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Alternatives to Formaldehyde in the Embalming of Cadavers in the Teaching of Anatomy

Alternativas al Formaldehído en el Embalsamamiento de Cadáveres en la Enseñanza de la Anatomía

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SUMMARY: Currently used embalming solutions continue to present formaldehyde at low concentrations and its identification as a carcinogenic and irritant agent has promoted the search for other formulations, such as N-Vinyl-2-Pyrrolidone (NVP2), which are effective and less harmful. The objective of this study was to evaluate the efficacy of the use of NVP2 in the preservation of cadavers, to assess the appearance, texture, flexibility and microbicidal effect according to the different formulations used in veterinary anatomy teaching. A preliminary study phase was carried out with 16 cryopreserved mice, divided into 2 groups according to the climatic conditions of exposure: room temperature (n=8) or refrigerator chamber at 4 ºC (n=8), 2 of them being excluded at the beginning of the study. Six different preservation solutions were used, depending on the percentage of NVP2 (5 % or 10 %) and the proportion of ethanol (30 % or 50 %) or the use of distilled water. Appearance, texture, flexibility and antimicrobial effect were evaluated according to the formula used, humidity and temperature were recorded in both groups and the pH of the embalming solutions was determined. Environmental conditions had an influence on the state of preservation and antimicrobial effect, being better in preservation at 4 ºC. The solutions with NVP2 at 5-10 % + 30-50 % ethanol obtained better results for most of the variables analyzed and showed fungicidal efficacy and ease of dissection similar to the cryopreserved cadaver. The preservation solutions with NVP2 offer physical-mechanical characteristics and dissection capacity similar to the fresh and/or cryopreserved model, with adequate antimicrobial efficacy under controlled conditions.

KEY WORDS: Embalming; N-Vinyl-2-Pyrrolidone; Cadaver; Tissue preservation; Bacterial growth; Biomechanical phenomena.

INTRODUCTION

Embalming is the process of artificial preservation of human and/or animal corpses through the use of chemical substances of natural or synthetic origin, which seek to reduce the presence and growth of microorganisms responsible for organic decomposition of tissue, by autolysis and putrefaction, achieving long-term preservation of the macroscopic anatomical structure of organs and soft tissues (Balta *et al*., 2015; Alsharif *et al*., 2017; Shrestha *et al*., 2019; Srivastava & Nagwani, 2019; Ogaili *et al*., 2023), depending on the chemical formula used and the extrinsic and intrinsic conditions to which the body is exposed (Zhou & Byard, 2011).

Historically, the Egyptian civilization is known as a pioneer in the process of artificial mummification (6000 BC) (Pal Singh Batra, 2010; Abdel-Maksoud & El-Amin, 2011; Toy & Secgin, 2022). However, the discovery of the circulatory system (1578-1657) prompted the idea of the development of cadaveric preservation techniques by intravascular infusion. Subsequently, the discovery of formaldehyde, an effective and cheap preservative, provided an alternative to the embalming techniques used to date (Beltrán Guerra, 2009), which were improved with the addition of glycerol to the solution to reduce the dehydration state of the body (Toy & Secgin, 2022).

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At present, and due to the harmful and adverse health effects related to the use of formaldehyde, formulations with low concentrations of formaldehyde are used, and formulations without formaldehyde or with low concentrations of formaldehyde are being developed: Thiel, Modified Larssen, Cambridge, Saturated Salt Solution (SSS), Imperial College London, Zinc Chloride, Solution with thymol, ethanol and glycerol, Genelyn and N-Vinyl-2-Pyrrolidone (Srivastava & Nagwani, 2019; Kaliappan *et al*., 2023).

The objective of this preliminary work is to evaluate the efficacy, in terms of appearance, texture, biomechanical qualities and antimicrobial effect, of a formaldehyde- and phenol-free cadaveric preservation formulation exposed to different storage conditions for use in anatomy teaching.

MATERIAL AND METHOD

Cryopreserved and previously sacrificed mice from another research project, directed by Dr. Eva Barrio Ollero, were used after having received authorization from the animal research ethics committee.

The experiment began with a total of 16 mice (n=16), which were divided into two groups with the same number of individuals, according to the climatic conditions of exposure: refrigeration chamber at 4 ºC (n=8) and environment (n=8).

Mice that had been previously eviscerated or showed incipient signs of organic decomposition were excluded from the study.

Study variables

Once the samples of each group have been prepared, the following variables of interest will be recorded, related to the environmental conditions of exposure, physical and biomechanical characteristics of the tissue, the appearance of initial signs of organic decomposition and frank data of growth of microorganisms.

- Temperature (ºC): in the dissection room where the study group is exposed to the environment, it will be measured twice a week using a thermometer, and in the cold room, continuous monitoring will be carried out by means of an integrated device.

- Humidity (%): the frequency of measurement will be the same as for the temperature in the room and in the refrigerating chamber, using a digital hygrometer.

- pH: a single weekly control will be performed in all the models of both groups exposed to different formulations, using a colorimetric pH reagent strip submerged in the embalming liquid of the models.

- Microbiological and fungal analysis (number of colonies): samples will be taken with a sterile swab from the skin surface, inside the thoracic and abdominal cavity in cases where visible signs of incipient decomposition are observed. The samples will be collected by independent swabs and will be cultured in separate media according to the anatomical location of origin. For the analysis of bacterial growth, Chocolate Agar (non-selective, growth of gram +, gram - and fungi), CNA (gram + specific) and MacConkey (gram - specific) will be used. For yeast and fungi culture, Sabouraud medium will be used.

- Color or appearance: 3 examiners will evaluate the similarity of the 24 study mice to live mice in terms of color and physical appearance. The qualitative Likert scale will be used with 5 possible answers according to the level of agreement with the statement made. The following statement will be read “the examined mouse presents physical appearance and color similar to a live mouse” and then after visual examination it will be given a value between 1 and 5, being 1 totally disagree and 5 totally agree.

- Texture, softness or degree of hydration: it will be evaluated by manual touch with protective gloves and evaluation of the fold sign in all the mice. The Likert scale will be used with the following statement “the mouse is correctly hydrated and has a soft touch”, the possible answers being the same as in the previous section.

- Stiffness: given the small size of the models and the difficulty of joint analytical evaluation, it will be evaluated by flexion-extension movement of the trunk of the 24 mice. The Likert scale will be used with the statement “the mouse does not present stiffness to the flexion and extension movement of the trunk”, the possible answers to mark being the same as in previous sections.

- Tissue mobility (ease of dissection): the soft tissue of the abdominal wall will be dissected with an axial and longitudinal section. The Likert scale will be used with the statement “the dissection of the soft tissue in the abdominal wall was easy” and 1 of the 5 possible options mentioned above will be answered.

In any case, the duration of the measurements extracted from the different models will be determined by the viability of the samples.

Table I. Assignment of mice from the 2 study groups to the different embalming formulas.

CONSERVATION FORMULAS	ENVIRONMENTAL (N=8)	REFRIGERATION CHAMBER A 4º (N=8)
NVP2 5 % (100 ml) + distilled water (100 ml)	2*	2*
NVP2 10 % (100 ml) + distilled water (100 ml)	2	2
NVP2 5 % (100 ml) + Ethanol 30 % (100 ml)	1	1
NVP2 5 % (100 ml) + Ethanol 50 % (100 ml)	1	1
NVP2 10 % (100 ml) +Ethanol 30 % (100 ml)	1	1
NVP2 10 % (100 ml) + Ethanol 50 % (100 ml)	1	1

*One mouse from each condition was excluded for reasons of poor condition.

A total of 6 preservation solutions were used in this study, with different concentrations of the fixative compound NVP2 (5 % or 10 %), in combination with distilled water and alcohol (ethanol) and using different proportions of the alcohols (30 % or 50 %), as shown in Table I.

Each embalming condition was used in 1 model of both groups (environment or refrigeration chamber), except for the formulation of distilled water with NVP2 at 5 % and 10 %, which was administered in 2 mice of each group, which were used as controls.

Given the technical difficulty in these models due to their size, intra-arterial injection of the preservative solution was not performed, so the procedure consisted of the immersion of each mouse in 1 bucket containing 200 ml of the solution to be studied and subsequent exposure to the climatic situation corresponding to each group. Likewise, for the measurement of the study variables (with the exception of pH), the models were removed from the container and returned after titration.

RESULTS

The initial sample of mice (n=16) was divided equally into the 2 climatic exposure groups and each mouse was numbered according to the conservation formula applied to it to facilitate subsequent recognition (Table II).

Microbiological analysis

The microbiological analysis of the samples was carried out by the Microbiology Service of the Hospital Clínico Universitario "Lozano Blesa", obtaining the following results.

The two animals immersed in NVP2 5 % and distilled water were excluded due to the presence of decomposition. Of the other two control specimens (NVP2 10 % and distilled water), bacterial overgrowth was only observed in the one stored in the refrigerated chamber, as shown in Table III.

In mice exposed to NVP2 (5 % or 10 %) and ethanol

Table II. Sample of mice using NVP2 and ethanol preservation formylation AD- distilled water. Mice 1,2,7,8- controls with NVP2 (5% and 10%) + distilled water.

	Mice 1 y 2	10-ene-25		NVP2 5% + AD*
Sarcophagus	Mice 3 y 4	10- ene-25		NVP2 10% + AD
	Mouse 5	10-ene-25	Infiltration 0.8 ml	NVP2 5 % + Ethanol 30 %
	Mouse 6	10-ene-25	Immersión in 100 ml	NVP2 5 % + Ethanol 50 %
	Mouse 7	10-ene-25		NVP2 10 % + Ethanol 30 %
	Mouse 8	10-ene-25		NVP2 10 % + Ethanol 50 %
	Mice 9 y 10	10-ene-25		NVP2 5 % + AD*
Chambert	Mice 11 y 12	10-ene-25		NVP2 10 % +AD
	Mouse 13	10-ene-25	Infiltration 0.8 ml	NVP2 5 % + Ethanol 30 %
	Mouse 14	10-ene-25	Immersión in 100 ml	NVP2 5 % + Ethanol 50 %
	Mouse 15	10-ene-25		NVP2 10 % + Ethanol 30 %
	Mouse 16	10-ene-25		NVP2 10 % + Ethanol 50 %

* One of the controls assigned to the NVP 5% + distilled water solution (mice 1 and 9) showed clear signs of decomposition prior to immersion and had to be withdrawn from the study, leaving 22 mice in the project (n=14).

Table III. Microorganisms detected in mice embalmed with NVP2 and ethanol C- refrigerated chamber at 4º; T- room temperature.

Mouse	Sample Collection	Date: 22.02.2025	Date: 01.03.2025
Mouse 5 (NVP2 5% + Ethanol 30%, T)	Skin	*Bacillus lentus*	*Bacillus lentus*
Mouse (NVP2 10% + Ethanol 30%, T)	Skin	*Bacillus lentus* *Staphylococccus epidermidis*	*Bacillus lentus*
Mouse 11 (NVP2 10% + distilled water, C)	Skin	*Corynebacterium amycolatum* *Staphylococccus epidermidis* *Bacillus lentus*	*Corynebacterium amycolatum* *Staphylococccus epidermidis*

(30 % and 50 %), bacterial overgrowth was detected in the cases (2/6) where the percentage of alcohol was 30 % and room temperature. In the remaining cases, there was no evidence of bacterial overgrowth (50 % ethanol at room temperature and chamber and independent of the percentage of NVP2).

After the bacterial overgrowth of *Bacillus lentus*, *Staphylococcus epidermidis* and *Corynebacterium amycolatum*, it was decided to analyze the immersion cuvettes of the affected mice, the gloves of the responsible researchers and the table where the research was carried out. Overgrowth of *Bacillus lentus* was observed in 1 of the 3 cuvettes evaluated and *Staphylococcus epidermidis* was observed in one of the gloves used, and on the table where the investigation was carried out, so cross-contamination was suspected.

On the other hand, no bacterial or fungal overgrowth was observed in the rest of the models subjected to ethanol preservation solutions.

Humidity, temperature and ph recording

It was not necessary to monitor these parameters in the refrigerating chamber, due to the presence of sensors and devices responsible for maintaining both variables, avoiding large oscillations and maintaining stable temperature values of around 4.4º and 41 % humidity.

Humidity values remained stable (around 35 %), with an average oscillation throughout the study of 7 %, with minimum peaks of 26 % and maximum peaks of 40 %, the largest increase recorded being 14 % in the last month.

The temperature analysis also showed stability in the values detected, with an average of 19.08 ºC. The average variability over the course of the study was of 1.08 %. The average variability throughout the experiment was 0.65º, with minimum temperatures of 18º and maximum temperatures of 19.7 ºC, with the greatest change recorded being 1.7 ºC at the beginning of the project.

The pH value remained stable in all the preservation solutions used in the trial, with values between 7 and 8, regardless of storage at room temperature or in the refrigeration chamber.

Appearance, hydration, stiffness and ease of dissection

Sample preservation status. The upper margin of Figure 1 shows the 5 mice stored at room temperature (NVP2+ distilled water, 5 % or 10 % NVP + 30 % or 50 % ethanol), which showed an inadequate appearance with a blackish color. Likewise, the lower end shows the models kept in the refrigerator chamber, being the specimens exposed to NVP2 and distilled water, as well as NVP2 at 5 % and 30 % ethanol in which a darkened appearance of the tissues was appreciated with the naked eye, although with a slight edematous appearance. On the contrary, mice with NVP2 at 5 % and 50 % ethanol, as well as those with NVP2 at 10 % with 30 % or 50 % ethanol, showed a more similar aspect to cryopreserved mice, being those submitted to 50 % ethanol in combination with NVP2 at 5 % or 10 % in which a better preservation state was appreciated (Fig. 1).

The results of the physical-mechanical analysis of the models whose solution contains ethanol as an alcoholic product were also represented qualitatively and are shown in Figures 2, 3 and 4.

When preserved at room temperature, they showed a good appearance, especially those preserved in high concentrations of NVP2 and ethanol (10 % and 50 %, respectively). Similarly, in cold storage, better physical condition was observed in all samples, reaching almost maximum scores (4.5/5) in all formulations with a combination of NVP2 and ethanol, regardless of the percentage of both.

The behavior of the flexibility and stiffness variables was indifferent to the storage temperature used. Thus, the best scores were obtained in the combinations of 50 % ethanol with NVP2 (5 % or 10 %) and 30 % ethanol with NVP2 10 %, followed by those subjected to low alcohol concentration and NVP2 (30 % and 5 %, respectively), while the worst scores were obtained in the models with NVP2 with distilled water.

Regarding the ease of dissection, no differences were identified in relation to the climatic condition or the preservation solution used. In all models the dissection procedure was similar to the cryopreserved model and much simpler than in those submitted to formalin solution.

Fig. 1. Mice subjected to preservation solutions with NVP2 and ethanol. From left to right: NVP 10 % +AD, NVP 5 %+ ethanol 30 %, NVP 5 %+ ethanol 50 %, NVP 10 %+ ethanol 30 % and NVP 10 %+ ethanol 50 %. Above: ambient temperature. Bottom: refrigeration.

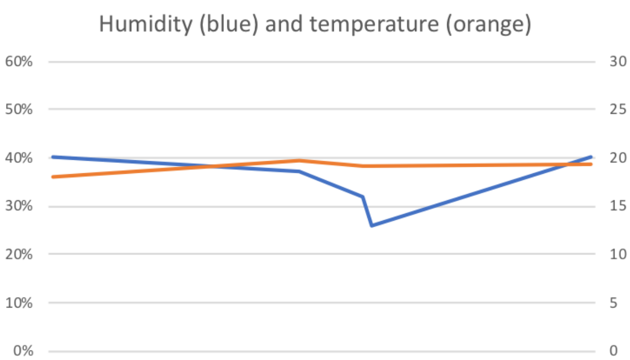

Fig. 2. Humidity and temperature in sarcophagus in ethanol models (ambient temperature).

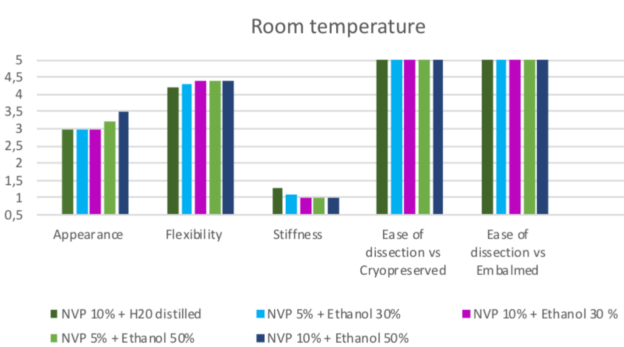

Fig. 3. Appearance, flexibility, stiffness and ease of dissection in samples subjected to ethanol solution at room temperature.

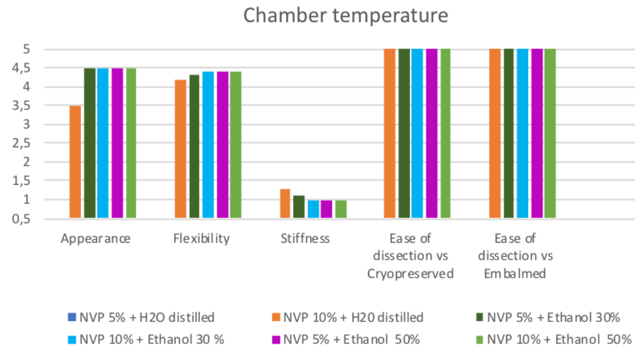

Fig. 4. Appearance, flexibility, stiffness and ease of dissection in samples subjected to ethanol solution at room temperature.

DISCUSSION

In recent years, different mild or low-formaldehyde embalming solutions have been developed, which have tried to reduce the health risk to exposed personnel and achieve to maintain biomechanical qualities of the tissue similar to the fresh and/or cryopreserved cadaver, given the increasing use of cadaveric material in the formation and training of clinical, surgical and diagnostic skills (Srivastava & Nagwani, 2019). However, although in smaller quantities they still contain formaldehyde in their composition so they are still not risk-free and also have certain drawbacks that hinder their routine use. For example, the Thiel method requires trained personnel and expensive equipment, contains flammable substances, has a limited dissection time, the elasticity of tendon tissue is not considered comparable to that of fresh cadaver tissue and its use deteriorates the histological state of the tissue (Kocbek & Rakusa, 2021; Nagase *et al*., 2022; Rakusa & Kocbek Saherl, 2023). Others such as Larssen's solution require expensive refrigeration equipment to maintain very low temperatures (-20 °C) (Yegin *et al*., 2018) and the use of saturated saline solution presents less biocidal power,evidencing the presence of bacterial growth and physical deterioration with the appearance of subcutaneous edema (Hayashi *et al*., 2014).

Recently, N-Vinyl-2-Pyrrolidone has been introduced as a formalin-free fixative, preservative and antiseptic in preservation solutions, with promising results in terms of soft tissue and organ dissection, appearance and joint mobility similar to the fresh and/or cryopreserved cadaver, adequate long-term microbicidal effect and good microscopic preservation (Kaliappan *et al*., 2023). In terms of safety, NVP2, unlike formaldehyde, has not been identified as a carcinogen by the International Agency for Research on Cancer, although the product safety data sheet lists severe oculo-cutaneous irritant effects and specific organ toxicity in humans (lung, kidney, liver, spleen, blood) and reproductive toxicity in animals. Therefore, preventive measures such as the use of face mask, latex gloves, goggles, face shield and proper room ventilation are advised (Nagase *et al*., 2022). However, we believe that more and longer-term studies are needed to assess the toxicity risks of NVP2.

There is currently no established protocol for use and the appropriate technical conditions for use are unknown. All published studies have used the "Preserve" solution containing 100 % pyrrolidone and < 0.1 % N-n-dibutylphenylenediamine, but have used different concentrations of NVP2 (5 %, 10 % or 21.5 % respectively) in combination with different compounds such as ethanol (50 %, 70 %) or H2O. Likewise, there are also discrepancies in terms of the immersion solution used, with 30 % ethanol solution being used in some cases and 5 % preserve in others. However, in all the research conducted there is consensus on the storage mode at 4 °C and they report good preservation state in environment, although the details are not specified (Haizuka *et al*., 2018; Maruyama *et al*., 2019; Mroz˙ek *et al*., 2022; Kaliappan *et al*., 2023).

In agreement with previous studies, similar conditions in terms of NVP2 concentrations (5 % and 10 %) and combination with ethanol (50 %) were used in our trial. Two studies confirm the possibility of adequate long-term preservation of carcasses fixed with 10 % NVP2 and 50 % ethanol in the environment (Nagase *et al*., 2020, 2022). It is true that in our study the best appearance in models preserved under these climatic conditions was in those fixed with these same proportions; however, the state of preservation was considered suboptimal (3.5/5) as opposed to the value achieved in cold preservation (4.5/5). Likewise, embalming at 4 ºC made it possible to achieve the same state using lower doses of both compounds (NVP2 5 % and ethanol 30 %).

However, the storage conditions of the samples in the environment are unknown in the published studies (Nagase *et al*., 2020, 2022) and it would be of interest to know if there is the use of extractor devices or ventilation equipment, as well as the control exercised on the opening of windows to the outside, since these are external factors that can maintain more stable temperature and humidity values and of which nothing is mentioned in this regard. However, it is true that Nagase *et al*. (2020, 2022) adopt measures of protection against light and use of nitrogen gas to reduce oxidative processes in open containers, which are measures that have not been adopted in our study.

The appearance of the models was positively evaluated in all the studies analyzed. However, in our case the samples that maintained a more similar appearance to the cryopreserved model were those exposed to controlled climatic conditions (4 ºC) and subjected to a high content of NVP2 and ethanol (10 % and 50 %) as in the study by Nagase *et al*. (2020), however, we also obtained favorable results with lower concentrations of both compounds (NVP 5 % and ethanol 30 %). On the contrary, formulations composed of NVP2 10 % and water were insufficient to maintain an adequate state of the models unlike what was reported by Haizuka *et al*. (2018), Kaliappan *et al*. (2023) and Maruyama *et al*. (2019).

On the other hand, all authors who employed NVP 10 % reported the presence of an epidermal detachment phenomenon and visible retraction of thoraco-abdominal cavity, intercostal spaces and orbital fossae, which they attribute possibly to the lipolysis process associated with the use of NVP2 (Mroz˙ek *et al*., 2022; Nagase *et al*., 2022; Kaliappan *et al*., 2023). This effect was also observed in our study and was more pronounced the higher the amount of NVP2 and alcohol (NVP 10 % and ethanol 50 %).

Similarly, Haizuka *et al*. (2018) and Mroz˙ek *et al*. (2022) describe connective tissue transparency and easy identification of tendinous and vasculo-nerve structures with the use of NVP2 at 5 and 10 %. In our study, subcutaneous fat reduction was more evident at higher doses of NVP2 (10 %), but it was not possible to discern small anatomical structures such as those reported by these authors despite subjecting the models to the same conditions, possibly due to the size of the samples.

Regarding flexibility and distensibility, all authors agreed that fixation with NVP2 offers better results than using formalin solutions and even compared it to body mobility in life (Haizuka *et al*., 2018; Nagase *et al*., 2020; Mroz˙ek *et al*., 2022; Nagase *et al*., 2022). In our assay, we obtained the same result when comparing these variables with Cambridge models regardless of the concentration of NVP2 and alcohol used, suggesting that the use of NVP2 might be more suitable for teaching use. However, we did find differences when compared with the cryopreserved model depending on the formula used, the type of alcohol and the climatic condition used being indifferent.

Regarding stiffness, Nagase *et al*. (2020) and Maruyama *et al*. (2019) reported that at high doses of NVP2 (20 %) and ethanol (70 %) they found lower tissue distensibility than at lower doses. Regarding this, in our trial the best results were obtained with doses of NVP2 at 10 % and ethanol 50 % coinciding with these authors, which could suggest the existence of a limiting dose in both compounds from which the biomechanical effects on the tissue are reversed. It should also be noted that we also obtained the same results with lower concentrations of NVP2 and alcohol

(NVP 5 % and ethanol 30 %), which could indicate that it is possible to maintain the same efficacy at less toxic doses and keeping the tissue more hydrated. On the contrary, we observed that tissue laxity was considerably reduced when H2O was used instead of alcohol. In addition, the aforementioned authors cautioned that other factors such as the individual's weight and the percentage of fat and muscle tissue could also have an influence on these parameters (Maruyama *et al*., 2019).

Regarding stiffness, Nagase *et al*. (2020) and Maruyama *et al*. (2019) reported that at high doses of NVP2 (20 %) and ethanol (70 %) they found lower tissue distensibility than at lower doses. Regarding this, in our trial the best results were obtained with doses of NVP2 at 10 % and ethanol 50 % coinciding with these authors, which could suggest the existence of a limiting dose in both compounds from which the biomechanical effects on the tissue are reversed. It should also be noted that we also obtained the same results with lower concentrations of NVP2 and alcohol (NVP 5 % and ethanol 30 %), which could indicate that it is possible to maintain the same efficacy at less toxic doses and keeping the tissue more hydrated. On the contrary, we observed that tissue laxity was considerably reduced when H2O was used instead of alcohol. In addition, the aforementioned authors cautioned that other factors such as the individual's weight and the percentage of fat and muscle tissue could also have an influence on these parameters (Maruyama *et al*., 2019).

In our study, there was no evidence of fungal growth with any of the formulas used, so it seems that NVP2 alone has an optimal fungicidal action. However, unlike what was observed by these authors, in our research we did find growth of bacteria such as *Bacillus lentus*, *Staphylococcus epidermidis*, *Corynebacterium amycolatum* and Enterococcus faecalis depending on the percentage of NVP2 or alcohol and climatic conditions of exposure. Thus, at room temperature only solutions with high doses of ethanol (NVP 5/10 % + alcohol 50 %) had an adequate antimicrobial effect coinciding with that reported by Nagase *et al*. (2020, 2022) but differing from that stated by Haizuka *et al*. (2018) who reported that from 4 % NVP2 without the use of alcohol adequate disinfection was achieved. However, variability in weather conditions depending on the geographical area or the use of control devices could explain these discrepancies. Similarly, in this study there was no bacterial overgrowth in any of the formulations with cold storage (NVP2 5/10 % + alcohol 30/50 %) as also reported by Mroz˙ek *et al*. (2022), so it seems that cold storage reduces the required effective dose of both compounds.

Regarding the shelf life of the samples, all studies report adequate long-term preservation viability, however there are discrepancies in the time elapsed until the appearance of signs of decomposition, so that some speak of 11 (Maruyama *et al*., 2019; Kaliappan *et al*., 2023), others of 24 (Haizuka *et al*., 2018; Nagase *et al*., 2020) and some up to 37 months (Mroz˙ek *et al*., 2022). In our case, after 12 months post-procedure, all mice had undergone decay processes.

Limitations of the study

Due to ethical issues this project has been performed in the first instance in mice already sacrificed from another project, but this has limited the assessment of parameters conditioned by size, as well as the availability of the models to carry out the two experiments of the study simultaneously. For this reason, in future studies it would be advisable to repeat this trial in larger animal models. In addition, another limitation of this project is the qualitative description of the variables analyzed, and more objective measurements that validate the conclusions of this study would be desirable.

Likewise, given the scarcity of publications on the subject, it is recommended for further research to compare the use of NVP2 with respect to other mild preservation solutions, as well as to identify the optimal climatic, technical and storage conditions for fixation and preservation with NVP2. It is also necessary to investigate possible long-term toxic or carcinogenic effects of this new substance and finally to clarify its antimicrobial efficacy.

CONCLUSION

Although further studies are needed, NVP2 preservation solutions offer physico-mechanical characteristics and dissectability similar to those of the fresh and/or cryopreserved model, with adequate antimicrobial efficacy under controlled conditions.

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RESUMEN: Las soluciones de embalsamamiento utilizadas actualmente siguen presentando formaldehído en bajas concentraciones, y su identificación como agente cancerígeno e irritante ha impulsado la búsqueda de otras formulaciones, como la N-vinil-2-pirrolidona (NVP2), que son eficaces y menos dañinas. El objetivo de este estudio fue evaluar la eficacia del uso de NVP2 en la preservación de cadáveres, para evaluar la apariencia, la textura, la flexibilidad y el efecto microbicida de las diferentes formulaciones utilizadas en la enseñanza de la anatomía veterinaria. Se realizó una fase preliminar de estudio con 16 ratones criopreservados, divididos en 2 grupos según las condiciones climáticas de exposición: temperatura ambiente (n=8) o cámara frigorífica a 4 ºC (n=8), siendo 2 de ellos excluidos al inicio del estudio. Se emplearon seis soluciones de conservación diferentes, en función del porcentaje de NVP2 (5 % o 10 %) y la proporción de etanol (30 % o 50 %) o del uso de agua destilada. Se evaluó aspecto, textura, flexibilidad y efecto antimicrobiano según la fórmula empleada, se registró la humedad y la temperatura en ambos grupos y se determinó el pH de las soluciones de embalsamamiento. Las condiciones ambientales influyeron en el estado de conservación y efecto antimicrobiano, siendo mejores en la conservación a 4 ºC. Las soluciones con NVP2 al 5-10 % + 30-50 % de etanol obtuvieron mejores resultados para la mayoría de las variables analizadas y mostraron eficacia fungicida y facilidad de disección similar al cadáver criopreservado. Las soluciones de preservación con NVP2 ofrecen características físico-mecánicas y capacidad de disección similares a las del modelo fresco y/o criopreservado, con adecuada eficacia antimicrobiana en condiciones controladas.

PALABRAS CLAVE: Embalsamamiento; N-vinil-2-pirrolidona; Cadáver; Preservación de tejidos; Crecimiento bacteriano; Fenómenos biomecánicos.

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