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Can a tannic acid-based solution substitute formalin as a fixative for macroscopic specimens?

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ABSTRACT

Introduction: The most famous pedagogic tool to teach anatomy is the dissection. Despite recent models with fresh cadavers, dissection mainly occurs in formalin fixed specimens. However, this substance is known to be toxic. Several alternative solutions appeared in the last decades, although most of them are used in microscopic studies, as such, the macroscopic environment remains in need of a new alternative capable to replace the formaldehyde. The study conducted herein proposes the use of this solution as an option for formaldehyde replacement. **Material and Methods:** 8 Wistar rats were fixated in a 10% formalin solution and 8 rats were fixated in a tannic acid alcohol-based solution (0.25%). These animals were dissected by 14 medical students. Afterwards, the students received a questionnaire to evaluate aspects such as odor, texture and color of the specimen and flexibility of the joints. The toxicity of each compound was analyzed by "in silico" tests for cutaneous and respiratory systems. **Results:** The results showed superiority of the tannic acid alcoholic solution over the regular formalin solution in several parameters analyzed, except in relation to the color of the specimen. The toxicity of skin and respiratory tract was absent in tannic acid alcoholic solution. **Conclusion:** The tannic acid may be a substitute to formaldehyde-based solutions, as it has been shown to decrease risk factors to individuals that manipulates fixed corpses and tissues.

Keywords: alternative solution, dissection, formaldehyde, tannic acid, toxicity.

INTRODUCTION

Despite the appearance of several complementary tools to aid anatomical teaching, dissection is still the most popular and effective method, as it discuss anatomical relationships, function, form as well as its clinical significance. The differential, however, lies in the fact that dissection trains the individual in spatial appreciation and orientation by the psychomotor activity, as well as cognitive development, which is essential for acquisition of medical skills, experience and surgical techniques [1-6].

Dissection is mainly performed in embalmed cadavers, and several universities commonly use a regular 10% v/v formalin solution (RFS), although some institutions are opting for less aggressive solutions (4-5% v/v of formaldehyde) [6-8]. This solution is based on aqueous solution of formaldehyde, which is the most famous fixative, used in more than 80% of US and UK histology laboratories, and in as much as 65% of them in other parts of the world [9].

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However, formaldehyde is classified as a carcinogenic substance (group 1) by the International Agency for Research on Cancer and, therefore, represents a risk to anyone handling the solution [10, 11]. Generally, institutions rely on ambient air exchange (downdraft airflow, away from the worker) or by air filtration with facemasks worn by workers near the cadavers to minimize exposure to the formaldehyde vapors [12].

Another alternative is to change the embalming solution to less toxic compounds. This has been subject to many different researches with the purpose of replacing formaldehyde in gross anatomy laboratories [6, 12, 13]. As such, fixatives containing alcoholic and nonalcoholic solutions, with or without acetic acid [14], zinc-based solution [15], less than 10% formalin solution [6], saturated salt-solution [6, 16], combinations of diethylene glycol and monoethylene glycol [13] and Larsen's, Thiel's and even the usage of fresh frozen cadavers have been studied recently [6, 8, 13, 17].

The work presented herein aims to study the effects of a tannic acid based solution in Wistar rats. Furthermore, another goal of this study was to compare this solution with formaldehyde through dissection and evaluation of the tissue by medical students.

MATERIAL AND METHODS

Materials

The TA of highest purity available was obtained from Sigma-Aldrich Co. The 10% formaldehyde buffered solution was bought from Merck-Millipore Co.

Animals

The study design and experimental protocols were approved by the Animal Care and Use Committee of the University of Sao Paulo (protocol number 2851/2012), which is in accordance with the Guide for the Care and Use of Laboratory Animals. The experiments were performed according to guidelines of the Brazilian College for Animal Experimentation (COBEA). Sixteen Wistar rats (350-460g) were utilized as experimental model for gross dissection and obtained from laboratory animal facility of Fluminense Federal University. The euthanasia was performed by lethal intraperitoneal injection of pentobarbital.

Experimental design

Immediately after death (n = 8 per group), the two fixatives solutions, regular formalin (RF-10%) and 300 mL Tannic acid alcohol-based solution (TAAS-0.25%) were perfused via the left ventricle, using a peristaltic pump (60 – 80 mm/Hg). After injection, both groups were kept immersed on each fixative solution for 30 days, at room temperature, before gross dissection.

Analyzed parameters of dissection

A blinded evaluation of fixed specimens was carried out by fourteen medical students, who have been dissecting for 2 or more years, twice a week at the Fluminense Federal University dissection program. The rats were randomly and blindly distributed and were only marked with the letter A for the RF and the letter B for the TAAS group. Analysis of the odor, color, texture and flexibility of joints and skin of tissues was performed according to Silva, Matera [7].

The evaluation process was based on detailed questionnaire answered by the students (Figure 1). The quality for these aspects was graded as 0 (inadequate for dissection), 1 (reasonable quality for dissection, but adjustments in protocol are needed), or 2 (good quality for dissection).

In silico assessment

The in silico assessment of genotoxic effects was performed using the ACD / Labs I-Lab 2.0 server. The cutaneous and respiratory study was performed using the PredictorTM ADMET software (Simulations Plus Inc., Lancaster, CA, USA). Both programs use libraries of molecules including chemical structural information (2D or 3D) and experimental data to create statistical models that are used to predict the properties of the studied molecules as well as their toxicity.

Figure 1. Questionnaire used in this study.

Data assessment
Subject: Dissection Program (College of Medicine, Fluminense Federal University)
Name:
E-mail:

The quality for each aspect is: 0 (inadequate for dissection), 1 (reasonable quality for dissection, but adjustments in protocol are needed), or 2 (good quality for dissection).. Please, answer the objective following questions based on these grades.

1- On your opinion, the color of fixed tissues contributes for the dissection process?
(Yes)
(No)

2- The color of chemically preserved specimens.
Group A= (0) (1) (2)
Group B= (0) (1) (2)

3- The texture of skin and internal disposition of organs in chemically preserved specimens:
Group A= (0) (1) (2)
Group B= (0) (1) (2)

4- Did you feel any odor of chemical compound during dissection?
(Yes)
(No)

5- The odor in chemically preserved specimens during dissection:
Group A= (0) (1) (2)
Group B= (0) (1) (2)

6- Does the flexibility help the dissection in your opinion?
(Yes)
(No)

7- Flexibility of joints and skin:
Group A= (0) (1) (2)
Group B= (0) (1) (2)

8- Are you in favor of the use of cadavers for surgical technique teaching?
(A) Yes
(B) No

9. Point out the vantages and disadvantages of both fixative methods:

10. What type of chemically preserved specimen did you preferred for dissection?
(A) Group A
(B) Group B

The cutaneous toxicity: The program interprets as sensitizer compound or substance, the one, which induces allergic skin reactions in mice. A mathematical model is implemented to predict qualitatively the molecule that has the potential to induce cutaneous allergic reactions, and is based on a database of 298 molecules tested experimentally by the local lymph node murine assay (LLNA), which has been a recommended method and validated for the determination of relative potency of a chemical compound to be irritating. The end point of LLNA is the EC3, which is the estimated chemical needed to produce a 3-fold stimulation of draining lymph node cell proliferation in rats compared with controls, being used to divide concentration in classes and non-sensitizing compounds sensitizers. Compounds with EC3 lower or equal to 10 % and sensitizers are considered as sensitizer, and those above 10% as non-sensitizers. For this evaluation we used the TOX_SKIN program module.

The respiratory toxicity: The mathematical model implemented to qualitatively predict whether the molecule is toxic when inhaled was based on a library containing 314 molecules identified by inhalation studies with rats as sensitizers or not. Most molecules were obtained from AOEC (Association of Occupational and Environmental Clinics). For this evaluation was used the TOX_RESP program module.

Statistical Analysis

Results are expressed as means (%) obtained by the answered questionnaires. The chi-square test was used to observe significant differences ($p < 0.05$) between answers. The statistical analysis was performed with the IBM SPSS 21 version.

RESULTS

Macroscopic analysis

The macroscopic analysis was performed by qualitative and quantitative evaluations (observation and gross dissection, respectively), being the quantitative analysis performed blindly. The parameters selected for analysis (color, texture, odor and flexibility) were unanimously considered important for dissection procedures by all respondents.

The color of chemical preserved specimens appeared different for skin, muscle and organs as expected, with TAAS darker than RFS (Figure 2).

The TAAS specimens were softer and more flexible, different from RFS ones. The odor was absent in TAAS preserved specimens. All qualitative parameters described above were classified as good, based on the grade created and previously described.

The results exhibited a superiority of TAAS, when compared to formaldehyde for some relevant aspects for dissection. The TAAS texture (very significant for initial incisions and skin dissection) was classified by 71.42% of responders as good for dissection, while the RFS specimens were rated as reasonable for dissection and in need of adjustments by 64.28% of the students. The odor and flexibility were classified as good for dissection by 92,85% and 85,71% of respondents, whereas 42,85% and 35,71% of them thought that RFS needed adjustments for these aspects. All these results were statistically significant ($p < 0.05$) according to the chi-square test.

In silico assessment

a) Genotoxic effect

The mathematical model implemented to predict whether the molecule has mutagenic potential is based on a database of 8607 molecules experimentally tested by the Ames test, obtained the CCRIS (Chemical Carcinogenesis Research Information) and GENE-TOX (Genetic Toxicology Data Bank). The design of 2D structure of tannic acid and formaldehyde in the model showed that formaldehyde has a 62% probability of being mutagenic, while the tannic acid had a probability of only 3% (Table 1).

b) Cutaneous and respiratory toxicity

The molecules of formaldehyde and tannic acid were submitted to the same mathematical models mentioned above, with the prediction of skin and respiratory toxicity of formaldehyde 83% and 96%, respectively, which indicates high respiratory toxicity as expected. The result of cutaneous toxicity of formaldehyde demonstrated to be non-sensitizer. For tannic acid, predicted cutaneous and respiratory toxicity was 42% and 36% respectively, indicating that this molecule has a moderate probability of be toxic for both cutaneous and respiratory ways.

DISCUSSION

The carcinogenic and irritative properties of the formaldehyde are known and have been vastly studied [6, 8, 11, 12, 16]. A few studies observed is genotoxic effects, and, due to its high solubility in water, the formaldehyde can be rapidly absorbed by the respiratory and gastrointestinal tract, and has been shown to cause tumors in rodents. Moreover, formaldehyde exposure can cause central nervous system damage, immune system disorders, blindness, dermatitis and several respiratory diseases [6, 8, 11, 18, 19].

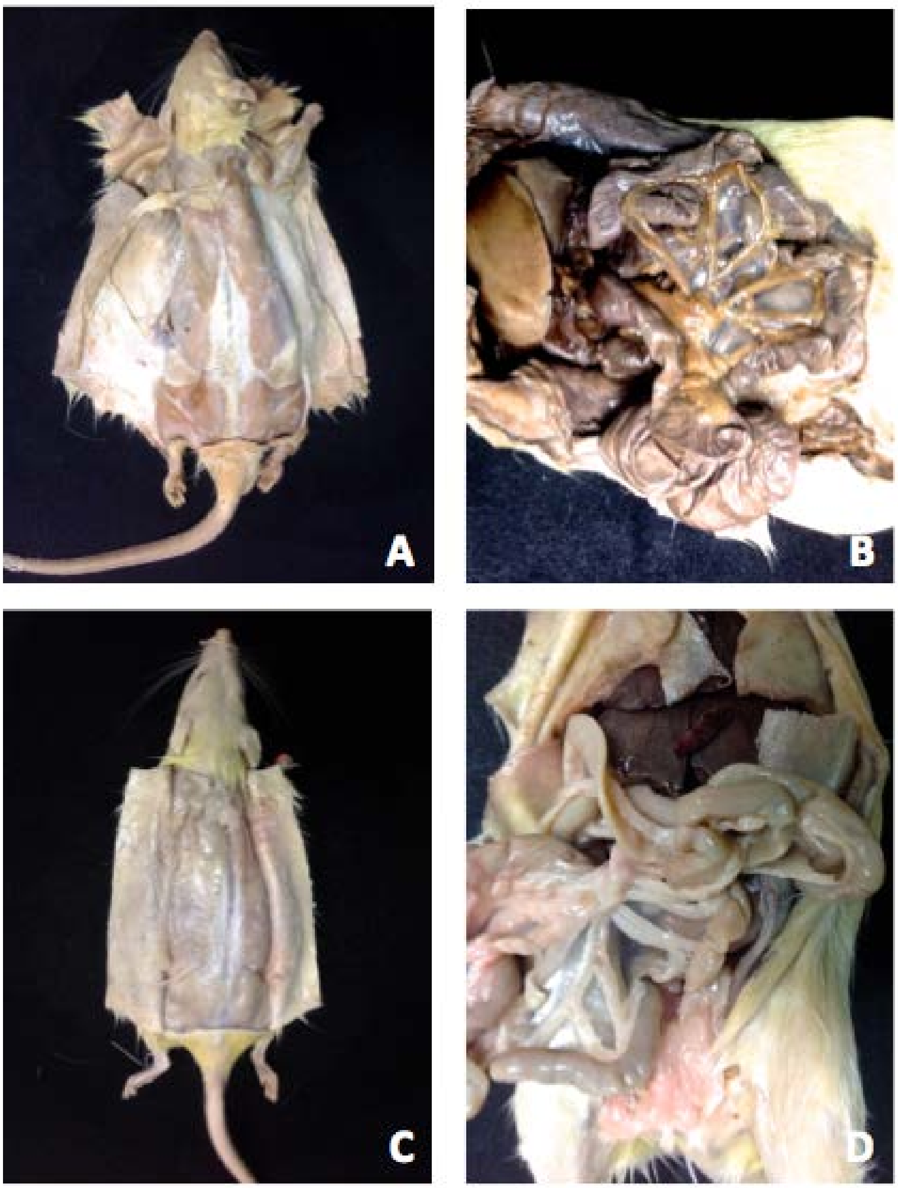

Figure 2. Photograph of the preserved specimens fixed with TAAS (A and B) and RFS (C and D).

Furthermore, these effects negatively impact the students' perception of anatomy, since they often experience irritative symptoms and thus opt to stay away from the anatomy laboratory and the cadaver [6, 11, 20]

Despite these risks, formaldehyde-based solutions are still among the most used due to its potent antimicrobial properties (fungicide/bactericide) and its low cost [6, 11]. However, several institutions among the globe are researching other embalming fluids with the purpose of replacing the formaldehyde.

These new compounds aim to reduce the exposure to carcinogenic substances while maintaining cadaveric integrity, joint mobility and other aspects related to the color and texture of the structures to be dissected [6, 8, 14, 16, 17].

Several of these have been tested in histological specimens [9, 14, 15], while a few are already being used to reproduce similar or better effects than formaldehyde without the downside of toxic exposure to vapors, such as the saturated salt solution, Thiel's solution and mixtures of diethylene glycol and monoethylene glycol [6, 13, 16]. Despite that, these solutions have a few limitations, such as the maintenance of the cadaver in cold temperatures [13], massive hair loss (in animal cadavers), rigid joints and tissues and edematous subcutaneous tissue [6, 16].

TA has characteristics compatible for both microscopic and macroscopic fixation purposes [21], which lead us to test it as an alternative to formaldehyde.

The work presented herein observed that the TAAS showed a significant capacity to preserve macroscopic specimens, as it was odorless and preserved the flexibility of the entire specimen, thus allowing and enhancing the dissection procedures in comparison to formaldehyde-based solutions.

The use of TA in association of glutaraldehyde is common for ultrastructural analyses of elastin fibers under electron microscopy, and more recently for bioprosthetic heart valves and vascular grafts [21-25], mainly by its properties to form multiple bonds extracellular compounds, such as elastin and collagen [21, 23, 24].

The knowledge of TA properties to preserve tissues comes from ancient Egypt, where methylgallate and inositols were identified in different parts of a 40-year old body of an unknown Egyptian mummy #90001255 (100BC) from the Guimet Natural History Museum (Lyon, France), indicating the general use of vegetable tannins for mummification [26].

Some features were analyzed to test the capacity of TAAS to preserve entire specimens, and only the color seemed to be a disadvantage in comparison with formalin. The modification of color is generally used to control the process of fixation, although alternative fixatives do not change color as formalin does (the Thiel's method produces more realistic colors) [6, 27] As such, the change in color may not be interpreted as a negative feature, since the observed color is closer to alive specimens, thus, more prone to simulate real characteristics.

All other parameters of the TAAS were better than formalin, including cadaveric flexibility (one of the most important parameters for dissection). The specimens showed a perfect condition of flexibility on skin and joints, keeping the “life like” appearance of them. This type of fixation that maintain the flexibility can be acquired with other fixative kits, as: Thiel`s solution, saturated salt solution and other compounds [6, 8, 13, 16].

Benkhadra, Bouchot [28] and Hayashi, Naito [6] studied cadaveric fragments fixed in Thiel`s solution, but did not observe any modification of collagen in either muscle or tendon fibers that may explain the flexibility. The TAAS has the capacity to stabilize not only collagen, but elastin as well, which may be the answer for this good flexibility and texture. This is of great importance, since known modified Larssen and Laskowski solutions have produced different results regarding cadaveric flexibility, as pointed out by Silva, Matera [7].

The analysis of toxicity should also be considered as significant in addition to the aforementioned parameter. Despite several alternative solutions being more famous nowadays, few or even none of them has been critically analyzed in this matter. It was observed on the present study that the toxicity of skin and respiratory tract is probably absent in TAAS.

This feature is important mainly for dissection rooms that do not have enough fume hoods or air ventilation, as the exposure to toxic vapors is higher. Moreover, the use of just one compound diluted in alcohol should make this solution cheaper than other alternatives, as they generally use a lot of secondary components

CONCLUSIONS

Owing the number of positive characteristics of this fixative, TAAS seems to be as good for macroscopic as for microscopy. It is less toxic than formaldehyde and odorless. Therefore, could be a real alternative for the use of RFS, decreasing risk factors to the ones manipulating fixed corpses and tissues..

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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RESUMO

Uma solução à base de ácido tânico pode substituir a formalina como fixador de amostras macroscópicas?

Introdução: A ferramenta pedagógica mais famosa para ensinar anatomia é a dissecção. Apesar dos modelos recentes com cadáveres frescos, a dissecção ocorre principalmente em amostras fixadas em formalina. No entanto, esta substância é conhecida por ser tóxica. Várias soluções alternativas surgiram nas últimas décadas, embora a maioria delas seja utilizada em estudos microscópicos; portanto, o ambiente macroscópico continua necessitando de uma nova alternativa capaz de substituir o formaldeído. O estudo realizado aqui propõe o uso desta solução como uma opção para a substituição do formaldeído. **Material e Métodos:** Oito ratos Wistar foram fixados em solução de formalina a 10% e 8 ratos foram fixados em solução à base de álcool com ácido tânico (0,25%). Esses animais foram dissecados por 14 estudantes de medicina. Posteriormente, os alunos receberam um questionário para avaliar aspectos como odor, textura e cor da amostra e flexibilidade das articulações. A toxicidade de cada composto foi analisada por testes “in silico” para sistemas cutâneo e respiratório **Resultados:** Os resultados mostraram superioridade da solução alcoólica do ácido tânico sobre a solução formalina em vários parâmetros analisados, exceto em relação à cor da amostra. A toxicidade da pele e do trato respiratório estava ausente na solução alcoólica do ácido tânico. **Conclusão**: O ácido tânico pode ser um substituto das soluções à base de formaldeído, pois demonstrou diminuir os fatores de risco para indivíduos que manipulam cadáveres e tecidos fixos.

Palavras-chave: solução fixadora alternativa, dissecção, formaldeído, ácido tânico, toxicidade.