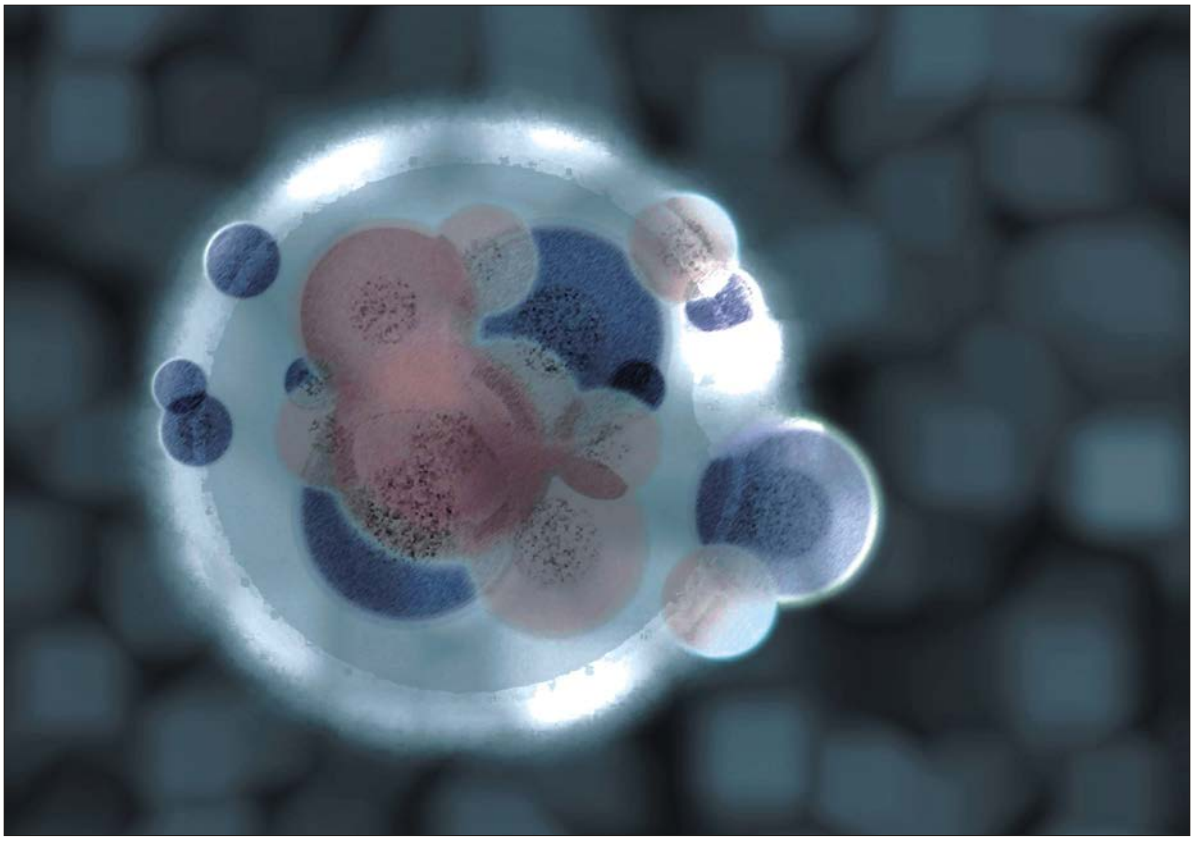

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Histopathology of Toxicity Induced in Broiler Chickens by Excess Sodium Compounds

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Abstract: An experimental study was conducted to investigate the effects induced in broiler chickens by excess sodium from different compounds, namely sodium chloride (SC), sodium bicarbonate (SB) and sodium phosphate (SP), added to the drinking water. Higher and lower doses of the these compounds were administered to the treated birds. All the high-dosed birds died during the first 10 days post-treatment. Histological examination revealed significant myocardial alterations in all groups, however these were more pronounced in the SC-group while SB-and SP-groups showed marked renal changes. Erythrocyte deformability was decreased in all treated birds and probably was related to the increased mean corpuscular volume (MCV) of erythrocytes.

Key words: Broiler chickens, sodium, toxicity

INTRODUCTION

Different sodium compounds such as sodium chloride[1-5] and sodium bicarbonate[6-9] have been used experimentally to induce disease conditions in broiler-chickens. Also, sodium overdoses from sodium citrate, sodium iodide, sodium sulphate and sodium hydroxide have been shown to produce edema in birds[6]. In natural cases of edema diseases of birds, salt toxicity is also suspected[10]. The toxicity of these compounds is attributable to their sodium content which is the toxic ion in salt toxicity[4,11]. It has been found that deleterious effects of sodium bicarbonate toxicity is similar to that of salt poisoning[8,12].

Reduced erythrocyte deformability contributes to the development of disease manifestations in salt poisoning or sodium bicarbonate toxicity[5,9]. Also, alterations of the cardio-pulmonary hemodynamics have a role in development of these manifestations[13,14] .

In the present study pathological changes induced in broiler chickens by salt poisoning, as well as by sodium bicarbonate and sodium phosphate toxicities are investigated from the pathological aspects in an attempt for more understanding of the relevant pathogenic mechanisms.

MATERIALS AND METHODS

Experiment 1: A total of 40 male, day-old commercial chicks were divided into 2 equal groups of 20 each and caged separately and during period of acclimatization they received drinking tap water and appropriate diet *ad libitum*. At one week of age sodium chloride (NaCl) (SC) was added to the drinking water at two different levels, group one had a level of 7.5 g L^{-1} drinking water and group two received a level of 15 g L^{-1}. At days 3, 7, 14, 21 and 28 post-treatment, 4 birds from each group were weighed and sacrificed. The experiment was terminated when birds were 5-week-old. Birds that died during the course of the experiment were also necropsied.

Experiment 2: Forty male, day-old commercial chicks were allotted into 2 equal experimental groups which were housed separately in cages. Drinking water and starter commercial ration were supplied *ad libitum*. When the birds were one week of age. sodium bicarbonate (SB) was added to the drinking water of both groups. Drinking water for group one contained 0.75% (7.5 g L^{-1}) sodium bicarbonate (0.20% Na^+). while that for group two contained 1.5% (15 g L^{-1}). Timing for recording body weights and sacrifice was similar to that in experiment 1. Dead birds during the experimentation period were also necropsied.

Experiment 3: Forty day-old male commercial chicks were randomly divided into two experimental groups of 15 birds each and kept separately in cages. During first week of age the chicks received drinking water and feed *ad libitum*. Sodium phosphate was added to the drinking water from day 7 at the levels of 7.5 and 15 g L^{-1} for group one and two, respectively. The remainder of experimental design was carried out as in experiment one and two.

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Three groups of 10 age-matched birds each served as control for the three experiments and received normal drinking tap water and diet without addition of sodium source. Body weights of control birds were recorded for purpose of comparison. In the three experiments blood from treated and control birds was collected. At each time interval, two age-matched controls were also sacrificed.

Hearts were removed at necropsy and RV:TV ratio was calculated by dividing the weight of RV (including the right atrioventricular valve RAV) by the weight of RV+LV (TV)[4]. RV was classified as hypertrophied (RVH) if the RV:TV ratio exceeded the value of 0.25.

Erythrocyte deformablility was determined according to the method described[9] using a vertical filtration apparatus for measurement of filtration time of 10% erythrocyte suspension passing through a polycarbonate filter with 5 μm pores. Filtration index was calculated following the formula of Mirsalimi *et al.*[5] and accordingly high index indicates lower erythrocyte deformability.

Histopathology: Tissues collected from experimental and control birds were fixed in 10% neutral buffered formalin and processed routinely for paraffin embedding, sectioned and stained with heamatoxylin and eosin (HE). Periodic acid Schiff reagent (PAS), Azan, phosphotungstic acid haematoxylin (PTAH), von Kossa's stains were applied on selected tissue sections.

Data for the experimental groups were analyzed using Student's t-tests and values were expressed as means and differences were considered significant for $p<0.05$.

RESULTS

Clinical and gross necropsy findings: Most of birds had watery feces throughout the experimentation period. Initially, body weight was increased and after that it gradually decreased with apparent anorexia. Within 10 days post-treatment all high dosed birds were died after being comatosed.

Carcasses of both dead and sacrificed birds were emaciated and severely congested with subcutaneous edema. All necropsied birds showed severely congested and edematous lungs, pale and swollen kidneys and petechial haemorrhages and excess mucus over the mucosa of proventriculus and intestine. Control birds showed no clinical signs or gross necropsy abnormalities.

RV:TV ratios and erythrocyte deformability: Erythrocyte deformability was significantly decreased in all experimental groups as indicated by the increased filtration index.

In birds that showed gross RVH, RV:TV ratios exceeded the value of 0.25 (ranged from 0.23-0.30). Compared with controls, body weights of the intoxicated birds were decreased significantly especially those birds received sodium chloride (Table 1). Significant decrease of body weights were recorded as early as day 7 of treatment. However, body weights of the treated birds were initially increased at 3 days post-treatment. RV:TV ratio was significantly increased from day 7 as manifested by SB-treated birds compared with controls. Thereafter, the increased ratio was reflected grossly as right ventricular dilation in the intoxicated birds (Fig. 1). Gross renal abnormalities indicating kidney disease in the treated birds were observed as early as day 3 and birds in SB and SP-groups manifested more severe kidney damage.

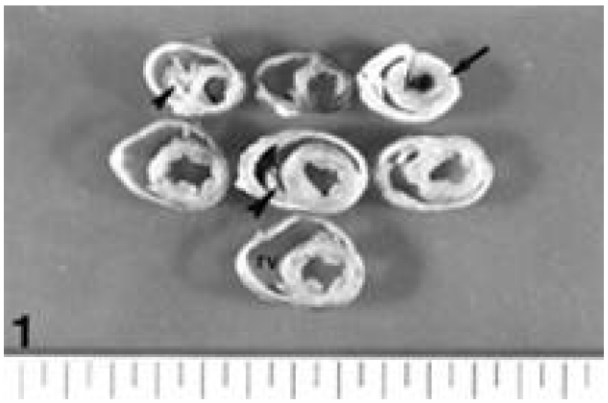

Fig. 1: Transverse sections at the level of the right atrioventricular valve of the hearts of control (arrow) and treated (all others) birds. Note the dilated right ventricle (rv) and the hypertrophied right atrio-ventricular valves (arrowheads) in the hearts of the treated birds

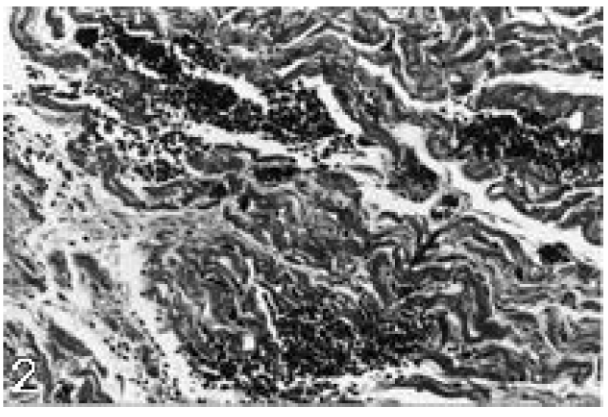

Fig. 2: Heart showing focal haemorrhages (arrows) between the disarranged myofibers. SC-treated bird at day 21 of treatment. HE.X100

Histopathology: Hearts, lungs and kidneys of the treated birds showed the most outstanding histological changes. The examined heart tissues of the treated birds showed

Table 1: Mean body weights (BW), RV : TV ratios and incidence of kidney disease in the different experimental groups at the different intervals post-treatment

	Day 3				Day 7				Day 14				Day 21				Day 28			
	SC*	SB	SP	C	SC	SB	SP	C	SC	SB	SP	C	SC	SB	SP	C	SC*	SB	SP	C
BW	155	167	150	148	250	280	260	300	540	580	565	650	830	975	940	1020	840	960	930	1130
RV:TV ratio	0.24	0.25	0.23	0.21	0.27	0.28	0.26	0.24	0.30	0.29	0.30	0.22	0.32	0.30	0.28	0.20	0.30	0.29	0.27	0.20
Kidney disease (No.)	1	1 (2)	1 (2)		1 (2)	3 (2)	1 (2)		4	3	3		4	4	3		4	4	3	

-Sodium chloride, SC; Sodium bicarbonate, SB; Sodium phosphate, SP; C, Control
-Figures in parentheses are those of high-dose groups
-Underlined figures indicate significant differences ($p<0.05$)

Table 2: The main histologic changes in the heart, lung and kidney of the treated birds expressed as mean scores

	Day 3			Day 7			Day 14			Day 21			Day 28		
Feature	SC	SB	SP	SC	SB	SP	SC	SB	SP	SC	SB	SP	SC	SB	SP
Heart															
Haemorrhages and edema	++*	++	++	+++	++	++	+++	++	++	+++	++	+++	+++	+++	+++
Disarrangement of myofibers	+	+	+	++	++	+	++	++	++	+++	++	++	+++	++	+++
Loss of cross striations[1]	++	++	+	+++	++	++	+++	++	++	+++	++	+++	+++	++	++
Myolysis	+	+	-	++	+	+	+++	+	++	+++	+	++	++	+	++
Calcification[2]	+	-	-	+	-	-	++	-	-	++	+	++	++	+	++
Lung															
Diffuse congestion and edema	++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Collapsed parabronchi	+	-	-	++	+	+	+++	++	+++	++	+	+	++	++	-
Changes of parabronchial epithelium[3]	+	++	+	++	++	++	++	++	+++	+++	++	++	+++	++	+
Changes of blood vessels[4]	+	++	+	++	++	++	++	++	++	+++	++	++	+++	++	++
Kidney															
Congestion and edema	++	+++	+	+++	+++	++	++	+++	++	++	+++	++	+++	++	++
Tubular dilation and necrobiosis[5]	++	++	++	++	+++	++	+++	+++	++	+++	+++	+++	++	+++	++
Proliferative glomerulitis	+	+	++	+	+++	+++	++	+++	++	++	+++	++	++	+++	++
Urate deposits or urate granulomas[6]	+	+	+	+	+++	++	++	+++	++	+++	+++	+++	++	+++	+++
Calcification	+	-	-	-	++	++	++	++	+++	+++	++	+++	++	++	+++

-, Not detected, +, mild, ++, moderate, +++ marked or severe
1) Demonstrated by PTAH stain 2) Confirmed by von Kossa stain 3) Consisting of epithelial flattening or hyperplasia
4) Pulmonary arterial branches showed marked perivascular and adventitial edema, medial hypertrophy and vacuolation and endothelial cell swelling or proliferation
5) Many of the dilated tubules contained proteinaceous material which also was seen in glomerular spaces
6) Urate deposits were seen as amorphous intratubular substance, basophilic spherical crystals or feathery crystals

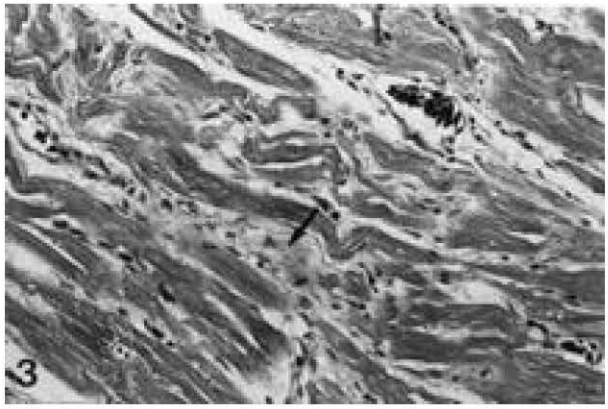

Fig. 3: Wavy myocardial fibers (arrow) showing loss of cross striations. Some myofibers have completely disarrayed myofilaments. SB-treated bird at day 21 of treatment. HE.X200

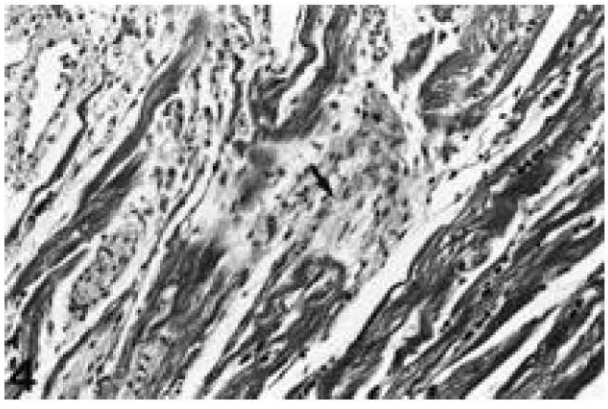

Fig. 4: Focal myolysis (arrow) in the heart of SC-exposed bird at 14 days of treatment. Note the proliferated sarcolemmal nuclei at the site of myolysis. HE.X200

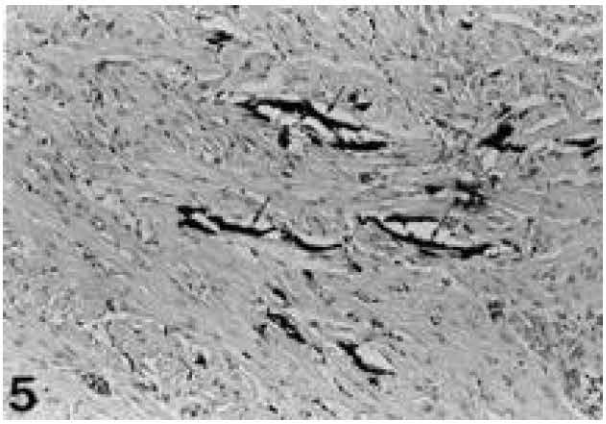

Fig. 5: Focal calcification (arrows) in the heart of SC-exposed bird at 21 days of treatment. Von kossa stain. X200

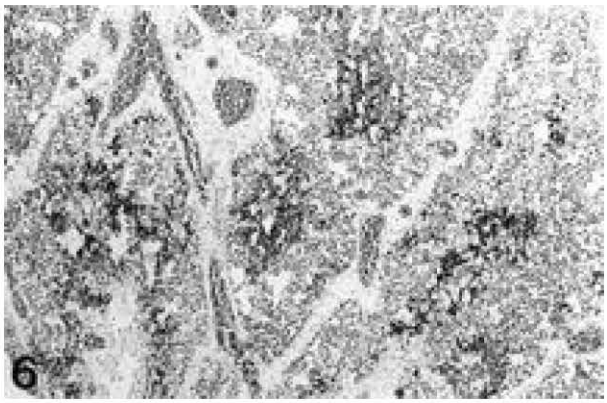

Fig. 6: Lung showing diffuse congestion and edema. All the vasculature is distended and the edematous fluid (*) apparently separates the parabronchi. HE.X100. SB-treated bird at day 14 of treatment

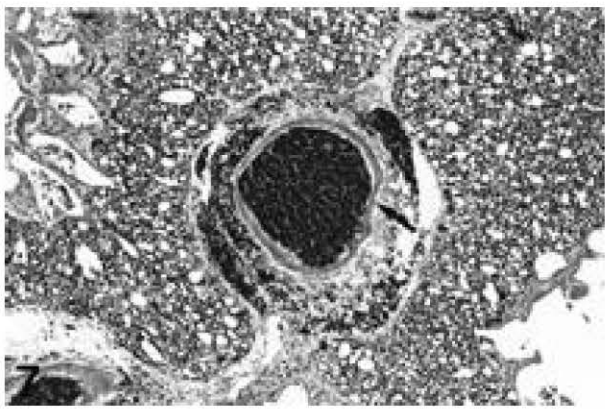

Fig. 7: Markedly distended pulmonary arterial branch (arrow) in the lung of SC-treated bird showing severe congestion. Note the perivascular and adventitial edema. Day 21 of treatment. HE.X100

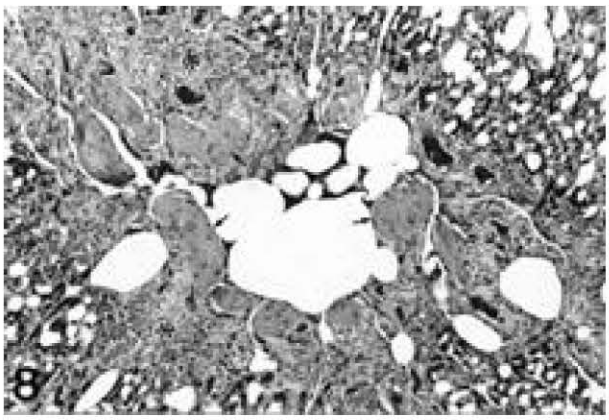

Fig. 8: Marked hypertrophy of parabronchial muscles (arrows) in the lung of SB-exposed bird at day 21 of exposure. The parabronchus is collapsed and the parabronchial epithelium (*) is hyperplastic. HE.X200

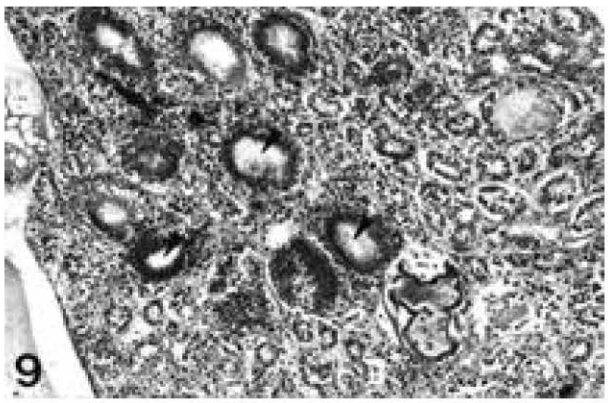

Fig. 9: Varied-sized focal urate deposits (arrowheads) in the kidney of SC-exposed bird at day 21 of treatment. HE.X100

hemorrhage and edema between the myocardial fibers (Fig. 2), disarrangement of myofibers (Fig. 3), loss of myofiber cross striations, focal myolytic changes (Fig. 4) and calcified deposits (Fig. 5). Lungs of treated birds disclosed diffuse congestion of vasculature and edema between parabronchi (Fig. 6 and 7) and many collapsed parabronchi (Fig. 8) which had altered parabronchial epithelium. Also, pulmonary blood vessels revealed histological changes such as adventitial edema and endothelial cell swelling and proliferation. Renal histological changes were prevalent in SB and SP-treated birds. Renal tissues of intoxicated birds revealed congestion and edema in the intertubular tissue. Renal tubules were dilated and had degenerated lining epithelium. Glomeruli possessed proliferated endothelial, epithelial and mesangial cells which filled the glomerular

spaces. Interstitial urate deposits and also urate granulomas were observed (Fig. 9). Calcified deposits were a feature in the renal tissues of treated birds. Control birds showed no comparable histological changes. Scoring of the histological changes observed in the different experimental groups at the different intervals is shown in Table 2.

DISCUSSION

The present results indicated that the three employed sodium compounds can induce pathological effects in the broiler chickens. The initial increase in body weights of the treated birds may attributed to fluid retention and decreased feed intake may explain the decreased body weights thereafter. Presently, RV:TV ratio was increased in the treated birds and this was ascribed to right ventricular hypertrophy (RVH) which reflects a compensatory change of the heart to increase the right ventricular pumping. RVH occurs as an attempt to increase the cardiac output in response to the increased volume load on the heart[15].

Sodium toxicity is known to result in hypervolaemia and increased volume load on heart and subsequently right ventricular hypertrophy (RVH) and right ventricular failure (RVF)[4,11,13,14]. In present study, the coincidental pulmonary congestion and RVH may support the fact that pulmonary capillary beds in birds are less expandable and it is the limiting factor in pulmonary circulation when more blood is pumped through the lung[16]. In such case, the coordination between the cardiac and pulmonary sides is disturbed. In other words, the cardio-pulmonary haemodynamics are compromised[14,17].

Currently, the three employed compounds caused decreased erythrocyte deformability which may be ascribed to the increased mean corpuscular volume (MCV). Erythrocyte swelling leading to increased MCV is known to accompany the increased plasma Na^+[5,9,11,14]. Decreased erythrocyte deformability increases blood viscosity[9] which undoubtedly elevates the work load on the heart due to increased resistance to blood flow within the pulmonary tissue. Increased work load, in addition to the existed volume load, due to hypervolaemia, finally lead to RVH.

The pathological changes of the left ventricles were relatively less severe in the present cases, especially in cases that showed marked RVH. This may indicate the higher susceptibility of RV to overload since it is a volume pump and rapidly hypertrophies in response to increased volume load pump and rapidly hypertrophies in response to increased volume load[18]. Right ventricular failure (RFV) is the terminal sequel to increased volume load[19].

The demonstrated pulmonary edema in the present cases may interfere with pulmonary circulation and previously was considered as an evidence of heart failure[20]. Pulmonary edema interferes with pulmonary circulation and becomes a part of the cardio-pulmonary complications[10,15].

According to the present observations, sodium chloride toxicity can provoke more severe cardiac pathological changes while the toxicity of sodium bicarbonate and sodium sulphate causes marked renal changes. The three sodium compounds may induce pathological effects through their effect on the cardio-pulmonary system. The resultant hypervolaemia overloads the heart and subsequently RVH and RVF developed.

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