U of K. J. Vet. Med. & Anim. prod. Vol. 5, No.1,2014 (94-111)

ASSESMENT OF EFFICACY OF THE TISSUE FIXATION SATURATED SODIUM CHLORIDE SOLUTION

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المستخلص

أجريت هذه الدراسة لفحص وتقييم فعالية محلول كلوريد الصوديم المشبع كمثبت للأنسجة . أخذت عينات للدراسة النسيجية والنسيجية المرضية من ضأن ذبح بسلخانة كرري بإمدرمان وذلك من الجلد والدماغ والرئة والأمعاء والكلية والطحال والكبد وغدد لمفية وبنكرياس وعضلات قلبية وعضلات هيكلية . حفظت هذه العينات في محلول كلوريد الصوديم المشبع وكذلك في 10% فورمالين كشاهد لمدة يومين و15 يوم و30 يوم . جهزت الأنسجة وقطعت وصبغت الشرائح النسيجية بصبغتي الهيماتوكسلين والإيوسين وتم تقييمها بواسطة المجهر الضوئي . أوضحت النتائج أن الشرائح التي جهزت من عينات الجلد والرئة والدماغ والغدد اللمفية والعضلات القلبية والعضلات الهيكلية قد ثبتت بصورة جيدة في كل فترات التثبيت الثلاث (2و15و30 يوم) مقارنة مع العينات التي ثبتت في الفورمالين. الشرائح التي جهزت من عينات الكبد والكلية ثبتت تثبيتا جيداً لمدة 2 و 15 يوم . شرائح الأمعاء والطحال ثبتت بصورة جيدة لمدة يومين , أما تثبيت البنكرياس لم يكن جيداً وظهر علي متنه التحلل الذاتي في كل فترات التثبيت الثلاث (2 ،5 و30 يوم). من جانب آخر فقد أوضحت شرائح العينات المرضية التفاصيل النسيجية للآفات المرضية بصورة جلية.

Abstract

The present study was conducted to examine and assess the efficiency of saturated sodium chloride solution as a tissue fixative. Specimens for histological and histopathological investigations where obtained from slaughtered sheep at karrari slaughterhouse in Omdurman. These specimens were taken from skin, brain, lung, liver, kidney, spleen, lymph nodes, pancreas, intestines, cardiac muscles and skeletal muscles. They were fixed in saturated sodium chloride solution as test fixative as well as in 10 % formalin (control) for 2, 15 and 30 days. They were processed and sectioned. The slides were stained with hematoxylin and eosin. The slides evaluated using the light microscope. The results showed that sections of skin, lung, brain, lymph nodes, skeletal muscles and cardiac muscles were well fixed in saturated sodium chloride solution compared with the control sections fixed in formalin for the three fixation periods (2, 15 and 30 days).sections of liver and kidney fixed for 2 and 15days showed good fixation .The spleen and intestine sections showed good results (fixation) in samples fixed in saturated sodium chloride solution for 2

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days. However, the pancreas showed autolysis in the parenchyma tissue and lost its histological details in section fixed for 2, 15 and 30 days. On the other hand, the sections of pathological lesions examined, showed clearly the histopathological details.

Introduction

The objective of fixation is to preserve cells and tissue constituents in as close a life –like state as possible and to allow them to undergo further preparation procedures without change (Carson et al *2009)* Fixation arrests autolysis and bacterial decomposition and stabilizes and hardens the tissue for convenient handling in subsequent stages of tissue processing. Furthermore it brings out differences in refractive indexes and increases the visibility or contrast between different tissue elements .Fixation should also provide for the preservation of tissue substances and proteins .Fixation is , therefore ,the first step and the foundation in a sequence of events that culminates in the final examination of a tissue section .The method of fixation should be selected immediately once the specimen is presented. The fixative should be selected with caution avoiding fixatives that may affect future studies. The most widely –used chemical fixative in laboratories worldwide and considered as a general purpose fixative is 10% formalin. This agent rapidly penetrates tissue, arrests enzymatic action .It also cross –links proteins, leading to tissue hardening (Pearse, 1980) .However this chemical has many side effects. It is irritant, corrosive and may cause allergic sensitization. It is now well established as a human carcinogen (NTP, 2011a). Because of the toxic hazards of formalin and other fixatives many laboratories have thought of a safer alternative. An alternative that is able to produce a morphological picture similar if not better than that produced by formalin. Saturated sodium chloride solution has been sought as one of the candidates as a safe alternative. This chemical was used by Alsaraj(2010) who fixed specimens taken from spleen, kidney and liver in this solution for 24 hours and he obtained good results.

The aim of the present study was to test the efficiency of saturated sodium chloride solution as a tissue fixative in various organ tissues for different fixation periods.

Materials and Methods

Samples from different organs of sheep (skin, liver, lung, brain, kidney, spleen, small intestine, pancreas, lymph nodes, cardiac muscle and skeletal muscle) were collected from national karrari abattoir. The samples were excised from apparently normal or condemned organs.Four samples (1cm ^{3} each)were excised from each organ. Three samples were fixed in 15 times volume of saturated sodium chloride solution (SSCS) and kept for 2 days, 15 days and 30 days as fixation periods. The fourth sample was fixed in 10% neutral buffered formalin and served as control. Tissue processing was made using automatic tissue processor (LEICATP1020).Stain with H&E and evaluated by light microscope.

Results

The results of fixation periods for different organs fixed in saturated sodium chloride solution (**SSCS**) were summarized in table 1. Evaluation was based on the histological and histopathological description for each organ at different fixation periods (2, 15 and 30 days) as compared to formalin fixed sections .

Table 1: Results of fixation periods for different organs fixed in saturated sodium chloride solution.

Organs	Fixation time		
	2 days	15days	30 days
1- Skin	√	√	√
2- Skeletal muscle	√	√	√
3-Cardiac muscle	√	√	√
4-Lung	√	√	√
5- Lymph node	√	√	√
6- Brain	√	√	√
7- Liver	√	√	*
8- Kidney	√	√	*
9- Small intestine	√	*	*
10- spleen	√	*	*
11- Pancreas	*	*	*

√ Good fixation
* Poor fixation

Skin :

The skin sections showed the keratinized epidermis E with under lying thicker layer the dermis D,the appendages of the skin ,hair follicles H and sebaceous gland S .Each hair follicle has an associated bundle of smooth muscle .The sebaceous gland S was found embedded in the dermis ,their nuclei were condensed with irregular shapes .The skin and skin appendages, sections were very clear as compared to the control groups at the three different fixation periods (Fig. 1,2,3 and4).

Skeletal muscle :

The sections of skeletal muscle showed the fibers with the nuclei located peripherally, the fibers were surrounded by connective tissue. The longitudinal sections showed light and dark cross striation, where as the transverse sections exhibited myofibril bundles and peripheral nuclei.

No changes were observed in sections of the muscles fixed in (**SSCS**) for the three fixation periods when compared with the control group fixed in formalin (Fig.5and6).

Cardiac muscle :

The sections showed the myofibers, a single central nucleus and the characteristic dark staining inter calated disks D. The sections were similar to those fixed in formalin after the three fixation periods (Fig.7 and 8).

Lung :

The sections illustrated the characteristic features of the lung, its alveoli lined by extremely thin simple squamous cells ,bronchioles B which exhibited epithelium , thin lamina propria , a layer of smooth muscle and adventitia .

The microscopic details of the lung at the three fixation periods did not differ from sections fixed in formalin (Fig.9 and 10).

Lymph node :

The sections showed aggregations of lymphocytes intermeshed with lymphatic sinuses supported by reticular fiber framework.

Lymphatic nodules exhibited germinal centers G , in which the cells were more loosely aggregated and the developing lymphocytes had lager and lighter nuclei with more cytoplasm than the small lymphocytes.No differences with formalin fixed sections at the three fixation periods were noted.(Fig11 and 12).

Brain :

The cerebral cortex sections revealed axons, pyramidal cells P which exhibited variable sizes as well as astrocytes.The histological details were well differentiated and recognized at 2 , 15 , and 30 days fixation periods as compared to formalin sections. (Fig.13 and 14).

Liver :

The sections fixed in saturated sodium chloride showed the classical hepatic lobule with the central vein C in the center and radiation of hepatic cells H and the sinusoids .The histological details were well differentiated in samples fixed for 2 and 15 days as compared to formalin fixed sections.(Fig.15 and 16).

Kidney :

The sections of the renal cortex revealed the greater details of the renal corpuscle G {a glomerulus and a glomerular capsule} the glomerulus with a tuft of capillaries supported by connective tissue Fig. And the associated collecting tubules. Sections prepared from samples fixed for 30 days lost the details when compared with those fixed in formalin(Fig .17 and 18).

Small intestine :

The mucosa of the small intestine showed the columnar cells, goblet cells and the intestinal glands which extend deep through the lamina propria to the muscularis mucosa. The lamina propria L exhibited as dark stained layer contained an abundance of connective tissue cells and lymphoid cells. The sections were adequately fixed and well stained in 2days of fixation and appeared similar to those obtained by formalin fixation (Fig. 19 and 20).Thereafter, the sections showed some degree of autolysis .

Spleen:

Histological sections of the spleen showed a dense connective tissue capsule C and the extended connective tissue trabeculae T ,aggregation of lymphoid follicles L with in the white pulp W ,and the red pulp R formed of diffuse cellular mesh work .The section was well stained after 2 days of fixation. However ,it lost the histological details thereafter i.e.,at 15 and 30 days of fixation. (Fig .21 and 22)

Pancreas :

The sections prepared from samples fixed for two days showed autolysis confined to pancreatic parenchyma which was changed to a homogenous substances lacking any sort of cellular structures or details ,However the lobulation was well defined and the demarcation between the different lobules L was very clear .(Fig .23 and 24)

Pathological findings:

Sarcocystspp :

The section showed the spindle elongated shape with septa and thickened wall. There was no evidence of any de generation or inflammatory reaction in muscle fibers surrounding the sarco cysts , resembling the section which fixed in formalin. (Fig.25 and 26).

Pulmonary hemorrhage:

The section showed RBCs in the alveolar spaces and interstitial tissues. Presence of inflammatory cells and emphysema . The section was well stained and adequately fixed as compared to the control (Fig.27 and 28).

Pulmonary edema:

The section showed intra alveolar accumulation of pinkish homogenous fluid , thickened alveolar walls the section was well stained and fixed like the formalin section(Fig.29 and 30).

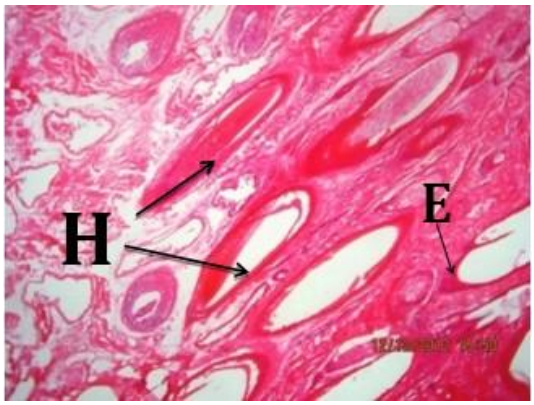

Fig 1 . skin fixed in formalin, E epidermis H hair follicles (H&E) X 100 (2days)

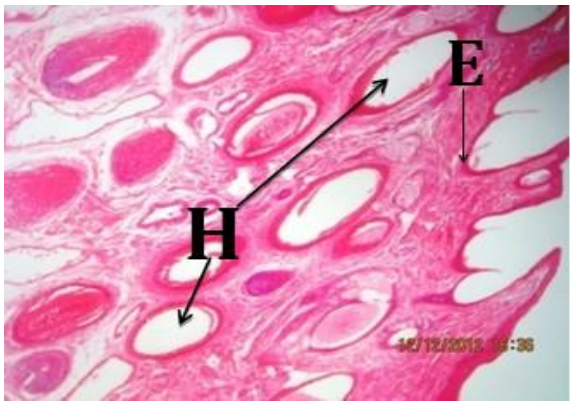

Fig 2 .skin fixed in saturated sodium chloride solution, E epidermis H hair follicles (H&E) X 100.(2days)

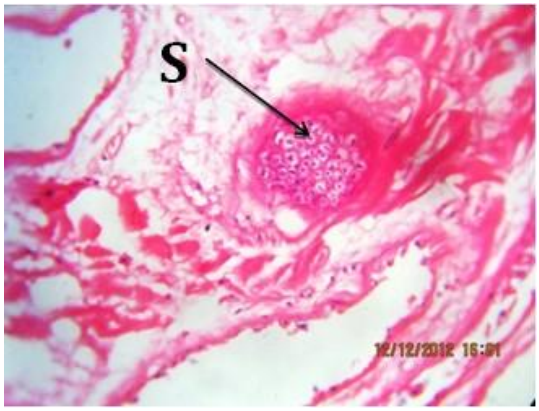

Fig 3.skin fixed in formalin, S sebaceous gland (H& E) X 400(15 days)

Fig 4. skin fixed in saturated sodium chloride solution S sebaceous gland (H&E) X 400(15 days)

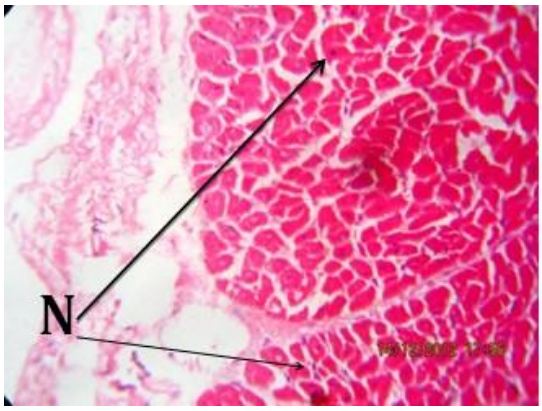

Fig 5 . muscle fixed in formalin **N** nuclei (H&E)X100 (15days)

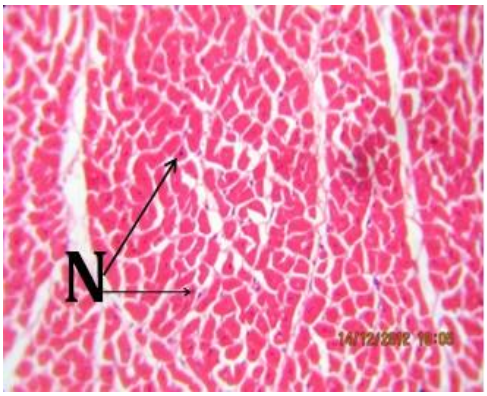

Fig6.muscle fixed in Saturated sodium chloride **N**nucli (H&E) X100 (15days)

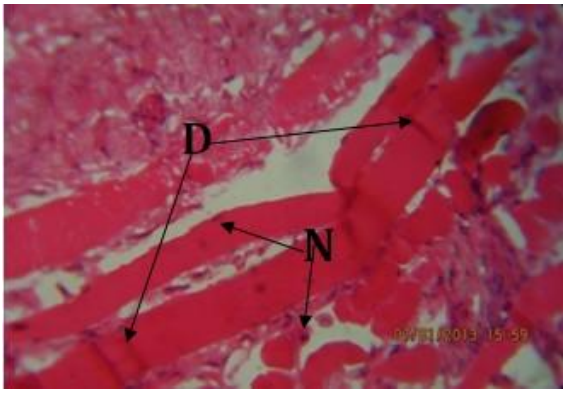

Fig7 cardiac muscle fixed in formalin D intercalated disk **N** nuclei (H&E)X 400 (2days)

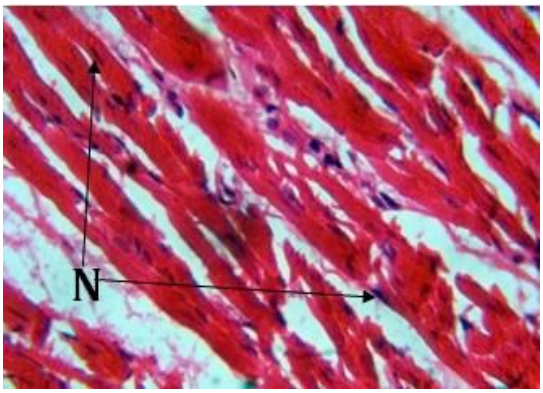

Fig 8 cardiac muscle fixed in saturated sodium chloride solution **N** nuclei (H&E)X

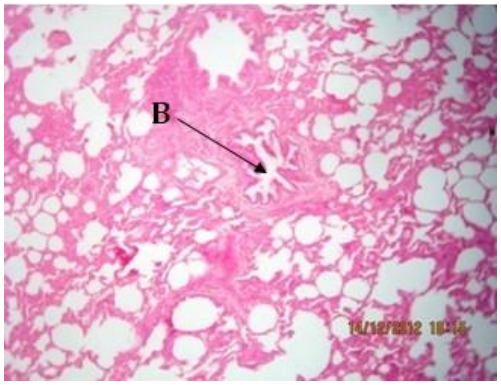

Fig 9 lung fixed in formalin **B** bronchiole (H&E)X 100 (15days)

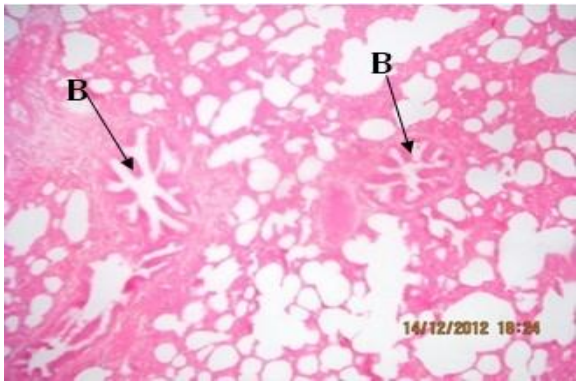

Fig 10 lung fixed in saturated sodium chloride solution**B** bronchiole (H&E)X 100

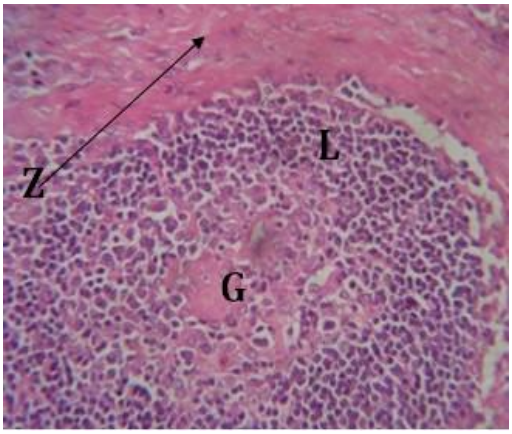

Fig 11 lymph node fixed in formalin Z marginal zone G germinal zone L lymphocytes (H&E)X 400 (2days)

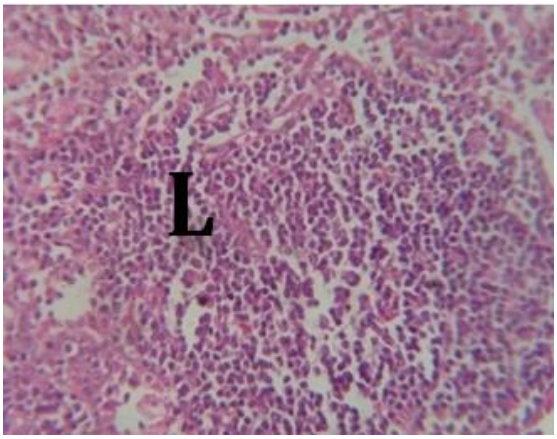

Fig12 lymph node fixed in saturated sodium chloride solution **L** lymphocytes (H&EX) 400 (2days)

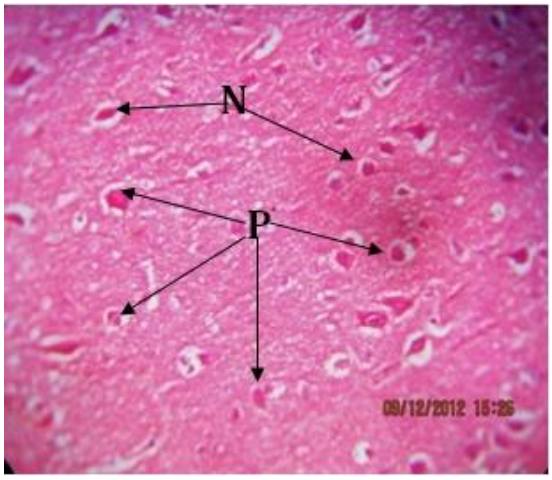

Fig 13 brain fixed in formalin P pyramidal cells N neuron (H&E)X 400 (30 days)

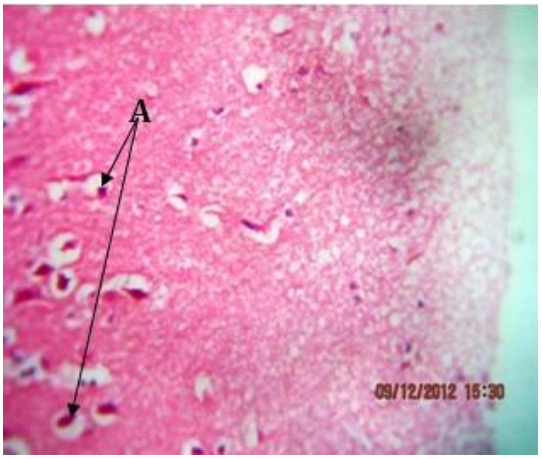

Fig14 brain fixed in saturated sodium chloride solution A astrocyte (H&E) X 400

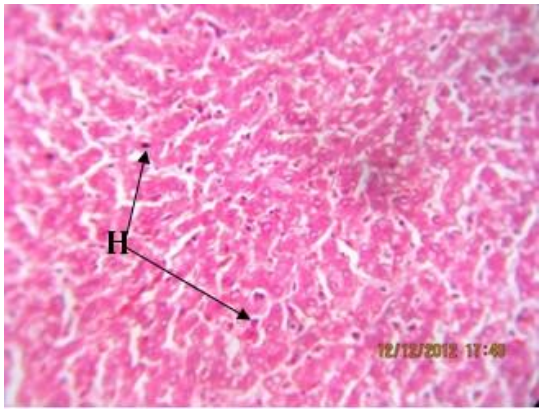

Fig 15 liver fixed in formalin H hepatocytes (H&E)X 100(15days)

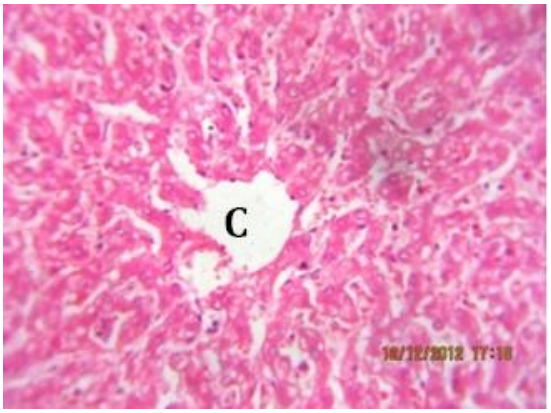

Fig16 liver fixed in saturated sodium chloride solution **C** central vein (H&E)X 100(15 days)

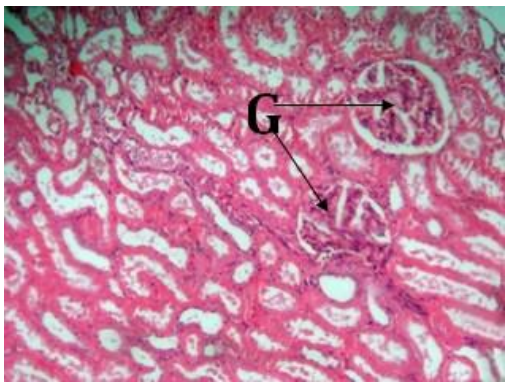

Fig 17 kidney fixed in formalin G glomeruli (H&E) X 100(15 days)

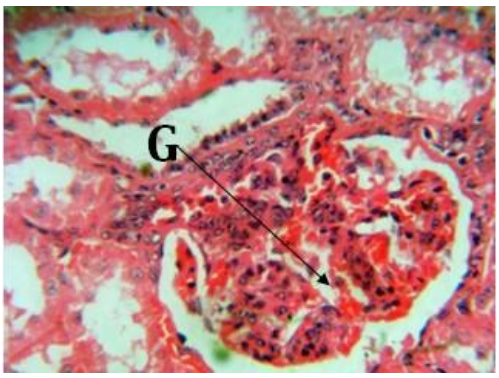

Fig18 kidney fixed in saturated sodium chloride solution G glomeruli (H&E) X400(15 days)

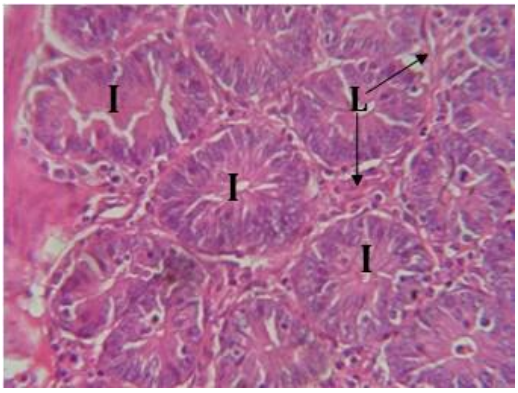

Fig 19 small intestine fixed in formalin I intestinal gland L lamina propria (H&E) X400 (2days)

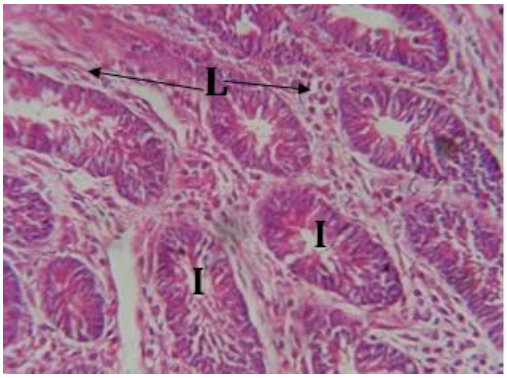

Fig 20 small intestine fixed in saturated sodium chloride I intestinal gland L lamina propria (H&E) X400(2days)

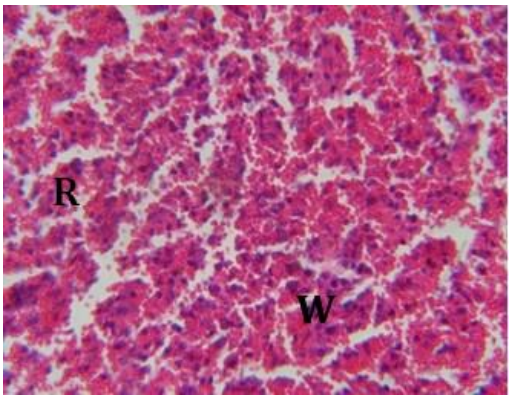

Fig 21spleen fixed in formalin **R** part of red pulp **W** part of white pulp (H&E) X 400(2days)

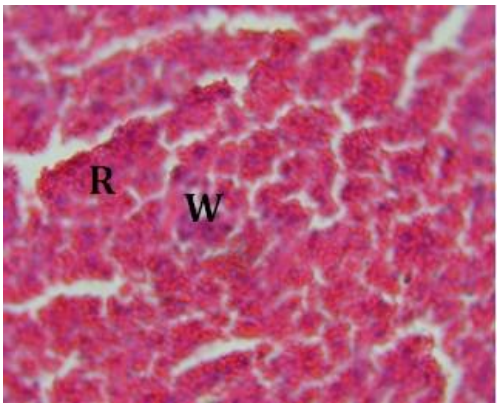

Fig 22 spleen fixed in saturated sodiumchloride **R** part of red pulp **W** part of white pulp (H&E) X 400(2days)

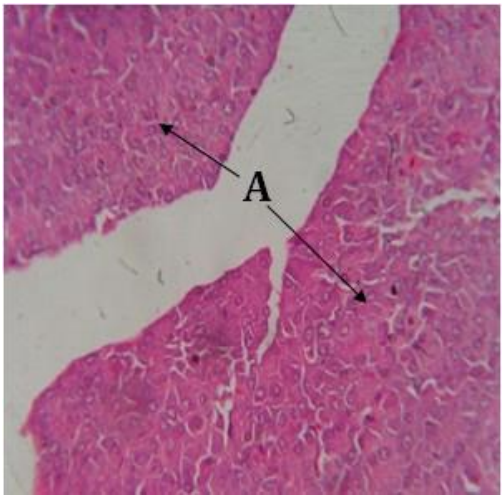

Fig 23 pancreas fixed in formalin **A** acini (H&E)X 400 (2days)

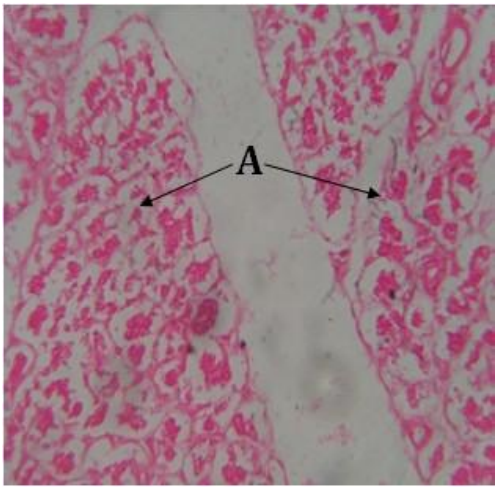

Fig 24 pancreas fixed in saturated sodium chloride solution **A-**auotolysed acini (H&E)X 400 (2days).

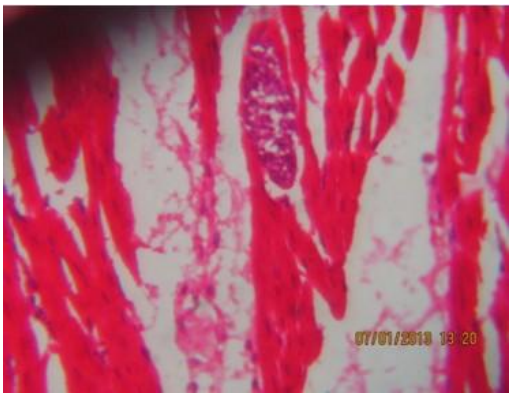

Fig 25 sarco cyst in muscle fixed in formalin(H&E)X 400. Note no degeneration or inflammation surround the cyst.

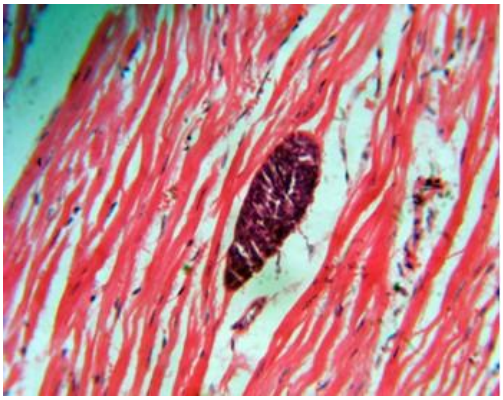

Fig 26 Sarco cyst in muscle fixed in saturated sodium chloride solution(H&E)X 400.Note presence of tissue cyst with no inflammatory reaction.

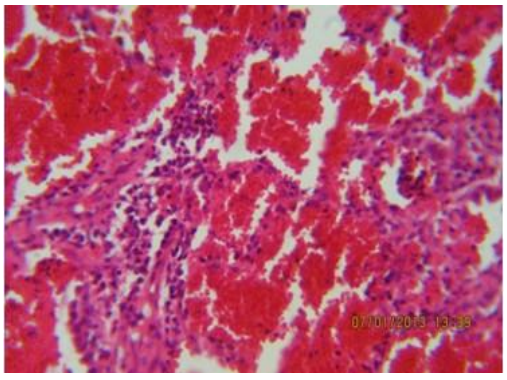

Fig 27 Hemorrhages in lung fixed in formalin (H&E)X 400 .**Note** the infiltration of inflammatory cells.

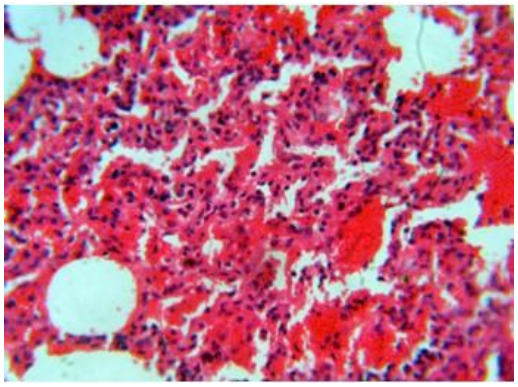

Fig 28 Hemorrhages in lung fixed in saturated sodium chloride solution (H&E) X400.Note also the infiltration of inflammatory cells and emphysema.

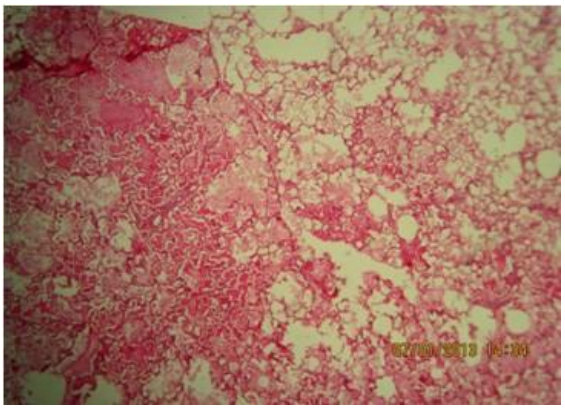

Fig 29 Edema in lung fixed in formalin (H&E) X 40

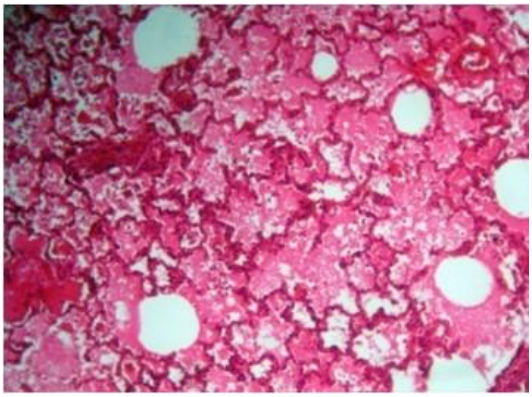

Fig 30 Edema in lung fixed in saturated sodium chloride solution (H & E) X 100

Discussion

There is no universal or ideal fixative that has been identified to serve all requirements .Fixatives are therefore selected on the basis of their ability to demonstrate a specific feature of a specific tissue (Grizzle et al, 2001). Each fixative has specific properties and disadvantages and their many different effects emphasis the necessity for careful consideration and selection of the appropriate fixing reagent, when studies of specific cellular substances or working in the field are planned (Hopwood et al, 1989). Most of the popular fixatives have disadvantages which the user should be well conversant with .For example formaldehyde is now known to be a human carcinogen. It causes nasopharangeal cancer, sinonasal cancer and myeloid leukaemia (NTP, 2011; OSHA, 2011) .It is an immediate irritant to the eyes, upper respiratory tract and the skin. Safety precautions should include proper ventilation and exhaust, limited or restricted exposure periods and thorough washing if spilt on tissue surfaces such as the skin (Eltoum et al, 2001b).Glutaraldehyde which is not normally used for routine histopathology penetrates very slowly and it is recommended that tissue be less than 1mm in thickness in at least one dimension (Bozzola and Russell , 1992).Mercury is highly toxic and can be absorbed through the skin and is an accumulative poison .Mercury salts has consequent problems with disposal .It must not be disposed into sewage system. It needs to precipitate the mercuric salts with thioacetamide and disposed as mercuric sulphide which could be disposed safely (Carson, 2007).Alcohols can cause distortion of nuclear details and shrinkage of cytoplasm , as well as it is volatile. Acetone should not be used on some tissue processors because it will adversely affect seals and components of the equipments , however it is flammable and volatile and generally not used on automatic tissue processors.(Drury and Wallington, 1980).Acetic acid is good in nucleic acid fixation, but it lyses red blood cells. Picric acid can hydrolyzes nucleic acids, so it should be avoided if DNA or RNA are to be demonstrated. It has the risk of explosion by heating or percussion of the dry substances so, it must be stored wet with water (Drury and Wallington, 1980).Because of the toxic hazards of formalin and other fixatives, saturated sodium chloride has been sought of as a safer alternative. ALSaraj in Iraq.(2010) published the only article that dealt with saturated sodium chloride as a tissue fixative. He fixed rabbit tissues obtained from liver, kidney, and spleen for 24 hours prior to tissue processing and staining. His results revealed that saturated sodium chloride solution could be used as a safe tissue fixative agent. The results of the present study ,showed that skin ,lungs ,lymph nodes, brain, skeletal and cardiac muscles specimens were well fixed in saturated sodium chloride solution for three fixation periods of(2,15,and 30 days) as compared to the control sections fixed in formalin . The liver and kidney showed good fixation in samples fixed for 15 days. The spleen and small intestine showed good results in samples kept in the fixative for 2 days and there after showed some degree of distortion. The pancreas showed autolysis in the

parenchyma tissue and lost its histological details in sections fixed for 2 days. The histopathological studies of some lesions revealed the obvious details of the sections under investigation. Needless to say that this study differed from that of AlSaraj in investigating the histology of more number of the organs, used different species and some pathological lesions kept in saturated sodium chloride for three different and longer periods of 2,15 and 30days.The technique of using saturated sodium chloride is simple, easy to prepare and apply and the required simple equipments can be found in any laboratory. Besides, the whole method does not take more than a few minutes. It is not hazardous, no need to strict guidelines to limit the exposure of workers to it in the workplace. Its disposal is simple with no harm to anybody. The last but not the least it is cheap and available everywhere.In the light of the above, it is anticipated that using this method will help promote the diagnostic efficiency and quality in veterinary clinics and dispensaries in different states of Sudan, particularly those in remote rural areas. The veterinarian can take specimens for histopathological examination and fix them in saturated sodium chloride solution when no formalin or any other fixative is available.The exact mechanism by which the sodium chloride acts on the tissue is unclear and no reports were made to explain its precise action on the tissue. Therefore it could be classified as unknown mechanism fixative. In conclusion Saturated Sodium chloride solution provides a good alternative method of fixation in specimens prepared for histological and hispathological studies. It is safe, cheap and available, with no deleterious effects on tissue morphology and without pigmentation.

References

A .Alsaraj . **(2010**), Use of saturated sodium chloride solution as a tissue fixative .Iraqi Journal of Veterinary Sciences.p.53 -58 ,v. 24(1) .

BozzolaJJ, and Russell LD. **(1992),** Electron microscopy: principles and techniques for biologists. Boston: Jones and Bartlett.

Carson FL. **(2007),** Histotechnology. 2nd ed. Chicago: ASCP Press.

Carson, Freida L , Christa, Hladik .(**2009).** Histotechnology : Aself – International Text (3 ed.). Hong Kong : P . 2 .

Drury RAB, and Wallington EA. **(1980)** Carleton's histological technique. . 5th ed. New York: Churchill Livingstone.

Eltoum I, Fredenburgh J, and GrizzleWE **(2001a);**Advanced concepts in fixation: 1. Effects of fixation on immunohistochemistry, reversibility of fixation and recovery of proteins, nucleic acids, and other molecules from fixed and processed tissues. 2. Developmental methods of fixation. J Histotechnol 24;201-210.

Grizzle WE, Stockard C, and Billing P. **(2001)** .The effect of tissue process variables other than fixation on histochemical staining and immunohisto chemical detection of antigens. Journal of histo technology 24: 213 – 219.

Hopwood D, Slidders W, Yeaman GR. **(1989)** Tissue fixation with phenol-formaldehyde for routine histopathology.Histochem J;21:228-234.

NTP. **(2011a);** Addendum to the 12th Report on Carcinogens. USA Department of Health and Human Services, National Toxicology Program.

NTP. **(2011b);** Report on carcinogens, Twelfth Edition. National Toxicology Program, USA Department of Health and Human Services, 2011;

OSHA **(2011);** Occupational Safety and Health Administration. Standard Number: 1910. 1048: Formaldehyde. United States Department of Labor, Occupational Safety and Health Administration, 2011.

Pearse AGE.(**1980),** Histochemistry .Theory and applied .4thed Edinburgh :Churchill Livingston.