Morphological and Histological Changes in Lungs and Thyroid Gland Due to Exposure of Formalin in Albino Rats

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Abstract: formalin is used as disinfectant for preservation of museum specimens, preservation of surgical and pathology specimens, as a fumigating agent in operation theatres, for preservation of cadavers in anatomy department, in plastic industries, in dyeing and hardening of celluloid.
Formalin has many side effects because of which the importance of this study increases many times. The person working in the atmosphere of fumes of formalin may have respiratory irritation and have rhinorhoea and discharge of water from eye on direct exposure.
The present study is based on universal use of formalin in various industries including the various departments of medical sciences. The aim of study is to observe changes in Lungs and Thyroid gland in albino rats due to inhalation exposure of formalin fumes.
In this study, 100 albino rats were taken and divided into 4 groups A, B, C and D. the group D is retain as control group and the remaining 3 groups were subdivided into subgroups.
Except D group rats, all other rats were exposed with Formalin for different time intervals and changes were observed, recorded and discussed in relevant headings.

Keywords: formalin, formaldehyde inhalation, rat lungs

I. Introduction
Formalin is 40% aqueous solution of FORMALDEHYDE which is a colorless gas having a strong, pungent and irritating odour.
It gives off vapor at room temperature. The vapors are highly pungent, respiratory irritant and produces rhinorhoea and discharge of water from eye on direct exposure. There are irritation of eyes and air passages on direct exposure. If it is swallowed it produces burning pain from mouth to stomach, nausea, vomiting, contracted pupils and flushing of face.
Lethal oral dose of formalin is 30-90 ml and fatal period ranges from 1-2 days.
Patient died due to formalin intake. On autopsy, shows that gastric mucosa is red, inflammed and eroded with extravasations of blood or it may be hard and tough like leather. The intestines and lungs are congested. Liver may show fatty degeneration and kidneys may be inflammed.
The present study is based on fact that the formalin is being used universally in various field. Person working in rubber industries, dyeing, all laboratory workers, all medical students, teachers and staff working in Anatomy and Pathology department remain exposed to hazardous and deleterious effect of formalin.
The aim of present study is to observe changes in lungs as well as thyroid gland due to inhalation exposure of formalin fumes.

II. Material And Method
In present study, 100 Albino rats have been used. Average weights of them were ranging from 100-160 gms. They were divided into 4 groups, namely A, B, C and D. The group D was retained as control. The groups A B and C were subdivided further into two subgroups as follows. In present study, sex was not considered during observation.
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<table>
<thead>
<tr>
<th>S.NO.</th>
<th>GROUPS</th>
<th>SUBGROUPS</th>
<th>NO. OF ANIMALS PER SUBGROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>A&lt;sub&gt;1&lt;/sub&gt;</td>
<td>15 Animals</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A&lt;sub&gt;2&lt;/sub&gt;</td>
<td>15 Animals</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>15 Animals</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>15 Animals</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>C&lt;sub&gt;1&lt;/sub&gt;</td>
<td>15 Animals</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C&lt;sub&gt;2&lt;/sub&gt;</td>
<td>15 Animals</td>
</tr>
<tr>
<td>4</td>
<td>D</td>
<td></td>
<td>10 Animals in control group.</td>
</tr>
</tbody>
</table>

Exposure

Iron case containing animal subgroups were kept in wooden boxes. These wooden boxes were having holes. Formalin was placed in a beaker in wooden box for direct inhalation exposure and holes were provided for proper aeration.

III. Dose And Duration

Commercial Formalin (40% formaldehyde in water w/v) was used. 50 ml formalin was given to each subgroup in a beaker. Formalin was changed after every 10 days. Duration of exposure in different subgroups was as follows.

<table>
<thead>
<tr>
<th>S NO</th>
<th>SUBGROUP</th>
<th>DURATION OF EXPOSURE (IN HOURS/DAY)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Exposed 3 hr/day for one month</td>
</tr>
<tr>
<td>2</td>
<td>A&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Exposed 6 hr/day for one month</td>
</tr>
<tr>
<td>3</td>
<td>B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Exposed 3 hr/day for two months</td>
</tr>
<tr>
<td>4</td>
<td>B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Exposed 6 hr/day for two months</td>
</tr>
<tr>
<td>5</td>
<td>C&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Exposed 3 hr/day for three months</td>
</tr>
<tr>
<td>6</td>
<td>C&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Exposed 6 hr/day for three months</td>
</tr>
<tr>
<td>7</td>
<td>D&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Control group, Not exposed to formalin</td>
</tr>
</tbody>
</table>

Weight of animals of each subgroup was recorded prior to commencement and completion of experiment including the control group.

Feeding Of Animals – During experiment, rats were provided rat chow, black grams, carrot and water ad-libitum. Their local hygiene was also maintained up to possible extent. Same food was also provided to control group.

Dissection Of Animals – After the completion of related period of individual animals were anaesthetized by inhalation of ether. They were sacrificed after 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> month and dissected from the dorsal aspect by giving median incision and the individual organs like Lungs and Larynx (for Thyroid gland) was excised and fixed into 10% formalin for 24 hrs.

Processing – organs fixed into 10& formalin were subjected to manual processing which consists of all step including dehydration, wax impregnation, block preparation, microtomy, dewaxing of section, staining, mounting of slides and microscopy. All necessary and precautionary steps have been taken into account.
Observations –

### Table -1 Parameter – Behavioural Changes

<table>
<thead>
<tr>
<th>Sub – Groups</th>
<th>A1</th>
<th>A2</th>
<th>B1</th>
<th>B2</th>
<th>C1</th>
<th>C2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Restlessness On Fresh Exposure For 20 Minutes</td>
<td>Restlessness On Fresh Exposure For 20 Minutes</td>
<td>Restlessness For About 20 Minutes Less Exit</td>
<td>Relatively Lesser Respiratory Rate Increased</td>
<td>Less Restlessness Respiratory Rate Increased</td>
<td>Restlessness Was Of Lesser Degree Increased</td>
<td></td>
</tr>
<tr>
<td>Uncoordinated Locomotion</td>
<td>Uncoordinated Locomotion</td>
<td>Increased Respiratory Rate Do Not Urinates On Exposure</td>
<td>Sluggishly Acting Uncoordinated Locomotion</td>
<td>Sluggish Behaviour</td>
<td>Difficulty In Breathing</td>
<td></td>
</tr>
<tr>
<td>Increased Respiratory Rate Urinated On Fresh Exposure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine Output</td>
<td>Normal</td>
<td>Normal Morphology</td>
<td>Swollen Lungs With Thickened Margin</td>
<td>Swollen Lungs With Thickened Margin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table -2 Parameter – General Health Changes

<table>
<thead>
<tr>
<th>Sub – Groups</th>
<th>A1</th>
<th>A2</th>
<th>B1</th>
<th>B2</th>
<th>C1</th>
<th>C2</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Skin Changes Feeding Habits Altered Only At Time Of Exposure Urine Output Normal</td>
<td>No Skin Changes Feeding Habits Altered Only At Time Of Exposure Urine Output Normal</td>
<td>No Skin Changes Feeding Habits Altered Only At Time Of Exposure Urine Output Normal</td>
<td>Slight Discoloration Thickness Of Skin Of Fore Limb And Hind Limb Were Noted Feeding Reduced Reduced Urine Output In 10 Animals</td>
<td>Discolouration Of Fur From Turbid To Yellow Area Of Alopecia Specially In Back Region Thick Keratinized Skin Of Non Fur Area Reduced Urine Output In All Animals</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Restlessness On Fresh Exposure For 20 Minutes Uncoordinated Locomotion Increased Respiratory Rate Urinated On Fresh Exposure</td>
<td>Restlessness On Fresh Exposure For 20 Minutes Uncoordinated Locomotion Increased Respiratory Rate Urinated On Fresh Exposure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine Output</td>
<td>Normal</td>
<td>Normal Morphology</td>
<td>Swollen Lungs With Thickened Margin Decreased Elasticity (Recoil Tendency)</td>
<td>Swollen Lungs With Thickened Margin Decreased Elasticity</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table – 3 Parameter – Weight

<table>
<thead>
<tr>
<th>Sub – Groups</th>
<th>A1</th>
<th>A2</th>
<th>B1</th>
<th>B2</th>
<th>C1</th>
<th>C2</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Weight Loss</td>
<td>No Weight Loss Recorded</td>
<td>Weight Loss By Gns. In Animals</td>
<td>Weight Loss By 10 Gns. In 9 Animals Rest 6 Animal Showed No Weight Loss</td>
<td>Weight Loss By 15 Gns. In All Animals</td>
<td>Weight Loss By 15 Gns. In 8 Animals And 20 Gns. In 7 Animals</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lungs</td>
<td>Normal Morphology</td>
<td>Normal Morphology</td>
<td>Slightly Swollen Lungs</td>
<td>Swollen Lungs With Thickened Margin Increased Elasticity (Recoil Tendency)</td>
<td>Swollen Lungs With Thickened Margin Decreased Elasticity</td>
<td></td>
</tr>
</tbody>
</table>

### Table – 4 Parameter – Morphological Changes

<table>
<thead>
<tr>
<th>Morphological Changes In</th>
<th>Sub – Groups</th>
<th>A1</th>
<th>A2</th>
<th>B1</th>
<th>B2</th>
<th>C1</th>
<th>C2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lungs</td>
<td>Normal Morphology</td>
<td>Normal Morphology</td>
<td>Slightly Swollen Lungs</td>
<td>Swollen Lungs With Thickened Margin Decreased Elasticity (Recoil Tendency)</td>
<td>Swollen Lungs With Thickened Margin Decreased Elasticity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroid Gland</td>
<td>Normal Morphology</td>
<td>Normal Morphology</td>
<td>Normal Morphology</td>
<td>Normal Morphology</td>
<td>Normal Morphology</td>
<td>Normal Morphology</td>
<td>Normal Morphology</td>
</tr>
</tbody>
</table>
### Table – 5 Parameter – Microscopic Changes

<table>
<thead>
<tr>
<th>Microscopic Changes In</th>
<th>Sub – Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1</td>
</tr>
<tr>
<td>Lungs</td>
<td>Normal Microscopic Finding (Fig-1)</td>
</tr>
</tbody>
</table>

**Figure 1:** Normal lung showing alveoli and their wall

**Figure 2:** Congestion and hyperemia
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**Figure 3:** Slight emphysematous changes with increased alveolar macrophages and congestion.

**Figure 4:** Congestion, emphysematous changes, lymphoid follicle

**Figure 5:** Many lymphoid follicles, emphysematous changes in bronchial mucosa, slightly irregular, infiltration of macrophages, lymphocytes and eosinophils
From preceding observations (various tables) and their comparative study with review of literature, it is evident that formalin fumes seem to be hazardous to the general health and behavioural changes to albino rats. Histology of organ shows some changes (pathology to the lesser or greater extent).

**Behavioural Changes:**

In present study it is evident that rest showed acute symptoms on exposure. Animal subgroups exposed for shorter duration and for lesser time period in a day (ex – subgroup A1, A2 and B1) showed restlessness on each fresh exposure of formalin for about 20 mins. The same findings were recorded by DICKSON et. al. (1987) and Wouterson et. Al. (1987)

Present study showed that animal were excited, showing increased respiratory rate and discharged urine at once on exposure and then gradually settle down. But animals exposed to longer duration (like B2, C1 and C2 subgroups) also showed such type of restlessness however relatively of lesser degree, but their duration of tachypnoea (increased respiratory rate) was increased and after settling they become sluggish. Similar

**Figure 6:** Marked hyperplastic lymphoid follicle with increased alveolar macrophages.

**Figure 7:** Interstitial pneumonia

**IV. Discussion**

From preceding observations (various tables) and their comparative study with review of literature, it is evident that formalin fumes seem to be hazardous to the general health and behavioural changes to albino rats. Histology of organ shows some changes (pathology to the lesser or greater extent).
observation has been reported by SALDIVA et. al. (1985). This paradox can be explained on the basis of environmental adaption especially animal of C2 subgroup. In later period of present experiment showed difficulty in breathing and some animal of C2 subgroup were extremely sluggish. WOUTERSON et. al. (1987) and TILL et. al. (1989) also observed uncoordinated locomotion and difficulty in breathing.

General Health:

Regarding general health of animals of subgroups A1, A2 and B1, there were not any significant skin changes (table 2) and feeding habit of animals were changed transiently i.e. they restored to normal on removal of formalin except that some animals showed decreased food intake during exposure but their feeding was restored to normal range on removal of formalin (A1 and A2 subgroup). There was also slight decrease in urine output in animals of B1 subgroup. Urine output in B2, C1 and C2 subgroups gradually reduced considerably. TILL et. al. (1989) also reported a slight temporary increase in density of urine of rats. At lower doses of formalin (given in drinking water) while at higher doses oliguria was noticed.

Some skin changes were observed in B2 group table-2 which includes slight discoloration of fur and thickness of skin of fore limb and hind limb (keratinization). Aooelman et al (1988) has found adverse effect of formaldehyde in higher dose group manifested as reduced urine output, growth retardation and rhinitis changes were pronounced in animals exposed to longer duration (e.g. group C1 and C2) (Table-1). They were in the form of discoloration of fur from turbid to yellow tendency to fall of hairs easily on slight manual manipulation (C1) and occasional area of diminished, skin was thick in non fur area and they showed significant oliguria. Woutersen et al. (1987) also observed lowered urine production in high obsess observation grouped in a study done on 140 wistar rats.

Weight Loss:

Observation on weight loss and growth retardation of animals of subgroup A1, A2 and B1 did not showed significant changes (Table-3) except about 33% animal showed weight loss (B1). Weight loss and growth retardation was more between (5-10 gm) in animal of subgroup C1 and C2. They showed weight loss in 60% and 100% respectively. Thus, it is showing hazardous effect on general health of individual due to chronicity of exposure of formalin fumes. Aooelman et al (1988), Til et al (1989), Vorrueg et al. (1983) made the similar observation.

Morphological Changes:

Present study regarding morphological changes in different organ’s showed that lungs showed slight swelling in B1 subgroup (Table-4). Decreased elasticity (recoil tendency) was observed in B2 and C1 sub-group (Table-4). The animals exposed for longer duration revealed tough consistency, rounded margins and slight loss of spongy nature of lung tissue (C2) (Table-4). The animal exposed to shorter duration showed normal morphology of lungs in comparison with control group (Table-4). These finding have not been reported by any author till now.

Histological Findings:

The histological changes in lungs were the earliest due to formalin fumes. The earliest histological findings were congestion, in A2 Sub-group animal of B1 and B2 showed congestion, emphysematous changes with appearance of lymphocytes in lungs. No. of lymphoid follicle were more in B2 Sub-group. The findings were more marked in sub-group C1 and C2 (Table-5), with infiltration of macrophages, lymphocytes and eosinophils and several lymphoid follicles. The lungs of C2 Sub-group there were dilated and emphysematous alveoli with localized pneumonitis congestion with more aggregation of eosinophils (lymphocytes + neutrophils). Follicles were also seen along the bronchial. The bronchial lining in the low dose group was insignificant while in higher dose group, there has been focal hyperplasia lining mucosa.

Gross et al (1970) showed after one month of formalin inhalation massive proliferation of alveoli, and macrophages with interstitial pneumonia. Similarly Piment et al (1976), Avila (1971) also observed chronic interstitial fibrosis with formalin inhalation. Corrin & Price (1972) studied the electron microscopy of lung exposed to formalin vapour and reported increase in alveoli exposed to formalin globuler cells which are found to be type II granular pneumocytes.
Martin (1973) showed a mixed infiltrate in the inflammatory foci comprising of macrophages, eosinophils, neutrophils, and lymphocytes in alveola wall. Similar finding were noted in present study. Johnson & Ward (1974) showed that neutrophil infiltration may be a response against pulmonary injury produced by formalin vapour.

Animal of sub-group A1, A2, B1 and B2 showed normal splenic histological finding. Occasional black pigmentation and congestion with normal white pulp were noted in animal (increased red pulp) (increase cellularity) with slight reduction in white pulp area were noted down in C2 sub-group (Table-5) Vorgeo et al (1993) also demonstrated increased cellularity of spleen. No abnormal Histological finding observed in thyroid gland.

V. Conclusion

From the present study this is evident that the formalin has a definite damaging effect on general health, behavior of animals and at the histological level.

1. Behavioral changes were noted almost in all sub-groups which were indicating the acute response of animals. Besides these behavioral changes in some of the sub-group which were exposed for longer duration showed paradoxical behavior as was not expected due to exposure, indicated towards the environmental adaptation of animal up to certain extent.

2. Feeding habits were also compromised in animals of longer duration. Though weight loss was not remarkable finding but general body health like weak and yellow fur, Keratinization of skin and decreased urine output were the common presentation.

3. Morphological changes in Thyroid gland did not showed any kind of change in any sub-group.

4. Principal organ of impact were lungs. They showed changes even in animals who were exposed for shorter duration. The lung showed lymphoid hyperplasia with follicle formation with mild to moderate emphysematous changes. Parenchymal epithelium in short duration group dis not showed any change while on prolong exposure occasional area of hyperplasia of bronchial lining with seen. Increase in alveolar macrophages with increased interstitial stroma is also seen in these cases. Spleen was the organ affected least and thyroid was the least.

Thus it can conclude that formalin can cause a deleterious health derangement both on acute as well as chronic exposure. The dose and duration of exposure are also important. Very higher doses over shorter duration may kill the individual. Lesser doses for longer period, may cause permanent disorganization of general health especially, respiratory function, blood gases (due to diffusion defect).

Finally the lung shows chronic pulmonary restrictive disease like pattern. In total formaldehyde (formalin) has a potential for, increased mortality and morbidity. Hence there must be adequate precaution during it’s use and some legislation are working in that atmosphere for a long period. The Brazilian legislation has considered formaldehyde (formalin) as a definite environmental pollutant and fixed its threshold limit value (TLV) up to 1.9 ppm for 8 hr. a day for 5 days in a week.

Bibliography


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