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Original Research Article

Estimate the shrinkage and weight loss of the tissues during gum Arabic preservation process

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Abstract

Plastination process is a modern safe preservation process of biological tissues and succeeded in providing semi-original anatomical specimens for teaching medical students, but it relatively cost. In our previous study by Satte et al, in 2017, we used gum Arabic solutions as safe and inexpensive for the preservation of biological tissues, and the produced specimens were maintained their original anatomical features for the long-term. The current study aimed to estimate the shrinkage and weight loss of tissues preserved by gum Arabic solutions compared with the same tissues preserved by silicone-S10, which serve as a control. This study was conducted on 144 specimens obtained from adult sheep, divided into eleven experimental groups and one control group, each group contains twelve specimens of four halves of kidney, heart, and brain. The test groups were preserved in eleven different concentrations of gum Arabic solutions, and the control group was preserved in silicone-S10 as a control. The shrinkage and weight loss of preserved tissues were measured during the preservation process. During gum Arabic preservation process, no significant (p≤ 0.05) showed between test groups 1 to 10 and control, and the produced specimens relatively normal in shape and size. Group 11 specimens were presented a significant (p>0.05), which have the highest level of shrinkage and weight loss among all groups. To conclude: The specimens preserved by gum Arabic solutions (1-10) are realistic, maintained their general shape and size and can be used for teaching anatomy for medical students instead of relatively costive silicone-S10 plastinated tissues.

Keywords: Heart, Gum Arabic, Preservation, Shrinkage

INTRODUCTION

Recently, there is a considerable concern toward the direct technicians and students' exposure to formaldehyde during gross anatomy practical sessions. Therefore, medical institutes directed to the plastination of a real tissue, which is a worthy procedure for avoiding the risky agents used in ordinary specimen preparation. Previously, Dr. von Hagens in (1977) applied the plastination process by silicone-S10 impregnation in medical and veterinary medicine anatomy education but the materials used are relatively costive. The final plastinated specimens are realistic and relatively normal in shape and size (Von Hagens et al., 1987; Grondin, 1998). The measurements of the tissue shrinkage and weight loss during the plastination process are important

Figure 1. How the measurements of the shrinkage areas were applied in the kidney.

to evaluate the shape and size of produced specimens. Meanwhile, some tissues shrinkage and weight loss were presented during the plastination process (Brown et al., 2002). The gum Arabic solutions were used for the preservation of biological tissues, and the produced specimens were maintaining their anatomical features for the long term (Satte et al., 2017).

Gum Arabic is a natural polymer produced from wild trees of Acacia Senegal or Seyal which mainly growing in the African region. The structure of the gum Arabic is composed of high weights of glycoprotein and polysaccharide; therefore, these substances are water-soluble (Shanmugam et al., 2005). Gum Arabic was used in food and pharmaceutical industries as a long-term stabilizer (Garti and Reichman, 1993). Gum Arabic solution produced from a mixture of gum Arabic powder, glycerin, and distilled water, however, these material are inexpensive and available. The gum Arabic and some local materials such as natron and herbs were used traditionally by ancient Egyptian to preserve cadavers (Abdel-Maksoud and El-Amin, 2011). Pure gum Arabic solutions have some poor qualities such as absorption, flexibility, and viscosity (Duaqan and Abdullah, 2013; Alkarib et al., 2015). Some available plasticizers such as ethylene glycol, glycerin, polyethylene, and glycol are used to improve absorption, flexibility, and viscosity of gum Arabic solution (Alkarib et al., 2016; Wyasu and Okereke, 2012).

MATERIALS AND METHODS

A total of 72 adult sheep organs (24 hearts, 24 kidneys, and 24 brains) were possessed from a slaughterhouse in Najran city, Saudi Arabia. The ethical of the animal was taken and controlled by the experimental animal research and ethics committee in Najran University, Saudi Arabia. The organs were transferred in an icebox to dissection room and washed with water to clean the organs surfaces blood spots. Each organ was cut longitudinally into two halves. The specimens were divided into 12 groups with each group includes 12 specimens (4 halves of kidneys, 4 halves of hearts and 4 halves of brains). Each group of specimens was maintained in a plastic box with tight lids and then fixed in 10% formalin for three days (Srisuwatanasagul et al., 2010; Satte et al., 2017).

During the fixation phase, the specimen's dimensions were measured in centimeters using Vernier caliper (150X0-05X1\128, Raider, Shanghai, China) and also the weights were counted in gram using the digital weighing machine (AE80004083, Adam, London, The United Kingdom). Each specimen has been marked by pinpoints in order to calculate the distance between the markers and we have also numbered all samples (1-144) for the purpose of individual measuring. The measurement method was performed as follows: The kidney surface shrinkage area was the distance between the upper and lower pole (Figure 1). The heart surface shrinkage area was the distance between apex and base (Figure 2). The brain surface shrinkage area was the distance between frontal and occipital lobes of the cerebral hemisphere (Figure 3) (Satte et al., 2017).

After fixation, the specimens were dehydrated in 3 changes of pure acetone (7566/12, Arabian Pipecoating Company, Jubail, Saudi Arabia) for 10 days at room temperature. The acetone is replacing the tissues fluid

Figure 2. How the measurements of the shrinkage areas were applied in the heart.

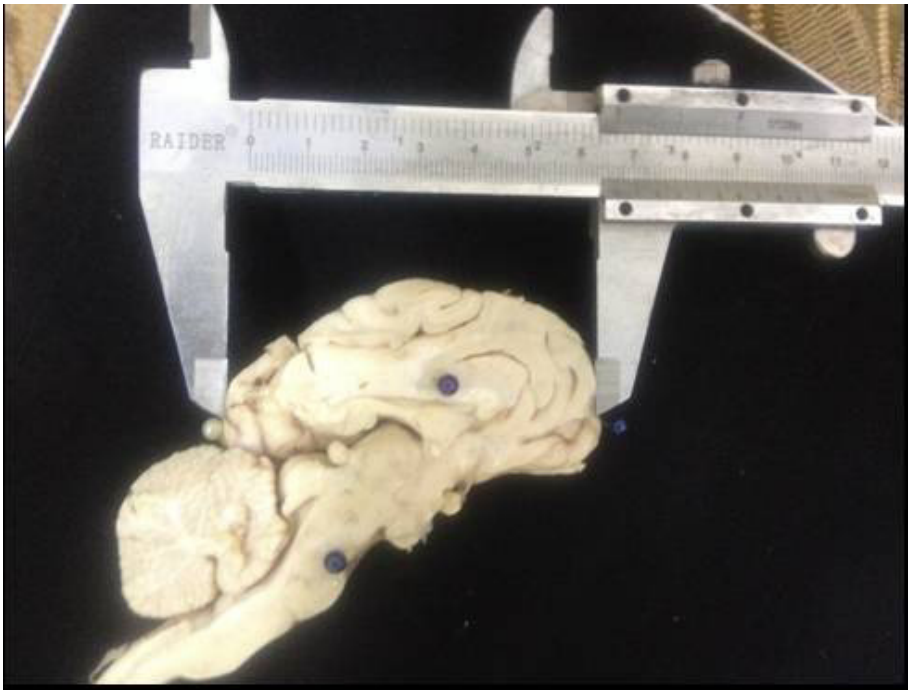

Figure 3. How the measurements of the shrinkage areas were applied in the brain.

and dissolved the fat available in the tissues. The concentration of acetone was measured by using a hydrometer (9598115900, Fisher brand, Waltham, United States of America). When the final acetone condensation remained 99 % or above, the specimens were considered dehydrated (Dejong and Henry, 2007; Elnady, 2016).

Eleven gum Arabic solutions of different concentrations were prepared from gum Arabic power (6-14600-000191, Natural Gum, Khartoum, Sudan), distilled water and glycerin (Chiangrai Agro-Industry, Chiang Rai,

Table 1. Impregnation solutions quantities

Solution No	Gum g/L	Glycerin%	Water %	Test & control groups
1	100	75	25	Group 1
2	100	80	20	Group 2
3	100	85	15	Group 3
4	100	60	40	Group 4
5	100	40	60	Group 5
6	100	30	70	Group 6
7	80	85	15	Group 7
8	90	85	15	Group 8
9	110	85	15	Group 9
10	50	90	10	Group 10
11	227	10	90	Group 11
12	A mix of silicone-S10 and catalyst silicone-S3 at ratio 100:1			Group 12 (control)

Figure 4. Submerged groups of specimens in the vacuum chamber.

Thailand). The silicone-S10 (NC27261, Silicones Corporation, Peoria, United States of America) was mixed with catalyst-S3 (NC27260, Silicones Corporation, Peoria, United States of America) at 100:1ratio and was used as a control. Therefore, two liters of each solution were kept in plastic containers of three liters capacity (Table 1) (Satte et al., 2017; Suganthy and Francis, 2012).

After dehydration, every eleven groups of specimens was 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 the force impregnation process. The submerged specimens for each group were covered by the stainless grid to avoid the samples floating.

After submerged, forced impregnation was used to replacement the acetone in the specimens with the gum Arabic solutions (for experimental groups) and a curable polymer (for the control group). The immersed groups of specimens were transferred to the vacuum chamber (800-362-8491, Mopec, Michigan, United States of America), and connected it to a vacuum pump (HP200D11001, Mopec, Michigan, United States of America) for forced impregnation at room temperature (Figure 4). 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

Figure 5. Excess gum Arabic and silicone-S10 solutions were allowed to drain.

vacuum pressure was progressively decreased to reach 6 mmHg. The vacuum was worked for four days (five hours daily) for the experimental groups and one week for the control group. Impregnation was considered completed when there are no air bubbles coming out from the specimens (Von Hagens, 1979).

After forced impregnation, the specimens were taken from the solutions, and excess gum Arabic solution and polymers were allowed to drain (Figure 5). The specimens in each group were rearranged on the steel plate. One day after force impregnation, the experiment specimens were allowed to harden by atmospheric air at room temperature. The control group was transferred to close curing gas chamber at room temperature and cured with catalyst S6 (two times daily, 10minutes each) until the specimens were hardened up during three days (Suriyaprapadilok and Withyachumnarnkul, 1997).

After the preservation process, the shrinkage surface area and weight loss of each group specimens were measured after a week, a month and three months in a similar manner to that taken during the fixation phase. The obtained data were analyzed using Statistical Package for the Social Sciences, version16 (Software development, Chicago, United States of America) software program. The data were expressed as a mean percentage of tissue shrinkage and weight loss. One-way analysis of variance (ANOVA) was used to test the significant difference between different groups with the level of significance set at P0.05)

RESULT

Compared with control, the gum Arabic preserved tissues (kidneys, hearts, and brains) in the present study showed a relatively insignificant ($p>0.05$) degree of shrinkage and weight loss during the preservation process in the first (10) test groups. Whereas, the preserved specimens in group 11 showed a significant ($p<0.05$) (Table 2, 3).

When compared in between the test groups, group 10 specimens were showed less shrinkage and weight loss than other groups (1-9) (Table 2, 4). The mean percent shrinkage in group 10 was 13.9%, 13.6%, and 28% in kidney, heart, and brain respectively while the mean percent weight loss was 53.1%, 50.4% and 61.8% in kidney, heart, and brain respectively.

The Average of the means percent shrinkage of the specimens preserved by gum Arabic solutions (test

Table 2. The mean shrinkage% (in centimeters) for the kidneys in between test groups (1-11) and control (12) during the preservation process

Groups .No	Kidneys	Hearts	Brains
1	14.7[a]	12.8[a]	30.3[a]
2	18.9[a]	17.9[a]	29.3[a]
3	19[a]	18.9[a]	28.8[a]
4	17.7[a]	17.3[a]	30.8[a]
5	18.7[a]	22.1[a]	28.9[a]
6	20.7[a]	16.5[a]	31.9[a]
7	17.9[a]	16.3[a]	30.1[a]
8	21[a]	14.7[a]	28.4[a]
9	19.4[a]	14.2[a]	30.5[a]
10	13.9[a]	13.6[a]	28[a]
11	33.8[b]	33.2[b]	42.6[b]
12	20.9[a]	15.7[a]	30[a]

[a]=the mean difference is no significant ($p>0.05$). [b]= the mean difference is significant ($p\leq 0.05$).

Table 3. The average mean shrinkage% (in centimeters) for all test groups (1-11) and control (12 during the preservation process.

Groups .No	Kidneys	Hearts	Brains
Control (12)	20.9[a]	15.7[a]	30[a]
Test groups (1-11)	19.7[a]	17.8[a]	30.7[a]

[a]=the mean difference is no significant ($p>0.05$). [b]= the mean difference is significant ($p\leq 0.05$).

Table 4. Mean weight loss% (in gram) for the kidneys in between test groups (1-11) and control (12) during the preservation process

Groups No.	Kidneys	Hearts	Brains
1	57.3[a]	52.4[a]	64.2[a]
2	55.1[a]	53.4[a]	66.6[a]
3	58.2[a]	54[a]	62.9[a]
4	55.4[a]	53.2[a]	62.6[a]
5	61.3[a]	54.9[a]	63.9[a]
6	61.4[a]	58[a]	64.9[a]
7	56.3[a]	52.1[a]	63.2[a]
8	54.6[a]	53.7[a]	63.6[a]
9	53.6[a]	52.1[a]	62.3[a]
10	53.1[a]	50.4[a]	61.8[a]
11	76.5[b]	72.3[b]	76.5[b]
12	55.8[a]	52.1[a]	62.1[a]

[a]=the mean difference is no significant ($p>0.05$). [b]= the mean difference is significant ($p\leq 0.05$).

Table 5. The average weight loss% (in gram) for all test groups (1-11) and control (12 during the preservation process.

Groups No.	Kidneys	Hearts	Brains
Control	55.8[a]	52.1[a]	62.1[a]
Test groups (1-11)	58.3[a]	54.9[a]	64.6[a]

[a]=the mean difference is no significant ($p>0.05$). [b]= the mean difference is significant ($p\leq 0.05$)

groups) was 19.9%, 17.8%, and 30.7% in the kidneys, hearts, and brains respectively while the average of the means percent weight loss was 58.3%, 54.9%, 64.6% in the kidneys, hearts, and brains respectively. (Tables 3 and 5).

Group 11 specimens were exhibits the highest level of

shrinkage; 33.8%, 33.2%, 42.6% in the kidneys, hearts, and brains respectively, while weight loss means percentage; 76.5%, 72.3%, 76.6% in the kidneys, hearts, brains respectively (Tables 2 and 4).

DISCUSSION

Previously, most of the studies revealed that the tissues shrinkage and weight loss has been mainly associated with dehydration and impregnation during the plastination process (Ameko et al., 2013b; Brown et al., 2002). The present study showed an insignificant percentage ($P>0.05$) of shrinkage and weight loss during the preservation process among the first (10) test groups specimens that treated with gum Arabic solutions. Meanwhile, test group (11) specimens were presented a significant percentage ($p< 0.05$) of shrinkage and weight loss compared with the control and all the other (1-10) test groups.

We added more plasticizer agent (glycerin) to the gum Arabic solution number (10) and this accepted it more viscosity and high quality of plastic liquid, and vice versa we decrease the glycerin and increase the water in gum Arabic solution number 11, which accepted it less viscosity and low quality of plastic liquid (Alkarib et al., 2016). These findings were presented in parallel with results of the current study, when we impregnated the high-quality gum Arabic solution (as in solution number 10) in the specimens (as in group 10), the produced samples were presented less shrinkage and weight loss while opposite results were recorded in group 11 specimens, which have a high level of shrinkage and weight loss among other groups.

In previous studies showed that the total percent shrinkage of the kidneys, hearts, and brains during silicone-S10 plastination process was 19.72%, 6.98%, and 40.9% respectively (Pereira-Sampaio et al., 2011, Ameko et al., 2013a, Asadi et al., 2013). Therefore, these reports are related to our findings that the average of the total percent shrinkage during gum Arabic preservation process was 19.7%, 17.8% and 30.7% in kidneys, hearts, and brains respectively. The researchers reported that the average percent weights loss of the hearts during silicone-S10 plastination was 47% (Darawiroj et al., 2010). In our study, the average of the total mean percent weight loss of the hearts during gum Arabic preservation was 54.9% while the kidneys and brains were 58.3% and 64.6% respectively. Economically and anatomically, the gum Arabic solution number 10 is excellent in preservation process of biological tissues in compared with others test groups, because it contains less gum Arabic powder (decrease the cost) and their preserved specimens have obvious less shrinkage and weight loss among the other groups.

CONCLUSION

In our results, the preserved biological tissues in gum Arabic solution are semi-original and relatively maintained their shape and size, and then can be used for teaching anatomy in the medical field instead of current use of relatively costive silicones plastinated tissues.

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