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EFFECT OF FORMALIN EXPOSURE IN THE LIVER, KIDNEY AND SPLEEN OF ALBINO RATS: A MORPHOLOGICAL AND HISTOLIGICAL STUDY

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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.

KEYWORDS: rhinorhoea, Lungs and Thyroid gland.

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 fatalperiod ranges from 1-2 days.

Patient died due to formalin intake. On autopsy, shows that gastric mucosa is red, inflamed and eroded with extravasation 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 inflamed.

The present study is based on fact that the formalin is being used universally in various fields. 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.

MATERIAL AND METHOD

In present study, 100 Albino rats have been used. Average weight of them was 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 furtherinto two subgroups as follows.

In present study, SEX wasnot considered during observation.

S.NO.	GROUPS	SUBGROUPS	NO OF ANIMALS PER SUBGROUP
1	A	A_1	15 Animals
		A_2	15 Animals
2	B	B_1	15 Animals
		B_2	15 Animals
3	C	C_1	15 Animals
		C_2	15 Animals
4	D	10 Animals in control group.	

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.

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.

S NO	SUBGROUP	DURATION OF EXPOSURE (IN HOURS/DAY)
1	A_1	Exposed 3 hr/day for one month
2	A_2	Exposed 6 hr/day for one month
3	B_1	Exposed 3 hr/day for two months
4	B_2	Exposed 6 hr/day for two months
5	C_1	Exposed 3 hr/day for three months
6	C_2	Exposed 6 hr/day for three months
7	D_1	Control group,Not exposed to formalin

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 1st, 2nd and 3rd 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 step by step as follows;

DEHYDRATION
WAX IMPREGNATION
BLOCK PREPARATION
MICROTOMY
DEWAXING OF SECTION
STAINING
MOUNTING OF SLIDES
MICROSCOPY
And finally PHOTOGRAPHY

OBSERVATION

TABLE -1PARAMETER: BEHAVIOURALCHANGES

SUB – GROUPS					
A1	**A2**	**B1**	**B2**	**C1**	**C2**
RESTLESSNESS ON FRESH EXPOSURE FOR 20 MINUTES UNCOORDINATED LOCOMOTION INCREASED RESPIRATORY RATE URINATED ON FRESH EXPOSURE	RESTLESSNESS ON FRESH EXPOSURE FOR 20 MINUTES UNCOORDINATED LOCOMOTION INCREASED RESPIRATORY RATE URINATED ON FRESH EXPOSURE	RESTLESSNESS FOR ABOUT 20 MINUTES LESS EXITED INCREASED RESPIRATORY RATE DO NOT URINATES ON EXPOSURE	RELATIVELY LESSER RESTLESSNESS RESPIRATORY RATE INCREASED MORE SLUGGISH BEHAVIOUR	LESS RESTLESSNESS RESPIRATORY RATE INCREASED SLUGGISHLY ACTING UNCOORDINATED LOCOMOTION	RESTLESSNESS WAS OF LESSER DEGREE INCREASED SLUGGISHNESS DIFFICULTY IN BREATHING

TABLE -2 PARAMETER: GENERAL HEALTHCHANGES

SUB – GROUPS					
A1	**A2**	**B1**	**B2**	**C1**	**C2**
NO SKIN CHANGES FEEDING HABITS ALTERED ONLY AT TIME OF EXPOSURE URINE OUTPUT NORMAL	NO SKIN CHANGES ALTERED FEEDING HABITS URINE OUTPUT NORMAL	NO SKIN CHANGES TRANSIENT CHANGE IN FEEDING HABITS 8 ANIMALS SHOWED REDUCED URINE OUTPUT REST WERE NORMAL	SLIGHT DISCOLOURATION THICKNESS OF SKIN OF FORE LIMB AND HIND LIMB WERE NOTED FEEDING REDUCED REDUCED URINE OUTPUT IN 10 ANIMALS	DISCOLOURATION OF FUR WITH TENDENCY OF FALL EASILY ON SLIGHT MANUAL MANIPULATION FEEDING REDUCED MAREDLY NOT RESTORED ON REMOVAL OF FORMALIN REDUCED URINE OUTPUT IN ALL ANIMALS	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

TABLE – 3 PARAMETER: WEIGHT

SUB – GROUPS					
A1	**A2**	**B1**	**B2**	**C1**	**C2**
NO WEIGHT LOSS	NO WEIGHT LOSS RECORDED	WEIGHT LOSS BY GMS. IN ANIMALS	WEIGHT LOSS BY 10 GMS. IN 9 ANIMALS REST 6 ANIMAL SHOWED NO WEIGHT LOSS	WEIGHT LOSS BY 15 GMS. IN ALL ANIMALS	WEIGHT LOSS BY 15 GMS. IN 8 ANIMALS AND 20 GMS. IN 7 ANIMALS

TABLE – 4 PARAMETER: MORPHOLOGICALCHANGES

PARAMETERS	SUB – GROUPS					
	A1	**A2**	**B1**	**B2**	**C1**	**C2**
LIVER	NORMAL MORPHOLOGY	NORMAL MORPHOLOGY	3 ANIMALS SHOWED SLIGHT YELLOWISH AREA ON LIVER SURFACE	SLIGHT MARGINAL SWELLING IN LOBES OF LIVER	SLIGHT HEPATOMEGALLY IN 6 ANIMALS FIRM CONSISTENCY IN ALL ANIMALS	FIRM CONSISTANCY HEPATOMEGALLY IN ALL ANIMALS
KIDNEY	NORMAL MORPHOLOGY	NORMAL MORPHOLOGY	NORMAL MORPHOLOGY	NORMAL MORPHOLOGY	RADISH PINK APPEARANCE IN KIDNEY'S OF ALL ANIMALS	REDISH PINK APPEARANCE IN KIDNEY'S OF ALL ANIMALS INCREASED SIZE OF KIDNEY IN 6 ANIMALS
SPLEEN	NORMAL MORPHOLOGY	NORMAL MORPHOLOGY	NORMAL MORPHOLOGY	NORMAL MORPHOLOGY	SLIGHT SPLENOMEGALY IN 8 ANIMALS REST SHOWED NORMAL SPLENIC MORPHOLOGY	APLENOMEGALY IN 8 ANIMALS OBLITERATION OF MARGIN IN 10 ANIMALS FIRM CONSISTANCY AND DISCOLOURATION IN 2 ANIMALS

TABLE – 5 PARAMETER: HISTOLOGICALCHANGES

PARAMETERS	SUB – GROUPS					
	A1	**A2**	**B1**	**B2**	**C1**	**C2**
LIVER	NORMAL MICROSCOPIC FINDING	SLIGHTLY CONGESTION DILATED CENTRAL VEIN	DILATED AND CONGESTED CENTRAL VEIN AND SINUSES AND PORTAL AREA	DILATED AND CONGESTED CENTRAL VEIN WITH FATTY CHANGE (FIGURE-3)	GENRALISED CONGESTION AND DILATED CENTRAL VEIN WITH FRESH BLOOD SLIGHT MILD FATTY CHAGE DISORGANISATION AND OCCASIONAL COLAPSED AREA AND BALOONING DEGENERATION (FIGURE-1)	FATTY CHANGE OF CELLCYTOPLASM WITH MARKED CONGESTION AND GENERALISED MILD COLAPSE OF LABULAR ARCHITECTURE (FIGURE-2)
KIDNEY	NORMAL MICROSCOPIC FINDING (FIGURE-6)	MILD CONGESTION	MILD CONGESTION (FIGURE-5)	MILD CONGESTION	CONGESTION AND PELVIS SHOWS MONO NUCLEAR INFILTRATION (FIGURE-4)	CONGESTION WITH LYMPHOCYTIC INFILTRATION
SPLEEN	NORMAL MICROSCOPIC FINDING	NORMAL MICROSCOPIC FINDING	NORMAL MICROSCOPIC FINDING	NORMAL MICROSCOPIC FINDING	CONGESTION BLACK PIGMENTATION NORMAL WHITE PULP (FIGURE-8)	MARKED CONGESTION RED PULP WHITE PULP SLIGHTLY REDUCED (FIGURE-7)

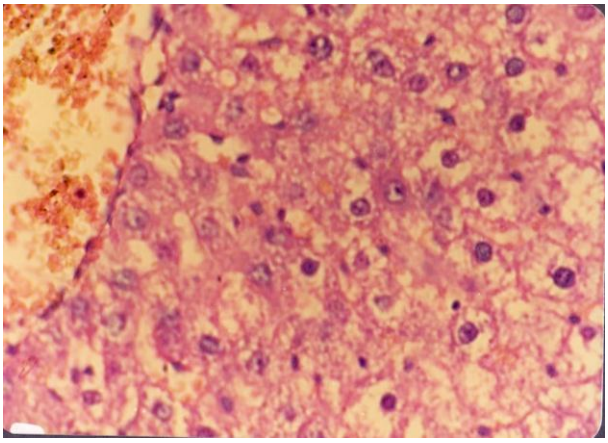

Figure 1: Dilated and congested central vein, ballooning degeneration with mild fatty changes at periphery in liver of exposed albino rat. (High power 40X, H&E)

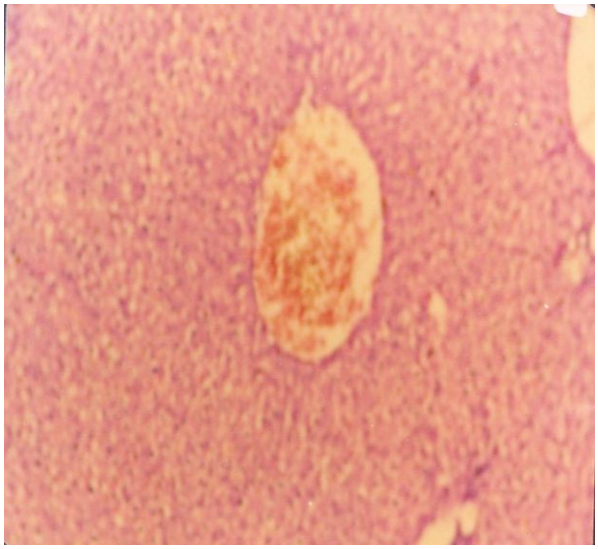

Figure 2: Mild Fatty changes, dilated central vein and slight disorganization in liver of exposed albino rat. (Low power 10X H&E)

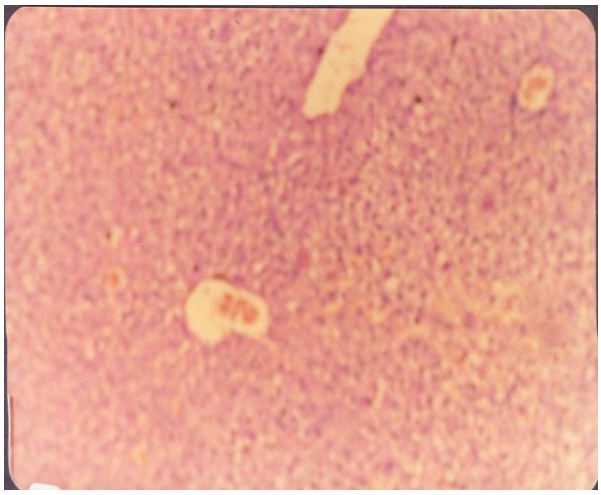

Figure3: Fatty changes, slight collapse and congestion in liver of exposed albino rat. (Low power 10X H&E)

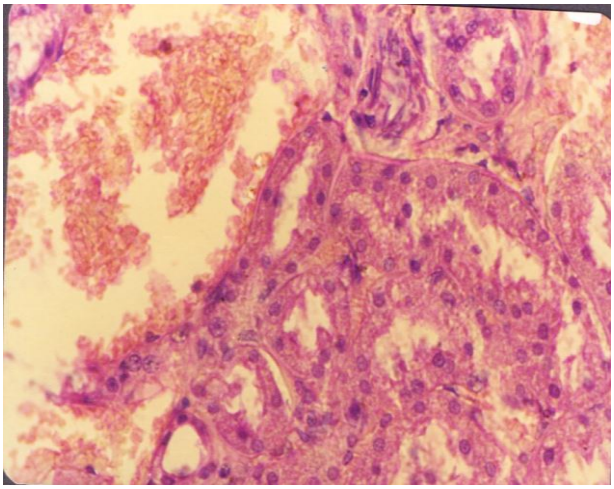

Figure4: Congestion and hemorrhages in kidney of of exposed albino rat. (High power 40X, H&E)

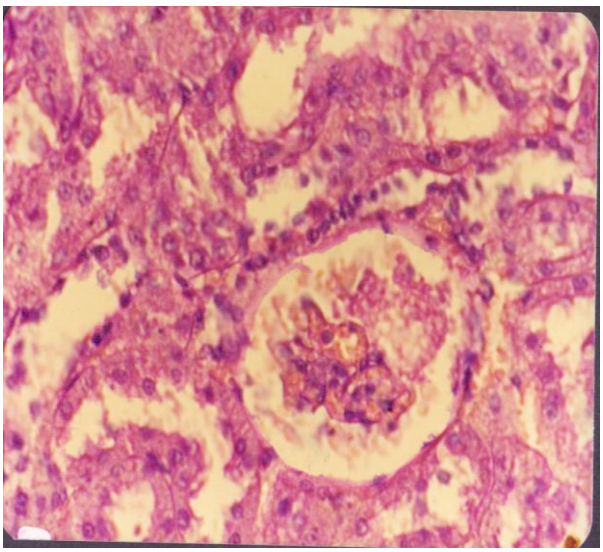

Figure5: Congestion in kidney of of exposed albino rat. (High power 40X, H&E)

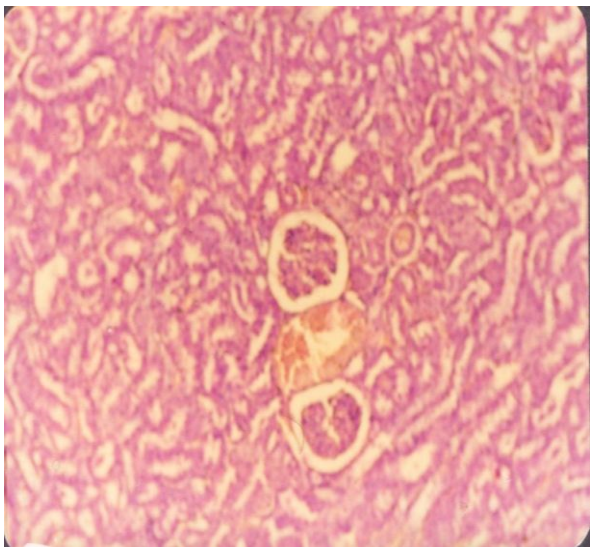

Figure6: Normal histological picture of kidney of Control group albino rat. (Low power 10X H&E)

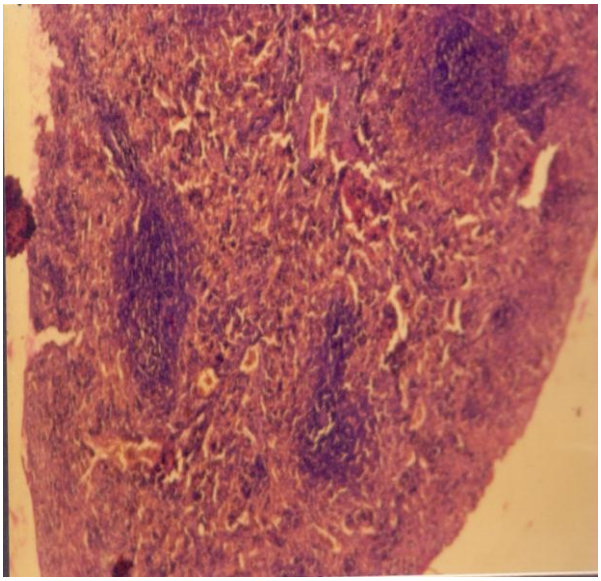

Figure 7: Congested spleen showing increased red pulp and slightly reduced white pulp in exposed albino rat. (Low power 10X H&E)

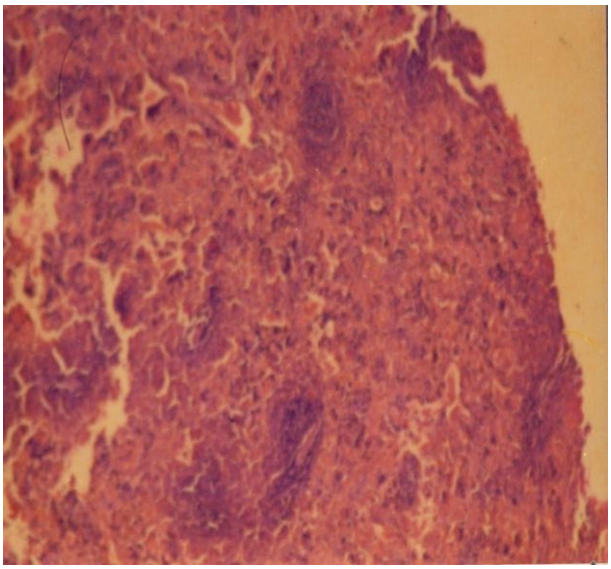

Figure 8: Congested spleen exposed albino rat. (Low power 10X H&E)

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).

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 C2subgroups) also showed such type of restlessness. However, it is relatively of lesser degree but their duration of tachypnoea (increased respiratory rate) was increased and after settling they become sluggish. Similar observation has been reported by SALDIVA et. al. (1985). (Table-1) 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 beaker (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. They have also observed relative increase in kidney weight in higher dose animal which was not seen in our group.

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). Bruze M et al. in (1985) studied the effect of phenol-formaldehyde resins and observed contact allergy with production of contact dermatitis but their feeding habit reduced were not restored to normal even after removal of formalin. These animals showed sign of loss of apetite and there was significant oliguria in 60% animal Aooelman et al (1988) has also found adverse effect of formaldehyde in higher dose group manifestead 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 show 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), Vorrgue et al. (1983) made the similar observation.

MORPHOLOGICAL CHANGES

Present study regarding morphological changes in different organ's in question 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.

Liver showed earlier changes in morphology like, there were yellowness of some area of liver surface (B1 subgroup) (Table-4), slight marginal swelling of lobes of liver (B2 subgroup) (Table-4). Hepatomegaly was observed in 45% animal of C1 sub-group with slight firm consistency. Hepatomegaly and firm consistency of liver was noted in all animals of C2 sub-group, similar has been reported by Vorguo et al. (1993).

Kidney was the organ affected only in sub-group C1 and C2 i.e. animal exposed to longer duration (3 hours & 6 hours in a day for 3 months). There were finding of reddish pink appearance of Kidney in animal of C1 sub group and increase size of Kidney in 45% animals (Table-4) Wauterson et. Al. (1989 reported relative increase in Kidney weight in female rates exposed for high doses of formaldehyde Kidney of rest of the animal sub-group were found normal as reported by Vorguo et. al. (1993).

In the present study splenomegaly was found in C1 (Table-4) in about 50% animals with obliteration of margins in 50% animals, firm consistency and sligh discoloration in two animals. It is suggested that spleen was affected only in animals that were exposed for longer duration. Splenic Vorguo et. al. (1993) reported the same finding. No Morphological abnormality could be detected thyroid glands.

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 fallicle were more in B2 Sub-group. The findings were more marked in sub-group C1 and C2 (Table-5), with infiltration of macrophages, lumphocytes 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 Pement 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 globular 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 alveoli 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.

Liver findings in present study were slight congestion and dilatation of Central Vein in A2 Sub-group (Table-5) Fig.). In Sub-group B1 and B2 there were dilated and congested central vein with Generalized congestion of sinuses with fresh blood, slight disorganization and occasional collapse area were noted in animal of C1 and C2 sub-group (Table-3). Mild fatty change of cell cytoplasm, ballooning degeneration, mild collapse of lobular architecture was noted down in C1 and C2 sub-group. Present finding telly with findings of Johns On & Ward (1974), Sharon (1979), Bories et al (1985), Woutersen et. al (1987).

Histological changes, in Kidney were late and except mild congestion which appeared late in animals of A2, B1 and B2 sub-group. Congestion and mononuclear infiltration in pelvis area were noted in animals of sub-group C1 (Table-5). Marked congestion with occasional lymphoid aggregation were note in animal exposed to longer duration C1 and C2 sub-group. Similar finding have been reported by Richet et al (1987), Aooelman (1988) and Till et. al (1989).

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.

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. Behavioural changes were noted almost in all sub-groups which were indicating the acute response of animals. Besides these behavioural 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, out put were chronically exposed to formalin as histologically Kidney appeared to be normal. This could be attenuated.
3. Morphological changes in Kidney, liver spleen and thyroid were not very significant. However hepatomegaly and splenomagely were noted in animal exposed for longer duration. This indicates formalin has harmful effect on these organs if they are subjected to expose for chronic period. Thyroid did not show 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 din 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 less 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. Liver metabolism may hamper permanently with total or partial loss of its synthetic property. As liver is a principal and most vital organ of the body, It's functional as well as anatomical changes may lead to increased morbidity and mortality of animals. Finally life span and PQLI (Physical quality of life index) maybe decreased markedly.

Involvement of Kidney will lead to the gross electrolyte imbalance in body and later on it will affect normal blood biochemistry, oncotic functions of blood. Reduced urine production of longer duration indicates susceptibility of animal towards the development of chronic renal failure. Splenic enlargement, though it was relatively of lesser degree, shows increased tissue destruction and increased lymphoid activity and increase in red pulp due to congestion.

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.

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