

## The Effect Of Formaldehyde On The Lymphoid Tissues Of Rats ( Quantitative And Histological Study)

**Ayad Al – Saraj\*; Elias Shaya\*\* And Maha Al Sammak\*\***

*\*Department of Dental Basic Sciences, College of Dentistry , Mosul University Mosul ,  
Iraq \*\*Department of Anatomy, college of medicine, Mosul University- Mosul, Iraq*

### **Abstract**

This study is concerned with the effect of formaldehyde on the lymphoid system by quantitative and histological analysis. For this purpose 4 groups , each of 8 male rats were examined , group I lefts as a control , while group II,III and IV had given oral administration of 10% formaldehyde (10 mg /kg body weight /day ) for 30, 60 and 90 days respectively . Two lymphoid organs , spleen and iliac lymph node were examined . The results showed significantly decreased of body weight during long period of formaldehyde exposure . In general a significant decrease were observed in organ weight , standardized weight , total white cell count , cell density and differential white cell count in the treated groups and especially during long period of exposure. Histologically, a significant decrease was observed in the number and the percentage of while pulp of spleen, and in the number of nodules and the percentage of the diameter of the cortex of the iliac lymph node. These results showed that the formaldehyde caused a marked involution of the lymphoid system and this might be due to decreasing in cell-mediated immunity and humoral immunity leading to immunosuppression.

**Key words :** Formaldehyde, Lymphoid tissues, Rats

### **Introduction:**

Formaldehyde is well known as a preservative , a sterilizer and embalming fluids. It is primary use in foam insulations , as adhesives in the production of particle board and plywood as well as in treating of textiles (1). Formaldehyde solution was used for irrigating the cavity of the hydate cyst to destroy scolices during surgical removal of the cyst . It has been also used for the inactivation of viruses in vaccine production (2).

There are 2.1 million workers are exposed to formaldehyde and the occupational health hazards are primarily due to its toxic effects after inhalation , direct contact and after ingestion .(3) The concentration of formaldehyde that is immediately dangerous to life health is 100 PPM and can cause death from throat swelling or from chemical burns to

lungs (4) Gastrointestinal toxicity after ingestion is most sever in the stomach and cause nausea , vomiting , sever abdominal pain , convulsions , loss of consciousness and circulatory failure (5) Occupational exposure

to formaldehyde was associated with moderate increase of pancreatic cancer (6) Formaldehyde has been classified as neurotoxic because the excessive and repeated airborne exposure to formaldehyde lead to impaired CNS –functions such as fatigue , somnolence and irritability .(7,8).

From the above introduction which indicates that exposure to formaldehyde has a serious health effects and is highly suspected to be toxic and human carcinogen, so question is raised about workers routinely exposed to formaldehyde as anatomists, embalmers, pathologists and industrial workers who had been occupationally exposed to formaldehyde – For this purpose, and because no one studied the affect of formaldehyde on the lymphoid system, the present study is designed to know the effect of formaldehyde on the lymphoid system . by quantitative and histological analysis.

### **Materials and Methods:**

Plan of the experiment :-

For this experiment thirty – two albino male rats *Rattus norvegicus* aged between 120- 130 days were isolated and maintained

on normal diet. All animals were examined daily for any clinical manifestations or apparent disease to exclude a pathological involvement of lymphoid tissues. The animals were placed in four groups each of eight animals according to their administration of formaldehyde as follows:-

Group I (control): rats were left without administration.

Group II: Rats had been given daily oral administration of 10% formaldehyde (10mg/kg body weight/day for 30 days).

Group III: Rat had been given daily oral administration of 10% formaldehyde (10mg/kg body weight/day for 60 days).

Group IV: Rat had been given daily oral administration of 10% formaldehyde (10mg/kg body weight/day for 90 days).

Removal of tissues:-

Each animal was anaesthetized with chloroform and the total weight recorded in grams. The abdominal cavity of each animal was opened through a median incision made through the whole length of the abdomen. The spleen was exposed and removed by cutting through the mesentery along the greater curvature of the stomach. The iliac lymph nodes usually three to four in number situated at the bifurcation of the great vessels were exposed and two nodes were removed.

Weighing of the tissues:

Each tissue was blotted dry on a filter paper and cleaned of fat. Then each tissue was weighed using digital microbalance and the total weight recorded in grams, then expressed per one hundred grams animal weight.

Preparation of cell suspensions:

Portion of spleen and one iliac lymph node were taken and weighed, then each placed in a petri dish containing fresh media. The remaining portion of spleen and the other lymph node were fixed in 10% formaldehyde for histological preparation. Each portion of the tissue in the culture media was teased out using a pair of iris forceps and fine scissors. After settling the supernatant fluid was pipetted into a graduated centrifuge tube and the diluent completed to 10ml. The cell suspensions were spun for eight minutes. The supernatant was discarded and the cell precipitates

were then resuspended in fresh diluent. The dilution of the spleen cells was in 8ml while the iliac lymph node cells in 1ml.(9)

Total white cell counts:-

All the total white cells counts were done using a Neubauer improved haemocytometer. The white cell count per one milliliter of suspension was obtained by recording the number of cells counted in the 4 large squares at the opposite corners of the haemocytometer grid, multiplying the figure by fifty thousand. The total tissue white cell count was obtained by using the dilution factor (eight for spleen and one for iliac lymph node) and the weight of the tissue from which the cell suspension was made.(10)

Differential white cell counts:-

After completion of the total white cell count, the cell suspension of each tissue was made again to five milliliters by adding fresh media and centrifuged again. The supernatant was discarded and the cell precipitates were resuspended again in fresh media. From each cell suspension smears were obtained and stained by May Grunwald-Giemsa's stain. The differential white cell count was done by examining at least four to five hundred cells of lymphocytes series in each smear. A cell with a densely stained nucleus filling the whole cell was designated as small lymphocyte. A larger cell with a densely stained nucleus and light blue cytoplasm was designated as a large lymphocyte and the lymphoblast was identified as the largest cell with a coarsely stained nucleus with obvious nucleoli and light blue cytoplasm. The number of large lymphocytes and lymphoblasts counted was expressed as a percentage of total lymphocyte count giving the percentage of the proliferating population (9).

Histology:

The portions of tissue used for histology were fixed in 10% formaldehyde and then procedure of preparing the paraffin section slides and staining by methyl green pyronin stain (to demonstrate lymphocytes) was done to prepare a stained histological sections for light microscope examination (Fig. 1 and 2). For spleen and iliac lymph node sections, the number of white pulps and nodules were counted in four

fields at X40 objective then recording the average number of each of them. The percentage of white pulp of spleen and the cortex of iliac lymph node were recorded by measuring the diameter of them in four fields at X40 object by using ocular and stage micrometers.(11)

#### Statistical Analysis :-

All the parameters were subjected to one way analysis of variance (ANOVA) directly by using tables of them. For each of parameters, the P value obtained from the eight animals in each four groups indicated wither or not a significant difference existed at 0.05 level between any of the four groups .

### Results:

#### Animal weight :-

There is no significant difference between group I and group II, but there is a significant decrease between group I and each of group III and IV , and between group II and each of group III and IV , and between group III and IV . ( table -1).

#### Spleen :-

##### Weight of tissue in mgs :-

There is a significant increase between group I and group II , and there is a significant decrease between group I and IV and between group II and each of group III and IV , but there is no significant difference between group I and III. (table -2).

##### Weight in gm/100 gm animal weight :-

There is a significant decrease between group I and IV and between group II and each of group III and IV and there is a significant increase between group I and group II, but there is no significant difference between group I and III . ( table-3).

#### Total tissue white cell count :

There is a significant decrease between group I and each of group II , III and IV ,and between group II and each of group III and IV , but there is no significant difference between III and IV . ( table-4).

##### White cell count per 100 mgs tissue :-

There is a significant decrease between group I and each of group IV and between

group II and each of group III and IV , but there is no significant difference between group I and II , and between group III and IV . (table - 5)

#### Differential white cell count :-

There is a significant decrease between group I and each of group III and IV , and between group II and each of group III and IV , but there is no significant difference between group I and II , and between group III and IV . (table -6).

#### Number of the white pulp :

There is a significant decrease between group I and each of group III and IV , and between group II and each of group III and IV, and between group III and IV ,but there is no significant difference between group I and II . (table -7).

#### Diameter of the white pulp :-

There is a significant decrease between group I and each of group III and IV, and between group II and each of group III and IV , and between group I and II , but there is no significant difference between group III and IV.(table-8).

#### Iliac lymph node:-

##### Weight of tissue in mgs:-

There is a significant decrease between group I and each of group III and IV, and between group II and each of group III and IV , and between group III and IV but there is a significant increase between group I and II .(table-9).

##### Weight in gm / 100-gm animal weight

There is a significant increase between group I and group II but there is a significant decrease between group I and each of group III and IV , and between group II and each of group III and IV , and between group III and IV .(table -10)

#### Total tissue white cell count :-

There is a significant decrease between group I and each of group III and IV, and between group II and each of group III and IV , and between group III and IV, but there is a significant increase between group I and group II. (table-11).

##### White cell count per 100 mgs tissue :-

There is a significant decrease between group I and each of group III and IV , and

between group II and each of group III and IV. There is a significant increase between group I and group II, but there is no significant difference between group III and group IV. (table – 12).

Differential white cell count :-

There is a significant increase between group I and II, but there is a significant decrease between group I and each of group III and IV, and between group II and each of group III and IV, and between group III and group IV – (table- 13).

Number of nodules :-

There is a significant decrease between group I and each of group III and IV, and between group II and each of group III and IV, but there is no significant difference between group I and II, and between group III and IV. (table –14).

Percentage of diameter of the cortex :

There is no significant difference between group I and group II and between group III and group IV, but there is a significant decrease between group I and each of group III and IV, and between group II and each of group III and IV. (table-15).

## **Discussion:**

The experiment deals with the effect of formaldehyde on the lymphoid system in adult male rats. For this purpose the spleen was examined because it is the largest lymphoid organ in mammals and is a major site of antibody formation particularly to blood – born antigens. The iliac lymph nodes also examined because they are visceral lymph nodes representing a deep group of lymph nodes, and they facilitate the proliferation of activated cells and amplification of the immune response.(12).

From each tissue removed five parameters were monitored. Firstly each of the two tissues (spleen and iliac lymph nodes) were weighed. The animals varied in their total weight so to standardize the weight it was expressed per one hundred grams of animal weight. Quantification of the body and organ weight, forms an integral part of any toxicology study, since it provides an initial assessment of over all

animal health status and many identify potential target tissues for toxic effects (13-14). Secondly the total lymphocyte count of each tissue was recorded so that thirdly with the tissue weight, the lymphocyte or white cell density of each tissue was calculated. This enable weight changes in the tissues examined due to variations in lymphocytes content to be distinguished from weight changes due to other causes as lymph. This gives an idea about their stimulation or inhibition. Fourthly, the determination of the proliferating lymphocyte populations designated as the differential white cell count allows an objective estimation of the functional activity to perform an immune response. Finally, a number of histological variables for each of the two organs mentioned were examined to compare with and to confirm the quantitative changes. In spleen the number of white pulp were calculated to observe the stimulation or inhibition effect on humoral component of immune system, while the diameter of white pulp was measured to know the effects on humoral or cellular components of immune system. In iliac lymph node the number and percentage of the cortex were studied to give an idea about the effect of formaldehyde on B – dependent area.

The data of the animal weight (table-1) shows a significant decrease in body weight in group III and IV and this may be due to feed refusal and decreased feed efficiency with long period of formaldehyde exposure. This decrease in body weight may be due to chronic lesion in liver with vacuolation and mitochondrial damage of hepatocytes (15).

The results of the total weight and standardized weight of spleen and iliac lymph node (table –2,3,9 and 10) show significant increase in group II in comparison with group I. This increase in spleen and iliac lymph node weight may be time dependent, so short period of formaldehyde exposure caused enlarged spleen and iliac lymph node, while long period of exposure as in group IV has lead to decreased splenic and iliac lymph node weight.

The result of the total white cell count and cell density of spleen and iliac lymph node (table-4,5,11 and 12) indicate that formaldehyde has decreased white cell count and cell density of spleen and iliac lymph

node . This decrease leading to cellular depletion and immunosuppression (12-15).

Except the total white cell count and cell density of the iliac lymph node in group II which show a significant increase . This increase may be due to enhanced immune response due to short period of formaldehyde exposure, and this could be a possible explanation about the significant increase in the total weight and standardized weight of the iliac node in this group (II).

The data of differential white cell count of the spleen and iliac lymph node (table-6 and 13 ) indicate significant decrease in group III and IV in the percentage of large lymphocytes and lymphoblasts which means long period of formaldehyde exposure affect the differential leukocyte count and lymphoblastogenesis . This leads to reduction in both B- cell and T-cell mitogenesis and loss of normal lymphocyte activity (12,13) .While the same data (table – 6, and 13) of group II shows significant increase in differential white cell count of spleen and iliac lymph node . This means that short time of formaldehyde exposure may lead to stimulation of lymphoblastogenesis.

In general all the histological parameters ( table-7,8,14 and 15) show a significant decrease in all three treated groups in comparison with control group and these results indicate a suppression in white pulp of spleen and the cortex of the iliac lymph node . This suppression due to inhibition of functional activity of B and T lymphocytes with resultant reduced humoral and cell mediated immune response (13,14).

From over all results and discussion is concluded that formaldehyde affect and modulate the immune system and this leads to suppression of both cell- mediated and humoral immunity. This affect on the immune system is time dependent , so long period formaldehyde exposure has lead to more immune suppression . The exact mechanism of this affect is unknown , but the formaldehyde could be affect the surface topography of lymphocytes by causing extensive mitochondrial damage , that may affect the way in which these cells function (15).

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**Table 1 :** weight of animals in grams

Animal number	Group I	Group II	Group III	Group IV
1	234.7	222.1	224.7	191.5
2	233.9	231.6	206.5	215.5
3	236.9	201.5	232.2	212.4
4	239.2	259.8	221.3	198.2
5	232.3	246.1	192.7	188.6
6	221.4	193.2	221.3	217.4
7	231.6	282.4	246.4	221.2
8	227.7	231.3	220.8	230.8
Mean	232.2	233.6	220.8	209.4

**Table 2 :** Spleen weight in mgs

Animal number	Group I	Group II	Group III	Group IV
1	352.5	331.7	282.1	310.4
2	247.3	389.2	297.7	265.7
3	2.778	391.8	298.6	253.2
4	362.1	397.2	351.7	274.7
5	261.4	541.4	398.1	310.2
6	323.7	581.4	421.2	240.3
7	269.8	410.2	351.4	319.4
8	310.2	417.4	341.7	278.7
Mean	300.6	432.5	324.8	281.5

**Table 3 :** Spleen weight in gm / 100 mg animal weight

Animal	Group I	Group II	Group III	Group IV
1	0.1610	0.1906	0.1655	0.1022
2	0.1129	0.1872	0.1508	0.0978
3	0.1245	0.1614	0.1320	0.1130
4	0.1448	0.1836	0.1488	0.0988
5	0.1595	0.1724	0.1529	0.1019
6	0.1510	0.1821	0.1591	0.0892
7	0.1449	0.1963	0.1514	0.0972
8	0.1440	0.1931	0.1581	0.1183
Mean	0.1427	0.1833	0.1523	0.1023

**Table 4:** Spleen – total tissue white cell count x 10

Animal number	Group I	Group II	Group III	Group IV
1	312.7	225.8	210.7	304.2
2	250.2	339.3	320.5	218.4
3	371.8	310.7	301.2	310.6
4	350.4	286.9	210.4	309.2
5	338.7	358.7	320.2	211.4
6	319.3	310.6	350.6	330.2
7	428.3	245.4	204.8	301.9
8	535.5	284.5	310.2	210.7
mean	363.3	295.2	278.5	374.5

**Table 5 :** Spleen -white cell count x 10<sup>6</sup> /100 mgs tissue

Animal number	Group I	Group II	Group III	Group IV
1	85.6	101.6	78.6	68.2
2	103.2	99.3	91.2	71.4
3	95.3	85.8	75.7	65.7
4	104.2	97.2	101.2	60.3
5	97.7	85.6	62.3	71.2
6	90.4	98.7	66.3	74.8
7	118.5	91.4	73.7	62.2
8	112.6	96.8	61.3	77.8
Mean	1009	94.5	76.2	68.9

**Table 6:** Spleen differential white cell count % of large lymphocytes and lymphoblasts

Animal number	Group I	Group II	Group III	Group IV
1	19.5	15.0	8.0	7.2
2	13.4	14.7	7.3	6.4
3	14.6	12.5	10.7	8.5
4	15.7	10.0	6.8	9.4
5	9.8	12.0	7.2	6.7
6	12.9	11.5	8.1	7.1
7	9.8	11.5	7.4	8.2
8	11.7	12.0	8.1	7.5
Mean	13.4	12.4	7.9	7.6

**Table 7 :** Number of white pulp in spleen

Animal number	Group I	Group II	Group III	Group IV
1	5.0	4.5	4.0	3.5
2	6.0	5.1	3.5	3.0
3	5.5	5.0	3.0	2.2
4	6.5	7.0	3.3	2.0
5	7.0	6.0	3.5	1.5
6	5.0	4.0	4.0	2.0
7	5.5	5.5	3.0	1.5
8	6.0	4.5	3.5	2.0
Mean	5.8	5.2	3.4	2.2

**Table 8 :** Percentage of white pulp

Animal number	Group I	Group II	Group III	Group IV
1	30.2	19.5	16.8	17.1
2	27.6	20.6	13.1	14.5
3	26.3	21.8	15.2	13.1
4	30.1	22.4	16.3	14.2
5	28.2	21.6	18.2	15.3
6	24.3	23.1	16.6	17.6
7	27.5	19.8	15.2	13.1
8	26.2	21.2	16.7	17.2
Mean	27.5	21.2	16.1	15.2

**Table 9 :** Iliac lymph node weight in mgs.

Animal number	Group I	Group II	Group III	Group IV
1	16.2	31.3	15.2	14.3
2	26.3	32.7	21.3	15.9
3	28.2	29.5	16.7	12.9
4	27.0	33.3	23.4	15.4
5	19.7	24.2	25.7	17.3
6	28.3	29.1	19.7	13.3
7	25.3	35.6	17.2	14.1
8	24.2	29.2	18.6	16.2
Mean	24.4	30.6	19.7	14.9

**Table 10 :** Iliac lymph node weight in gm /100 gm animal weigh

Animal number	Group I	Group II	Group III	Group IV
1	0.0152	0.0078	0.0077	0.0130
2	0.0085	0.0075	0.0131	0.0081
3	0.0088	0.0328	0.0135	0.0062
4	0.0075	0.0077	0.0129	0.0063
5	0.0126	0.0132	0.0126	0.0072
6	0.0222	0.0154	0.0123	0.0079
7	0.0152	0.0215	0.0118	0.0083
8	0.0146	0.0139	0.0115	0.0078
Mean	0.0130	0.0149	0.0119	0.0081

**Table 11 :** Iliac lymph node-total tissue white cell count X 10

Animal number	Group I	Group II	Group III	Group IV
1	21.32	39.53	15.94	10.87
2	21.42	38.03	23.66	15.15
3	44.71	47.95	22.60	18.92
4	24.52	36.25	23.36	19.38
5	26.81	35.51	22.37	12.95
6	25.29	33.52	23.53	12.39
7	55.93	39.47	21.41	12.78
8	25.35	30.07	23.21	15.28
Mean	30.66	37.54	22.01	14.71



**Table 12 :** Iliac lymph node- white cell count  $\times 10^6$  /100 mgs tissue.

Animal number	Group I	Group II	Group III	Group IV
1	118.3	124.6	62.5	93.2
2	112.5	129.8	104.2	82.5
3	121.4	131.6	112.3	94.3
4	103.6	135.5	68.5	87.5
5	110.7	115.2	93.2	82.6
6	97.9	109.5	95.4	91.4
7	93.7	122.9	88.5	92.2
8	101.4	125.3	89.3	79.8
Mean	107.4	124.3	89.2	87.93

**Table 13 :** Iliac lymph node differential white cell count % of large lymphocytes and lymphoblasts

Animal number	Group I	Group II	Group III	Group IV
1	24.3	28.3	26.2	13.3
2	21.5	36.2	19.7	11.2
3	25.3	27.4	23.3	12.6
4	19.5	22.5	21.2	17.4
5	15.2	17.3	13.4	18.9
6	19.3	14.2	22.6	13.1
7	17.6	24.7	19.5	14.8
8	19.7	26.3	18.7	13.7
Mean	19.3	24.6	20.5	14.3

**Table 14:** Number of nodules in iliac lymph node.

Animal number	Group I	Group II	Group III	Group IV
1	3.0	4.5	1.75	1.75
2	2.5	5.0	1.5	1.5
3	5.75	4.2	1.75	1.5
4	2.0	3.0	1.25	1.25
5	1.75	5.5	1.5	1.5
6	3.5	4.2	1.5	1.25
7	4.1	4.5	1.75	1.5
8	3.5	4.2	1.5	1.2
Mean	3.8	4.3	1.5	1.4

**Table 15:** Percentage of diameter of the cortex in iliac lymph node

Animal number	Group I	Group II	Group III	Group IV
1	30.2	27.7	27.5	24.3
2	32.7	28.9	26.3	23.7
3	25.4	30.1	22.4	22.3
4	27.8	42.2	23.7	24.2
5	27.2	24.6	24.6	23.6
6	30.6	31.3	26.7	31.3
7	31.6	30.4	23.9	20.9
8	28.9	29.5	25.3	24.2
Mean	29.3	30.5	25.1	24.2



Figure 1 :Section of the spleen of rat stained with methyl green pyronin G Showing the white pulp, (W) red pulp (R) X40

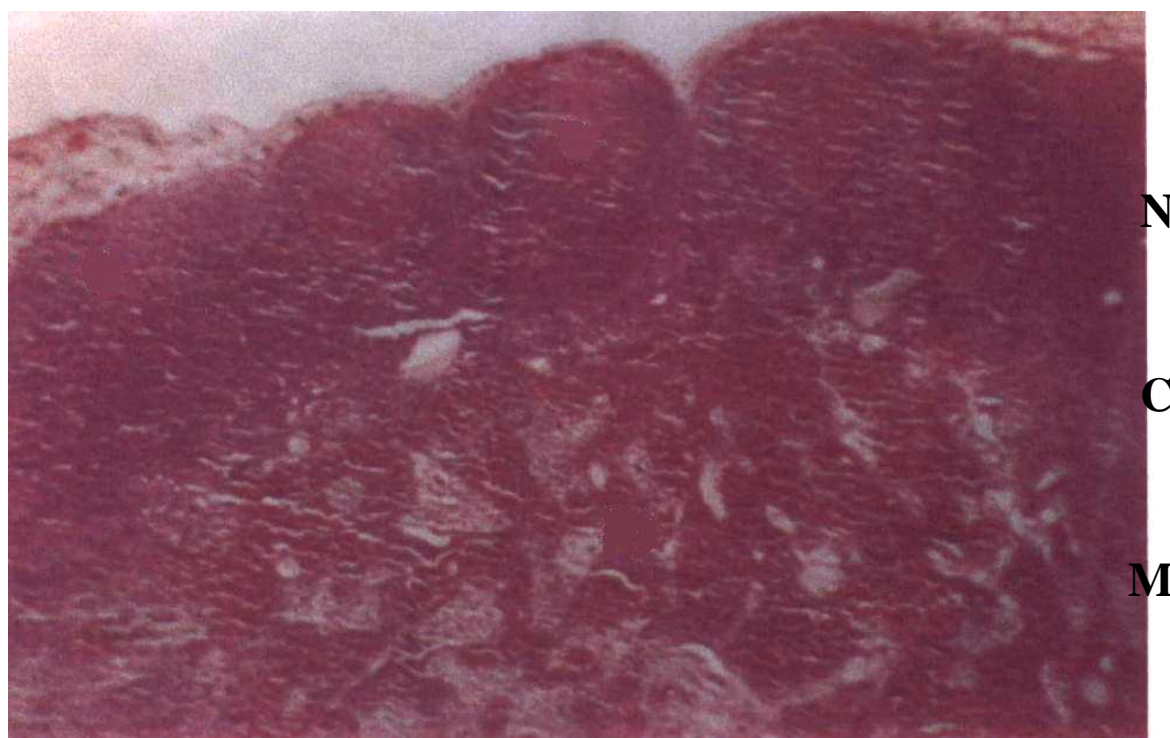


Figure 2 : Section of the iliac lymph node of rats stained with methyl green pyronin G Showing nodules ( N) Cortex ( C ) and medulla (M) X40.