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Studies on preservation of gross pathological cardiac lesions by Thermocol plastination

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ABSTRACT

Demonstration of gross lesions for educational purposes is often based on wet specimens, preserved in formalin, that emit hazardous formaldehyde vapors. This study is a pioneering attempt to plastinate pathological lesions in animal tissues using the economical Thermocol instead of costly resins, viz. silicon, epoxy and polypropylene. Six bovine heart samples, showing pathological lesions, were fixed with 5% formol saline, followed by dehydration in pure acetone, and impregnation with 15% Thermocol in organic solvent. Finally, curing of specimens was undertaken using Touchwood. Three gross lesions of the heart, viz. myocardial cyst, fibrinous pericarditis and endocardial/epicardial hemorrhages, were plastinated in the present study. The myocardial cyst could be fixed in seven days; the fibrinous pericarditis could be fixed in 10 days, whereas hemorrhages required 15 days for fixation. The heart samples were impregnated by 44-48 days. The color of the lesions became darker and prominent in the plastinated hearts. There was no change in the texture of the tissues due to plastination, whereas the consistency of the heart became harder compared to fresh heart tissue. There was an significant reduction in mean mass (42.95%) and mean volume (40%) in the plastinated specimens. The Thermocol plastinated specimens were non-toxic, dry, easy to handle, odourless and durable, without the disadvantages of traditional formaldehyde preservation.

Key words: heart; pathology; plastination; preservation; teaching aids; Thermocol

Introduction

Preservation of specimens with gross lesions is an important tool for teaching and research of pathological alterations in animal tissues. Animal tissues are traditionally preserved by saturation in formaldehyde-based solutions (DAWSON et al., 1990). However, use of 10% formalin as a preservative has serious disadvantages due to the harmful vapors of formaldehyde that lead to an offensive odor and irritation of the eyes and skin. Health hazards, such as carcinogenicity and contact dermatitis (JADHAV et al., 2016), and hypersensitivity (DIMENSTEIN, 2009) have also been reported with traditional tissue preservation methods. The high solubility of formaldehyde in water causes absorption into the respiratory and gastrointestinal tracts, and this has been

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demonstrated to cause nasal tumours in rodents (MERK and SPEIT, 1998). Furthermore, the jars used for specimen storage also require regular maintenance to avoid cloudiness of the fluid, which obscures details. In order to overcome the drawbacks of the traditional approach to organ preservation, a new technique, viz. plastination, has been developed. It is a process of tissue preservation by embedding tissues in synthetic material wherein the water and lipids in the tissues are replaced by costly resins, viz. silicon, epoxy, and polypropylene. Plastinated organs are easy to handle, can be stored in bags, and do not require any maintenance. They are non-toxic and do not have any health hazards, such as carcinogenicity or contact dermatitis, which often occur from formalin fumes. However, the Thermocol plastination approach has, so far, not been implemented by any Veterinary Pathology Department of any college in India, or most other countries, owing to the high cost of the plastination procedure. Lately, an economical method of 'Thermocol plastination' has been reported that is performed at normal atmospheric pressure and room temperature, incorporating recycled environment pollutants such as teacups, and Thermocol as an alternative to expensive resins for plastination of tissues (MUTTURAJ et al., 2014). Therefore, this pioneering attempt has been undertaken to demonstrate lesions in bovine heart samples, preserved by plastination, using economical Thermocol in place of costly resins.

Materials and methods

Collection of specimens. During post-mortem examinations, conducted at the Department of Veterinary Pathology, GADVASU, Ludhiana, heart samples (n=6) of bovines were collected and were abbreviated as HE 1, HE 2, HE 3, HE 4, HE 5 and HE 6. HE1 exhibited a myocardial cyst; HE2 and HE 5 revealed epicardial hemorrhages; HE3 and HE 6 showed endocardial hemorrhages and HE 4 showed traumatic pericarditis.

Preparation of the hearts. All heart samples incorporated in the study were prepared by removing blood clots, unwanted tissue and fat. Thereafter, the heart samples were washed in running tap water for proper cleaning.

Fixation. All heart samples were fixed in 5% formol saline. One liter of 5% formol saline was prepared by dissolving 9g of sodium chloride and 50ml pure formalin (40% formaldehyde) in 950 ml of tap water. The quantity of fixative used was 10 times more than the volume of the specimen. The heart tissues designated HE1 and HE2 were fixed for 7 days; HE 3 and HE 4 were fixed for 10 days, and HE 5 and HE 6 were fixed for 15 days.

Dehydration. The fixed specimens in each group were washed in running tap water for four hours to remove excess formalin. Thereafter, dehydration of the heart samples was carried out by using three changes of 100% acetone (with the tissue volume and acetone in a ratio of 1:5), at room temperature. The first treatment of acetone solution was for three days; the second treatment of acetone was for seven days, and finally, the third treatment of acetone was extended until the concentration of acetone (as recorded by an Alcoholmeter) remained at 98% or above for two consecutive days.

Impregnation. The dehydrated hearts were air dried for fifteen minutes and then impregnated with the solution (constituted by dissolving 15 g of Thermocol along with 5 g of Petroleum Jelly per 100 ml of Chloroform). Impregnation was carried out at normal room temperature and at normal atmospheric pressure. The end point of impregnation was determined by the complete immersion of the heart in the impregnation solution (RAMAKRISHNA and LEELAVATHY, 2019).

Curing. Excess impregnation solution was mopped up, and the heart tissue samples were air dried at room temperature. The heart samples were cured using touchwood, twice, at an interval of 24 hours. After curing, the heart samples were air dried at room temperature for one hour.

Study of alterations in the hearts due to Thermocol plastination. The color of the plastinated tissues was recorded, their consistency and texture were felt, and the mass and volume of each heart was measured. The color was recorded by taking digital pictures, consistency and texture were felt by tactile sensation, mass was measured on a digital weighing machine, and volume was measured by the fluid displacement method. The color, consistency, texture, volume and mass of

each heart sample was compared with the fresh heart sample and with every stage of plastination.

Histopathological studies. Histopathological alterations were studied in the impregnated heart tissues, wherein the impregnated tissue was directly embedded in paraffin wax, and blocks were made. Sections of tissue with thickness of 5 µm were cut and stained by the routine Hematoxylin and Eosin-Phloxine (H&E-Phloxine) method (CULLING, 1974), and observed under a light microscope. The histopathological alterations in the impregnated tissues were compared with histopathological alterations in tissue samples collected from the same heart samples (viz. HE1, HE 2, HE 3, HE 4, HE 5 and HE 6) that were processed by the traditional method of tissue processing.

*Statistical analysis***.** The paired T test was used to detect differences in the mean mass and mean volume of the fresh and plastinated specimens. The value $P \leq 0.05$ was taken as significant. The data were analyzed using SPSS software**.** Microsoft Offices Excel was employed for percentage analysis.

Results

Unplastinated tissue. The mass, volume, color (Fig. 1a, 2a, 3a), consistency and texture of the fresh heart tissues exhibiting gross lesions are shown in Table 1.

Table 1. Mass, volume, color, consistency and texture of fresh hearts and their lesions

S. No.	Case No.	Gross Lesions	Mass (g)	Volume (ml)	Color of tissue	Color of lesions	Consistency of tissue	Consistency of lesions	Texture of tissue	Texture of lesions
1	HE1	Myocardial cyst	294.64	286	Red	Yellowish white with orange foci	Slightly hard	Soft	Smooth surface with irregularly raised areas	Smooth with granules
2	HE2	Epicardial hemorrhages	514.09	445	Pink	Red	Slightly hard	Slightly hard	Smooth	Smooth
3	HE3	Endocardial hemorrhages	398.58	418	Creamy brown	Red	Slightly hard	Slightly hard	Smooth	Smooth
4	HE4	Fibrinous pericarditis	1575.42	1272	Creamy	Creamy	Slightly harder	Slightly harder	Rough	Rough
5	HE5	Epicardial hemorrhages	473.34	448	Pink	Red	Slightly hard	Slightly hard	Smooth	Smooth
6	HE6	Endocardial hemorrhages	278.37	316	Creamy brown	Red	Slightly hard	Slightly hard	Smooth	Smooth

Fixed hearts. After fixation of the heart samples, all tissues exhibited loss of pink color. The myocardial cyst (HE1) was fixed in 7 days (Fig. 1b), the fibrinous pericarditis (HE4) was fixed in 10 days (Fig. 2b), and the endocardial/epicardial hemorrhages (HE5 and HE6) were fixed in 15 days. The hemorrhages fixed in HE-2 and HE-3 disappeared during dehydration, which indicated that 7-10 days duration was not sufficient to fix hemorrhages in hearts. After fixation, the mass and volume were reduced in all the heart samples (Table 2). The consistency of the tissues and lesions became harder, but no alterations were observed in the texture of the heart tissues and the lesions in the hearts.

Table 2. Mass, volume, color, consistency and texture of fixed hearts and their lesions

S. No.	Case No.	Gross Lesions	Mass (g)	Volume (ml)	Color of tissue	Color of lesions	Consistency of tissue	Consistency of lesions	Texture of tissue	Texture of lesions
1	HE1	Myocardial cyst	290.00	280	Creamy brown	White with yellowish foci	Slightly harder	Slightly harder	Smooth surface with irregularly raised areas	Smooth with granules
2	HE2	Epicardial hemorrhages	505.00	435	Light brown	Brown	Slightly harder	Slightly harder	Smooth	Smooth
3	HE3	Endocardial hemorrhages	390.00	403	Light brown	Brown	Slightly harder	Slightly harder	Smooth	Smooth
4	HE4	Fibrinous pericarditis	1540.00	1225	Creamy	Creamy	Slightly harder	Slightly harder	Rough	Rough
5	HE5	Epicardial hemorrhages	460.00	425	Light brown	Brown	Slightly harder	Slightly harder	Smooth	Smooth
6	HE6	Endocardial hemorrhages	270.00	300	Light brown	Brown	Slightly harder	Slightly harder	Smooth	Smooth

Dehydrated hearts. Bovine heart samples were dehydrated within 13 days (Table 3). However, the mass and volume of the fixed heart tissues was further reduced by dehydration. In HE2 and HE 3, the color of healthy tissues was maintained after dehydration, however, the color of the lesions disappeared during dehydration (Table 4). This indicated that the duration of fixation was not sufficient to fix hemorrhages in the heart. The color of the lesions in HE1 (Fig. 1c), HE4 (Fig. 2c), HE5 and HE6 was maintained.

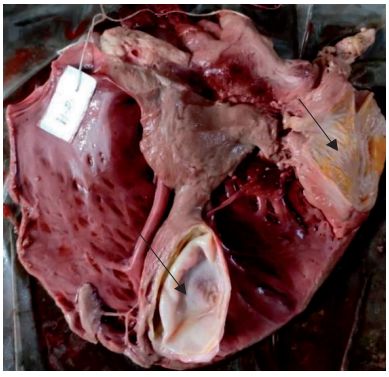
Table 3. Changes in the concentration of acetone during dehydration of heart samples

S. No.	Case No.	1st Treatment				2nd Treatment								3rd Treatment			
	Days	0	1	2	3	0	4	5	6	7	8	9	10	0	11	12	13
1	HE1	100	90	86	84	100	98	97	96.5	96.5	96	95.5	95.5	100	99.5	99	99
2	HE2	100	91	87	85	100	98	97	97	96.5	96.5	96	95.5	100	99.5	99	99
3	HE3	100	90.5	87	85.5	100	98.5	97.5	97.5	97	96.5	96.5	96	100	99	98.5	98.5
4	HE4	100	91	87	85	100	98	97	97	96.5	96.5	96	95.5	100	99.5	99	99
5	HE5	100	90	86	84	100	98	97	96.5	96.5	96	95.5	95.5	100	99.5	99	99
6	HE6	100	90.5	87	85.5	100	98.5	97.5	97.5	97	96.5	96.5	96	100	99	98.5	98.5

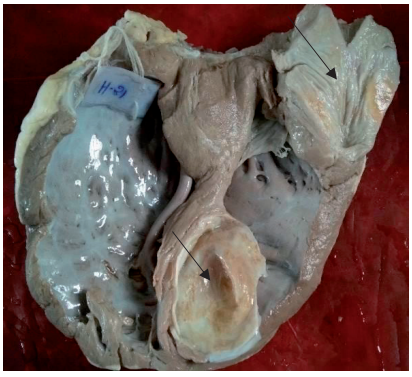
Table 4. Mass, volume, color, consistency and texture of dehydrated hearts and their lesions

S. No.	Case No.	Gross Lesions	Mass (g)	Volume (ml)	Color of tissue	Color of lesions	Consistency of tissue	Consistency of lesions	Texture of tissue	Texture of lesions
1	HE1	Myocardial cyst	241.28	242	Creamy brown	White with yellowish foci	Slightly harder	Slightly harder	Smooth surface with irregularly raised areas	Smooth with granules
2	HE2	Epicardial hemorrhages	417.53	374	Light brown	Lesions disappeared	Slightly harder	Slightly harder	Smooth	Smooth
3	HE3	Endocardial hemorrhages	325.29	355	Light brown	Lesions disappeared	Slightly harder	Slightly harder	Smooth	Smooth
4	HE4	Fibrinous pericarditis	1289.89	1074	Creamy	Creamy	Slightly harder	Slightly harder	Rough	Rough
5	HE5	Epicardial hemorrhages	389.42	377	Light brown	Brown	Slightly harder	Slightly harder	Smooth	Smooth
6	HE6	Endocardial hemorrhages	229.98	267	Light brown	Brown	Slightly harder	Slightly harder	Smooth	Smooth

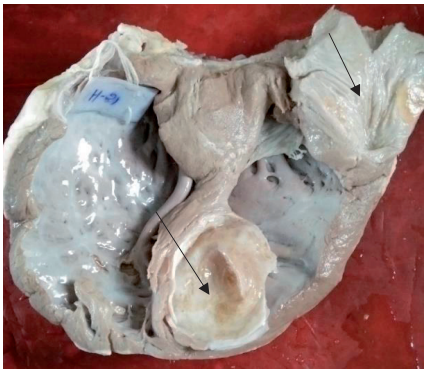
Thermocol plastinated hearts. The color of all heart samples became darker (Fig. 1d and Fig. 2d) during impregnation but reverted back to a lighter color during curing, thereby prominently revealing the lesions (Fig. 1e, Fig. 2e and Fig. 3e). During impregnation, the heart samples initially floated but were later immersed in the impregnation solution. Later, they sank gradually to the bottom of the impregnation solution in 44 to 48 days (Fig. 4a, 4b and 4c) indicating the completion of impregnation (Table 5). Although consistency of the plastinated tissues became hard after curing, there was no alteration in the texture of the plastinated hearts (Table 6). The volume of the dehydrated hearts was also further reduced from 22.9 to 26.06%, with a mean reduction of 24.06% (Fig. 5).



a) Myocardial cyst in the fresh heart (HE1)

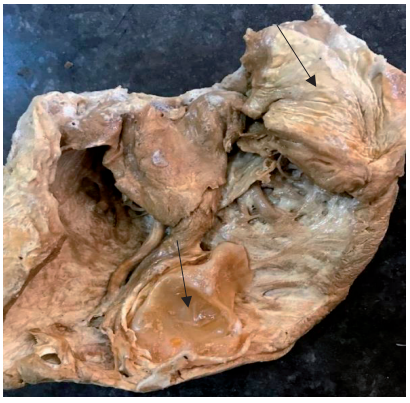


b) Myocardial cyst in fixed heart (HE1)



c) Myocardial cyst in dehydrated heart (HE1)

d) Myocardial cyst in impregnated heart (HE1)



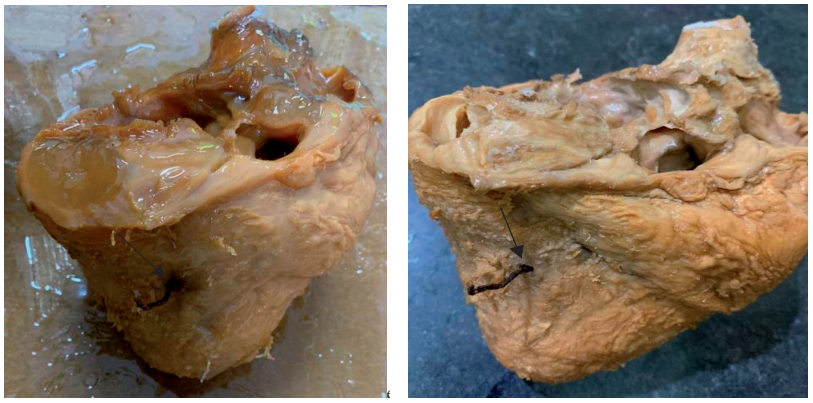
e) Traumatic fibrinous pericarditis in cured heart

Fig. 1. Myocardial cyst at fresh (a), fixation (b), dehydration (c), impregnation (d) and curing (e) stage of the Thermocol plastination

a) Traumatic fibrinous pericarditis in fresh heart (HE4)

b) Traumatic fibrinous pericarditis in fixed heart (HE4)

c) Traumatic fibrinous pericarditis in dehydrated heart (HE4)



d) Traumatic fibrinous pericarditis in impregnated heart (HE4)

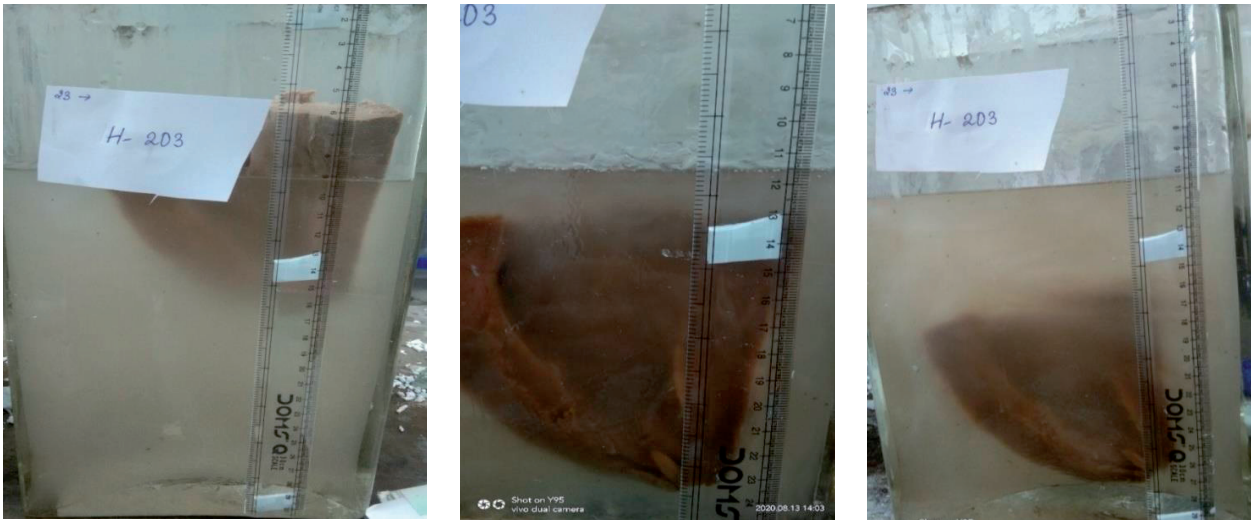
e) Traumatic fibrinous pericarditis in cured heart (HE4)

Fig. 2. Traumatic fibrinous pericarditis at fresh (a), fixed (b), dehydrated (c), impregnated (d) and cured (e) stage of the Thermocol plastination

a) Endocardial haemorrhages in fresh heart (HE6)

b) Endocardial haemorrhages in plastinated heart (HE6)

Fig. 3. Endocardial haemorrhages in heart at fresh (a) and cured (b) stage of the Thermocol plastination



a) Heart floating in impregnation solution

b) Heart immersed in impregnation solution

c) Heart dipped in impregnation solution

Fig. 4. Impregnation of heart showing floating (a), immersion (b) and dipping (c) in the impregnation solution of the Thermocol plastination

Table 5. Duration of impregnation of the hearts

S. No.	Case No.	Gross lesions	Days
1	HE1	Myocardial cyst	46
2	HE2	Epicardial hemorrhages	48
3	HE3	Endocardial hemorrhages	46
4	HE4	Traumatic fibrinous pericarditis	47
5	HE5	Epicardial hemorrhages	44
6	HE6	Endocardial hemorrhages	44

Table 6. Mass, volume, color, consistency and texture of plastinated hearts and lesions thereof

S. No.	Case No.	Gross Lesions	Weight (g)	Volume (ml)	Color of tissue	Color of lesions	Consistency of tissue	Consistency of lesions	Texture of tissue	Texture of lesions
1	HE1	Myocardial cyst	180.96	183	Creamy brown	Creamy with light brown foci	Hard	Hard	Smooth surface with irregularly raised areas	Smooth with granules
2	HE2	Epicardial hemorrhages	316.90	283	Light brown	Lesions disappeared	Hard	Hard	Smooth	Smooth
3	HE3	Endocardial hemorrhages	247.22	273	Light brown	Lesions disappeared	Hard	Hard	Smooth	Smooth
4	HE4	Fibrinous pericarditis	980.31	810	Light brown	Light brown	Hard	Hard	Rough	Rough
5	HE5	Epicardial hemorrhages	293.23	288	Light brown	Black	Hard	Hard	Smooth	Smooth
6	HE6	Endocardial hemorrhages	172.02	202	Light Brown	Brown	Hard	Hard	Smooth	Smooth

Quantitative analysis of alterations in mass and volume due to Thermocol plastination of the hearts. It was found that the mean mass and mean volume of the fresh heart samples, at the stage of collection, reduced significantly by the end stage of plastination. A graphical representation of alterations in mean mass and mean volume is shown in Fig. 5.

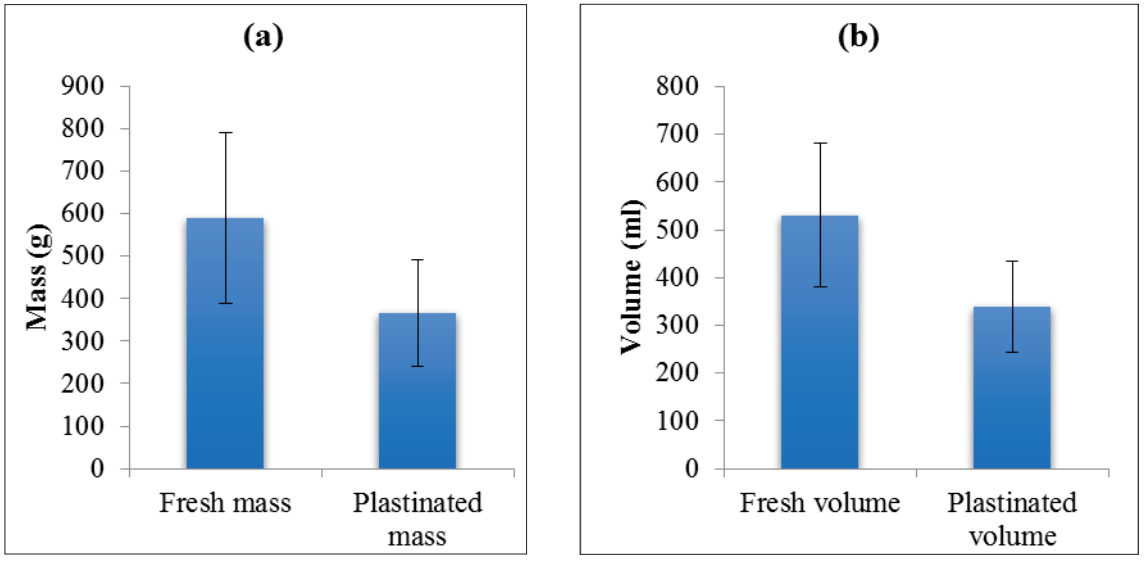
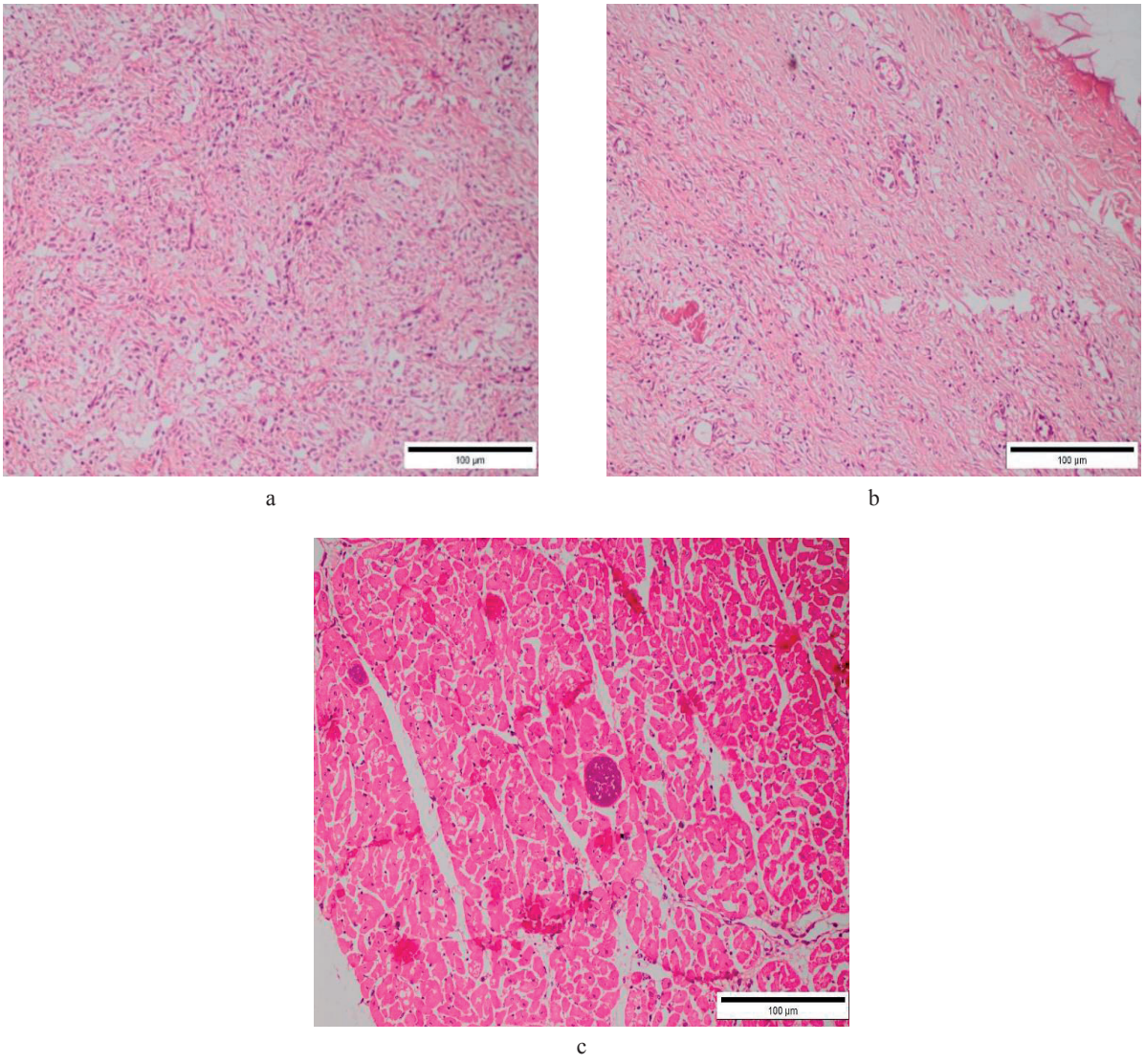


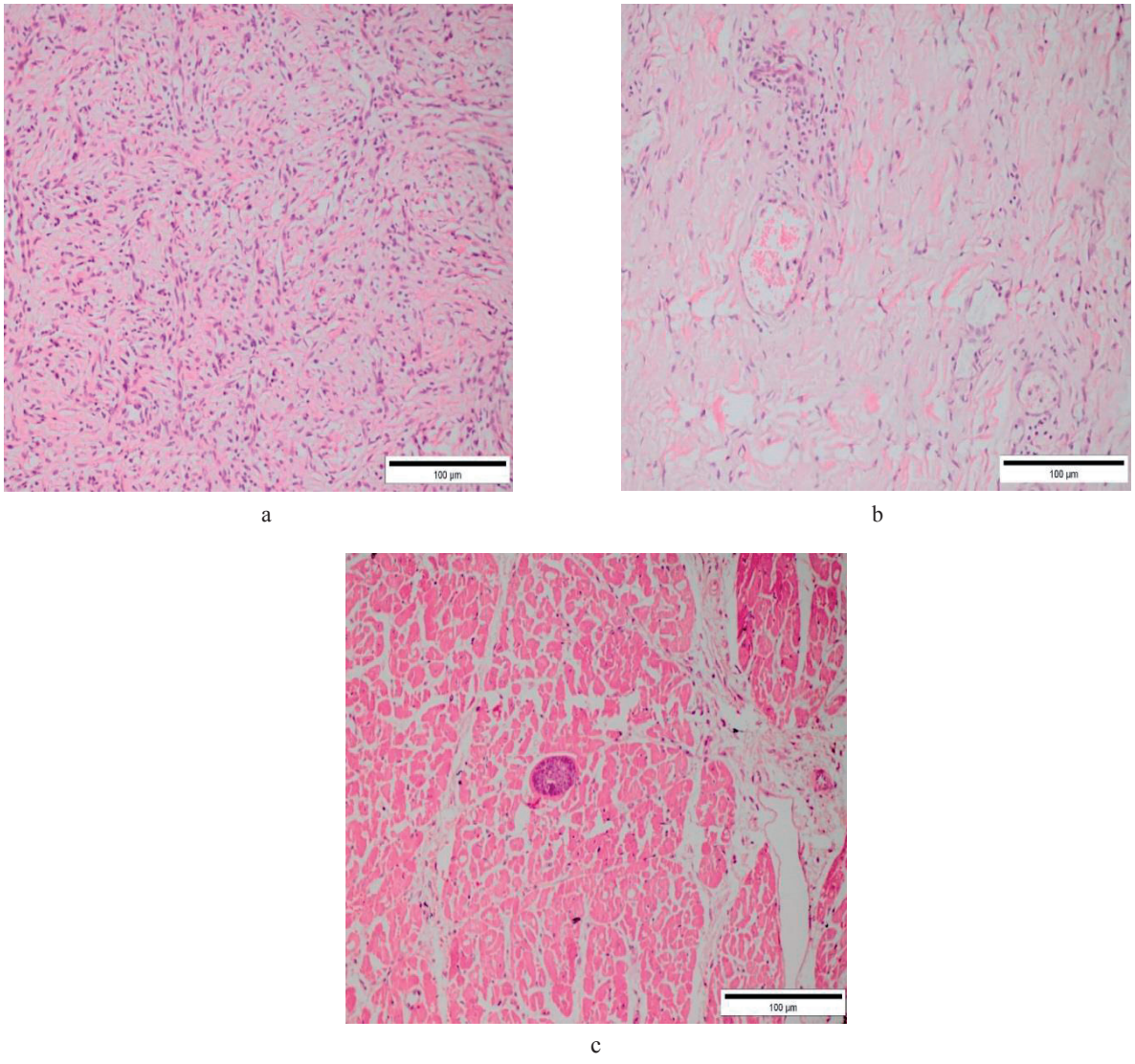
Fig. 5. Comparison of mean mass (a), and mean volume (b) of fresh heart samples with plastinated heart samples

*Histopathological studies***.** The processing of the impregnated tissues revealed preservation of the morphological architecture of tissue sections. Comparison of histopathological analyses of fibrinous pericarditis, epicardial and endocardial hemorrhages and myocardial cysts in impregnated tissues with the corresponding heart tissues processed by the routine approach revealed characteristic fibers (Fig. 6a) and mononuclear cell infiltration (Fig. 6b) in fibrinous pericarditis; red blood cells in congestion and epicardial/endocardial hemorrhages, and cyst wall and surrounding fibrosis in myocardial cysts. Histopathological examination of the impregnated tissues also revealed sarcocysts in the myocardial muscles (Fig. 6c). Comparison of histopathological observations on slides of impregnated tissues with slides of routine histopathological preparations of the same tissue collected before processing the tissues for Thermocol plastination (Fig. 7a, Fig. 7b and Fig. 7c), revealed that the slides of impregnated tissues were more intensely stained and more prominent than on the slides of fresh heart tissue samples, despite the fact that both types of slides were stained with the same Hematoxylin and Eosin stain.



a b c

Fig. 6. Histopathological alterations in impregnated tissue after embedding in wax: a) characteristic fibers in fibrinous pericarditis tissue (H&E, Bar=100µm); b) mononuclear cell infiltration in fibrinous pericarditis tissue (H&E, Bar=100µm); c) sarcocysts in myocardial muscle (H&E, Bar=100µm)



a b c

Fig. 7. Histopathological alterations in fresh tissue after routine tissue processing: a) characteristic fibers in fibrinous pericarditis tissue (H&E, Bar=100µm); b) mononuclear cell infiltration in fibrinous pericarditis tissue (H&E, Bar=100µm); c) sarcocysts in myocardial muscle (H&E, Bar=100µm)

Discussion

No study has been reported in the published literature on the preservation of pathological lesions by the economical Thermocol plastination method used in the present study. Most studies on plastination of healthy tissues have largely used costly silicon resins (HAGENS, 1979; STOYANAV et al., 2015; HAYAT et al., 2018). The epoxy resins used by plastinators are known to be skin, eye and mucous membrane irritants (HOLLADAY et al., 2001). Therefore, we suggest that this economical technique can be usefully applied for preservation of pathological tissues. While most studies have used plastination for the preservation of whole organs, only a few studies have reported plastination for the preservation of gross lesions in animal tissues (ALPAR et al., 2005). However, such studies have

used expensive SR10 polymer with 1% catalyst SH03. Further, such approaches have not conducted detailed studies of the alterations in mass, volume, color, consistency etc. as undertaken in the present study.

In the present study, it was found that bovine hearts can be dehydrated in 13 days by using 1:5 tissue acetone with three changes, as compared to earlier studies that used 1:10 acetone with six changes (BROWN et al., 2002). Thus, the desired results were obtained in the present study by using half the volume of acetone and half the number of acetone changes.

The duration of 2-4 weeks for impregnation, as indicated by RAMKRISHNA and LEELAVATHY (2019), was found to be related to the initial immersion of tissues. The present study revealed that complete impregnation could only be accomplished with complete immersion of the tissue in Thermocol solution, which in our study took 44-48 days.

BROWN et al. (2002) reported 19.6 ± 7.01 % shrinkage, as indicated by loss of volume calculated by the fluid displacement method at room temperature. The present study reported 15.58% loss in the volume of the dehydrated heart samples, as calculated by the fluid displacement method at room temperature. AMEKO et al. (2013) reported 58.2 % loss of mass in plastinated guinea pigs even when using costly resins at room temperature in a vacuum, whereas in the present study, plastinated heart samples exhibited a loss of 40% of mass at room temperature and normal atmospheric pressure.

The plastinated tissues did not significantly impact the study of gross lesions in the plastinated heart samples. This was precisely due to the shift in focus of plastination, from anatomical preservation of whole organs to the focus of plastination on pathological gross lesions in animal tissues. Thus, the standardization of plastination for preservation of pathological gross lesions by using affordable Thermocol, at room temperature and at normal atmospheric pressure, is a useful approach for preservation of pathological gross lesions in animal tissues.

The decrease in mass and volume of the plastinated hearts used in our study did not alter the color and tissue texture. The color of the lesions became even more prominent. The prominence in color was helpful in detecting gross lesions with ease. It ensured the longevity of the patho-histological lesions and ensured convenience in teaching undergraduates using plastinated samples of gross lesions in animal tissues. Further, due to the feasibility of histopathological analysis of plastinated tissues, this approach is highly useful for teaching and research purposes.

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SAŽETAK

Prikaz makroskopskih lezija tkiva u nastavne svrhe najčešće se temelji na mokrim preparatima čuvanim u formalinu koji je, zbog isparavanja formaldehida, opasan za zdravlje. Ovo je istraživanje pionirski pokušaj plastinacije patoloških lezija tkiva u životinja upotrebom ekonomičnog termokola umjesto skupe smole, odnosno silikona, epoksidne smole i polipropilena. Šest srca goveda, sa znakovima patološke lezije, fiksirano je u 5 %-tnoj otopini formola, nakon čega je uslijedila dehidracija čistim acetonom i impregnacija 15 %-tnim termokolom u organskom otapalu. Na kraju je provedeno učvršćivanje i sušenje preparata. Plastinirane su tri makroskopske srčane lezije: cista miokarda, fibrinozni perikarditis i endokardijalna/epikardijalna hemoragija. Cista miokarda može se fiksirati za 7 dana, fibrinozni perikarditis za 10 dana, dok hemoragija zahtijeva 15 dana fiksacije. Uzorci srca impregnirani su 44 do 48 dana. Lezije su potamnjele i postale istaknutije na plastiniranim srčanim organima. U teksturi tkiva nije bilo promjene, dok je konzistencija srca postala tvrđa u usporedbi sa svježim srčanim tkivom. U plastiniranih preparata utvrđeno je smanjenje prosječne mase od 42,95 % i prosječnog volumena od 40 %. Termokol plastinacija preparata nije opasna za zdravlje ljudi, suha je i jednostavna metoda, bez mirisa, trajna i bez nedostataka koje ima tradicionalna metoda prepariranja organa pomoću formaldehida.

Ključne riječi: srce; patologija; plastinacija; prepariranje; nastavna pomagala; termokol