Effect of formaldehyde vapor on the blood constituents of male rabbits

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Abstract

The present experimental study was designed to investigate the effect of formaldehyde on blood constituents of rabbit males, Twenty four adult males were randomly subdivided into 3 groups (I, II, III) and exposed to vapour of 10% FD (12 ppm) in cages for the following periods: 2, 4 and 6 months; beside, 8 rabbits were exposed to vapour of distilled water as a control group. Blood parameters examination showed no morphological changes, but with a significant increase in lymphocytes and esonophils percentage. Significant decrease in neutrophil, red blood cell (RBC) and platelets counts was detected. The present study concluded that formaldehyde of such concentration and exposure time have an effect on blood constituents of rabbit males.

Keywords: Formaldehyde; Rabbits; Blood.
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تأثير بخار الفورمالديهايد في مكونات دم ذكور اللأرانب

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الخلاصة

صممت ألدراسة ألمختبرية ألحالية على أساس دراسة تأثير ألفورمالديهايد على دم الأرانب. تم اختيار ٢٤ من ذكور الأرانب البالغين قسموا إلى ثلاثة مجاميع، كل مجموعة تضم ٨ أرانب، وضعوا في اقفاص و عرضوا الى بخار الفورمالديهايد وبتركيز (١٢ جزء لكل مليون) و لمدة ٢، ٤، ٦ أشهر على التعاقب. اما مجموعة السيطرة (٨ ارانب) فقد عرضت الى بخار الماء فقط. أوضح فحص مكونات الدم عدم و جود أي تغير في شكل خلايا الدم، مع زيادة معنويه في نسبة الخلايا اللمفية و الحمضية ونقصان معنوي في نسبة كريات الدم العدلة والحمراء والعدد الكلي لخلايا الدم البيض و ألأقراص الدموية. نستنتج من هذه الدراسه ان الفورمالديهايد بهذه الجرع و الفتره الزمنيه للتعرض احدث تأثيرات سلبيه في مكونات الدم لذكور الارانب.

Introduction

Formaldehyde was introduced in 1893 by Blum as a fixative and embalming fluid. It is well known as a preservative, a sterilizer and embalming fluids; and approximately 2.1 million workers in United State (USA) are exposed to formaldehyde (1).

Several epidemiological studies have analyzed the potential association between formaldehyde and cancer risk in humans. These have been reviewed in three consecutive monographs of the International Agency for Research on Cancer (IARC, 2) which classified formaldehyde as (Group 1) 'carcinogenic to humans' (2). More recently, there has been increased concern and scientific debate regarding the potential for exposure to formaldehyde in air to cause lymphohematopoietic cancers in humans, particularly leukemias (3-7). Although other studies show no such effects (8). The studies reporting associations have shortcomings, including poor disease classification and unverified estimates of exposure. The animal studies generally reported neither hematotoxicity nor leukemia (9) associated with formaldehyde exposure. Although a few

animal studies reported changes in one or more hematology parameters and leukemia (10), and a few human study findings were consistent with hematotoxicity from exposure to formaldehyde (11-12). From the above introduction the present study was designed to examine the potential for formaldehyde in air to induce hematotoxicity or leukemia in rabbits.

Materials and methods

Thirty two adult rabbit males were used with weight range between 875-1622 grams. They were kept under the same environmental conditions. The temperature was maintained at 22- 32 ^{o}C Dry absorbent, bedding materials, like wood particles was provided in all cages. All animals were allowed free access to food and water. Antihelmintic drug (ivermectin 2 mg/kg subcutaneous) was also given against internal and external parasites. All the animals were observed for 10 days before the beginning of the experiment to exclude any possibility of abnormal behavior and disease.

The animals were randomly assigned irrespective of age and weight into four groups; Control group, Exposed group I, Exposed group II and Exposed group III. Each group include 8 animals, and all the rabbits of each group were weighted before and after exposure.

All groups (except control one) were exposed to the vapour of 10% formaldehyde for the following periods; Group I for 2 months, group II for 4 months and group III for 6 months.

Formaldehyde,10% solution, was placed in steel containers covered by nylon mesh and filled periodically. All the animals were exposed to the same constant surface area of 10% formalin solution. While the rabbits of the control group were exposed to a vapour of distilled water, under the same condition of housing, feeding and duration of the exposure. Analytic procedure (13) was applied several times during the exposed period of this work to determine the concentration of formaldehyde (ppm) in the atmosphere of the exposed cages. From this analytic measurement the concentration of formaldehyde in the exposed cages was found to be (12 ppm).

Routine venous blood samples were collected from the exposed groups (before exposure) as a pre-exposed sample as well as from the control group from the auricular vein by using sterile disposable plastic syringes.

For routine hematological laboratory test, whole blood samples were collected into chemically clean disposable plastic tubes, each containing a dry amount of dipotassium salt of Ethylene Diamine Tetraacetic Acid (EDTA). The following parameters were estimated according to the standard methods for hematological investigation (14); Haemoglobin estimation (Hb) using spectrophotometer (Drabkin's reagent). Packed cell volume (PCV) using microhaematocrit method. Total white blood cells (WBC) count using manual method. Differential leukocyte counts (DLC) using (Leishman`s stain) manual method. Platelet count using manual method.

The significance of difference of blood parameters among the groups was assessed using one way analysis of variance (ANOVA) with Duncan's multiple range test. The values are expressed as mean and standard deviation at the $P<0.05$ were considered significant (s) (15).

Results

Physical observations

All rabbits were still alive until the end of the study, during the beginning of formaldehyde exposure, the animals showed aggressive behavior and movements, while at the end of the exposure time the animals were very calm, limited their movements and gathered at one corner of the cage to avoid the area of maximum formaldehyde concentration.

Frequent face washing, sneezing, coughing, crouching position, lacrimation and serous nasal discharge were observed specially among the animals of exposed group I.

The exposed animals showed lower appetite for food and water intake as well as a significant decrease in body weight (Table – 1).

Blood

Hemoglobin (Hb)

As shown in (Table – 1) there is no significant changes between control group and group I. There is a significant decrease between group I and group II and group III.

The packed cell volume (PCV)

The results represented in (Table – 1) showed no significant changes between control group and group I. While a significant decrease was noticed between group II and group and group III.

Platletsts

There was no significant changes between control group and group I, but there was a significant decrease (thrombocytopenia) noticed between group III and group II when they compared with control group and group I (Table – 1).

White blood cells (WBC) count

The results represented in (Table - 1) showed no significant changes between control group and group II; but there was a significant increase between exposed group I and control group and exposed group II, and a significant decrease in (leukocytopenia) between exposed group III and control group, exposed group I and exposed group II.

Differential leukocytes count (DLC)

Neutrophil

The results which are shown in (Table - 1) revealed a significant decrease between control group and the three exposed groups (group I, group II and group III). The results also showed no significant changes between the three exposed groups.

Eosinophil

There is a significant increase between the control and the three exposed groups (I, II and III) from other side. Eosinophils of all exposed groups show no significant changes between them (Table – 1).

Basophil

The basophil showed (Table – 1) no significant changes between control group and all other (exposed I, exposed II and exposed III) groups.

Lymphocyte

As shown in (Table – 1) there is a significant increase between the control and the three exposed groups (I, II, III). The results also showed no significant changes between the three exposed groups.

Monocyte

The results which are shown in (Table – 1) indicated no significant changes between control group and all exposed groups.

Blood cells morphology

The red blood cells appeared normochromic normocytic in all groups (control and all exposed groups). All leucocytes in control group and the three exposed groups were mature cells.

Table (1): The different blood parameters between exposed groups and control group.

Parameter	F	P-Value	Duncan's test			
			Control	Group 1	Group 2	Group 3
Weight grams	4.31*	0.013	A	B	B	B
Hemoglobin g/L	11.02**	0.000	A	A	B	C
PCV %	15.12**	0.000	A	A	B	C
platelets count X 10^9 / L	13.65**	0.000	A	A	B	C
white blood cell X 10^9 / L	53.75**	0.000	A	B	A	C
Neutrophil %	4.41*	0.012	A	B	B	B
Eosinophil %	14.67**	0.000	A	B	B	B
Basopbil %	2.33N.S.	0.96	A	B	B	B
lymphocyte %	4.34*	0.012	A	B	B	B
Monocyte %	0.41N.S.	0.745	A	A	A	A

* Sign. level at P < 0.05, ** Sign. level at P < 0.01.

Table (2): The mean and the stander deviation of different blood parameters of the control group and exposed groups (pre-exposed and post –exposed).

parameter	Control group	Group 1		Group 2		Group 3	
		pre	post	pre	post	pre	post
	Mean ±std	Mean ±std	Mean ±std	Mean ±std	Mean ±std	Mean ±std	Mean ±std
Weight grams	1243.7±179.8	915.4±405.1	952.2±411.2	1205.9±232	1133±223.5	1274±79	1263±73
Hemoglobin g/L	121.62±6.23	125±9	125.5±10.15	128±8	112.75±8.96	126±8.82	99.25±13.12
PCV %	0.39±0.04	0.4±0.05	0.40±0.05	0.42±0.05	0.34±0.03	0.41±0.04	0.27±0.05
platelets count X 109/L	368.1±44.24	365±37	373±28.7	401±70	314.62±30.3	394.5±33.57	271±42.14
white blood cellX109/L	6.95±0.81	7.3±1.3	9.75±0.65	6.7±0.5	7.15±0.70	6.68±0.81	5.23±0.71
Neutrophil %	44±6.5	42±3.7	33.12±7.37	44±7.4	34.62±7.99	43.87±7.12	34.6±5.34
Eosinophil %	1.3±0.51	1.5±0.53	3.37±0.74	1.2±0.46	3±0.75	1.25±0.46	3.12±0.64
Basopbil %	3±1.5	3±0.75	2±0.93	2±0.75	2±0.75	2.75±0.70	1.75±0.70
lymphocyte %	45±5.3	45±3.3	55.25±7.64	42.8±6.3	54.75±7.04	42.5±5.31	53.87±4.76
Monocyte %	6±2.1	8±1.2	6.5±1.41	6.5±1.6	5.62±1.4	8.25±1.48	6.12±1.24

Discussion

Rabbits showed aggressive behaviour and movement at the beginning of the exposure time, this could be due to the irritant effect of formaldehyde, specially the mucous membranes of the nasal cavities and eyes or could be due to its effect on the central nervous system. Neuropsychological effect such as loss of concentration and mood alterations were noticed in man and in animals exposed to formaldehyde (16). The present work noticed lower food and water intake with a significant decrease in the body weight of the formaldehyde- exposed animals. This could be due to the stress and irritation of the animals as a result of formaldehyde-exposure. This result is in agreement with that reported by (9).

The current study found a significant increase in the number of esonophil and lymphocytes with a significant decrease in the number of neutrophil between the control group and the three exposed groups, but there was no significant changes with regards to basophil and monocytes between all exposed and control groups.

The significant decrease in the number of neutrophil through out this study, agrees with the results of (17) who noticed a significant decrease in polymorphonuclear neutrophil granulocytes in occupational workers exposed to formaldehyde.

Also (18) mentioned an increase in esonophil number in students who were exposed to formaldehyde. Esonophil number increase might be due to the allergic reaction and increase incidence and severity of asthma as a result of formaldehyde exposure.

In general, the present study showed no morphological changes in the blood cells, and a significant decrease in the number of red blood cells (erthrocytopenia), white blood cells (leukopenia) and platelets (thrombocytopenia) of all exposed groups and specially in group III, this could be due to long time of formaldehyde exposure to this group. Recently, there are large epidemiological studies among industrial workers, which noted a significant association between formaldehyde exposure and the incidence of leukaemia (3-7).

They referred the care to the disposition and toxicity of inhaled formaldehyde in experimental animals and humans, particularly as it shows to affects blood or bone marrow, specially among embalmers, pathologist and anatomist.

Nurses in Taiwan showed decrease in WBC counts after exposure to formaldehyde (19). A recent study in China showed that formaldehyde was associated with lowered T lymphocytes in the blood of exposed workers (20). Several studies in the Chinese literature reported that occupational formaldehyde exposure was associated with a decrease in WBC counts and possibly other cell counts such as platelets (21), which is consistent with our findings.

A question arises as to how it reaches the blood and bone marrow to elicit toxic effects. Several studies have reported increased chromosomal damage in the form of aberrations and micronuclei in circulating peripheral blood lymphocytes of workers exposed to formaldehyde (20,22).

Formaldehyde may act on bone marrow directly or, alternatively, may cause leukemia by damaging the hematopoietic stem or early progenitor cells that are located in the circulating blood or nasal passages, which then travel to the bone marrow and become leukemic stem cells. To test these hypotheses, we recommend that future studies apply biomarkers validated for other chemical leukemogens to the study of formaldehyde (23).

The present investigation suggests that formaldehyde has a direct and indirect effect on blood, but this effect is interfered with different factors such as the concentration of formaldehyde, time exposure, route of administration, individual variations (humans or animals), orders or species of animals, age, sex, and race.

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