SIGNIFICANCE TEST TO A DATA MATRIX OF NANORESERVORIES OF TANNINS EXTRACTED FROM TENUIFLORA MIMOSA (TEPEZCOHUITE) ENCAPSULATED IN TIO2 ANORESERVOIRS

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Abstract. The action of the active principles of tepezcohuite (condensed tannins) was used to value the healing capacity in aseptic surgical wounds of 1 centimeter diameter in New Zealand rabbits, a sample of 6 rabbits was taken, to which 3 healing treatments and isotonic saline solution were applied, the objective is to demonstrate which treatment is the best healing. We applied the Hotelling test, comparing the samples 2 by 2, to know if there are significant differences between the methods. The treatments used were nanoreservoirs of Tan/TiO2-150 in weight of tannins in 60% and condensed tannins, TiO2 and the isotonic saline solution (I. S.S.).
Key words: *Nanoreservoirs of Tannins, Mimosa, Tenuiflora, Test Hotelling, TiO2, Tepezcohuite, Isotonic Saline Solution (I.S.S.).*

For years, alternative methods of application have been developed to improve the evolution of burn wounds such as the application of hydroactive gel, hydrocolloid occlusive dressings, hydrofiber dressings with ionic silver, stretch bandages, among others, however, most of they are inappropriate because they present a very slow healing activity or because of their high economic cost, translating into a great burden for the health services and for the patient [1-5]. In the Mexican Republic there are numerous species of plants and trees that are used for therapeutic purposes, among them the tepezcohuite tree, the name of the roots of the Nahuatl language that comes from the word tepezcuahuitl, which means "tree of the hill that bleeds". This was used by the Mayan Civilization as a cure for skin diseases, such as wounds, burns, dermatosis, acne [6, 7].

Nanoreservoirs of TiO_2 were synthesized by encapsulating active substances such as condensed tannins extracted from tenuiflora mimosa (Tepezcuohuite). The nanoreservoirs obtained were applied to shallow non-chronic surgical wounds on the back of New Zealand rabbits. Preliminary results show a 90% closure evolution in wounds with Tan/TiO_2-150 nanoreservoirs compared to isotonic saline, tannins and TiO_2.

EXPERIMENTAL REPORT

Two nano-reservoir syntheses were made independently by the sun-gel technique. The nano-reservoir of TiO2 and the nano-reservoir of TiO2 with 150 mg of condensed tannins, which are labeled as Tan/TiO2-150. The synthesis was carried out with the help of a reflux system with constant agitation at 70°C reaction temperature. All nano-reservoirs were synthesized under the same conditions separately, the only variable being the mass concentration of condensed tannins in mg. The TiO2 and Tan/TiO2-150 nano-reservoirs are characterized by Varian UV-VIS spectrophotometer model Cary 100, which has a diffuse reflectance coupled integration sphere coated with MgO as Labsphere certified reflectance standard with 100% reflectivity and an IR spectrophotometer with Fourier transform (Varian model Scalibur Digilab). The rabbits were classified in treatments A, B and C, and isotonic saline, as shown in table 1.

Aseptic surgical wounds 1cm in diameter were made to 6 New Zealand rabbits, clinically healthy with an average weight of 2 to 3 Kg, on the back of these, anesthetized intravenously in the marginal vein of the ear with sodium pentobarbital, 1 mL/2.5 kg weight. The determination of the closure area of the aseptic surgical wounds was made with the help of a cellophane paper and marker. The edges of the wounds are delimited with intervals of two days; the cellophane paper is placed on millimetric paper in order to quantify how many squares each circle occupies measured in mm [1-3]**.** The procedure of the physical injury in the back of the rabbits is approximately 2 cm in diameter; it consists of shaving the dorsal part to each rabbit and 6 surgical wounds were made in the back; 3 in the left part and 3 in the right part. [3]**.** Surgical injuries caused to each group were verified from the beginning of treatment until 15 days after the first application of the treatment. See Table 2**.** The wounds located on the left side of the back of each group are given 0.5 ml of isotonic saline solution (I. S. S.) as a control. The wounds on the right side of the back, corresponding to each group, were applied 30 mg as follows: condensed tannins (group A), tan-con/TiO_2-150-70 nanomaterial (group B) and TiO_2-70

Table 1. Description of the treatments carried out on New Zealand rabbits

Treatment "A"	Treatment "B"	Treatment "C"
Rabbits 1, 2	Rabbits 3, 4	Rabbits 6, 7
nanoreservoir Tannins y S.S.I.	nanoreservoir Tan/TiO_2-150 y S.S.I.	TiO_2 y S.S.I.

Table 2. Comparative table of the healing process of six rabbits, measured in mm^2

	Rabbit 1		Rabbit 2		Rabbit 3	
Day	S.S.I	Tan	S.S.I.	Tan	S.S.I.	Tan/TiO_2
	X_1	X_2	X_3	X_4	X_5	X_6
0	25	25.2	26.7	26.3	28.7	26.7
1	24.5	24.7	25.3	24.1	27.1	24.1
2	21.8	22	22.3	19.7	24.6	19.7
3	15.7	16	17.8	15.3	21	15.3
4	14.5	14.6	16.1	13.7	17.2	13.7
5	12.3	12.8	14.6	11.4	13.8	11.4
6	11	10.8	12.4	8.7	11.7	8.7
7	9.7	8.3	8.9	6.8	9.5	6.8
8	8	7.4	6.1	4.1	8	4.1
9	6.7	6.1	4.8	2.7	5.8	2.7
10	5.6	5	3.5	0.6	3.5	0.6
11	4.9	4.5	2.7	0	2.4	0.2
13	1.9	1.5	1.3	0	1.3	0
14	0.4	0.8	0.4	0	0.4	0
15	0	0	0	0	0	0

,	Rabbit 4		Rabbit 5		Rabbit 6	
Day	S.S.I.	Tan/TiO_2	S.S.I	TiO_2	S.S.I.	TiO_2
	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}
0	26.3	25.7	24.3	25	21	23.7
1	24.6	23.2	22.7	23.7	19.6	22.1
2	22.4	19.8	19.6	19.9	16.5	19.8
3	16	13.7	16.7	17.3	13.3	15
4	15.2	12.1	15.1	15.0	12.5	13.7
5	14.6	9.9	14.7	13.4	10.4	11.1
6	13.7	8.3	13.3	12.4	8.3	10.7
7	10.3	5.7	12.6	11.7	6.7	8.8
8	8	3.7	11	10.8	5.7	7.3
9	5.9	1.5	9.5	8.7	4.9	5.8
10	3.6	0.8	7.3	6.4	3.1	3.5
11	2	0	5.1	4	2.8	2.5
13	1.5	0	3	2	1.5	1.7
14	0.6	0	2.5	1.2	0.3	0.9
15	0	0	1.1	0	0	0.3

nanomaterial (group C) and I. S. S. The results of the tissue regeneration activity show that, on the tenth day after the first application of the treatment, the tan-con/TiO_2-150-70 nanomaterial presents total tissue regeneration, i.e. 100% healing and cell regeneration of surgical wounds corresponding to the application of condensed tannins (group A) and the TiO_2-70 nanomaterial (group C) and the isotonic saline solution (I. S. S.)**.** The great potential for tissue regeneration of the tan-con/TiO_2-150-70 nanomaterial in a short period of time may be due to the synergism of the condensed tannins presented in the mesh of the TiO_2-70 nanomaterial. Treatment with condensed tannins applied in surgical wounds to rabbits, an area of closure (granulation) is observed on the fourteenth day of 94%.

Significance test corresponding to the average values for 3 treatments applied to 6 rabbits of the New Zealand breed.

It is a question of demonstrating, through their stocking vectors, if there are significant differences in the samples, in order to conclude which healing treatment worked best, for which we will use:

The statistic $\boldsymbol{T^2}$:

$$\boldsymbol{T^2}=\boldsymbol{n_1 n_2}(\bar{\boldsymbol{x}}_{\boldsymbol{1}}-\bar{\boldsymbol{x}}_{\boldsymbol{2}})^{\boldsymbol{1}}\boldsymbol{C^{-1}}(\bar{\boldsymbol{x}}_{\boldsymbol{1}}-\bar{\boldsymbol{x}}_{\boldsymbol{2}})/(\boldsymbol{n_1}+\boldsymbol{n_2}) \quad (1)$$

$$F=(\boldsymbol{n_1}+\boldsymbol{n_2}\text{-p-1})\boldsymbol{T^2}/\{(\boldsymbol{n_1}+\boldsymbol{n_2}\text{-2)p}\} \text{ [8]}. \quad (2)$$

Where $\boldsymbol{n_1}, \boldsymbol{n_2}$ are the sample sizes, $(\bar{\boldsymbol{x}}_{\boldsymbol{1}}, \bar{\boldsymbol{x}}_{\boldsymbol{2}})$ are the mean vectors of each sample, p the number of variables and C the grouped matrix. These statistics are used to compare the samples 2 by 2 and be able to determine significant changes in these samples, we will do this by comparing the samples of rabbit 1 with rabbit 2, rabbit 3 with rabbit 4 and rabbit 5 with rabbit 6 and the other combinations and results are shown in table 3.

For the 2-in-2 test, it is necessary to know the mean vectors and covariance matrices. T and F statistics were programmed using MATLAB. The results are as follows:

For rabbit 1 and rabbit 2 samples, their mean vectors were calculated:

$$x=\begin{bmatrix}\boldsymbol{10.8000}\\ \boldsymbol{10.6467}\end{bmatrix} \qquad y=\begin{bmatrix}\boldsymbol{10.8600}\\ \boldsymbol{8.8933}\end{bmatrix}$$

their covariance matrices:

$$\text{covariance x}=\begin{bmatrix}\boldsymbol{67.2886} & \boldsymbol{68.7807}\\ \boldsymbol{68.7807} & \boldsymbol{70.5355}\end{bmatrix}$$

$$\text{covariance y}=\begin{bmatrix}\boldsymbol{84.6426} & \boldsymbol{83.5961}\\ \boldsymbol{83.5961} & \boldsymbol{83.7392}\end{bmatrix}$$

and its covariance matrix with clustered sample:

$$c=\begin{bmatrix} 75.9656 & 76.1884 \\ 76.1884 & 77.1374 \end{bmatrix}$$

its inverse matrix:

$$c^{-1}=\begin{bmatrix} 1.3997 & -1.3825 \\ -1.3825 & 1.3785 \end{bmatrix}.$$

And the results obtained when applying formulas (1) and (2) are:

T=34.0019 and F=16.3938.

Which are clearly high values and as F is greater than 1 and T is a large value, a significant difference is evident in the samples, although the same healing method has been applied to rabbits 1 and 2.

When comparing rabbit 3 and rabbit 4 samples, the following results were obtained:
averages vectors:

$$x=\begin{bmatrix} 11.6667 \\ 8.9333 \end{bmatrix} \qquad y=\begin{bmatrix} 10.9800 \\ 8.2933 \end{bmatrix}$$

covariances:

$$\text{covariance } x=\begin{bmatrix} 99.5367 & 90.8840 \\ 90.8840 & 84.4924 \end{bmatrix}$$

$$\text{covariance } y=\begin{bmatrix} 78.7596 & 77.5463 \\ 77.5463 & 79.2250 \end{bmatrix}$$

the covariance matrix of the grouped simple is:

$$c=\begin{bmatrix} 89.1456 & 84.2152 \\ 84.2152 & 81.8587 \end{bmatrix}$$

its inverse is:

$$c^{-1}=\begin{bmatrix} 0.3990 & -0.4105 \\ -0.4105 & 0.4345 \end{bmatrix}.$$

Then using formulas (1) and (2) we have to:

T=0.0399 and F=0.0192.

That they are very small values for both T and for F which is less than 1, therefore, we can conclude that when comparing rabbit samples 3 and rabbit 4 with the same treatment, there is no significant difference between one sample and the other, for rabbits 3 and 4.

When comparing rabbit 5 and rabbit 6 samples, the following results were obtained:
media vector:

$$x=\begin{bmatrix} 11.9000 \\ 11.4333 \end{bmatrix} \qquad y=\begin{bmatrix} 8.4400 \\ 9.7933 \end{bmatrix}$$

their covariances:

$$\text{covariance } x=\begin{bmatrix} 52.2457 & 56.9271 \\ 56.9271 & 62.2224 \end{bmatrix}$$

$$\text{covariance } y=\begin{bmatrix} 47.1626 & 53.0274 \\ 53.0274 & 60.0392 \end{bmatrix}$$

the covariance matrix with the grouped sample is:

$$c=\begin{bmatrix} 49.7041 & 54.9773 \\ 54.9773 & 61.1308 \end{bmatrix}$$

Table 3. Results of 2-in-2 sample comparisons

	rabbit 1 vs rabbit 2	rabbit 1 vs rabbit 3	rabbit 1 vs rabbit 4
T	34.0019	43.8290	30.5817
F	16.3938	21.1318	14.7447
	rabbit 1 vs rabbit 5	rabbit 1 vs rabbit 6	
T	3.8373	44.7703	
F	1.8501	21.5857	
	rabbit 2 vs rabbit 3	rabbit 2 vs rabbit 4	rabbit 2 vs rabbit 5
T	2.7290	1.9174	19.1709
F	1.3158	0.9232	9.2431
	rabbit 2 vs rabbit 6		
T	81.3324		
F	39.2138		
	rabbit 3 vs rabbit 4	rabbit 3 vs rabbit 5	rabbit 3 vs rabbit 6
T	0.0399	27.9687	73.5107
F	0.0192	13.4849	35.4427
	rabbit 4 vs rabbit 5	rabbit 4 vs rabbit 6	
T	21.3105	67.1489	
F	10.2747	32.3754	
	rabbit 5 vs rabbit 6		
T	113.5830		
F	54.7632		

its inverse is:

$$c^{-1}=\begin{bmatrix} 3.8320 & -3.4463 \\ -3.4463 & 3.1150 \end{bmatrix}.$$

And using formulas (1) and (2) you have to:

T=113.5830 and F=54.7632.

Which denotes a clear difference in its vector of stockings, which means that although it is the same treatment that is applied to rabbit 5 and rabbit 6, there are differences in the evolution of the healing process. The other comparisons are summarized in Table 3.

RESULTS

It can be observed in the results table that when comparing all rabbits by pairs, rabbits 3 and 4, the ones to whom the Tepezcohuite treatment was applied to, did not show significant differences, which can be interpreted that the scars disappeared, that is to say, when having a faster healing process, the wounds closed and therefore the differences disappeared. We can therefore conclude that the treatments used do show differences, but the Tepezcohuite treatment can be considered to have healed better, as seen in the photos, and that it statistically coincided with the application of the Hotelling test.

CONCLUSIONS

The nano reservoirs of Tan/TiO2-150, show faster closure in surgical wounds caused intentionally on the back of the rabbits, the evolution of healing (closure) of these presented 11 days after having caused the wounds. The tannins extracted from the Tepezcohuite bark show a better healing speed. The reference nano reservoir TiO2 presented an activity similar to tannins. While the isotonic saline solution presented scarring at 13 days, where scarring is partial. Therefore, according to the results the efficiency of the nano reservoirs in the speed of healing (closure) is established the following scheme: Tan/TiO2-150 > condensed tannins ≥ TiO2 > I.S.S.

When conducting an experiment, where it is a question of demonstrating the effectiveness of one treatment with respect to another, it is important to know if the data provided by the experiment has significant differences. It was possible to prove that out of the 12 samples of size 15, when comparing them to 2 in 2, it was only found that the samples of rabbits 3 and 4 did not have significant differences, because the evolution of cicatrization was evolving favorably in these rabbits and was much better than the other treatments, the other comparisons, if they demonstrated significant differences.

In conclusion, the Hotelling's significance test indicated that there are significant differences in the closure process in treatments A, C and S.S.I., but that in terms of the one that healed his wounds the fastest, it was rabbits 3 and 4 to which the nano tannin reservoir treatment was applied/TiO2-150, which is the B treatment.

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