ cells in some somniferous tubules, damage somniferous tubules and necrosis of spermatogonia cells and observed abnormal accumulation of inflammatory cells around the lamina properia, arrest in the spermatogenesis process and reduction the number of sperms in the lumen, figs (12,13).

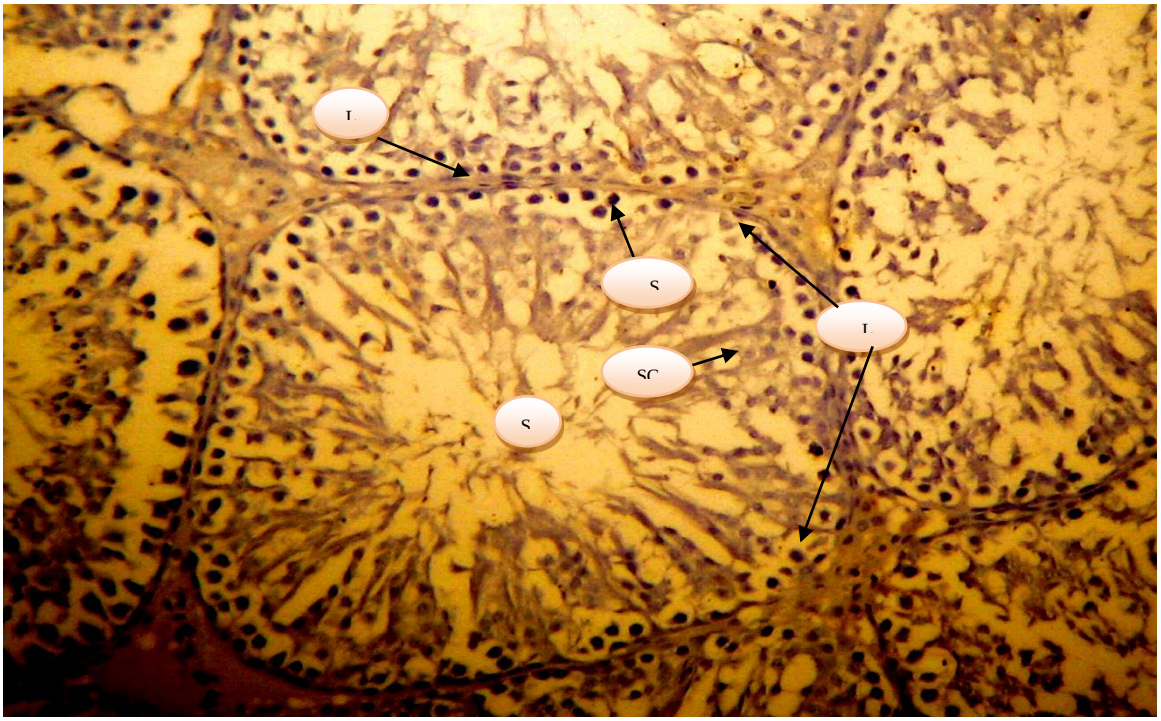

Figure (10): High power micrograph of rat testes tissue section in the control group stained with (Ki-67-ir) revealed strong Positive reactions in spermatogonia, sertoil, and lydig cells. Lamina properia(LP),Laydig cells(LC),Spermatogonia(SG),Sertoil cells(SC),Sperms(S).

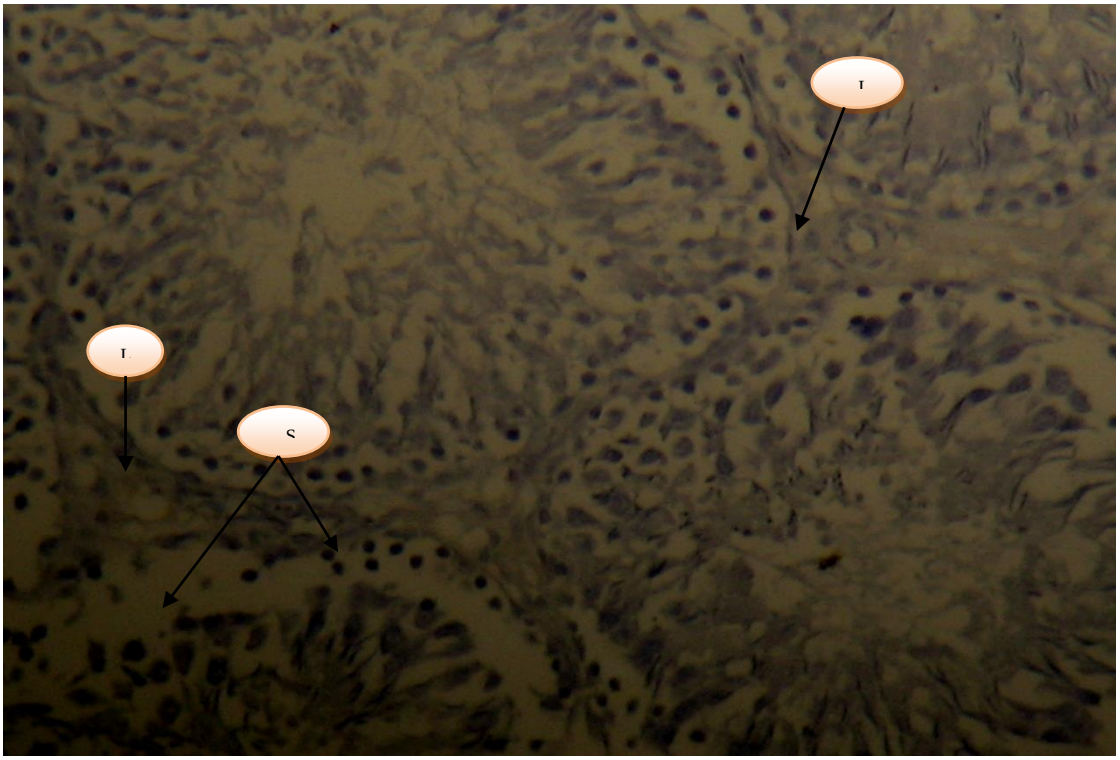

Figure (11): High power micrograph of rat testes tissue section after exposure to formaldehyde vapor (1 hour) and stained with (Ki-67-ir) revealed mild to moderate Positive reactions in spermatogonia, sertoil, and lydig cells.Lamina properia(LP), Laydig cells(LC), Spermatogonia(SG).

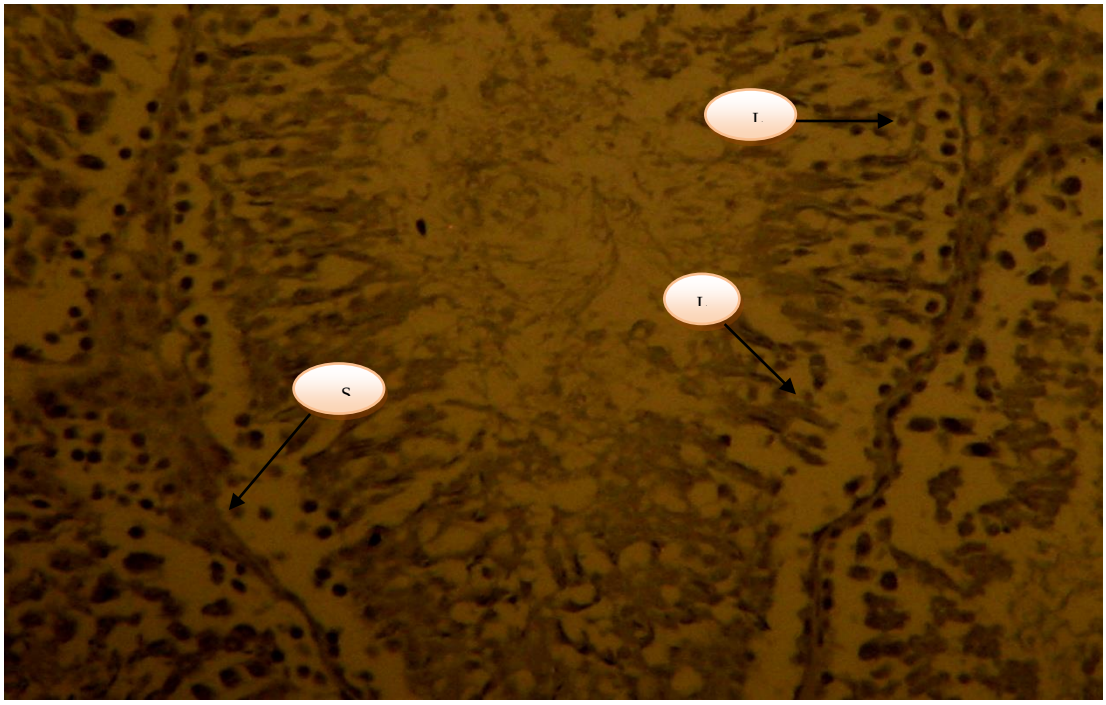

Figure (12): High power micrograph of rat testes tissue section after exposure to formaldehyde vapor (2 hours) and stained with (Ki-67-ir) revealed mild to faint Positive reactions in spermatogonia, sertoil, and lydig cells. Lamina properia(LP), Laydig cells(LC), Spermatogonia(SG).

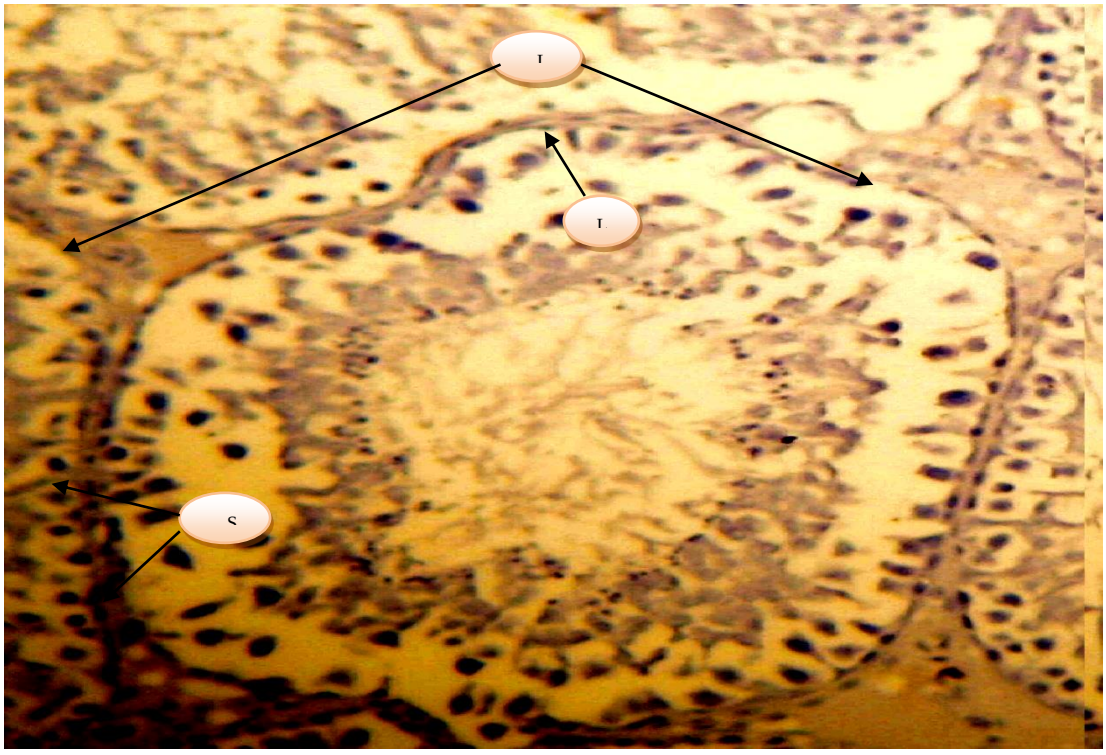

Figure (12): High power micrograph of rat testes tissue section after exposure to formaldehyde vapor (3 hours) and stained with (Ki-67-ir) revealed faint Positive reactions in spermatogonia, sertoil, and lydig cells. Lamina properia(LP), Laydig cells(LC), Spermatogonia(SG).

Discussion

The harmful effect of formaldehyde increases under conditions at room temperature and also becomes more toxic and slightly carcinogenic in high concentrations **(16).** The results showed an increase in the weight of the testes in all groups that were exposed directly to the formaldehyde vapor with an increase in the time of exposure and both right and left testes were seen massive swelling with congested and enlarged blood vessels and some of patchy hemorrhage.

Gololipour *et al* **(8)** reported that formaldehyde exposure can cause increases in reactive oxygen species (ROS) which leads to oxidative damage to the testis tissue and a decrease in the testicular antioxidant with increased lipid peroxidation which interferes with protein biosynthesis and DNA damage with cellular injury, also found the mass of the testes significantly higher in 15mg/kg in the treatment group with formaldehyde as compared with the control group.

Chowdhury *et al* **(9)** reported a decrease in testicular weights in rats after being subjected to intraperitoneal injection of formaldehyde (15mg/kg) daily for 60 days, while Zahra *et al* **(17)** found no significant difference in the weights of testes after administration intraperitonial (10mg/kg) formaldehyde for 40 days

The present study observed a significant decrease in red blood cells, hemoglobin concentration, hematocrit, MCV, and MCHC concentration and a significant decrease in platelets accounts after exposure to the formaldehyde vapor at different times (1,2,3 hours) for (20 days). These results agreed with Al-Saeed *et al* **(18)** who found a significant decrease in total blood picture after one week and two weeks of exposure to formaldehyde which attributed to inflammation in internal organs and led to anemia.

Several previous studies observed the hemotoxicity of formaldehyde which can alter the different types of blood cells, where the high doses of formaldehyde can increase the monocytes and decrease red blood cells and hemoglobin concentration.

Ki-67 immunoreactivity in the testes tissue sections in this study observed a strong positive reaction for Ki-67 stain in spermatogonia and sertoil cells in the control group, moderate positive reaction for the testes tissue sections after exposure to the formaldehyde vapor (1 hour)daily for 20 days with an increase in space between the germ cells in some seminiferous tubules, while the results observed faint positive reaction with Ki-67 stain in spermatogonia and sertoil cells with moderate to strong positive reaction in the lydig cells and accumulation of inflammatory cells around the lamina properia in the testes tissue sections after exposure to the formaldehyde vapor for (2-3 hours **(15).**

Gerdes *et al* **(19)** reported that Ki-67 Antigen can be identified by immune stain with a monoclonal antibody in all phases of cell proliferating, where the Ki-67 score or index represented the percentage of positively stained cells among the total number of the cells in the tissues. The proliferation-associated antigen Ki-67 is expressed in the nuclear matrix of cells during the late G1-, S-, G2-, and M phases of the cell cycle with a maximum in G2- and early M phases. The Ki-67 protein is absent in resting cells G0-phase of the cell cycle or when the cells are during the early G1-phase and DNA repair processes **(20).**

Guzman *et al* **(21)** found that the Ki-67 protein is present during all active phases of the cell cycle but is absent from resting cells making it an excellent marker for determining the growth of cell populations. The monoclonal antibody of Ki-67 has been developed and used in evaluating cellular proliferation rates of malignant tumors and evaluating multistage of the tumor in the tissue, where its presence during all active phases of the cell cycle other than in resting cells makes it an excellent marker for neoplasia **(22,23).**

Golalipour *et al* **(8)** found that exposure to the formaldehyde vapor for 2 hours /day for two days in the week caused abnormal changes in the testes tissues and disrupted the association between sertoil and germinal cells with increased space between the germ cells. Gnanadeepam *et al* **(7)** found adverse impacts with an increase in the time of exposure to the formaldehyde solution.

The study performed by Zhou *et al* **(24)** found exposure of the experimental animal to the formaldehyde vapor (10mg /M3) for two weeks caused severe atrophy of somniferous tubules with a decrease in the number of spermatogenic cells.

Formaldehyde administration in dose (200mg/kg) for 10 days caused degeneration and spermatogenesis arrest in the testes, and prolonged exposure to the formaldehyde can lead to focal hyperplasia of interstitial cells of lydig due to cessation of spermatogenic activity and tubular atrophy **(25).**

Gnanadeepam *et al* **(7)** showed that exposure to formaldehyde even in minimal doses harms the testes such as focal tubular necrosis with peritubular fibrosis which leads to the arrest of spermatogenesis.

In conclusion, the results observed exposure to the formaldehyde vapor even in low doses may be attributed to haemotoxic and cause dangerous effects on the hematological parameters. Ki-67 immunoreactivity in the testes tissue sections observed moderate positive reaction for the testes tissue sections after exposure to the formaldehyde vapor (1 hour)and a faint positive reaction with Ki-67 stain in spermatogonia and sertoil cells with faint positive reaction in the spermatogonia and sertoil cells to moderate or strong positive reaction in the lydig cells after exposure to the formaldehyde vapor for (2-3 hours) daily for 20 days with an increase in space between the germ cells in some somniferous tubules and accumulation of inflammatory cells around the lamina properia.

Conflict OF Interests

The authors declare that they have no conflicts of interest.

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