

## Use of saturated sodium chloride solution as a tissue fixative

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### Abstract

The present study was carried out to examine the capability of saturated sodium chloride solution as a fixative agent instead of formalin which is regarded as a carcinogenic material. For this purpose 3 rabbits were used and their livers, kidneys and spleens were exposed and removed. Neutral buffered formalin solution, saturated sodium chloride solution and distilled water were used as fixatives for specimens obtained from the first, second and third rabbits respectively. Routine histological technique was performed to prepare a stained histological sections for light microscopic examination. The result showed that the tissue sections which were obtained by using sodium chloride have the same histological features and without any artifacts when they compared with the results obtained using formalin fixation method. We conclude that the saturated sodium chloride solution can be used as a fixative agent in some circumstances when no any fixative agent is available.

**Keywords:** Sodium chloride, Formalin, Tissue fixative.

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### أستعمال محلول كلوريد الصوديوم المشبع كمادة مثبتة للانسجة

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### الخلاصة

اجريت هذه الدراسة لفحص قابلية محلول كلوريد الصوديوم المشبع على حفظ الانسجة بدلا من محلول مادة الفورمالين المسرطنة. لذلك تم استعمال 3 ارناب حيث تم بعد قتلها اخذ الكبد والكلى و الطحال منها. تم استخدام محلول الفورمالين المتعادل و محلول كلوريد الصوديوم المشبع و الماء المقطر كمواد حافظة لاعضاء الارنب الاول و الثاني و الثالث على التعاقب. استخدمت الطريقة الروتينية للحصول على الشرائح النسيجية لفحصها باستخدام المجهر الضوئي. اظهرت النتائج بان الشرائح النسيجية التي حصل عليها باستخدام محلول كلوريد الصوديوم المشبع لها نفس الصفات النسيجية عند مقارنتها مع تلك التي استخدم فيها محلول الفورمالين المتعادل. وبذلك يمكن ان نستنتج بان محلول كلوريد الصوديوم المشبع يمكن ان يستعمل كمادة حافظة للانسجة وخاصة في الظروف التي لايتوفر فيها اي مادة حافظة.

### Introduction

As early as 400 BC Hippocrates discussed the biological effects of mercury and alcohol as fixatives (1). 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 preparative procedures without change. Fixation arrests autolysis and bacterial

decomposition and stabilises the cellular and tissue constituents so that they withstand the subsequent stages of tissue processing. Fixation should also provide for the preservation of tissue substances and proteins. Therefore, it is regarded the first step and the foundation in a sequence of events that culminates in the final examination of a tissue section (2).

A large variety of fixatives is now available but no single substance or known combination of substances has the ability to preserve and allow the demonstration of every tissue component. It is for this reason that some fixatives have only special and limited applications, and in other instances, a mixture of two or more reagents is necessary to employ the special properties of each. The selection of an appropriate fixative is based on considerations such as the structures and entities to be demonstrated and the effects of short-term and long-term storage (3). Each fixative has advantages and disadvantages, some are restrictive while others are multipurpose. Ferdinand Blum has been credited as the first person to use formaldehyde as a tissue fixative. Formaldehyde, as 4% buffered formaldehyde (10% buffered formalin), is the most widely employed universal fixative particularly for routine paraffin embedded sections (4).

The aim of the present work is to test the possibility of using saturated sodium chloride solution as fixative and preserving agent during histological and pathological procedures and compared with the results obtained by using conventional formalin fixation.

#### **Materials and methods**

Three male rabbits were randomly assigned irrespective of age and weight into the following: First rabbit: specimens fixed in neutral buffered formalin (positive control rabbit). Second rabbit: specimens fixed in saturated sodium chloride solution. Third rabbit: specimens immersed in distilled water (negative control rabbit).

Each rabbit was anaesthetized with chloroform in an air-tight jar, then the animal was laid down on dissecting board. The liver, kidneys and spleen were exposed, removed and washed by water. Specimens of 5mm thickness of these organs were excised.

The fixation was made immediately after the removal of the above mentioned organs for 24 hours by using the following:

##### **First rabbit:**

Neutral buffered formalin fixative solution which composed of: Formaldehyde 37%, 100 ml. Distilled water 900 ml. Sodium phosphate monobasic ( $\text{NaH}_2\text{PO}_3$ ), 4 gm. Sodium phosphate dibasic ( $\text{Na}_2\text{HPO}_3$ ), 6.5 gm.

##### **Second rabbit:**

Saturated sodium chloride solution.

##### **Third rabbit:**

Distilled water.

Then procedure of preparing the paraffin section slides and staining by Harrie's Haematoxylin and Eosin (H&E) was performed to prepare a stained histological sections for light microscopic examination (5).

## **Results**

### **Liver: fixed in neutral buffered formalin**

There were no tissue artifacts such as shrinkage, precipitate and swelling, therefore the liver appears to be divided clearly into many lobules, each one is hexagonal in shape with central vein located at its center. The portal canal which consist of a bile duct, a branch of hepatic artery and a tributary of the portal vein, all appeared clearly and enclosed in a common investment of connective tissue (take pink color). The paranchymal tissue of the liver appeared to be arranged in one or two cell thickness plates converging from the periphery to the center of lobule and the sinusoids are intermingled in between these plates. The cytoplasm appeared to be acidophilic and the nucleus basophilic and located at the center of the cells. Some hepatocytes appeared to contain more than one nucleus, and each nucleus contains a prominent nucleolus (Fig. 1 and 2).

### **Liver: fixed in saturated sodium chloride solution**

The sodium chloride solution was preserve the tissue and cells without any shrinking or swelling and without distorting or dissolving cellular constituents. Therefore the liver lobule architecture is similar to those of the control group. The central vein being located at the center of the hexagonal liver lobule. The hepatocytes appeared normal and arranged in form of plates. The cytoplasm appeared to be acidophilic while the nuclei basophilic and some of hepatocytes were binucleated, this indicates that the tissue sections were taken the stained in good manner and without any precipitate (Fig. 3 and 4).

### **Liver: immersed in distilled water**

There was no section appear under microscope.

### **Kidney: fixed in neutral buffered formalin**

The cortex of the kidney showed clear cellular details of its all structures (renal corpuscles, convoluted and straight tubules) and stain well by (H &E) stain. There was no shrinkage or swelling of cells. While the medulla of the kidney also showed clear cellular details of it all structures (straight portions of tubules, thin segments of Henel's loop and collecting tubules). There were no tissue artifacts (shrinkage, precipitate and swelling) and all of sections appeared to be stained well (Fig. 5 and 6).

### **Kidney: fixed in saturated sodium chloride solution**

Sections taken from kidneys of this rabbit revealed that the general structure of the kidney is nearly similar to that of the control group and seemed to be normal i.e. preserved cortex and medulla architecture without any morphological changes of the cells such as swelling and shrinkage or any artifacts. Also the cellular details appeared clearly and stained well (Fig. 7 and 8).

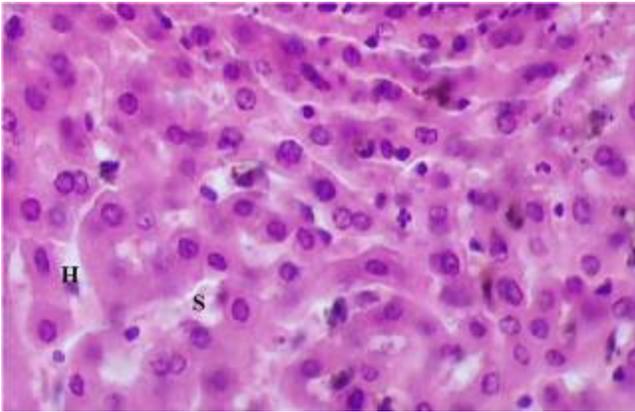


Figure 1: Photomicrograph of the liver of rabbit fixed in neutral buffered formalin showing hepatocytes (H) and the sinusoids (S) (H&E X400).

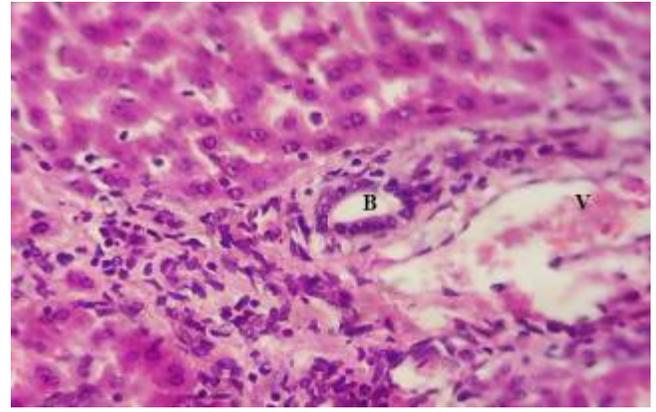


Figure 4: Photomicrograph of the liver of rabbit fixed in sodium chloride solution showing the small bile duct (B) and the terminal branch of the portal vein (V) in the portal tract (H&E X400).

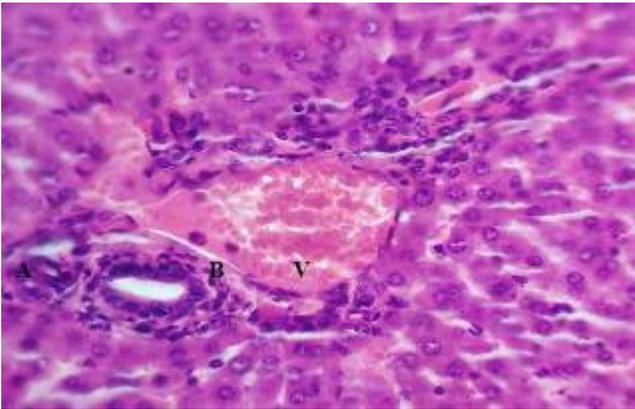


Figure 2: Photomicrograph of the liver of rabbit fixed in neutral buffered formalin showing the small bile duct (B), the terminal branch of the portal vein (V) and the terminal branch of hepatic artery (A) in the portal tract (H&E X400).

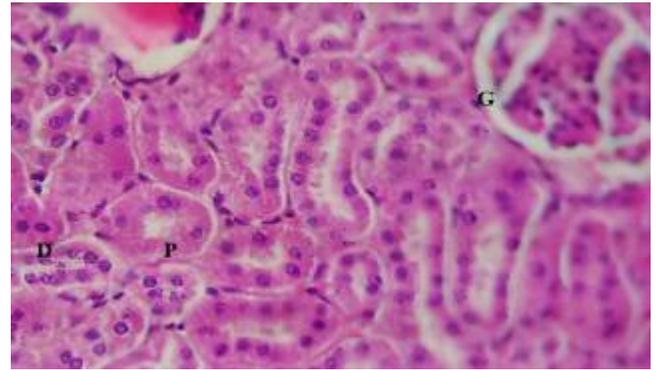


Figure 5: photomicrograph of the kidney (cortex) of rabbit fixed in neutral buffered formalin showing the glomerulus (G), proximal (P) and distal (D) convoluted tubules (H&E X400).

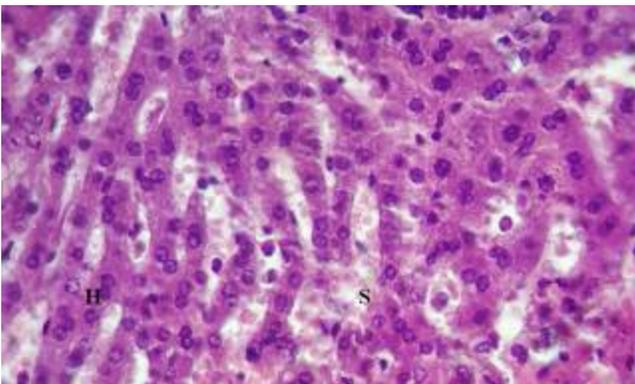


Figure 3: Photomicrograph of the liver of rabbit fixed in sodium chloride solution showing hepatocytes (H) and the sinusoids (S) (H&E X400).

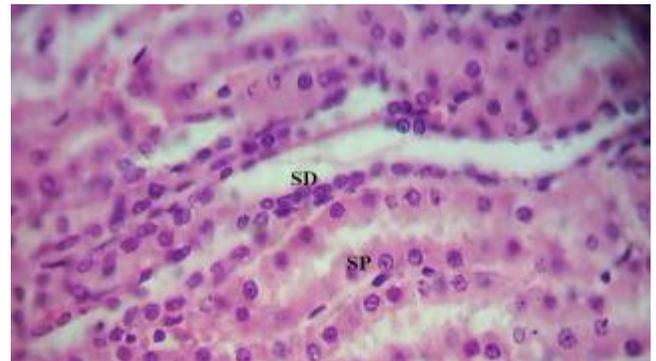


Figure 6: photomicrograph of the kidney (medulla) of rabbit fixed in neutral buffered formalin showing the straight segment of the proximal tubule (SP) and the straight segment of the distal tubule (SD) (H&E X400).

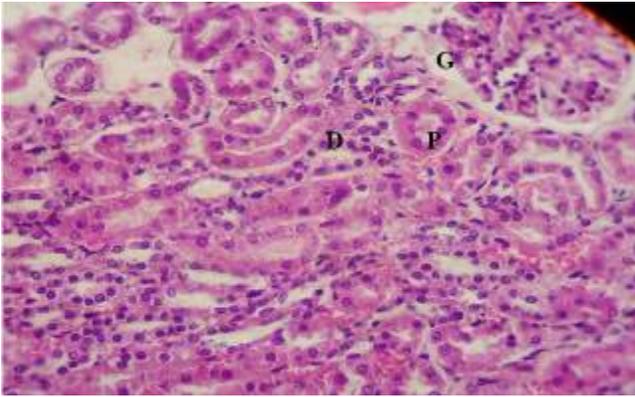


Figure 7: photomicrograph of the kidney (cortex) of rabbit fixed in sodium chloride solution showing the glomerulus (G), proximal (P) and distal (D) convoluted tubules (H&E X400).

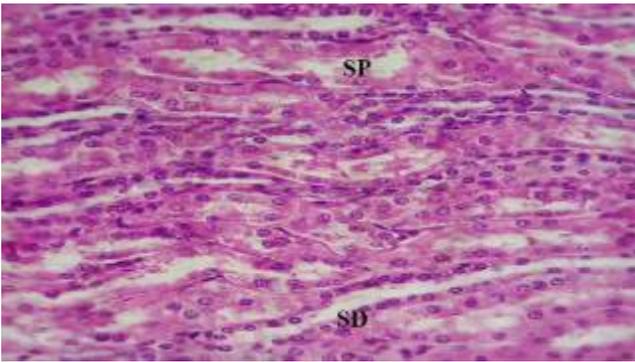


Figure 8: photomicrograph of the kidney (medulla) of rabbit fixed in sodium chloride solution showing the straight segment of the proximal tubule (SP) and the straight segment of the distal (SD) tubule (H&E X400).

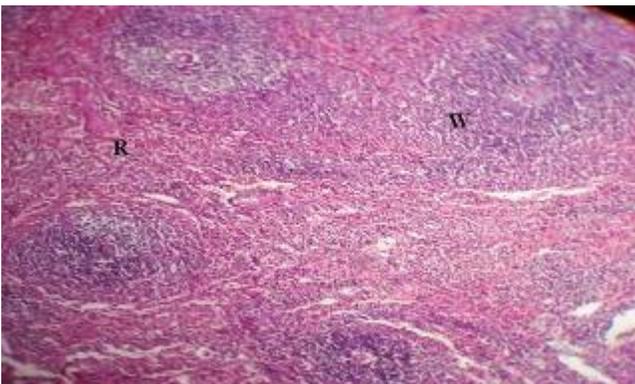


Figure 9: photomicrograph of spleen of rabbit fixed in neutral buffered formalin showing the white (w) and the red (R) pulps (H&E X100).

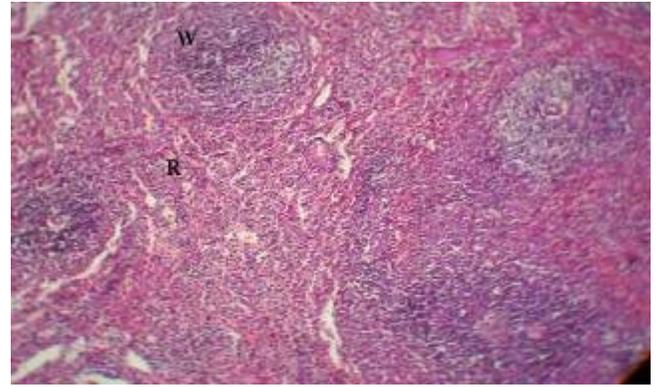


Figure 10: photomicrograph of spleen of rabbit fixed in neutral buffered formalin showing the central artery (A) of the white pulp and the sinusoids (S) of the red pulp. (H&E X400).

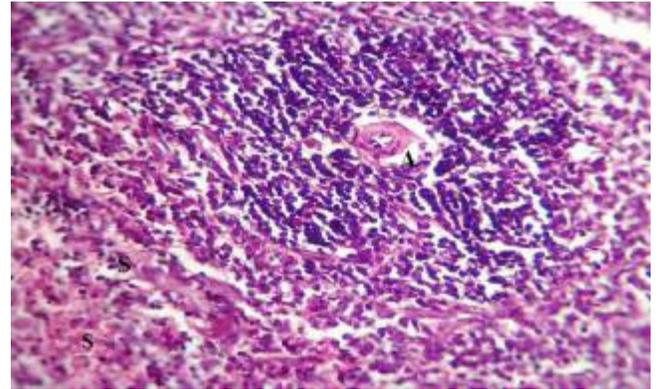


Figure 11: photomicrograph of spleen of rabbit fixed in sodium chloride solution showing the white (W) and the red (R) pulps (H&E X100).

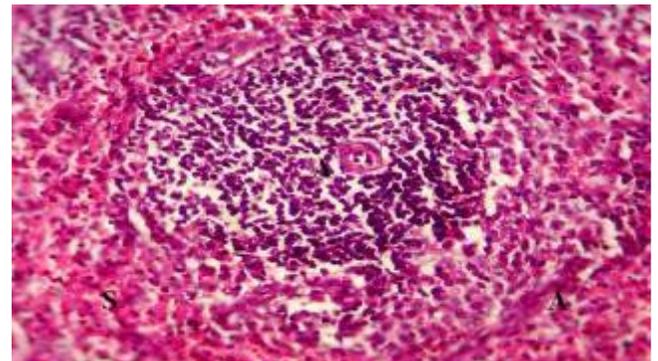


Figure 12: photomicrograph of spleen of rabbit fixed in sodium chloride solution showing the central artery (A) of the white pulp and the sinusoids (S) of the red pulp. (H&E X400)

**Kidney: immersed in distilled water**

There was no section appear under microscope

**Spleen: fixed in neutral buffered formalin**

Histological section throughout the spleen showed a well defined cellular structures arranged into white (splenic nodules and central artery) and red (splenic cords and sinuses) pulps without any artifacts (Fig. 9 and 10).

**Spleen: fixed in saturated sodium chloride solution**

Microscopically, there was a preserved normal histological sections architecture nearly similar to those of control group without any morphological (shrinkage or swelling) changes. The well defined histological structures indicates well stained and preserved sections (Fig. 11 and 12).

**Spleen: immersed in distilled water**

There was no section appear under microscope.

**Discussion**

A systematic study of the fixatives began in the latter half of the 19th century, however, it must be noted that fixation by itself introduces a major artifact. Much attention was focused on developing fixatives that would preserve cells and tissue constituents in as close a life-like state as possible while allowing them to undergo further preparative procedures without change (6).

The mechanisms by which fixatives act may be broadly categorized as dehydrants, heat effects, cross-linkers, and effects of acids and combinations of these. Agents that combine with proteins are called additives and those that precipitate proteins are called coagulants. At this time, it is accepted that no one fixative fulfils all of the aims of cell or tissue preservation: namely prevention of autolysis and preservation of physical and chemical properties of the tissue (7).

In aldehydes include formaldehyde (formalin) the tissue is fixed by cross-linkages formed in the proteins, particularly between lysine residues. This cross-linkage does not harm the structure of proteins greatly, so that antigenicity is not lost. Formalin penetrates tissue well, but is relatively slow and the standard solution is 10% neutral buffered formalin. A buffer prevents acidity that would promote autolysis and cause precipitation of formol-heme pigment in the tissues (8).

Formalin is used for all routine surgical pathology and autopsy tissues when (H and E) slide is to be produced. It is the most forgiving of all fixatives when conditions are not ideal, and there is no tissue that it will harm significantly. Most clinicians and nurses can understand what formalin is and it smells bad enough that they are careful handling it.

However previous workers have indicated that exposure to formaldehyde has a serious effects and is highly suspected to be a human carcinogenic (9,10).

A method to overcome the problems of formaldehyde is to use an alternative fixative that is better suited for the preservation of tissues.

A study conclude that the cheap saturated table salt solution can be used as an alternative to the formaldehyde in preserving the bodies of animals (11).

Fixation of skin and lymph node fragments in anhydric sodium chloride at room temperature for periods of weeks or months was found to preserve morphological structures and immunoreactivity (12).

Segments of rat's aorta were harvested, immersed in pulverized dehydrated sodium chloride, and stored for 1 week to 3 months. Thereafter, they were desalinated and transplanted. The tensile strength and maximum intraluminal pressures did not significantly differ from freshly harvested, transplanted aortic segments (13).

Human skin fragments can be preserved in anhydric sodium chloride at room temperature for periods of weeks or months and successfully transplanted, retaining normal morphological structure (14).

The result of the present study showed that the saturated sodium chloride solution can be used as a fixative for tissue in pathological or histological procurers. The tissue sections which obtained have the same histological features when they compared with the results obtained using conventional formaldehyde fixation methods. It penetrates tissue but is relatively slow and prevents autolysis as well as it cause no precipitation of pigment in the tissues nor morphological changes (swelling or shrinkage) of the cells.

The exact mechanism by which the sodium chloride act on the tissue is unclear and no one mentioned its precise action on the tissue. Therefore it need more investigations and can be classified as unknown mechanism fixative, such as mercuric chloride, picric acid.

We suggest that this new (cheap and safe) method of fixation can be considered as an alternative fixative procedure which can be used in emergency cases when no any fixative is available (for pathologist, veterinarian, biologist...etc) in such cases for tissue preservation.

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