SODIUM CHLORIDE POISONING IN IRAQI WATER BUFFALO *(Bubalus bubalis)* OF BASRAH GOVERNORATE, IRAQ

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ABSTRACT

Sodium chloride poisoning has been suspected in Iraqi buffalo (*Bubalus bubalis)* of Basrah governorate, A study was conducted on 523 animals. All animals drank natural water from the *Shatt al-Arab* sources. Seventy-six animals show different clinical manifestations, and twelve clinically healthy animals were used as controls. Diseased buffalo exhibited different clinical manifestations. A significant increase in PCV with alterations in indices of clotting factors was also explored. Serum analysis demonstrated increase in serum sodium and chloride concentrations in diseased buffalo. However, water analysis revealed elevation of electrical conductivity, sodium, chloride and total dissolved solids in the drinking water of diseased buffalo. The macroscopic appearance of the carcasses showed congestion and slight edema of the brain tissues, Moreover, the histopathological examinations revealed, spongiform-like encephalopathy with severe congested blood vessels and neuro-vascular thrombus was detected.

Key words; NaCl toxicity , diagnosis , healthy animals.

التسمم بكلوريد الصوديوم لدى الجاموس العراقي (*Bubalus bubalis*) في محافظة البصرة

كمال الدين مهلهل السعد عبد الباري عباس الفارس رحمن كاظم محسن
استاذ استاذ استاذ
كلية الطب البيطري , جامعة البصرة , العراق .فرع الطب الباطني والوقائي , فرع الجراحة والتوليد

المستخلص

تم الاشتباه بتسمم الجاموس بكلوريد الصوديوم في البصرة, العراق . اجريت هذه الدراسة على 523 من الجاموس المحلي في العراق . أظهرت ستة وسبعون من الحيوانات مظاهر سريرية مرضية مختلفة ، وعد اثني عشرة من الجاموس السوي سريرياً كمجموعة سيطرة .. اشارت نتائج الدراسة الى حدوث زيادة معنوية في معدلات خلايا الدم المرصوصة مع اختلاف معدلات عوامل تخثر الدم. أظهرت نتائج تحليل المصل زيادة كبيرة في تركيز الصوديم والكلوريد في الجاموس المريض كما كشف تحليل المياه عن ارتفاع كبير في معدلات التوصيل الكهربائي والصوديوم والكلوريد والمواد الصلبة الذائبة الكلية في مياه الشرب للجاموس المريض. بين المظهر العياني للحيوانات النافقة احتقان مع وذمة طفيفة في أنسجة المخ.فضلا عن ذلك ، كشفت الفحوصات النسجية، عن وجود اعتلال دماغي يشبه الإسفنجي مع أوعية دموية مزدحمة وخثرة وعائية.

الكلمات الافتتاحية: التسمم بكلوريد الصوديوم , ألتشخيص حيوانات سليمة

Received:12/3/2020, Accepted:21/6/2020

INTRODUCTION

Water is considered an indispensable nutrient and, it is crucial for animals have access to a sufficient level of good quality water to sustain acceptable production(1). The quantity and quality of water required vary from one animal species to another and among classes in similar species and impacted by the animal's environment, including factors like seasonal variations in pasture, types and availability, food and water content as well as ambient temperature (2,35). A main factor that affects the water quality is saline content, meaning, the level of total dissolved solids (TDS) in the water(5), The existence of elevated levels of some inorganic ions such as calcium, magnesium, sodium, chloride, sulfate and the bicarbonates in animals' drinking water may lead to adverse effects that cause impaired performance, poor health or even death.(21,31,32). The Basrah provenience of Iraq is the meeting point of two large rivers – the Tigris and Euphrates – which become one and flow into the Shatt Al-Arab. In a very short time, Basrah city has been exposed to a severe fresh water shortage because of low river level, which allowed salt water intrusion that has caused in excess of 100% increased salinity of the Shatt Al-Arab in recent months, as TDS, which is a measure of water purity, rose dramatically from more than 1,000 to approximately 6000 or even more. Even though there are numerous problem-related reasons, The issues emerged in the 1990s, when Turkey constructed many dams on the Euphrates river. Turkey's Southeastern Anatolia Project (GAP project), is currently completing the building of the Ilisu dam on the Tigris river, which is predicted to very substantially and adversely affect Iraq's fresh water availability situation. It will decrease freshwater levels in the Tigris river by a significant 25%. This effect will often be felt in the riverbed where Basrah is located. The salinity levels of Shatt Al-Arab will increase further when the fresh water level is low. As such, the actual effect of the Ilisu Dam, and stopping its construction, are most crucial to Basrah. The "Save the Tigris" campaign urges those involved to solve the problem of the Ilisu Dam, which will worsen the current fresh water issue in Basrah (1,6,15). The toxicity of sodium chloride salt has a relationship with water availability and known as "sodium ion toxicity water deprivation syndrome," When the sodium dose be sufficiently elevated, then sodium becomes a toxic matter in the water intake, (13). The objectives of the present study was to examine the clinical, histopathological and laboratory diagnosis, of the local Iraqi buffalo poisoned with sodium chloride in drinking water in the Basrah governorate.

MATERIALS AND METHODS

Experimental animals and design: This study was conducted on 523 Iraqi local buffalo of various ages, males and females representing different herds, raised indigenously in various parts of Basrah governorate, Iraq. All animals drank natural water from the *Shatt al-Arab* sources . Seventy-six (76) local buffalo showed different clinical manifestations, including anorexia , polyurea and polydypsia , and nervous manifestations, Twelve, clinically healthy local buffalo were regarded as controls. All animals were subjected to complete clinical examinations.

Blood sampling and hematologically assay: Blood samples (10mL) were collected via jugular and /or milk venipuncture. Part of the collect blood (2.5) mL was mixed with EDTA for total erythrocyte count (TRBc), PCV, hemoglobin level (Hb), TLC, Total platelet count (Pt) , MPV, and PDW assay using the GENEX, HEMATOLOGY ANALYZER USA. Giemsa-stained blood smears were used for differential leukocyte counts (33). An amount of 2.5 milliliter of blood mixed with Trisodium citrate was utilized for evaluation of the Prothrobmbin time , Activated patiel thromboplastine time, and Fibrinogen time, using commercial kits (Biolabo, France). Clotting time was also assessed based on Bush, (9).

Water analysis : Water was collected from *Shatt Al-Arab (Different main branches* sources), as it is the primary source of animal drinking water. Water acidity, electrical conductivity (EC) , Na, Cl, Ca, P, Ma. K and TDS were examined. Analyses were done. as similar day of water collection (11, 30).

Biochemical analysis : The concentrations of serum Cl, Ca, P, and Mg, were evaluated

using atomic absorption spectrophotometer with an autoanalyzer (Shimadzu Model AA 6200, Tokyo, Japan) and employing commercial kits (Ziest Chem Diagnostics, Tehran, Iran). Na and K values were analyzed using a flame photometer (Jenway, PFP 7 clinical and Essex, England).

Histopathological analysis : Diseased animals which had died slaughtered were subjected to histopathological examinations. The tissue samples were collected from different parts of the animal (brain, rumen, omasum, abomasum, and intestine), fixed with 10% neutral buffered formalin solution for 72 hrs, then trimming to appropriate sizes and rinsed, then dried and cleared in xylol. Finally, the samples were fixed in paraffin wax, and divided at 4-5 µ thickness, followed by staining with hematoxyline and eosin, and scrutinized under a light microscope (20).

Statistical analysis: Data were statistically analyzed using the student *t*-test (SPSS) program by comparing between control and diseased animals, The values represent the mean ± standard error of mean. * ($P<0.05$), (18).

RESULTS AND DISCUSSION

Diseased buffalo revealed different clinical manifestations such as anorexia, Polydipsia and polyuria,diarrhea and abdominal pain, Opisthotonos, nystagmus and tremor, Appearance blindness, Upward flexion of the head and /or circling, and Recumbency with or without convulsions, (Fig 1 and 2. Table 1).

Table 1. Clinical signs of diseased buffalo poisoned with sodium chloride in drinking water

Clinical signs	Diseased buffalo n=76	%
Anorexia	**67**	**88**
Polydipsia and polyuria	**65**	**85.5**
Diarrhea, abdominal pain	**56**	**73.6**
Opisthotonos, nystagmus and tremor	**45**	**60.5**
Appearance blindness	**28**	**36.8**
Upward flexion of the head and /or circling	**19**	**25**
Recumbency with or without convulsions	**13**	**17**

Fig 1. Upward flexion of the head

Fig 2.Recumbency with or without convulsions

Results of clinical examinations of diseased buffalo show a significant ($p<0.05$) increase in body temperature, respiratory and heart rates of diseased animals in comparison with controls (Table 2).

Table 2. Body temperature, respiratory and heart rates of diseased and control buffalo poisoned with sodium chloride in drinking water (Mean ± SE).

Parameters	Controls n=12	Diseased n=76
Body temperature C°	39.± 0.08	39.8 ± 1.4*
Respiratory rate/ min	21± 0.75	55.6± 10.6*
Heart rate/ min	75.2±0.74	97.8± 12.72*

**** (P<0.05).***

Data concerning hematological examinations of diseased buffalo and controls showed, a significant ($p<0.05$) increase in PCV values in NaCl-poisoned buffalo in comparison with controls, (Table 3).

Table 3. Hematological parameters of diseased and controls buffalo poisoned with sodium chloride in drinking water (Mean ± SE)

Parameters	Controls n=12	Diseased n=76
RBC $\times 10^6$	7.93±1.46	7.97±0.35
Hb g/dl	13.55 ± 1.77	13.4±1.22
PCV %	31.71 ± 3.87	41.93±3.45*
TLC $\times 10^3$	11.43±1.54	11.84±1.75
Neutrophiles $\times 10^3$	4.39± 0.16	4.42±0.73
Lymphocytes $\times 10^3$	5.55± 0.42	5.51±0.15
Monocytes $\times 10^3$	0.56± 0.03	0.54±0.05
Esinophiles $\times 10^3$	0.53 ± 0.12	0.58±0.14
Basophiles	0.08±0.03	0.08±0.04

**** (P<0.05).***

Results showed variations of attributes of clotting factor in diseased buffalo compared to those of controls, The results indicated a significant decline ($P<0.05$) in the mean values of total platelet count (Pt) and fibrinogen time, while significantly increased ($P<0.05$) platelet volume, PDW, CT, and pro-thrombin time and Appt (Table 4).

Table 4. The parameters of clotting factor of diseased and controls buffalo poisoned with sodium chloride in drinking water (Mean ± SE).

Parameters	Controls n=12	Diseased n=76
Total platelet count Pt x 10^3	556.8±22.6	240.5±46.3*
Mean platelet volume MPV/fl	9.3 ± 0.4	12.8 ± 2.4*
Platelet distribution width PDW %	15.7 ± 1.3	19.9 ± 2.7*
Clotting time CT/mint	3.6 ± 0.4	5.7 ± 1.7*
Prothrombin time Prt/sec	11.7 ± 1.2	26.9 ± 4.6*
Activated partieal thromboplastin time Appt/sec	49.8 ± 5.8	71.7 ± 6.3*
Fibrinogen time mg/100ml	366.5±12.3	268.3±56.1*

** (P<0.05).*

The outcomes of serum analysis indicated a significant (P<0.05) rise in serum sodium and chloride levels in diseased buffalo, However, values of calcium , phosphorus, potassium and magnesium were in their normal ranges in comparison with controls (Table 5).

Table 5. Serum analysis of Ions of diseased and controls buffalo poisoned with sodium chloride in drinking water (Mean ± SE).

Parameters	Controls n=12	Diseased n=76
Sodium (mmol/L)	141.40 ± 3.13	254.67± 10.48*
Chloride (mmol/L)	105.50 ± 2.14	180.66±13.828*
Calcium (mmol/L)	9.86 ± 0.53	9.92± 1.04
Phosphorus (mg/dL)	6.42 ± 0.33	6.23± 0.96
Potassium (mmol/L)	4.73 ± 0.56	4.88 ± 0.33
Magnesium(mg/dL)	1.76 ± 0.23	1.81± 0.53

** (P<0.05).*

On the other hands, water analysis revealed a significant increase of sodium and chloride ions in drinking water consumed by animals. However, values of other ions were increased slightly (Table 6).

Table 6 .Mean values of water analysis from *Shatt Al-Arab (Different main branches* sources) of Basrah province (Mean ± SE)

Parameters	Rang and mean of Normal values according to (WHO, 2008)*	Range and mean values from *Different main branches* sources of *Shatt Al-Arab*
Electrical conductivity mS/M	7.4 7.3-7.5	18.2* 17.64 - 18.76
Acidity Ph/IU	7.5 6.5- 8.5	8.05 7.7 – 8.4
Sodium mg/l	97.5 20-175	247.5 * 222-273
Chloride mg/l	112.5 25-200	6629* 6425- 6833
Magnesium mg/l	40 30-50	45 38-52
Calcium mg/l	150 100-200	172 132 - 212
Phosphate mg/l	2.7 0.40-5	3.25 1-5.5
Potassium mg/l	11 10-12	14.4 13-16
Total Dissolved Solids mg/l	900 300-1500	1382.5* 510-2255

*** (P<0.05).*WHO.2008(34).**

The macroscopic appearance of the carcasses showed congestion and slight edema of the brain tissues (Fig. 3), with severe congestion and thickening with petechial hemorrhages of the rumen (Fig. 4), omasum, abomasum, and

intestine, which contains dark fluid feces. Moreover, the histopathological examinations revealed spongiform like encephalopathy with severe congested blood vessels and neuro-vascular thrombus was detected, However, marked peri-vascular inflammatory cell infiltration was seen. Furthermore, necrotic neurons, and edematous fluid in the neural interstitium, and vacuolated neural interstitium were also detected (Figs: 5, 6, 7,and 8).

Fig 3. Congestion and slight edema of the brain tissues

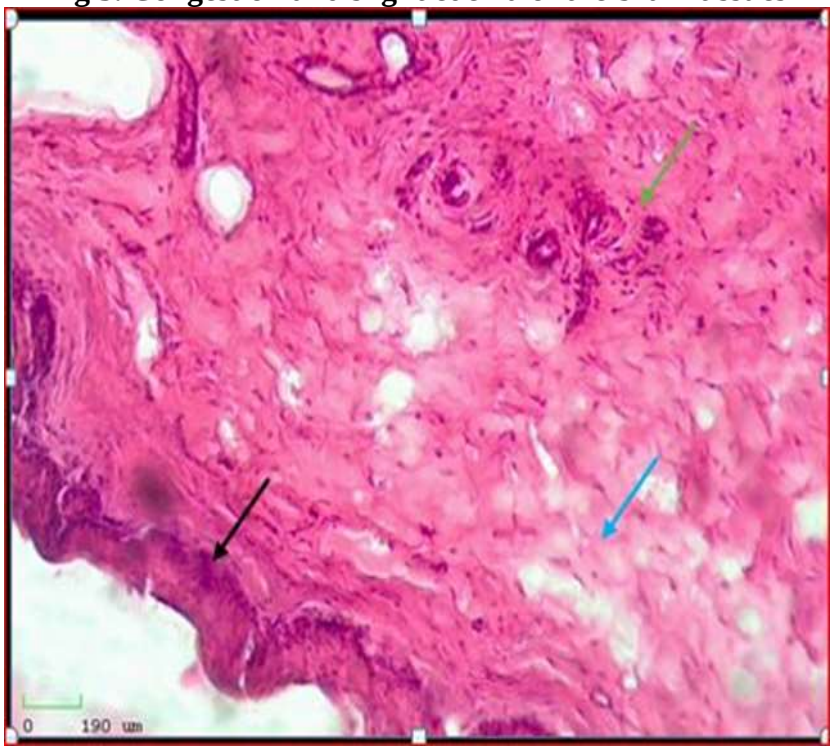

Figure 4: Histopathological section of rumen showed thickening of ruminal mucosal layer (black arrow), with edematous fluid aggregated in the sub-mucosal layer (blue arrow), and infiltration of inflammatory cells (green arrow). H&E stain. 10X

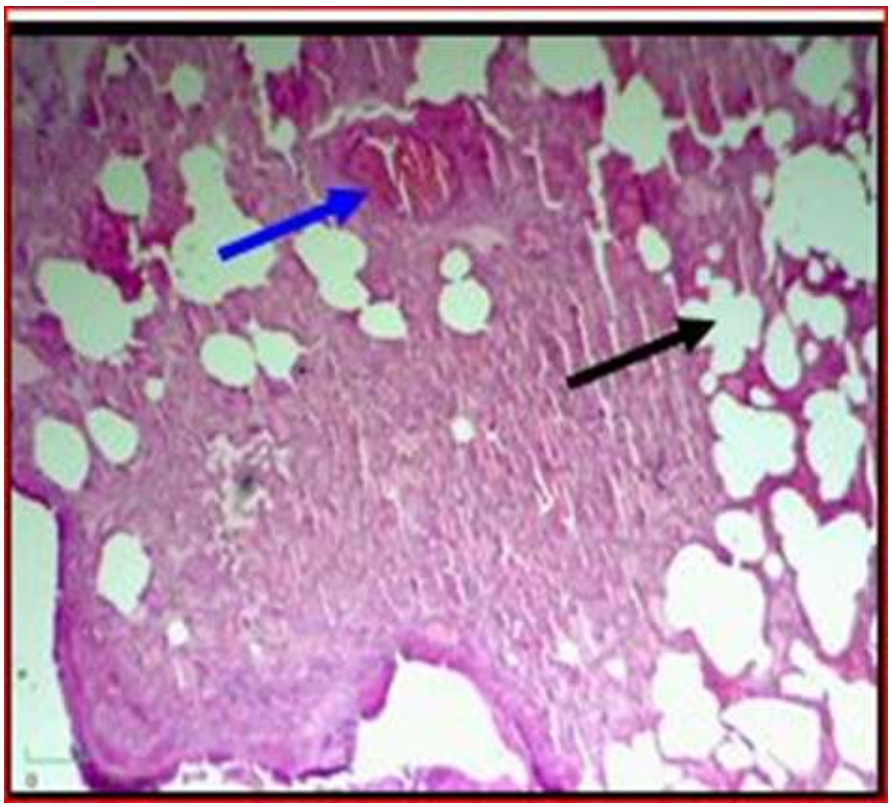

Fig. 5. Histopathological section of brain showing spongiform like encephalopathy (black arrow), and severe congested blood vessels (blue arrow). H&E stain. 40X

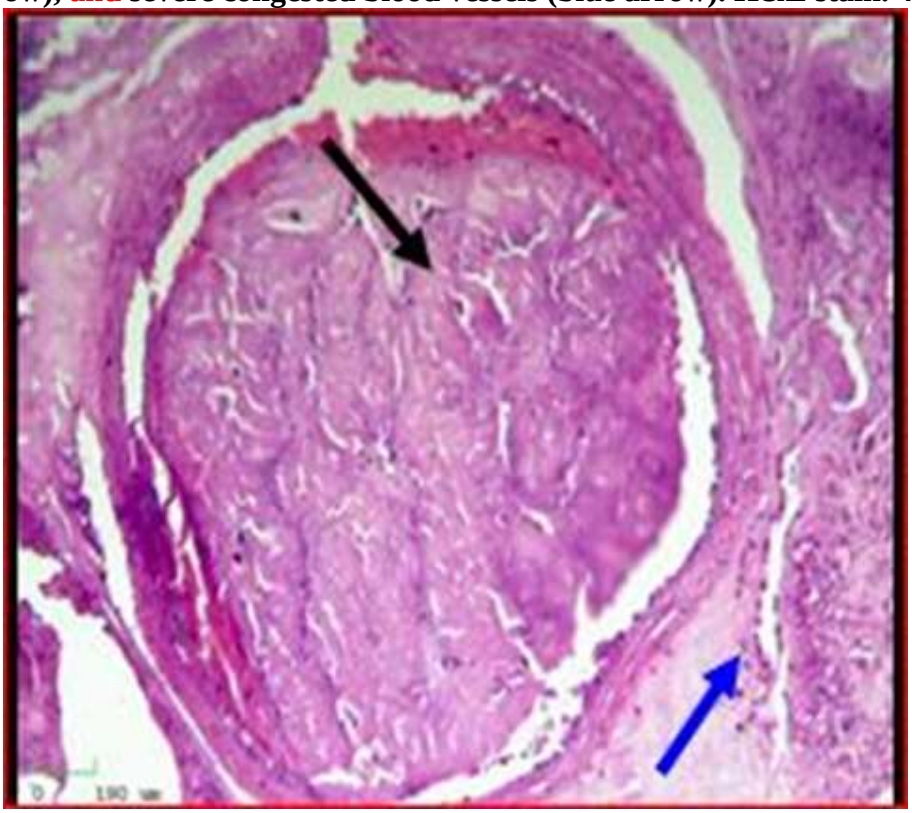

Fig. 6. Histopathological section of brain showed neuro-vascular thrombus (black arrow), and marked peri-vascular inflammatory cells infiltration (blue arrow). H&E stain. 40X

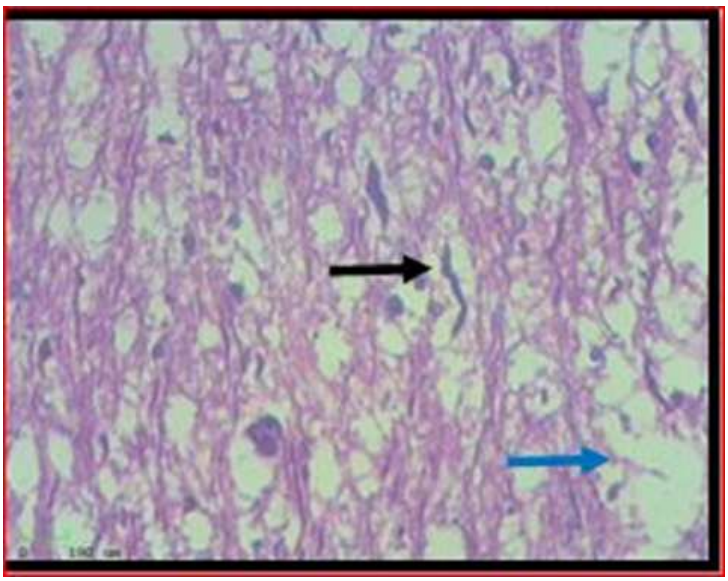

Fig.7. Histopathological section of brain showing necrotic neurons (black arrow), and an edematous fluid in the neural interstitium (blue arrow). H&E stain. 40X

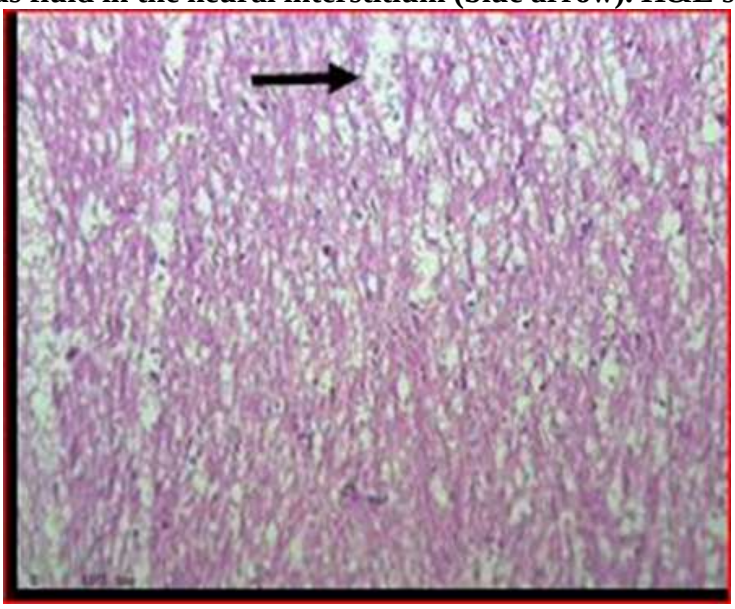

Fig.8. Histopathological section of brain showing vacuolated neural interstium (black arrow). H&E stain. 10X.

The current results showed clearly insufficient fresh water inflow from the Tigris and Euphrates into the Shatt al-Arab waterway and tests has indicated at least a three-fold increase in salinity which has affected the main important rivers of Iraq over the past 50 years . This has occurred because of irrigation of very intense agriculture upstream in Iraq compounded by evaporation. Additionally, the inadequacy of river water has resulted in the backflow of sea water into the Shatt al-Arab, thus adding to the further increase of TDS levels, which, significantly affect people's ability to maintain their farms and animals in their original locations (3). It is crucial to measure both the quality and quantity of water to enable active and efficient planning while careful observation of water supplies for livestock must also be carried out. This is because of poor water quality, livestock could drink less than they need or, occasionally, could cease drinking altogether. Insufficient intake of water will lead animals to eat less and adversely affect their body condition score and if they are lactating, there will be a reduction in their milk yield (4, 27). Sodium chloride poisoning can be the result of too much NaCl intake (direct salt poisoning) or might be caused by inadequate intake of fresh (indirect salt poisoning), however, it is usually a combination of these two factors (5,14,16). The level of NaCl in drinking water which satisfies the physiological requirements of the organism and does not

cause toxicity is less than 0.5%. It should be noted that higher in toxicity with NaCl was in dissolved form as it is than readily accessible to absorption, than the solid form (13, 23). It has been documented that, sodium and chloride maintain the osmotic balance in the body. The digestive tract totally absorbs the dissolved NaCl and distributes it in the whole body. Elevated blood osmolality creates thirstiness, induces water uptake and since it impacts the antidiuretic hormone (ADH) release, resulting in the organism retaining the water. This compensation mechanism will decrease the osmolality and it will have an effect when the animal has sufficient water available. Na begins to drain water out of the cells into the extracellular space, causing swelling. It also crosses the blood brain barrier and in acute cases, neurons are dehydrated leading to the intercellular matter swelling in the brain, and also causes alterations in the blood circulation of the brain and hemorrhages (19,31). Diseased buffalo exhibited various clinical signs, these data were in agreement with those reported by (7,12,32,35). In fact, if there is sufficient water, most animals are able to handle quite large doses by raising sodium excretion, On the contrary, if there is insufficient available water, acute toxicosis leads to dehydration, blindness, incoordination, convulsions, recumbency, and death. Furthermore, the mechanisms behind acute toxicity are linked to cells being dehydrated, shrinking issues, and edema. When extracellular sodium levels are raised. water is drawn out of the cell down the concentration gradient, causing cells to shrink, (26). Once ingested, approximately more than 85 to 95% of sodium and chloride are taken into the gastrointestinal tract, especially the small intestine, while big amounts of the two ions through recycling end up in the intestinal tract through salivary, pancreatic, and intestinal epithelial secretions and even the bile. The elevated intestinal level of sodium is needed for the transportation of glucose, amino acids, and other nutrients across the mucosa, Moreover, Cl is also released into the intestine to assist in originating a declining pH environment required for proteolysis. These secretions need to be reabsorbed further down the digestive tract to protect the elements (22,29). High NaCl intake with no corresponding intake of water can cause hypernatremia. This is because as the ratio of sodium to water is beyond normal, the plasma sodium level and the total body sodium are increased. As a result, the balance of extracellular and intracellular osmolality is disrupted, which can cause numerous clinical symptoms, such as extreme thirst, weakness, headache, irritability, pulmonary edema, hypertension, tachycardia, and also coma at high NaCl concentrations, as indicated in the current study. Furthermore, chloride, the other element of NaCl, is also needed for water balance and for plasma osmolality, thus, abnormalities in sodium metabolism occur together with abnormalities in chloride metabolism (21). It has been indicated that NaCl poisoning impedes the excretion of excess salt by the kidneys. Vacuolization of renal tubular cells and acute tubular necrosis may take place, besides, the most severe consequences of acute NaCl poisoning are evident in the nervous system due to hypernatremia, where, the brain cells are damaged due to dehydration following the significant osmotic shift of intracellular fluids to the extracellular space. Losing brain water can result in a rapid shrinking of brain volume, and in acute cases of intra-cerebral hemorrhage and this was also noted in the present study (13,35). Nonetheless, it has also been documented that , the impact of hypernatremia on brain cells is prevention of glycolysis. Depression in clinical cases might be the result of hypernatremia, dehydration, and acidosis combined. During states of hyperosmolarity, in this case caused by hypernatremia, the brain cells produce idiogenic osmoles which might provide protection of the brain tissue against harmful effects of hyperosmolarity. Therefore, these substances can elevate brain intracellular osmolarity during resolution of the hypernatremic state. Consequently, due to decrease of extracellular osmolarity, water penetrates the brain cells to cause brain swelling within the cranial vault, which can be shown clinically as convulsions (8,10,28). Changes in the indices of clotting factor which were indicated in the current study are obvious, as thrompocytopenia, and reduced

CT of the blood might reflect the petechial hemorrhages observed on the mucus membranes of the carcasses. Furthermore, the platelet count may be depressed because of the depressed activity of the bone marrow and thrombocytes sequestration, which may take place because the hemostatic mechanism is disorganized and aggravated by distributing intra-vascular coagulopathy, which might terminate with micro thrombosis and infarction of special organs including the brain, lungs and intestine (25) In the present study, different histopathological results were indicated which concurred similarly with those reported by (5,7,24). Kostić-Banović et al , (17) and Visscher et al. (32), mentioned that , "in the course of the first 48 hrs., diseased animals develop eosinopenia, eosinophilic cuffs around vessels in the cerebral cortex and adjacent meninges, and cerebral edema or necrosis. However, following 3-4 days, eosinophilic cuffs are usually no longer present." Moreover, Modra et al. (21), added that "Histopathological lesions in the brain included cerebral oedemic and meningitis, general congestion of the internal organs, disseminated micro bleedings and demyelination in the brain parenchyma of basal ganglia subarachnoid hemorrhages, and venous micro-thromboses in the brain." However, it has been postulated that, the oscillations of NaCl ions might result in demyelination lesions in these sensitive places (17). All those pathological lesions, especially of the brain would lead to deteriorations of the animal's health status which is invariably terminated by death .

CONCLUSIONS

It was concluded that poisoning of Iraqi buffalo with sodium chloride has substantially harmful effect on the diseased animals which invariably led to death.

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