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Development and Preliminary Assessment of Hemoperfusion Cartridge with Tannic Acid for Toxic Proteins' Precipitation: An In Vitro Model

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Abstract
Charcoal hemoperfusion (CHP) is one of the extracorporeal removal techniques that are used to remove toxins from the body. CHP generally is considered the preferred method for extracorporeal extraction of several toxins—toxins that are adsorbed by activated charcoal. Assessments of the tannic acid's protective effects on ophidian poisoning are associated with the toxic proteins' precipitation by tannic acid. The challenge in treating a snakebite lies in removing the injected poison with minimal damage to blood constituent proteins. An alternative is CHP, and this investigation proposed to develop a column for hemoperfuser cartridge, combining charcoal granules trapped between layers of polymeric material conjugated to tannic acid, using an in vitro model scaled to the Wistar rat, which can be tested in an animal model. The cartridge was evaluated using the 2^2 full factorial design, in duplicate, as a method to study the effects of granulated-charcoal size and tannic acid concentration on the hematologic profile (platelet and leukocyte counts) and biochemical profile (total serum protein and albumin dosages) of sheep blood. The results demonstrate that charcoal in hemoperfuser cartridge: (1) decreases the serum in sheep blood volume, as consequence, (2) increases the serum proteins' concentration, and (iii) exerts slight influence on albumin. The inclusion of tannic acid in hemoperfuser column precipitates some of serum proteins and albumin, decreasing their concentrations in the plasma serum. In conclusion, based on these effects we can suggest the use of 0.02 g tannic acid concentration and 8–20 mesh granulated charcoal in hemoperfuser cartridge for precipitating toxic proteins from snake venoms.

Keywords: Charcoal granules; Hemoperfusion; Snake venom; Tannic acid.

1. INTRODUCTION

Charcoal hemoperfusion (CHP) is one of the extracorporeal removal techniques that are used to eliminate toxins from the body [1, 2]. According to the Toxic Exposure Surveillance System (TESS), developed by American Association of Poison Control Centers, the number of patients who received hemoperfusion (normalized per million calls) decreased from 53 to 12 during 1985-2005. Theophylline was the most common toxin removed by hemoperfusion from 1985 to 2000, but carbamazepine became the most frequent toxin removed during 2001-2005 [1].

CHP is a method in which blood circulates through an activated charcoal-containing cartridge added to the circuit of a hemodialysis (HD) machine. CHP generally is considered the preferred method for extracorporeal extraction of several toxins—toxins that are adsorbed to activated charcoal [3, 4]. Like HD, CHP is very effective for toxins that are distributed in a small volume. Unlike HD, it can effectively remove toxins that are bound to plasma proteins [4].

Kuppusamy and Das [5] showed the protective effects of tannic acid and its related natural compounds on *Crotalus adamenteus* subcutaneous poisoning in mice. Pithayanukul *et al.* [6], reported in vitro investigation of the protective effects of tannic acid against the action of *Naja kaouthia* venom. Melo *et al.* [7] demonstrated the effectiveness of tannic acid in precipitating the constituent proteins of the complex mixture of snake venom (*Bothrops jararacussu* and *Crotalus durissus terrificus*).

The challenge in treating snakebite lies in removing the active poison with minimal damage to blood constituent proteins, which makes tannic acid intravenous administration not optional, even if the acid is able to precipitate the poison's proteins. An alternative to this method is CHP, and in this project, we proposed to develop a column for hemoperfuser cartridge, combining activated charcoal granules trapped between layers of semi-permeable polymeric material conjugated to tannic acid, in an in vitro model scaled to the Wistar rat, which can be tested in an animal model.

2. MATERIALS AND METHODS

2.1. Materials

The materials used are tannic acid (Sigma-Aldrich®, lot MKBV0516V), sheep blood (NewProw Prod Lab, lot 16318M), sodium heparin (5000 UI/mL, Cristália, lot 14107351), activated charcoal—4-8 mesh (Sigma-Aldrich®, lot SLBH5329V) and 8-20 mesh

(Sigma-Aldrich®, lot MKBR2258V), 5 mm thickness and 23 g/mL density polyurethane filter (Junseal®), 5 mL polypropylene syringe (BD Luer-LokTip™ Plastipak™), capillary hemoperfusion tube (Silastic® RX-50), sterile saline solution 0.9 % (JP, lot 16715), albumin monoreagent (Bioclin®, K040, lot 125), total protein monoreagent (Bioclin®, K031, lot 134), and normal and pathological controls—Biocontrol N (Bioclin®, K073, lot 52) and Biocontrol P (Bioclin®, K074, lot 39), respectively.

2.2. Cartridge Development

Charcoal granules are contained between two polyurethane polymer filter and into a 5 mL polypropylene syringe—specially modified (*made in house*). A mass of 0.01 or 0.02 g (Ohaus, Explorer, USA) of tannic acid impregnated on the tannic acid polymer filter column was kept in the oven (37°C) with air circulation (Tecnal®, TE-394, Piracicaba, Brazil). Carbon granules were prewashed with ultrapure water obtained from Purelab Option-Q Elga Milli-Q system, AV50, Araraquara, Brazil. The polyurethane membranes and syringes were cut, properly washed, and dried but not sterilized. The column was prepared with 2.50-2.75 mL of heparin and 0.9 % sodium chloride solution (1000 U/100 mL) after washing with 100 mL of heparinized saline. The active carbon granules are available commercially in different diameters, 4-8 mesh, and 8-20 mesh. Thus, it is possible to evaluate the effect of the charcoal granules' size in hemoperfusion and to select the best feature with bead diameter to compose the column under development. Each column received a mass of 1.42 g of activated carbon, as suggested in the work of Ryan *et al*. [8]. A column without treatment with tannic acid was built to be used as a reference. The system, including the column and polyurethane filters, was sterilized using ethylene oxide (Sterileno, Sorocaba, Brazil).

2.3. Simulated Hemoperfusion

An in vitro *experiment* of the simulated hemoperfusion is illustrated in Figure 1. Sheep blood was used to mimic the hemoperfusion, and the experiments were repeated by changing the activated carbon column and tannic acid in the multivariate experimental design. The blood was injected at a constant flow rate (3 mL/min) (Terumo Medical, TE-135, Brazil) in the hemoperfuser column test. The factors of interest for the development of hemoperfuser column study were varied as set out in selected design of experiments.

2.4. Analysis of Results

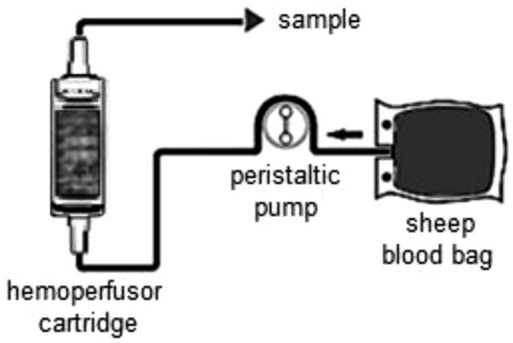
In order to evaluate the cartridge, the 2^2 full factorial design was selected in duplicate as a multivariate method to study the factors (average diameter of granulated charcoal and the amount of tannic acid which makes up the premembrane of the column for hemoperfusion), effects on the hematologic profile—platelet count (PLT) and white blood cells or leukocyte (WBC)—and effects on the biochemical profile—total serum protein (SP) and albumin (ALB) dosages in the sheep blood. The serum proteins' components were represented by the total SP, all protein fractions, and the ALB fraction.

Assays were performed in random order to avoid the occurrence of distortion in the statistical results. The effects of the main factors and the interaction's effects were calculated using the software Action Stat 3.1, as well as using ANOVA, variance, standard error, standard error of an effect, and a model of an independent test, providing data sufficient for the interpretation of the results. With the standard error, we can construct confidence intervals for the values of the effects, using the Student distribution ($\hat{\eta} - t_v \times s(effect) < \eta < \hat{\eta} + t_v \times s(effect)$), where η is the true value of an effect, that is, population and the caret indicates an estimated value obtained in this experiment. According to this equation, we only considered statistically significant with 95 % confidence an effect whose absolute value was greater than $t_4 \times s(effect)$ [9].

Hematologic profile was obtained by the hematological system (Roche®, Sysmex XS-1000i, Kobe, Japan) which provides platelet count (PLT) and total leukocyte count (WBC) of heparinized sheep blood, and commercial blood was used as control (Echeck). Blood samples were collected before and after simulated hemoperfusion [10].

Biochemical profile was obtained using Biuret (BIU) and bromocresol green (BCG) colorimetric tests. These tests were used to determine the total serum protein (SP) and albumin (ALB), respectively, with spectrophotometric readings (Perkin Elmer,

Figure 1: Simulated hemoperfusion illustration.



lambda 35, Brazil). As serum controls, the biocontrol kits N (normal) and P (pathological) were used. Three vials—B (baseline), S (sample), ST (standard)—were identified and followed in the procedure as shown in Table 1.

In the biuret method, tubes B, S, ST, and the normal (N) and pathological (P) controls were homogenized on the vortex and left to stand for 10 min. Further, a spectrophotometer B was used as a baseline, and analyses were taken at the wavelength 545 nm (absorbance), while the colors of the samples S, ST, N and P were stable (30 min). For calculations: $\text{SP(g/dL)} = \frac{\text{Sample Absorbance}}{\text{Standard Absorbance}} \times 4$. As the reaction follows the Lambert-Beer Law, the calibration factor can be used (Equation (1)), and SP concentration was calculated by Equation (2).

$$\text{Calibration Factor} = \frac{\text{Standard Concentration (4 g/dL)}}{\text{Standard Absorbance (nm)}} \quad (1)$$

$$\text{SP Concentration (g/dL)} = \text{Sample Absorbance} \times \text{Calibration Factor} \quad (2)$$

For the N batch used, the value of the concentration of SP was 5.3 g/dL, with a variation range set between 4.5 to 6.1 g/dL, and the P batch used an SP concentration of 8.8 g/dL, with a variation range set between 7.9 to 9.7 g/dL.

In the VBP test tubes, B, S, ST, and the N and P controls were homogenized by vortexing and left to stand for 5 min. Further, in a spectrophotometer, B was used as a baseline, and readings were taken at 630 nm, while the colors of the samples S, ST, N and P were stable (30 min). For calculations: $\text{ALB (g/dL)} = \frac{\text{Sample Absorbance}}{\text{Standard Absorbance}} \times 3.8$. As the reaction follows the Lambert-Beer Law, the calibration factor can be used (Equation (3)), and SP concentration was calculated by Equation (4).

$$\text{Calibration Factor} = \frac{\text{Standard Concentration (3.8 g/dL)}}{\text{Standard Absorbance (nm)}} \quad (3)$$

$$\text{ALB Concentration (g/dL)} = \text{Sample Absorbance} \times \text{Calibration Factor} \quad (4)$$

For the N and P batches, the values of ALB concentration were 2.8 and 4.5 g/dL, with a variation range set between 2.4 and 3.2 g/dL and between 4.0 and 5.0 g/dL, respectively.

3. RESULTS

The development of hemoperfuser-column design and assembly of the columns have been successfully made.

3.1. Hematologic and Biochemical Profiles

Hematologic profile (HP) of the sheep blood is represented by platelet count (PLT) and total leukocyte count (WBC), whereas these same tests were performed for all treated samples of sheep blood by simulated hemoperfusion in this study.

The biochemical methods—biuret to determine SP and bromocresol green to determine ALB—were validated for sheep blood in the spectrophotometer, and the results can be checked from the data in Table 2. SP values concentrations obtained were 5.37 and 8.86 g/dL for the N and P batches used, respectively, and ALB values of the concentration obtained were 2.91 and 3.90 g/dL for N and P batches used, respectively.

HP analysis results are showed in the Tables 3-5 by PLT and WBC values to sheep blood before and after simulated hemoperfusion.

Values of protein fractions concentrations—SP and ALB—for sheep blood treated by simulated hemoperfusion, initially without tannic acid and subsequently with tannic acid are exhibited on Table 4.

Table 1: Procedure for testing biuret and green bromocresol (BCG) methods in test tubes B (baseline), S (sample) and ST (standard), separately.

Biuret method			
	B	ST	S
S	...	...	50 μL
ST	...	50 μL	...
Biuret	2.5 mL	2.5 mL	2.5 mL
BCG method			
S	...	...	10 μL
ST	...	10 μL	...
BCG	2.5 mL	2.5 mL	2.5 mL

Table 2: Serum protein (SP) values in the BIU method and albumin (ALB) values in the BCG method.

Assay	BIU method				BCG method			
	Absorbance (λ = 545 nm)		SP_{Mean} (g/dL)	FC_{BIU}	Absorbance (λ = 630 nm)		ALB_{Mean} (g/dL)	FC_{BCG}
ST_1 ST_2 ST_3	0.233 0.234 0.233	Mean 0.233	...	17.17	0.417 0.430 0.424	Mean 0.424	...	8.96
N_1 N_2	0.313 0.312	Mean 0.313	5.37	...	0.323 0.327	Mean 0.325	2.91	...
P_1 P_2	0.513 0.519	Mean: 0.516	8.86	...	0.434 0.435	Mean: 0.435	3.90	...
S_1 S_2 S_3	0.409 0.408 0.411	Mean 0.409	7.02	...	0.372 0.374 0.370	Mean 0.372	3.33	...

Sheep blood sample (S), standard (ST), normal control (N), and pathological control (P).

Table 3: Platelet count (PLT) and leukocyte count (WBC) in sheep blood before the simulated hemoperfusion (*n* = 5).

HP	Assay						Mean ± DP
	1	2	3	4	5	6	
PLT ($10^3/\mu L$)	225	226	212	222	223	231	223.17 ± 6.31
WBC ($10/\mu L$)	5.07	5.23	5.51	5.49	5.66	6.03	5.50 ± 0.34

Table 4: Platelet count (PLT), leukocyte count (WBC) and protein fractions concentrations in sheep blood.

Assay in drawn order	Assay order	Granulated charcoal (mesh)	Tannic acid (g)	PLT Mean ($10^3/\mu L$)	WBC Mean ($10/\mu L$)	SP Mean (g/dL)	ALB Mean (g/dL)
After the simulated hemoperfusion without tannic acid (*n* = 3)							
4	1	8-20	0	275	5.83	11.15	3.53
3	2	4-8	0	272	5.87	11.07	3.55
2	3	8-20	0	264	5.69	10.93	3.51
1	4	4-8	0	269	5.73	10.88	3.51
After the simulated hemoperfusion with two levels of tannic acid (*n* = 3)							
4	1	8-20	0.02	216	5.84	7.28	1.86
4	2	8-20	0.02	221	6.03	7.26	1.81
3	3	4-8	0.02	224	5.85	7.35	1.99
2	4	8-20	0.01	213	6.07	7.13	2.02
1	5	4-8	0.01	211	5.92	7.10	1.98
3	6	4-8	0.02	224	5.67	7.44	2.05
2	7	8-20	0.01	206	5.86	7.33	2.06
1	8	4-8	0.01	212	5.56	7.31	1.96

3.2. 2^2 Full Factorial Design in Duplicate

Results of the influence of factors—charcoal granule size and amount of tannic acid—were evaluated by the effects on the PLT, WBC, SP, and ALB amounts after treatment by simulated hemoperfusion with 4 mL of sheep blood. Total serum protein was represented by SP and ALB, thus enabling the calculation of the main effects and interaction of factors selected for this study, summarized in Tables 5 and 6.

In Table 5, the results of the effects (PLT, WBC, PS and ALB) were considered statistically significant, with 95 % confidence, when the absolute value was greater than its respective $t_4 \times s(effect)$. But by ANOVA analysis only the concentration of tannic acid had significant difference compared to the model tested for PLT (P-value ≈ 0.008), and the concentration of tannic acid and interaction effects showed a significant difference for ALB (P-values ≈ 0.03 and 0.005, respectively).

Table 5: Results of 2^2 full factorial design in duplicate to study the effects of the diameter of granulated charcoal and amount of tannic acid from hemoperfusion column after simulated hemoperfusion.

Factors				**(−)**		**(+)**	
1 Granulated charcoal (mesh)				4-8 (A)		8-20 (B)	
2 Tannic acid (g)				0.01		0.02	
i	**1**	**2**	**12**	**HP**		**SP_{Mean} (g/dL)**	**ALB_{Mean} (g/dL)**
				PLT_{Mean} ($10^3/\mu L$)	**WBC_{Mean} ($10/\mu L$)**		
1	−	−	+	211.50	5.74	7.21	1.97
2	+	−	−	209.50	5.97	7.23	2.04
3	−	+	−	224.00	5.76	7.40	2.02
4	+	+	+	218.50	5.94	7.27	1.84
Effects				**PLT ± s(effect) ($10^3/\mu L$)**	**WBC ± s(effect) ($10/\mu L$)**	**SP ± s(effect) (g/dL)**	**ALB ± s(effect) (g/dL)**
1		Main		−3.75 ± 1.08	0.200 ± 0.06	−0.050 ± 0.038	−0.057 ± 0.011
2		Main		10.75 ± 1.08	−0.005 ± 0.06	0.115 ± 0.038	−0.077 ± 0.011
12		Interaction		−1.75 ± 1.08	−0.025 ± 0.06	−0.075 ± 0.038	−0.127 ± 0.011
$t_4 \times$ s(effect)				2.998	0.171	0.106	0.031

Experimental assay (*i*), hematologic profile (HP), platelet count (PLT), leukocyte count (WBC), serum protein (SP), albumin (ALB) and estimate, with four degrees of freedom in the Student's t distribution and 95 % confidence, the standard error of an effect ($t_4 \times$ s(effect)).

Table 6: Results of 2^2 full factorial design in duplicate to study the effects of the diameter of granulated charcoal and presence of tannic acid from hemoperfusion column after simulated hemoperfusion.

Factors:				**(−)**		**(+)**	
1: Granulated charcoal (mesh)				4-8 (A)		8-20 (B)	
2: Tannic acid (g)				0		0.02	
i	**1**	**2**	**12**	**HP**		**SP_{Mean} (g/dL)**	**ALB_{Mean} (g/dL)**
				PLT_{Mean} ($10^3/\mu L$)	**WBC_{Mean} ($10/\mu L$)**		
1	−	−	+	270.50	5.80	10.98	3.53
2	+	−	−	269.50	5.76	11.04	3.52
3	−	+	−	224.00	5.76	7.40	2.02
4	+	+	+	218.50	5.94	7.27	1.84
Effects:				**PLT ± s(effect) ($10^3/\mu L$)**	**WBC ± s(effect) ($10/\mu L$)**	**SP ± s(effect) (g/dL)**	**ALB ± s(effect) (g/dL)**
1		Main		−3.25 ± 1.56	0.07 ± 0.04	−0.030 ± 0.038	−0.097 ± 0.011
2		Main		−48.75 ± 1.56	0.07 ± 0.04	−3.675 ± 0.038	−1.597 ± 0.011
12		Interaction		−2.25 ± 1.56	0.11 ± 0.04	−0.097 ± 0.038	−0.087 ± 0.011
$t_4 \times$ s(effect)				8.640	4.320	0.114	0.106

Experimental assay (*i*), hematologic profile (HP), platelet count (PLT), leukocyte count (WBC), serum protein (SP), albumin (ALB) and estimate, with four degrees of freedom in the Student's t distribution and 95 % confidence, the standard error of an effect ($t_4 \times$ s(effect)).

Similarly, in Table 6 were are considered statistically significant, with 95 % confidence, the effects (PLT, WBC, SP and ACB) whose absolute value was greater than their respective $t_4 \times$ s(effect). But by ANOVA analysis only the concentration of tannic acid had significant difference to the model tested for PLT ($p \approx 9.7 \times 10^{-5}$) and for SP ($p \approx 1.1 \times 10^{-6}$), and the granulated charcoal size, concentration of tannic acid, and interaction effects showed significant difference for ALB ($p \approx 0.01$, 2.4×10^{-7}, and 0.02, respectively). ANOVA analysis showed no significant difference in the results of all WBC assays in the Tables 5 and 6. The Durbin-Watson test of independence does not reject the hypothesis that the statistical model used for the experiments was well adjusted ($p > 0.1$). Calculations of the main effects and interactions were made by determining the mean of results ($n = 2$) obtained for PLT, WBC, SP, and ALB in randomized trials (Tables 5 and 6). To simplify interpretation of the results obtained in this study, interactions graphics (Figure 2) and variables, diagrams of mean responses for all levels combinations of the variables may be observed in Figure 3.

4. DISCUSSION

In the assessment of the effects (Figure 2a) of the platelet count (PLT) results, the main effects whose absolute values were greater than $t_4 \times$ s(effect) = $2.998 \times 10^3/\mu L$ were considered statistically significant ($p < 0.05$) (Table 5). Wherein the Figure 3a

Figure 2: Interactions graphs of average responses of the variables.

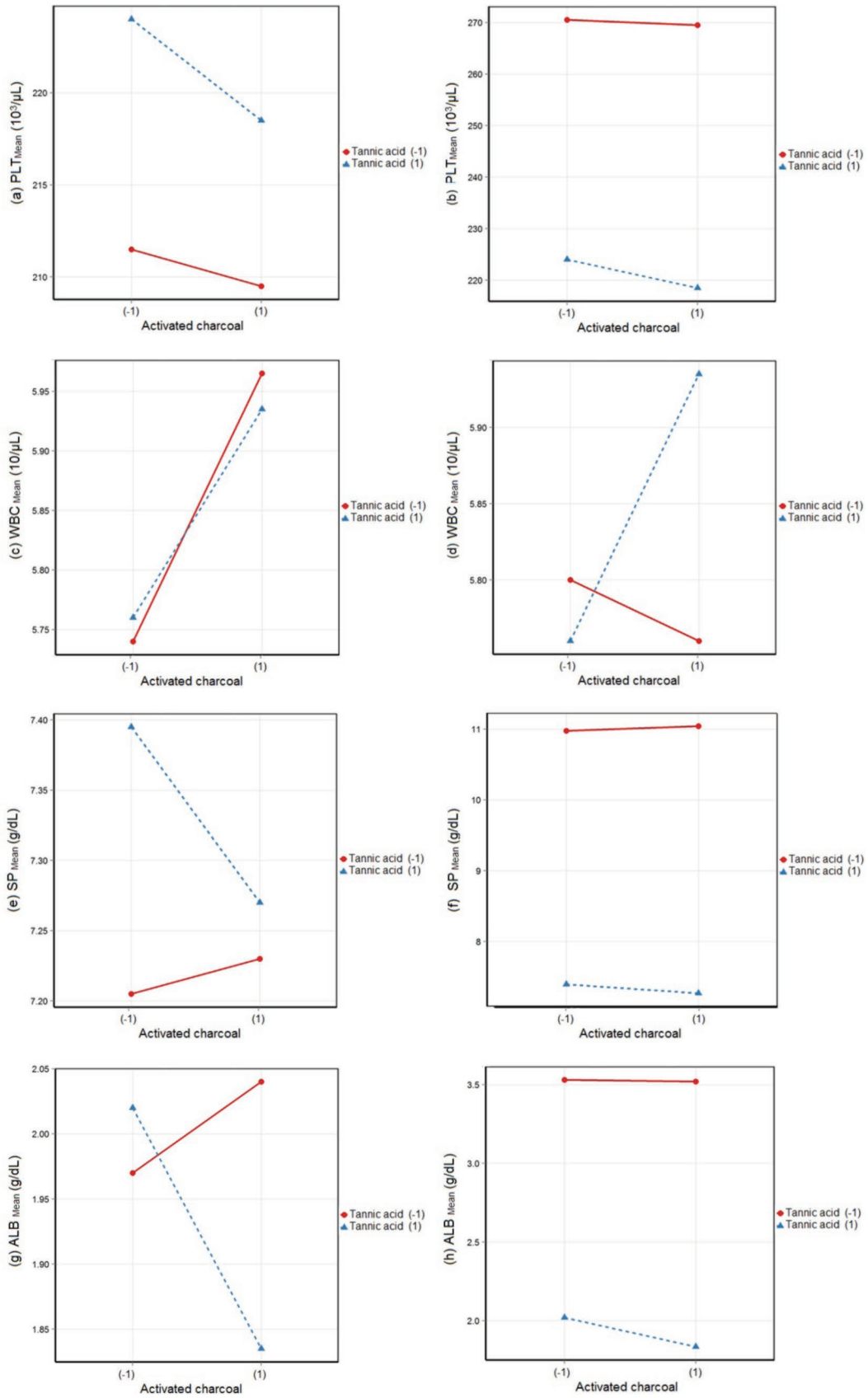


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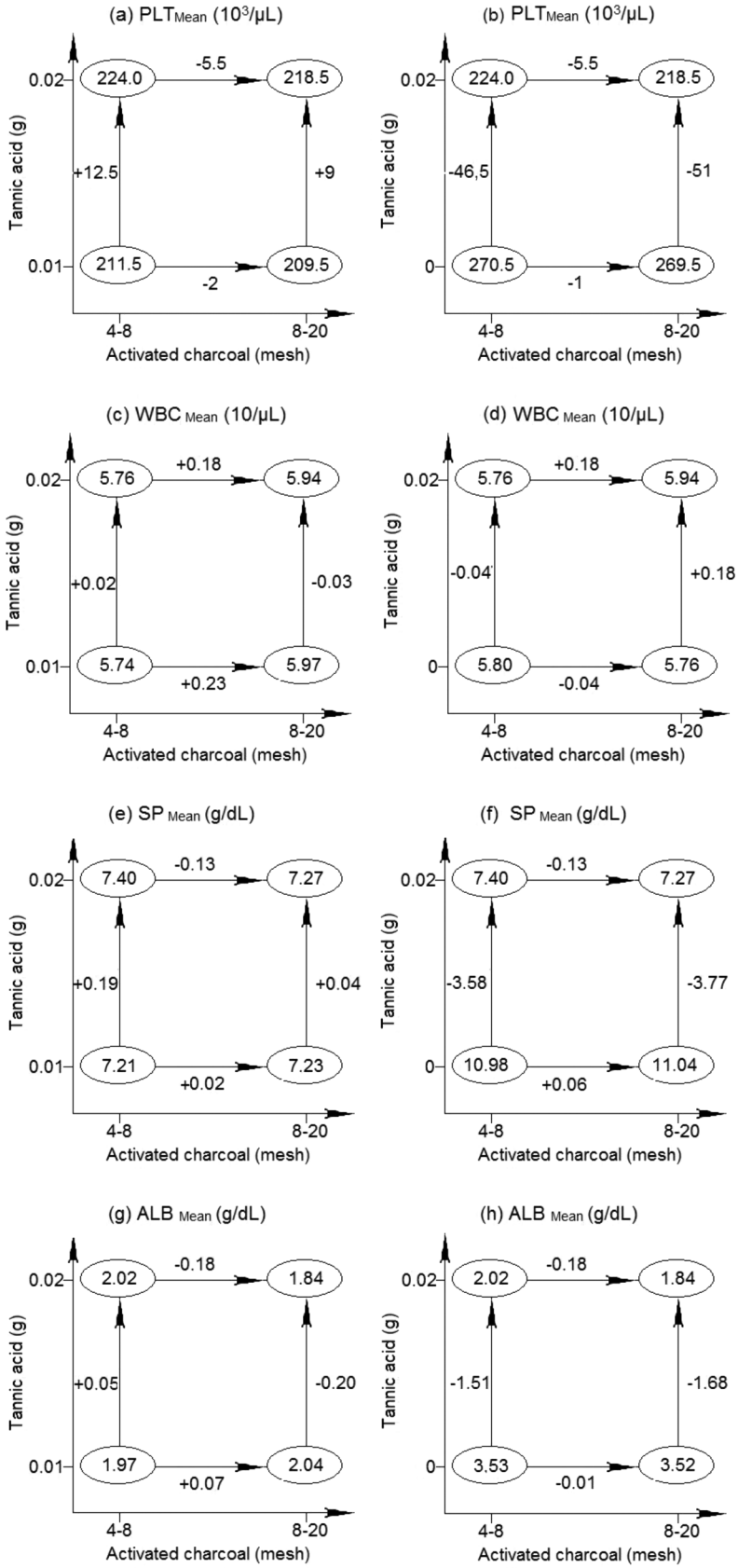
Figure 3: Diagrams of mean responses for all levels and combinations of the variables.



the diagram shows that: (1) by decreasing the diameter of the charcoal granule, PLT diminishes, but the effect is more pronounced with a higher concentration of tannic acid (0.02 g) than 0.01 g tannic acid. (-5.5 against -2); (2) by changing the 0.01 g tannic acid membrane to 0.02 g PLT increased, and this effect was greater for 4-8 mesh than for 8-20 mesh charcoal granule ($+12.5$ against $+9$); and (3) a smaller PLT value ($209.4 \times 10^3/\mu L$ on average) was obtained with 0.01 g tannic acid membrane and 8-20 mesh charcoal granules, although this last effect has no significant interaction.

In this work, we evaluated the effect of the tannic acid concentration (Figure 2b) on the membrane and the interaction effect thereof with the charcoal granule size for the results of platelet counts (PLT) (Figure 3b); the absolute values of these effects were much higher than $t_4 \times$ s(effect) $= 4.32 \times 10^3/\mu L$ (Table 6), indicating that the use of tannic acid significantly decreased PLT ($p << 0.05$) wherein: (1) the diameter of the charcoal granule did not affect PLT significantly; (2) replacing the membrane without tannic acid by 0.02 g tannic acid membrane decreased PLT, and this effect was more significant for 8-20 mesh than 4-8 mesh charcoal granule (-51 against -46.5); and (3) smaller PLT value ($218.5 \times 10^3/\mu L$ on average) was obtained with 0.02 g tannic acid membrane and 8-20 mesh charcoal granule.

In the effects' assessment ($p < 0.05$) of total leukocyte count (WBC) results, absolute values of the main effects were close to $t_4 \times$ s(effect) $= 0.171 \times 10/\mu L$ (Tables 5 and 6 show that in relation to $t_4 \times$ s(effect) $= 0.114 \times 10/\mu L$, the effect of the tannic acid concentration in the membrane, the charcoal granule diameter, and the interaction effect with the charcoal granules size thereof was not significant in the selected experimental condition ($p > 0.05$). Therefore, they are not determinative of CTL (Figures 3c and 3d); despite this, the interactions graphs, shown in Figure 2d, point to a possible interaction.

When evaluating the effects ($p < 0.05$) for the concentration of serum proteins' (SP) results (Figures 2e and 2f), the absolute values of the main effects were less than $t_4 \times$ s(effect) $= 0.106$ g/dL in Tables 5 and 6; therefore, they were not significant, and the effect of the concentration of tannic acid could be considered significant (Figure 2e and Table 5), although the ANOVA p value was 0.205, demonstrating that 0.01 and 0.02 g tannic acid concentrations had no significant difference in PS. However, the presence of tannic acid is significant for PS (Table 6 and Figure 2f), wherein the largest SP values (7.40 and 11.04 g/dL on average in the experimental conditions in Tables 5 and 6, respectively) were obtained with 0.02 g tannic acid membrane of 4-8 mesh charcoal granules (Figure 3e) and with a membrane without tannic acid of 8-20 mesh charcoal granules (Figure 3f). Therefore, there is evidence that SP decreases with increasing concentration of tannic acid. This is important to achieve the proposed toxic-protein precipitation in ophidian venom.

In the assessment of the effects ($p < 0.05$) for albumin (ALB) concentration results (Figures 3g-3h), the absolute values of the main effects and interactions effects were higher than $t_4 \times$ s(effect) $= 0.031$ g/dL in Tables 5 and 6, consequently considered significant. And (1) the charcoal granule diameter had slight influence on the ALB concentration; (2) by changing the 0.01 g tannic acid membrane by 0.02 g, ALB increased slightly to 4-8 mesh charcoal granules, and ALB decreased to 8-20 mesh charcoal granules ($+0.05$ against -0.2); this may be due to the smaller size granules having larger surface area thus adsorb more blood plasma; and (3) the highest ALB values (2.04 and 3.53 g/dL on average in the experimental conditions listed in Tables 5 and 6, respectively) were obtained with a membrane with low levels of tannic acid concentration, and there was slight significant influence of the charcoal granules size. Therefore, there is evidence that the ALB decreases with increasing concentration of tannic acid.

The ALB fraction was selected based on the fact that the association between serum albumin and clinical status, constituting a marker of severity; the lower serum levels were, the higher was the severity of the case [10].

Results of this research suggest that the hemoperfuser cartridge's carbon granules decrease blood plasma in sheep blood volume, thereby increasing the concentration of serum proteins and slightly influencing ALB values. The inclusion of tannic acid in hemoperfuser column precipitates some of serum proteins and albumin, decreasing their concentrations in the blood plasma.

As part of the tannic acid reacts with plasma proteins, it is suggested to increase its concentration in the hemoperfuser cartridge aiming, in future work, the precipitation of the ophidian-venom proteins.

5. CONCLUSION

The development of hemoperfuser column design and assembly of the columns have been successfully made. The hematologic and biochemical profile data were used as the study results of the effects of factors, charcoal granule size, and the amount of tannic acid in the column for hemoperfusion and precipitation of toxic proteins. Based on these effects, we can suggest a membrane with a concentration of 0.02 g of tannic acid and the use of 8-20 mesh granulated charcoal in hemoperfuser cartridge. Subsequently, our research group wants to investigate the precipitation of ophidian-venom proteins for animal models (Wistar rat) using the column model developed in this work.

Author Contributions

Valquíria Miwa Hanai Yoshida invented the cartridge-column concept and wrote the manuscript. Roberta L. Cavalcante developed the tannic acid membranes. Jessica Campanholi developed the circuit of the preliminary hemoperfuser. Élvio Franco de Camargo Aranha together with Valquíria Miwa Hanai Yoshida shared the cartridge-column concept and the production of the column in large scale. Maximilian Estevan Oliveira was responsible for validation of hematological analysis using sheep blood.

Edson Hideaki Yoshida was responsible for hematological analysis and interpretation. Yoko Oshima-Franco designed the global project and helped in writing the manuscript.

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Conflict of Interest
None.

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