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EFFECT OF THE LONG-TERM GUM ARABIC SOLUTIONS PRESERVATION ON HISTOLOGICAL STRUCTURE OF BIOLOGICAL TISSUES

Abstract: In our previous study by Satte et al., in 2017, gum Arabic solutions were used in the preservation of biological tissues, and the produced samples were maintained their macroscopic features for the long-term. The current study was planned to compare the microscopic structure of biological tissue preserved in gum Arabic solutions with those of unpreserved tissue as a negative control and tissue preserved in silicone-S10 as a positive control. The study was conducted on 5 µm thick tissue sections obtained from 39 specimens of adult sheep, divided into eleven experimental groups and two control groups (positive & negative); each group contained three specimens: kidney, heart, and brain. The experimental groups were preserved in eleven different concentrations of gum Arabic solutions, the positive control group was preserved in silicone-S10, and the negative control group was unpreserved tissue (fresh). From each group, five tissue sections of 5 µm thick were cut from kidney, heart, and brain and were prepared for subsequent hematoxylin and eosin staining for histological examination under a light microscope. The gum Arabic solutions preserved tissue sections revealed the histological architecture similar to that of the unpreserved and that observed in silicone-S10 preserved tissues. The present study concludes that; tissues preserved in gum Arabic solutions can be used in teaching histology for medical students.

Key words: histology, gum Arabic, preservation, biological tissues.

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1. Introduction

Each year new medical students make their entry in the medical and veterinary colleges. The difficulty of obtaining fresh organs and tissues to study anatomy has encouraged the used of preserved one. There are several methods for preservation of biological tissues, which has helped a lot in the study of anatomy for medical and veterinary students and researchers. In ancient times, gum Arabic and some local materials such as natron and herbs were used traditionally by Egyptian to preserve cadavers [1]. Century's later, formalin solutions have been used for fixation of tissues, but formalin has health hazards such as watery eyes; burning sensations in the eyes, nose, and throat; coughing; wheezing; nausea; and skin irritation for students and staff during practical section [2]. Recently, plastination was introduced as a modern and safe technique for preservation of cadavers by Von Hagens in 1979. During the plastination process, the tissues were fixed in formalin (5 to 20%), dehydrated in acetone, impregnated in curable silicone-S10 or epoxy resin and cured by silicone-S6. The silicones used in the plastination procedure are relatively expensive, not available and patented [4, 8, 18].

Excellent histological tissue sections similar to that of unpreserved histological sections were obtained from silicone-S10 plastinated specimens, however, simple compactness of cells was observed in sections prepared from silicone-S10 plastinated specimens [7, 8,].

The pure gum Arabic solution consists of natural polymers with weak physical properties such as viscosity and elasticity [10, 11]. Glycerin is used as the plasticizer agent to improve the absorption and viscosity of gum Arabic solution. Gum Arabic solution component are the gum Arabic powder, glycerin, and distilled water, and these materials are available and safe [10, 12]. Gum Arabic solutions were tested as an effective and safe material for the biological tissues preservation process, and the produced specimens were maintaining their original macroscopic features for the long term [14].

2. Material and methods

2.1 Preservation procedure

A total of 18 fresh organs (6 hearts, 6 kidneys, and 6 brains) of adult sheep were collected from an abattoir in Najran city, Saudi Arabia, and animal ethics were considered. The organs were transferred in an icebox to the dissection room at medical college, anatomy department, Najran University. First, the organs washed under running tap water to clean blood clots. Each organ was cut sagittally into two halves so that the total number of specimens became 36. The

specimens were divided into 12 groups, with each group containing 3 specimens (half kidney, heart, and brain). Each group of specimens was kept in plastic containers with tight lids and then fixed in 10% formalin (02144. CHEMANOL. KSA) for 3 days [14, 15].

After fixation, the specimens were dehydrated in three changes of pure acetone (7566/12. APCO. KSA) for 10 days at room temperature. The acetone is replaced by the tissues fluid and dissolved the excess fat. The concentration of acetone of the successive changes was measured by using a hydrometer (Fisher brand, USA, 9598115900). When the final acetone concentration remained at 99 % or above the specimens were considered dehydrated [16, 17].

Eleven gum Arabic solutions of different concentrations were prepared from pure gum Arabic powder (Acacia Senegal, Natural Gum, Sudan, 6-14600-000191), distilled water and pure glycerin (Chiangrai Agro-Industry Co. Ltd., Thailand 99.5% USP Grade). Two liters of each solution were kept in plastic containers of 3 liters capacity. The silicone-S10 (NC27261Silicones, Inc.211 Woodbine Street, High Point, NC 27260 USA) was mixed with catalyst-S3 (NC336-886-5018.) at 100:1ratio and was used as a control (Table 1) [5].

After fixation and dehydration, the first eleven groups of specimens were submerged in gum Arabic solutions, while group 12 specimens were submerged in the silicone-S10/S3 mixture as shown in table 1. The specimens were left in the different solutions for two days to equilibrate with solutions before force impregnation process. The submerged specimens for each group were covered by a stainless grid to avoid the samples floating [5, 14].

Forced impregnation was used for the replacement of acetone in the specimens with the gum Arabic solutions (for experimental groups) and a curable polymer (for the control group). The submerged groups of specimens were transferred to the vacuum chamber (Mopec, USA, 800-362-8491) connected to a vacuum pump (Mopec, USA, HP200D11001) for forced impregnation at room temperature. The vacuum caused the acetone to vaporize from the specimens creating spaces in the cell for the gum Arabic solutions and polymers to diffuse. The vacuum pressure was gradually decreased to 6 mmHg. The vacuum was maintained for 4 days (5 hours daily) for the experimental groups and one week for the positive control group. Impregnation was considered completed when there were no air bubbles coming out from the specimens [15].

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After forced impregnation, the specimens were removed from the impregnation solution, and excess gum Arabic solution and polymers were allowed to drain. The specimens in each group were then arranged on a stainless steel plate. The control group was transferred to close curing gas chamber at room temperature and cured with catalyst S6 (two times daily, 10minutes) until the specimens were hardened up for three days.¹⁷ The experimental specimens were allowed to harden by atmospheric air at room temperature for one week [14].

2.2 The histological procedure

From each group of experimental (preserved in gum Arabic) and silicone-S10 plastinated (as a positive control), a total of 3 small pieces of about 1 cm³ size were cut from kidney, heart wall, and brain after being stored for six months in the cabinet at room temperature. Subsequently, pieces of 1 cm³ size were cut from fresh (as a negative control) of each heart, kidney, and brain of adult sheep after the slaughter at an abattoir and fixed immediately after collection in 10% formalin (02144.CHEMANOL. KSA) for two days [6].

Specimens obtained from preserved gum Arabic tissues were submerged in three changes (70%, 80%, 90% and 100% ethanol) of alcohol for 12 hours per immersion to dissolved the impregnated gum Arabic solutions. Whilst, the collected specimens obtained from silicone-S10 plastinated tissues were submerged in alcohol (100% ethanol) for 48hours to dissolve the impregnated silicone. The fresh samples were dehydrated in 70%, 90%, 100% ethanol (32221. SIGMA-ALDRICH. Germany) for 12, 2 and 2 hours respectively.

after the tissues dissolved (for experimental and positive control samples) and dehydrated (for negative control sample), specimens were cleared in xylene (74132. APCO. KSA) for 30 minutes and embedded in molten paraffin wax (REF502004. McCormick. USA) for two hours in an embedding centre (Feica, Germany, 038837689). The embedded specimens were blocked and cut into thin sections of 5 µm thickness by using a microtome (Carl Zeiss, Germany, 3333000173). The tissue sections were transferred to water path heated at 50 °C for 2-3 minutes and then mounted on glass slides. The slides were placed on a heating plate (the UK, 1892447) at 70 °C for 15 to 20 minutes to adhere the tissue section to the glass slide [19, 22].

Five tissue sections mounted on glass slides were selected from each specimen and stained in hematoxylin (MOH784010004. SOMAT.KSA) and Eosin (17372-87-1. LOBA Chemie. Mumbai. India) [8].

The stained sections were examined under a light microscope (Carl Zeiss, Germany, 3333000173) and photomicrography images were taken for comparison.

3. Results:

The histological sections obtained from kidneys, hearts, and brains preserved in gum Arabic solutions showed good histological structures, and they are similar to that obtained from the same organs plastinated in silicone-S10 (positive control) and that obtained from unpreserved sections (negative control).

Histological sections obtained from gum Arabic solution preserved kidneys revealed clear renal corpuscles structure with obvious Bowman's capsule with its parietal layer of simple squamous epithelium followed by Bowman space, and the inner visceral layer made up of podocytes cells wrap around the capillaries of the renal glomeruli. The glomerular cells nuclei such as that of mesangial cells, and the glomerular capillaries endothelial cells that contact with blood are obvious. Also, renal tubules lining epithelia were visible, the proximal convoluted tubules lined with simple cuboidal cells with brush borders and the tubules lumens appeared narrow while the distal convoluted tubules are lined with simple cuboidal epithelium without brush border and appeared wide lumens. However, urinary spaces are slightly wider in negative control kidney sections compare to that in tissue sections obtained from kidney preserved in gum Arabic solution and that plastinated in silicone-S10 (Figures 1, 2, 3).

The heart tissue sections obtained from preserved gum Arabic solutions showed good stained cardiac muscle fibers connected by intercalated discs (densely staining regions at the end of muscle fibers) and with centrally located nuclei and similar to that of the unpreserved (negative control) heart sections (Figures 4, 5). The histological architectures of silicone-S10 plastinated heart sections showed deep compactness of cardiac muscles cells compared with that of the negative control and experimental groups (Figure 6).

Gum Arabic preserved brain tissue sections revealed well-stained neurons with clear outlines: the neuron cell bodies appeared with central nuclei and projected processes. Moreover, neuroglial cells nuclei were observed scattered among neurons. The histological architecture of gum Arabic preserved brain sections are similar to that obtained from unpreserved and plastinated silicone-S10 brains sections (Figures 7, 8, 9).

4. Discussions:

In this study, tissue sections obtained from gum Arabic preserved specimens showed very good microscopic details and can be used for histological studies; however, gum Arabic is cheap and safe natural products compared to silicone S10 which is

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relatively expensive synthetic materials with some health hazards [2, 17].

In previous studies, histological sections obtained from kidneys plastinated in silicone-S10 and stained with hematoxylin and eosin revealed clear and fine histological architectures as reported by researchers [7]. Therefore, these findings are similar to the tissue sections obtained from kidneys preserved by gum Arabic solution technique.

In current study tissue sections obtained from heart preserved in gum Arabic solution presented very good histological details, which is more clear than that obtained from silicone S10 plastinated heart sections, which agreed with the poor stained heart tissue sections reported in the previous study [9].

A previous study showed that the plastinated brain sections can be used for histological studies [23]. This agrees with our findings of tissue sections obtained from a brain preserved in gum Arabic

solutions, which showed the histological details such as neurons structures, supporting cells nuclei, and neurons boundaries, which are similar to that obtained from the negative control group.

The obvious wider of urinary space in negative control kidney sections compare to that of gum Arabic kidneys sections may be attributed to negative vacuum pressure used during organ impregnation in gum Arabic solutions and in silicone-S10, this agreed with reporters findings in tissue sections prepared from organs plastinated in silicone-S10 [7, 8].

Conclusion:

The outcome of the present study confirmed that gum Arabic solutions are an excellent medium for preservation of histological details of biological tissues, and is suitable for preservation of biological tissues for microscopic study for medical students.

Table 1. Impregnation solutions.

Solution No	Gum g/L	Glycerin %	Water %	Specimens group No
1	100	75	25	G1
2	100	80	20	G2
3	100	85	15	G3
4	100	60	40	G4
5	100	40	60	G5
6	100	30	70	G6
7	80	85	15	G7
8	90	85	15	G8
9	110	85	15	G9
10	50	80	20	G10
11	227	10	90	G11
12	Mix of Silicone-S10 and catalystS3 at ratio 100:1			G12 (positive control)

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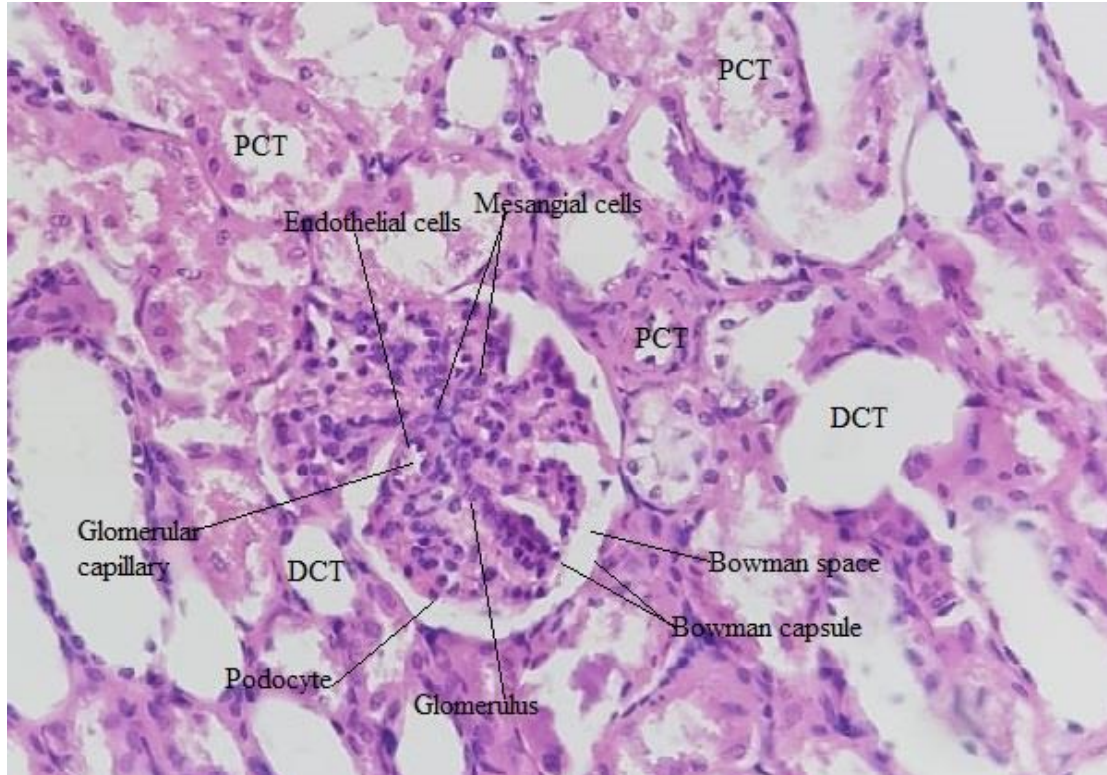


Figure 1: Histological features of unpreserved (fresh) kidney section. Photomicrograph, x40. Proximal convoluted tubules (PCT), distal convoluted tubules (DCT).

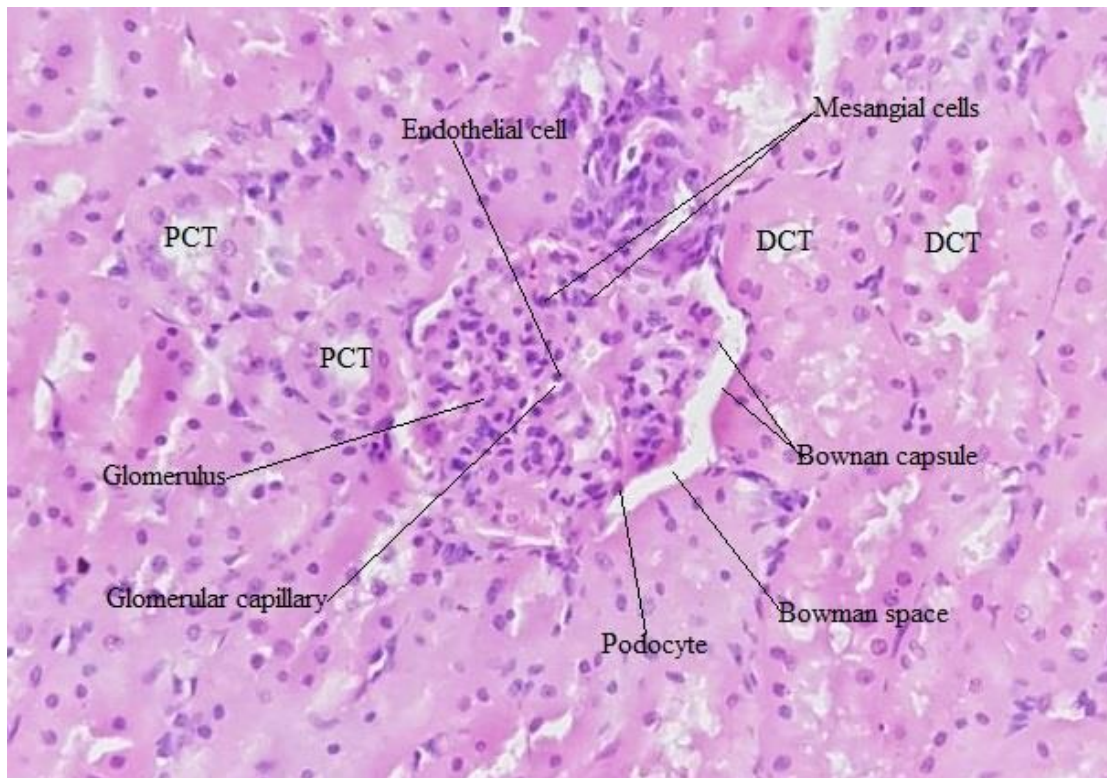


Figure 2: Histological features of gum Arabic solution preserved kidney section. Photomicrograph, x40. Proximal convoluted tubules (PCT), distal convoluted tubules (DCT).

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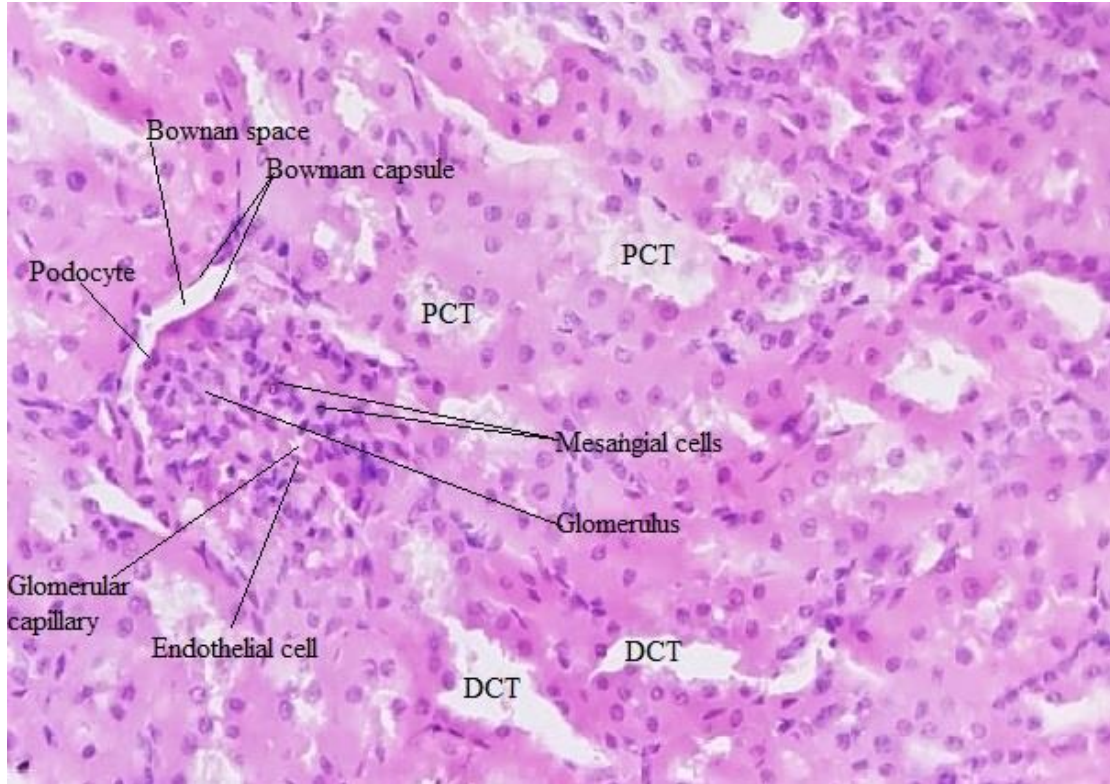


Figure 3: Histological features of silicone-S10 preserved kidney section. Photomicrograph, x40. Proximal convoluted tubules (PCT), distal convoluted tubules (DCT).

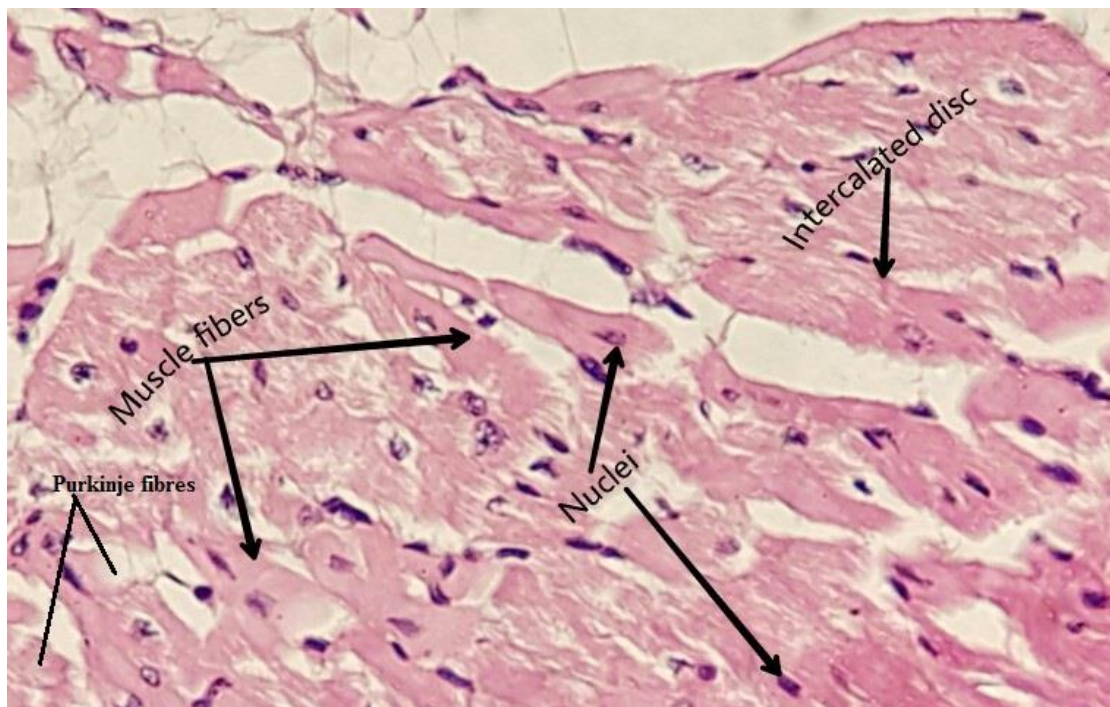


Figure 4: Histological features of unpreserved (fresh) heart section. Photomicrography, x40.

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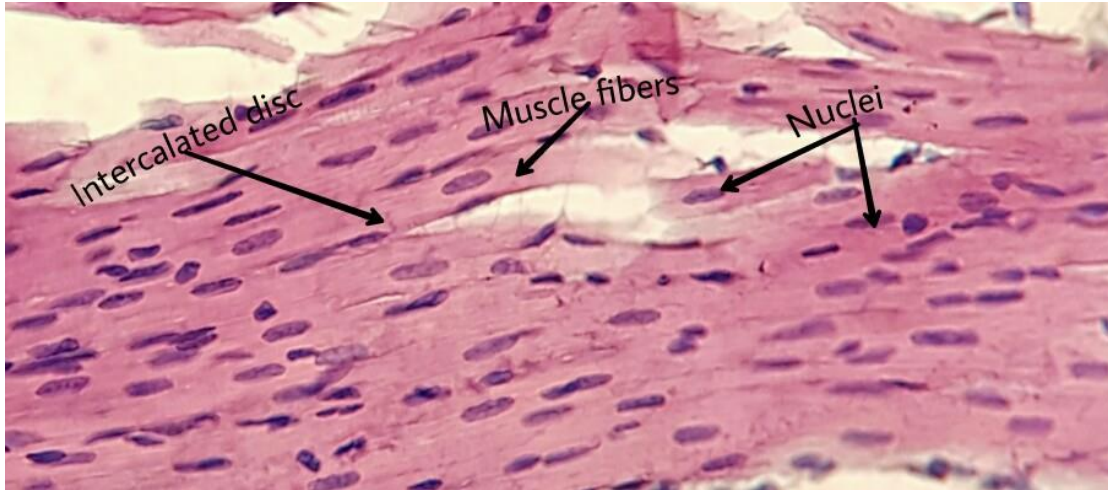


Figure 5: Histological features of gum Arabic solution preserved heart section. Photomicrography, x40

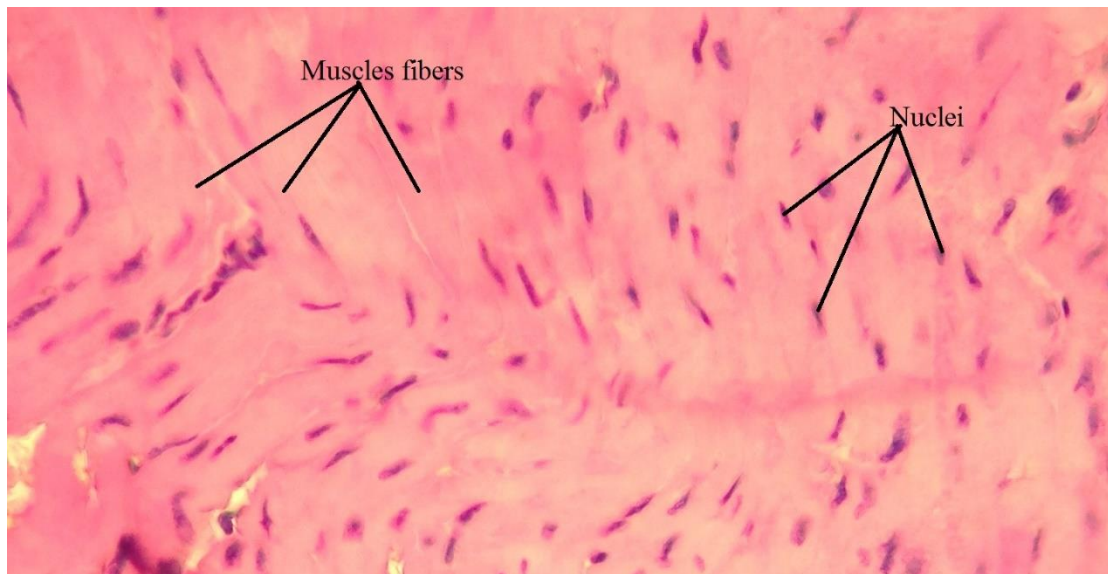


Figure 6: Histological features of silicone-S10 preserved heart section. Photomicrography, x40.

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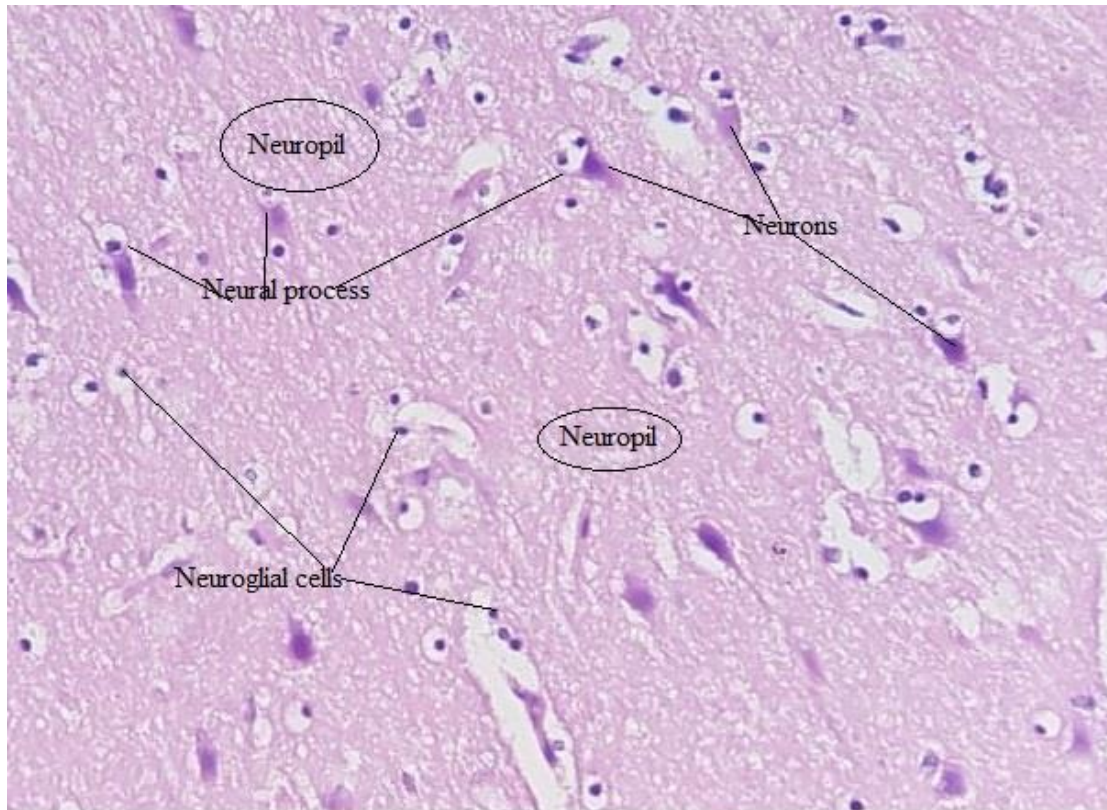


Figure 7: Histological features of unpreserved (fresh) brain section. Photomicrography, x40.

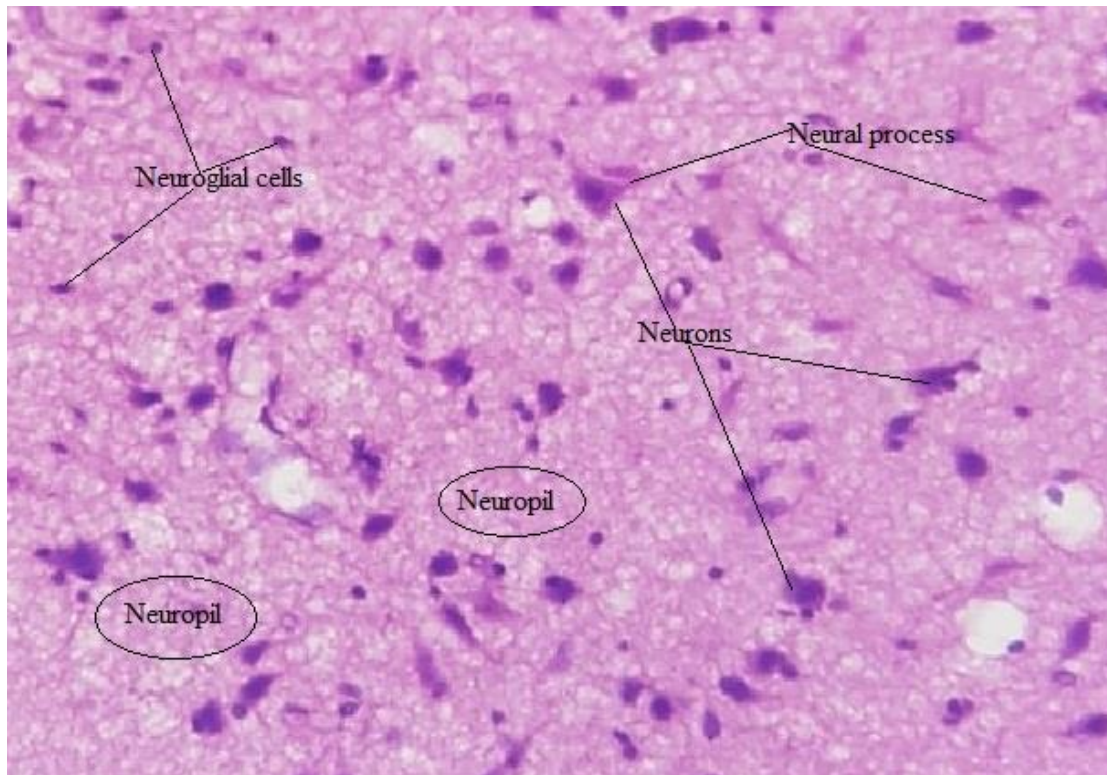


Figure 8: Histological features of gum Arabic solution preserved brain section. Photomicrography, x40.

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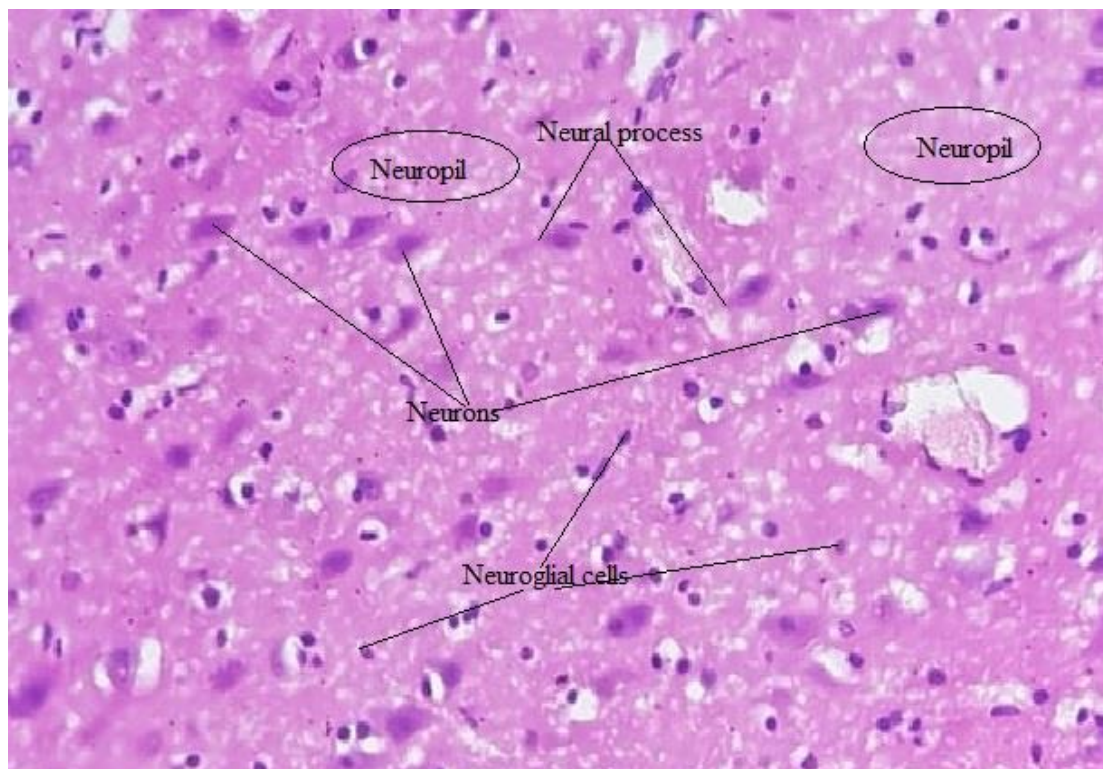


Figure 9: Histological features of silicone-S10 preserved brain section. Photomicrography, x40.

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