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Histopathological Changes in Kidney of Hatched Chicks on Exposure to Vapors of Different Concentration of Embalming Fluid during Incubation

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Abstract: *The study is aimed at observation of any teratogenic /adverse effects upon histology of the chick kidney when exposed to two different embalming fluid vapors containing different concentrations of formalin during their incubation period (21 days). 100 fresh fertilized eggs of Gallus gallusdomesticus were taken and divided in to three groups. group I contained 20 eggs as control group, not exposed to any embalming fluid but allowed to incubate and hatch in normal conditions. Group II and group III having 40 eggs each were exposed to vapors of embalming fluid containing 106 mmol/lt of formalin and 53mmol/litre of formalin respectively. The hatchability rate of Group I, II and III was 60%, 42.5% and 52.5% respectively. On dissecting the kidney of all 3 groups, no gross pathological changes were observed but on histopathological examination following pathological changes were observed. Glomerular enlargement, glomerular congestion, mesangial proliferation and increased bowman's space were maximum in group II followed by Group III. tubular changes like cystic dialatation and cloudy swelling in Proximal convoluted tubules were observed in group II and III but more prevalent in group II (17.64% and 41.17% respectively) . Diffused necrosis in group II was seen in 58.82% of cases while focal necrosis was observed in 38.09% cases in group III. Therefore it is concluded that embalming fluid containing more concentrations of formalin is nephrotoxic even in vapors form.*

Keywords: *Formalin, Embalming fluid, chick Kidney, Hatching, Teratogenic*

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I. Introduction

Embalming is a means of artificially preserving the dead human body to maintain as far as life like state and normal relationship of human anatomy. The embalming fluid consists of a combination of chemical substances that include preservatives, germicides, buffers, wetting agents, anticoagulants, dyes, vehicles, perfuming agents etc.(Azmani 1998).Although formaldehyde is an important constituent of embalming fluid, but its toxic effects are also well known due to its vapors. The side effects depend upon the duration of exposure and the concentration used (Dixit D 2008, Bedino JH 2004). It crosses the placenta and enters the fetaltissues, however effect of embalming fluid on human fetus is yet to be reported(Messier 1984). Therefore there was a need to evaluate the effect ofvapours of embalming fluid on kidney of experimental animal to assure its effects so that later these facts can be confirmed on mammalian model. With this aim present study was conducted on the developing chick embryos as it lacks placenta, so that it could be exposed freely and more easily than a mammalian embryo to a gaseous chemical agent (Magras I.N. 1996). Kidney is chosen as organ under study because chick kidney develops from metanephros and metabolism of formaldehyde is through kidney (Johnson OW, Muggas JN 1970). A close embryological association with human kidney further justified the study. Hence the study was aimed to observe & compare the effects of embalming fluid with variable concentration of formalin.

II. Materials And Method

Present study was conducted on 100fresh fertilized eggs of Gallus gallusdomesticus. Prior permission was obtained from the institutional animal ethical committee. Eggs were procured from a registered govt hatchery.Candeling was done on every third day to confirm fertilized eggs & their further development. Eggs not fulfilling the criteria of being fertilized on candeling, cracked or damaged eggs, too small or too large eggs were excluded from the study. The eggs were divided in to 3 groups, group I 20- eggs, group II- 40 eggs, group III- 40 eggs. Eggs from all 3 groups were taken and incubated for 21 days in manual incubator for hatching. During incubation ideal conditions of temperature (98.6-100.4^0 F), humidity(50-55%)) and eggs turning were maintained.(Hubbard incubation guide 2012)

During incubation eggs of group II &III were exposed to vapous of two embalming fluids with different composition (table 1) (O,SullivanE,Mitchell BS 1993). After hatching chicks were sacrificed and kidneys were dissected, processed and histological slides were prepared using hematoxylin and eosin stain. Slides of both the control and exposed groups were observed under light microscope and changes in different parts of kidney were noted and microphotographs were taken. The Histological changes of all three groups were confirmed by 2 senior pathologists and findings of all 3 groups were noted and compared.

III. Result

The hatchability rate of eggs was 60%, 42.5% and 52.5% in group I, II and III respectively. Glomerular changes like glomerular enlargement and hypercellularity,glomerular congestion, mesangialproliferation and increased bowman's spacewere more common in group II &III. However changes were more pronounced in group II(table 2 fig-5.4,5.9-----) Glomerular congestion was significantly higher in group II ((table 2 &5)Tubular Changeslike cystic dilatation and cloudy swelling in Proximal convoluted tubule were more common in group II (table 3 fig—5.10-----). Changes like vessel wall odema& thickening, formation of lymphoid aggregate and mononuclear infiltration were although observed in all three groups however changes were more frequent in group II (table 4 &5 fig -5.11+5.9----). Diffuse necrosis was significantly more prevalent in group II. While focal necrosis of nephrons was more frequently seen in group III (table no 4 , 5

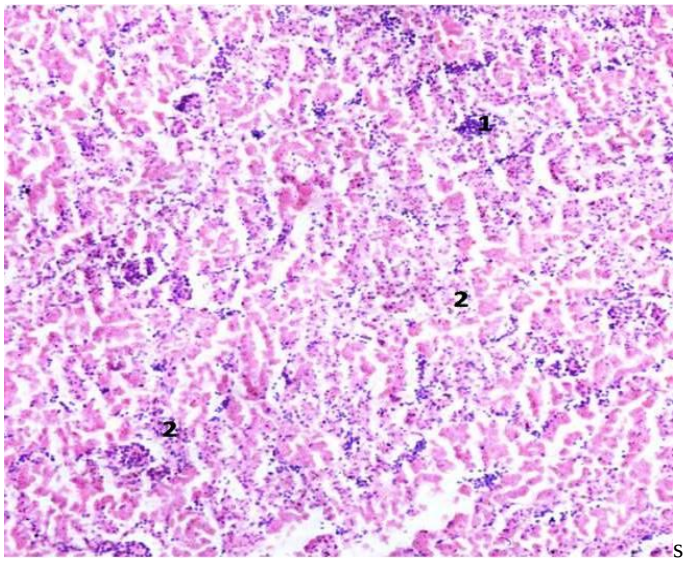

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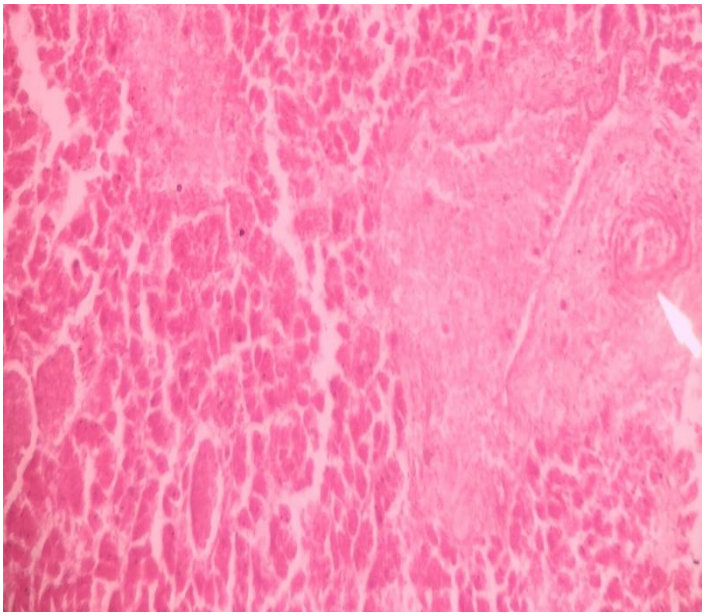
Fig 5.11



Fig=5.8

IV. Discussion

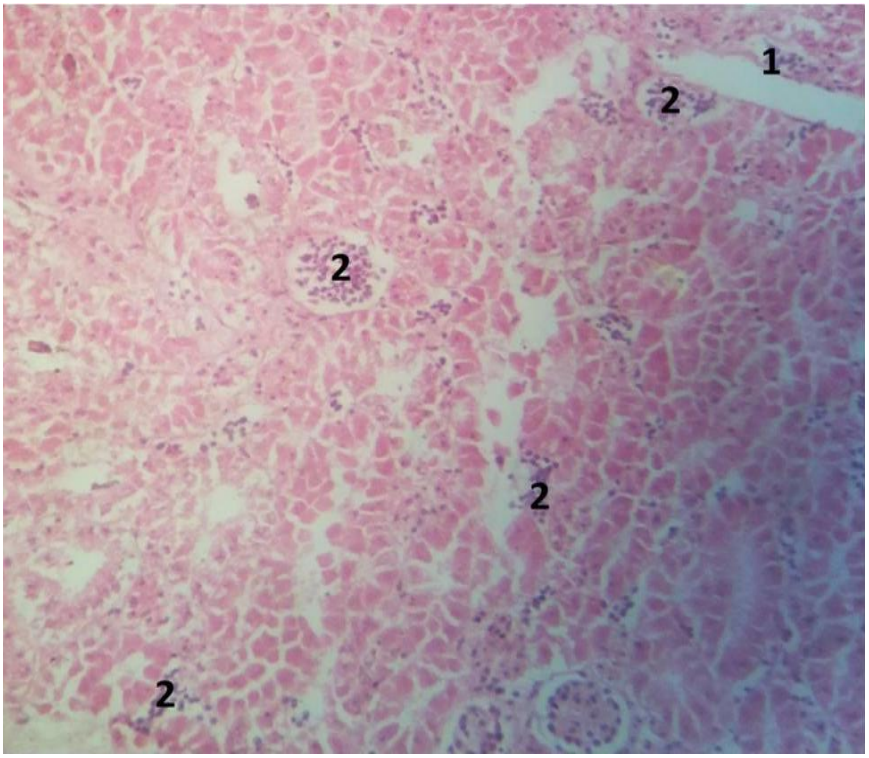


Fig-5.12

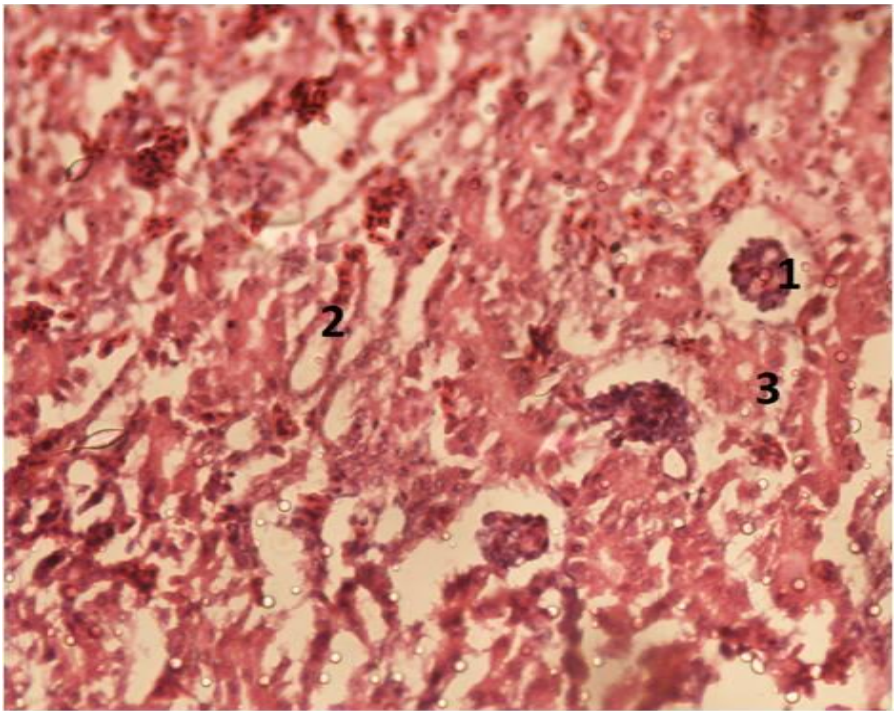


Fig 5.10The kidney of chick is metanephric in adults as is that in mammals.Chick kidney consists of three divisions cranial, middle and caudal lobes, and each lobe consist of smaller lobes. There are three distinct types of nephrons viz cortical, medullary and juxtamedullary. The cortical nephrons, are more numerous and are arranged on periphery and known as Reptilian type of nephron and they lack loop of Henle.While medullary nephron or Mammalian type have loop of Henle in them. Medulla of kidney consist of loop Henle and ProximalConvoluted tubule and distal convoluted tubules**.PCT** are lined with cubical epithelium. The medullary ducts run in large parallel tracts and are bound together by a thin connective tissue sheath, the whole assembly being termed the medullary cone (Johnson OW, Skadhauge E. 1975)All previous studies done on

chick embryo show effect of formalin and were done in poultry form setup to see the effects of fumigation on eggs but present study has been taken up to observe the effects of vapors of formalin containing embalming fluid on developing chick embryo. Embryogenesis of chick kidney is similar to mammals/humans and it lacks placenta so it can be exposed to embalming fluid vapors easily.

In chick kidney glomerular, tubular andother parenchymatouschanges were observed more in group II. In the present study, hatchability rate has decreased to 42.5% and 52.5% in group II &III.respectively. So we can conclude that increasing concentration of formaldehyde has a definite effect on the hatchability of the eggs. There are reports of a significant decrease in hatchability and embryonic mortality with higher duration and concentration of formaldehyde fumigation(Eliboletal 2003). Glomerular enlargement and hypercellularity were significantly higher in Group II when compared with Group I (p value = 0.0025). Although difference was not significant in Group III with respect to Group I and II (Table 5). Glomerular congestion and mesangial proliferation were seen in significantly higher number of cases in Group II in comparision to Group I and III. (table2)), though there was increase in Bowman's Space in Group II and III, but the difference was not significant (Table 2&5). The study done by Santos RF,Dias HM,Fujimoto RY(2012) have also reported increase in the Bowman's Space as one of the histopathological changes in the fish kidney when exposed to formalin. Degeneration of PCT, DCT and Collecting ducts were more prevalent in Group II and III. (Table 4). Similar changes have been reported by Bansal(2000) and Santos(2000) and they have reffered these as early ischemic changes. Inflammatory infilteration was seen in the form of mononuclear infilteratesand lymphoid aggregates was significantly more in group II (table 4). In the present studythe vascular changes like vessel wall edema & thickening were more prevalent in group II &III (table 4). These vascular changes can be due to formalin toxicity asBansal N,.et al (2011) and Santos et al2012 have also reportedformalin toxicity as the causative factor of marked vascular congestion. Diffused necrosis was seen in all three groups but it was seen only in one case in Group I. However, diffused necrosis was significantly higher in Group II even when compared with Group III (Table 4 &5) whereas, focal necrosis was more prevalent in Group III (38.90%). Hypoxia is one of the inducer of inflammatory response but prolonged exposure to toxic agents causing hypoxia can lead to chronic inflammatory response. Hence presence of inflammatory response in Group II and III in the present study can be explained on the basis of hypoxic injury caused by formalin vapors in embalming fluid. At the same time, diffused necrosis in Group II and focal necrosis in Group III are irreversible effects of ischemic injury. These changes are in accordance with the Fischer (1905) et alwho has reported acute degenerative changes in kidney of Guinea Pig,, 6-8 hours after administration of 3-6 cc of 0.1% formalin in the peritoneal cavity. Group II and Group III in present study also have shown degenerative changes but to a lesser extent in comparison to study done by Fischer et al. This difference is probably because of method of administration of formalin,as he injected formalin intraperitoneally while in present study there was diffusion of vapors through eggshell leading to effects on chick kidney. The above mentioned histopathologic changes were observed in chick kidney in the present study are collectively in accordance with the study done by Singroha et al(2012) who has seen the histopathological effects of Gentamycin, a known nephrotoxic agenton chick kidney This similarity, further strengthens the fact that formalin has got nephrotoxic potential. Formaldehyde is readily absorbed from the respiratory tract following inhalationand also from GI mucosa. It is a known local irritant and is notorious for causing vascular congestion in mucosa. After absorption it is rapidly distributed to all the tissue, which can explain presence of inflammatory response like vessel wall edema and thickeningin kidney of group II and group III. After absorption it is oxidized to formate and carbon dioxide in all the tissues of the body. Formate is quickly removed by the supporting blood supply of the region and it has been shown in rats that it is mainly excreted through kidney and CO_2 is removed through lungs and nominally in faeces(Upreti RK, Farooqui MY, Ahmed AE, Ansari GA 1987).

Formaldehyde as such is known to have toxic effects on humans as well as animals if used in higher concentration. Since it is nephrotoxic to developing chick it can also affect human fetus if a pregnant woman is chronically exposed to formalin vapors. Therefore it is suggested that embalming fluid with low formalin concentration should be used for embalming to reduce side effects not only in embalmers but also among Anatomy teachers and students, as they are constantly exposed to vapors of formalin in embalming fluid.

(Coleman&, Kogan 1998) have reported that low concentration of formaldehyde in embalming fluid can also give effective embalming results. They introduced a new embalming mixture with a0.50 to 0.75%.. formaldehyde and a very high salt content . In order to confirm the efficacy of the embalming, necropsy samples of a wide range of tissue and organs were taken for histological examinationNo indication of any fungal growth was observed. It proves that even with low formalin concentration excellent embalming qualities can be maintained without compromising health.

V. Conclusion

Therefore, it is concluded that kidney of chick shows hypoxic degnerative and necrotic changes when exposed to formalin containing embalming fluid during incubation. However these changes are less marked if

formalin concentration is decreased as in Group III. Therefore it is recommended that embalming fluid containing lesser formalin should be used which can also give effective preservation of cadaver as well as safety of the embalmers.Table 1: Composition of Embalming Fluids

Contents	Standardized Embalming Fluids	Experimental Embalming Fluid
Formaldehyde	106ml/L	53ml/L
Industrial Methylated Spirit	425ml	625ml/L
Distilled Water	248ml/L	48ml/L

Table 2: Glomerular changes in kidney as seen by light microscopy:

Group (n)	Glomerular enlargement and hypercellularity (%)	Glomerular Congestion (%)	Mesangial proliferation (%)	Increased bowmans space (%)
I(12)	(16.7)	(16.7)	(0)	(0)
II(17)	(76.4)	(78.98)	(47.05)	(17.65)

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III(21)	(52.38)	(52.38)	(38.09)	(19.04)

Table 3: Changes in proximal convoluted tubules in the kidneys as seen by light microscopy

Group (n)	Cystic dilatation n(%)	Cloudy swelling n(%)
I (12)	1 (8.3)	1 (8.3)
II (17)	3 (17.64)	7 (41.17)
III (21)	2 (9.5)	5 (23.81)

Table 4: Other changes in the kidneys as seen by light microscopy

Group (n)	Infiltration by mononuclear cells (%)	Vessel edema and thickening (%)	Formation of lymphoid aggregates (%)	Diffuse necrosis of the nephron(%)	Degeneration of DCT,PCT and CD (%)	Focal necrosis of the nephron (%)
I (12)	(16.7)	(16.7)	(0)	(8.33)	0 (0)	0 (0)
II(17)	(94.11)	(88.23)	(41.17)	(58.82)	8 (47.05)	3 (17.64)
III(21)	(66.66)	(76.19)	(38.09)	(23.81)	9 (42.85)	8 (38.09)

Table 5: p-values related to the comparison of histological changes in kidneys of the Group I, II and III.

Entity	**Changes**	**Group I vs Group II**	**Group I vs Group III**	**Group II vs Group III**
Glomerular changes	Glomerulus enlargement &hypercellularity	**0.0025**	0.0672	0.1812
	Glomerulus congestion	**0.0003**	0.0672	**0.0336**
	Mesangial proliferation	**0.0089**	**0.0299**	0.7430
	Increase bowmans space	0.2463	0.2713	1.000
Proximal convoluted tubules	Cystic dilatation	0.6221	1.000	0.6396
	Cloudy swelling	0.0926	0.3792	0.3073
Other changes	Infiltration by mononuclear cells	**0.0001**	**0.0118**	**0.0481**
	Vessel edema and thickening	**0.0003**	**0.0014**	0.4267
	Basophilic/Eosinophilic structure	**0.0280**	0.1329	0.4910
	Formation of lymphoid aggregates	**0.0230**	**0.0299**	1.000
	Degeneration of DCT,PCT and CD	**0.0089**	**0.0122**	1.000
	Diffuse necrosis of the nephron	**0.0080**	0.3792	**0.0458**
	Focal necrosis of the nephron	0.2463	**0.0299**	0.4524

sP<0.05 – Statistically significant

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