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Adjust of linear and nonlinear models in the study of neutralization of snake venom *Bothrops pauloensis* by aqueous extracts *Jathropha curcas* and *Hedychium coronarium*

Ajuste de modelos lineares e não-lineares no estudo da neutralização da peçonha da serpente *Bothrops pauloensis* pelos extratos aquosos *Jathropha curcas* e *Hedychium coronarium*

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Content

ABSTRACT:

In Brazil, the botropic snakes are responsible for most of the envenomation, causing local and systemic effects. Many of these effects cause irreversible damage, even when treated conventionally. A possible alternative that may assist in reducing these harmful situations is to use medicinal herbs according to popular culture. Many studies have approaching this theory and achieving promising results. The aim of this study is to demonstrate the ability of Jatropha curcas and Hedychium coronarium extracts to inhibit the coagulation activity induced by the venom of Bothrops pauloensis.

RESUMO:

No Brasil, as serpentes são responsáveis pela maior parte dos acidentes por envenenamento, causando efeitos locais e sistêmicos. Muitos destes efeitos causa danos irreversíveis, mesmo quando tratada convencionalmente. Uma possível alternative para ajudar a reduzir estas situações prejudiciais é usar ervas medicinais de acordo com a cultura popular. Muitos estudos têm demonstrado esta teoria e conseguido resultados promissores. O objetivo deste estudo é verificar a capacidade dos extratos de Jatropha curcas e Hedychium coronarium em inibir a atividade de coagulação induzida pela peçonha de Bothrops

Key-words: *Bothrops pauloensis, Jathropha curcas, Hedychium coronarium,* Inhibition, Statistical modeling.

pauloensis.
Palavras-Chave: *Bothrops pauloensis*, *Jathropha curcas*, *Hedychium coronarium*, Inibição, Modelagem estatística.

1. Introduction

Brazil's snake fauna is considered one of the richest in the world, being currently known 371 species belonging to 10 families: Anomalepididae, Leptotyphlopidae, Typhlopidae, Aniliidae, Tropidophiidae, Boidae, Colubridae, Dipsadidae, Elapidae and Viperidae (BÉRNILS, 2012). Of these species, 15% are considered venomous, they are distributed in the families Viperidae and Elapidae (BERNARDE, 2011).

The snakebites accidents have medical importance because of its high frequency and severity, and recently they were introduced by the World Health Organization in the list of neglected tropical diseases (OMS, 2008). In Brazil, according to data of the Ministério da Saúde occur on mean 24,000 cases of accidents by snakes accidents per year, with lethality close to 0.4%. Between 2000 and 2009 was notified in the Southeast an mean of 6000 cases of cases per year accidents per year, Minas Gerais is the most responsible state for this statistic with 50% of all reported cases (BRASIL, 2015).

The species of *Bothrops* snakes, which accidents are called botropic, are currently distributed in five genres: *Bothriopsis*, *Bothrocophias*, *Bothrops*, *Bothrops*, *Rhinocerophis* (BERNARDE, 2011) and are responsible for 90% of accidents in the country (BRASIL, 2015).

The botropic envenomations are characterized in general by immediate local reactions and systemic effects. Systemic effects involve changes in blood coagulation and cardiovascular system, and local reactions characterized by hemorrhage, necrosis and severe inflammation with prominent edema and pain (LUNA; SILVA; PEREIRA, 2011). These pathological phenomena are complex due to additive or synergistic effects of active enzymes and toxins.

Therefore, many researchers have been searching for medicinal herbs as an alternative therapy because of its antiophidic role (IZIDORO *et al*. 2003). According to MATSUDA *et al*., (2002) of the plant *Hedychium coronarium* has been isolated some types of diterpenes, which may be used to neutralize the pathological symptoms derived from ophidian accidents. BORGES *et al*., (2005) observed that the sap extract of *Musa paradisiaca* presents neutralizing properties on various effects caused by ophidian venoms. According to the literature, several other plants have had their antiophidian potential described, such as: *Marsypianthes chamaedrys* (MAGALHÃES *et al*., 2011), *Plathymenia reticulata* (FARRAPO *et al*., 2011), *Hypericum brasiliense* (ASSAFIN *et al.*, 2011) among other. These plants presented inhibition statistically satisfactory of some toxic parameter induced by the venom of snakes, however, can be observed many times, the incorrect use of statistical tools, especially for multiple comparisons, for example, use them to test level effects of a quantitative factor, where the correct would be to adjust regression.

According CARDELLINO & SIEWERDT (1992), in a review of the published works in the Revista da Sociedade Brasileira de Zootecnia between the years 1984 and 1989, 260 articles were using some kind of comparison test of means, of these 64.2% were utilized incorrectly. Currently, this is becoming a common practice in areas that have little contact with the theory of statistics.

Thus many scientific papers leave in low priority the statistical rigor in the planning of their experiments, "sinning" in the non use of a range of tools for quantitative analysis or misuse of the same. In this way, a well prepared planning and the correct use of statistical methods applied in the context in question, showing the step by step of its application may assist researchers and students in the biomedical area, especially with regard to statistical analysis.

Based on the importance of statistical rigor and in the correct way of using their tools, the aim of this paper is to present an application of linear and nonlinear regression models to assess the efficiency degree of venom neutralization of *Bothropos pauloensis* by the aqueous extracts of

Hedychium coronarium and *Jathropha curcas.*

2. Methods

2.1. Crude venom

The crude venom of *Bothrops pauloensis* was acquired along the Empresa Proteínas Bioativas (Serpentário Bioagents Ltda.), Batatais-SP. After collection, the venom was immediately vacuum desiccated at room temperature and stored at -20 ° C until the moment of use.

2.2. Plant extracts

To obtain the extracts, firstly the leaves of each plant (*Hedychium coronarium* and *Jathropha curcas*) were removed, washed with distilled water, triturated in a blender where the mixture filtered and the liquid content were centrifuged at 10.000rpm for 30 minutes. Each supernatant was frozen at - 80 ° C and then lyophilized. The final product dehydrated was hermetically stocked until the moment of use.

2.3. Coagulant activity

The coagulant activity induced by the venom of *Bothrops pauloensis* was analyzed according to PEREIRA *et al*., (2010). For inhibition assays of this biochemical parameter were used aqueous extracts of *Hedychium coronarium* and *Jathropha curcas*, previously incubated for 30 minutes with 25mg of the aforementioned crude venom the respective extracts in the ratios of 1: 0; 1: 1, 1: 5, 1:10, 1:20, 1:30, 1:40 and 1:50 (m/m; venom/extract), with a Completely Randomized Design (CRD) with 3 repetitions and 8 treatments. The time required for the formation of the fibrin in the form of coagulum was measured in seconds for the two extracts, and the inhibition of activity was observed in accordance with the mean increase of the coagulation time in relation to controls.

2.4. Statistical analysis

The achieved results went through adjustments in regression models between concentration levels of extract (x) and coagulation time (y) (seconds). To the extract *Hedychium coronarium*, it is observed that the phenomenon under study shows a linear behavior (Figure 1). While for the aqueous extract *Jathropha curcas* a nonlinear behavior (Figure 2), that is to say, as increasing the concentration of extract, plasma coagulation time becomes longer, but not linear, which justifies the adoption of nonlinear models for the study of coagulation time for different concentrations of aqueous extract of *Jathropha curcas.*

The following models were fitted: $y = \beta_0 + \beta_1 .x + \varepsilon$ (model A) for the *Hedychium coronarium* extract; $y = a.\left[1 - \exp\left(-b.x\right)\right] + \varepsilon$ (model B) and $y = c - d.\exp\left(-e.x\right) + \varepsilon$ (model C) to extract *Jathropha curcas.*

Where β_0 and β_1 are parameters of the model A; *a* and *b* are parameters of the model B, *c*, *d* and *e* are parameters of model C; ε is a vector of errors with normal distribution with zero mean and variance σ^2.

To estimate the confidence intervals of the estimated parameters of the models, will be used the covariance matrix (DRAPER; SMITH, 1998):

$$\hat{V}(\hat{\theta}) = \left(X'X\right)^{-1}.\hat{\sigma}^2 = \left(X'X\right)^{-1}.MSError = \begin{bmatrix} \hat{V}(\hat{\theta}_1) & \hat{C}ov(\hat{\theta}_1,\hat{\theta}_2) & \hat{C}ov(\hat{\theta}_1,\hat{\theta}_j) \\ \hat{C}ov(\hat{\theta}_1,\hat{\theta}_2) & \hat{V}(\hat{\theta}_2) & \hat{C}ov(\hat{\theta}_2,\hat{\theta}_j) \\ \\ \hat{C}ov(\hat{\theta}_1,\hat{\theta}_j) & \hat{C}ov(\hat{\theta}_2,\hat{\theta}_j) & \hat{V}(\hat{\theta}_j) \end{bmatrix}$$

with, $j = 1,2,\quad ,p$ and p is equal to the number of parameters.

Therefore, the standard error of the estimate of the parameter θ_j is given by:

$$s\left(\hat{\theta}_j\right) = \sqrt{V(\hat{\theta}_j)}.$$

Thus, the confidence interval θ_j is defined as:

$$IC\ (\theta_j): \hat{\theta}_j\ \pm\ t_{(\text{D.F.Error},\ \alpha/2)} \cdot \sqrt{\hat{V}(\hat{\theta}_j)}.$$

Selecting the most appropriate model to explain the nonlinear relation between the levels of extract concentration and coagulation time will be based on the accuracy of the adjustments and must meet the following criteria: adjusted coefficient of determination (*Adj.* R^2), residual standard deviation (RSD) and criteria of Akaike information (AIC), and the expressions of the evaluators are:

$$Adj.\ R^2 = 1 - \frac{MSError}{MSTotal},\ DPR = \sqrt{MSE}\ \text{ e }\ AIC = \ln\left(\hat{\sigma}^2\right) + \frac{2\left(p+1\right)}{n}$$

wherein: *MSError* is the mean square error, *MSTotal* is the mean square total, *n* the number of observations, *p* the number of parameters in the model and $\hat{\sigma}^2$ the estimated variance of residuals.

3. Results

The mean time and standard errors of the plasmatic coagulation induced by the snake venom *Bothrops pauloensis* and after inhibition of this activity by the aqueous extracts of *Hedychium coronarium* and *Jatropha curcas* are shown in Table I. The mean and standard errors results were obtained from 3 tests. In comparison to the positive control (only venom) there was a dose-dependent increase in the time of the plasma coagulation. The positive control was working with plasma and 25mg of crude venom induced the plasma coagulation, whose time was 8.67 seconds, while the negative control containing plasma incubated with 1250mg of each extract kept the plasma incoagulable. For inhibition tests (crude venom + vegetable extract) there was a dose-dependent increase in the time of the plasma coagulation. For the *Hedychium coronarium* extract as shown in Table I, the variation in coagulation time was negligible in all tested proportions. This behavior can be related to the low concentration of the antiophidian principle of this plant. As for *Jatropha curcas* extract the coagulation time was higher (Table I), so that from 1:20 ratio (m/m) there was complete inhibition of the coagulation. The sensitivity of the device records the coagulation for 120 seconds, after this period the plasma is considered incoagulable. In cases where the time for clot formation exceeds this time limit, as observed in this study, the crude venom is considered to be inhibited in its entirety. Royston and Pereira et al., observed similar results using extracts of *Casearia mariquitensis* and *Hedychium coronarium* respectively.

Table I. Inhibition coagulant activity of the bovine plasma citrated plasma

by the *Hedychium coronarium* and*Jatropha curcas* extracts

Treatments (Venom/extract)	*Hedychium coronarium*	*Jatropha curcas*
	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$
Peçonha (25mg)	8.67±1.67	8.67±1.67
(1:1 m/m)	13.6±1.51	17.33±0.52
(1:5 m/m)	11.8±0.11	71.57±4.13
(1:10 m/m)	17.77±0.62	95.73±1.75
(1:20 m/m)	15.63±0.59	120±0
(1:30 m/m)	18.27±1.24	
(1:40 m/m)	16.13±1.87	
(1:50 m/m)	22.87±0.54	
Extract (1250mg)	120±0	

$\bar{X}$: mean time of coagulation; $S_{\bar{X}}$: standard error of the mean time of coagulation.

In the Table II it is presented the variance analysis for the variable "coagulation time" for the aqueous extract *Hedychium coronarium*.

Table II. ANOVA for the variable answer "coagulation time" (seconds) in different concentrations of the aqueous extract *Hedychium coronarium*.

Causes of Variation	DF	SS	MS	F	p-value
Concentration	7	394.22	56.32	13.501	<0.000**
Error	16	66.74	4.17		
Total	23	460.96			

** Significant to 1 % of probability; DF: Degree of Freedom; SS: Sum of Square; MS: Mean Square; F: value of the F statistic.

4. Discussion

At the significance level of 1%, the treatments are significant, i.e, there is a significant difference between extract concentration levels, and coagulation time. From Table III we have the assumptions of the model on CRD (Table II) which are satisfied by the test of Shapiro-Wilk the p-value was greater than 5%, indicating normality. Analogously, there is the p-value from statistic of Levene's test that is greater than 5%, which show that the variance is constant for

the prevalence studied. Thus, we have reliable results on the inference made by analysis of variance for the effect of the concentrations of *Hedychium coronarium* aqueous extract.

Table III. Results of the Shapiro-Wilk test for residuals and Levene for the model of delineation entirely randomized n = 24. (CRD) (Table I) with n = 24.

Test	Statistic	p-value
Shapiro-Wilk	0.97	0.67
Levene	2.567	0.06

In Table IV the decomposition is shown in linear components, quadratic referring to the sum of squares of treatments (concentrations of *Hedychium coronarium* aqueous extract).

Table IV. ANOVA to the decomposition of the sum of square of concentrations of *Hedychium coronarium* aqueous extract.

Causes of Variance	DF	SS	MS	F	p-value
Linear Regression	1	263.73	263.73	63.23	<0.000**
Quadratic Regression	1	1.62	1.62	0.389	0.542NS
Deviation	5	128.87	25.77	6.18	0.002**
Error	16	66.74	4.17		
Total	23	460.96			

** Significant to 1 % of probability; NS = non significant.

Each mean square is tested by error and the null hypothesis is that the mean population for comparison is zero. If only the linear effect is significant, it is concluded that the increase in response to successive levels of the factor (concentration of *Hedychium coronarium* aqueous extract) is constant. Therefore, the analysis shows that the linear effect (or 1st degree) is meant to the extract concentrations. The component of 2nd degree non-significant indicates that it would not bring any improvement to the adjustment. The linear model can explain about 65% of the total variation, which according to Anderson et al., (ANDERSON; SWEENEY; WILLIAMS, 2007), for data in the biological sciences, values equal R^2 or greater than 0,60 are often considered useful. It can also be seen, Table V, that the confidence intervals for the parameters do not contain zero, which shows their significance.

Table V. Statistics referring to the composition of the regression model in estimated 1st degree.

Parameters	Estimates	99% de confidence		(1)R^2	(2)*Adj.* R^2
		LI	LS		
β_0	11.923	9.354	14.493	66.90	65.39

$\beta_1(x^*)$	1.881	0.903	2.859		

(*)*x*: level or dose of extract concentration; (1) R^2: coefficient of determination and (2)Adj. R^2: Adjusted coefficient of determination

To check the normality assumptions, homogeneity of variance and independence of residuals for the linear model were made the Shapiro-Wilk test, Levene and Durbin-Watson respectively. The test results are shown in Table VI.

Table VI. Results of the Shapiro-Wilk test, the Durbin-Watson and Levene for the residue of the regression model of 1st degree with n = 24.

Test	**Statistic**	**p-value**	**Durbin-Watson test**	
			Statistic – D(1)	**p-value**
Shapiro-Wilk	0.964	0.533	1.7263	0.1806

From Table VI, we have that the p-value of the Shapiro-Wilk statistic is greater than 5%, which indicates no rejection of *H*0, in other words, it is acceptable the normality assumption of the residuals to the model of 1st degree. The same was observed for Levene's test, wherein the p-value was greater than 5%, which indicates no rejection of *H*0, which means, it is permissible the assumption of the constant variance for all levels of concentration of the extracts. Similarly, through the Durbin-Watson test can be observed the residuals independence (ROYSTON, 1983).

Therefore, the fitted model, Figure 1, to predict the coagulation time depending on the level (or dose) of concentration of the *Hedychium coronarium* aqueous extract is: **Time = 1.8812 Dose + 11.923.**

This result differs from that found by (PEREIRA et al., 2010), where it fitted a model of the 2nd degree for the same aqueous extract (*Hedychium coronarium),* ***Time = 74,0128 + 24,2132 Dose – 3,3766 Dose2***, and yet, it has that the coagulation time observed in the work of Pereira et al. (2010) were much higher than the estimated model in Figure 1. A justification for this sharp decrease in coagulation time was the way how this extract was processed, as in the case of this work was done a scraping on plant leaves, and whit this was eliminated the high power of inhibition of *Hedychium coronarium* what had been observed by (PEREIRA et al., 2010).

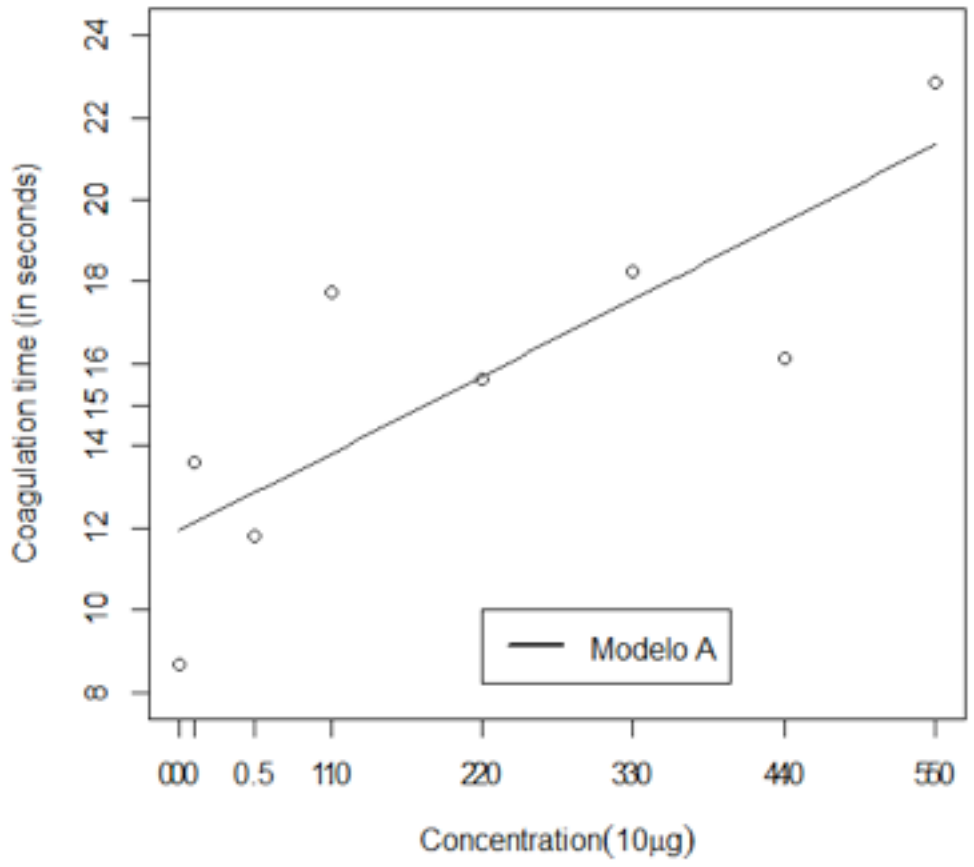

Figure 1. Observed values and adjust of the linear model between concentration levels of the *Hedychium coronarium* plant extract versus coagulation time (in seconds)

Table VII presents the analysis of variance for the variable "coagulation time" for the *Jathropha curcas* aqueous extract.

Table VII. Analysis of variance for the response variable "coagulation time" (seconds) at different concentrations of *Jathropha curcas* aqueous extract.

Causes of Variance	DF	SS	MS	F	p-value
Concentration	7	46786.8	6683.8	767.01	<0.000**
Error	16	139.4	8.7		
Total	23	46926.2			

** Significant to 1 % of probability

To the level of significance of 1%, the treatments are significant, i.e., there is a significant difference between the levels of the *Jatropha curcas* extract concentration and coagulation time. Therefore, the results allow concluding that the level of extract concentration influences the coagulation time.

Table VIII. Estimated values of the evaluators of adjust quality of the exponential asymptotic models with 2 and 3 parameters for *Jathropha curcas* extract.

Exponential asymptotic models	(1)*p*	(2)*Adj.* R^2	(3)*SRD*	(4)*AIC*
Model B	2	0.9900	4.526	144.4902
Model C	3	**0.9924**	**3.945**	**138.7787**

(1) p: number of parameters; (2) *Adj.* R^2: adjusted coefficient of determination;
(3) *SRD* stantard residual deviation; (4) *AIC*: Criteria of information of Akaike.

According to the results shown in Table VIII it can be inferred through intermediate of the estimates of evaluators of adjust quality, that the exponential asymptotic model with 3 parameters provided better adjust compared to the model with two parameters, because it has a higher adjusted coefficient of determination and lower standard residual deviation. As for the Akaike Information (AIC), used in selecting the most appropriate model, it allows to affirm, according to the results shown in Table VIII, that the model with 3 parameters is the closest to be considered correct in describing the phenomenon, once that it showed the lowest value for this criterion. In relation to the coefficient of determination it is observed that the model with three parameters can capture 99.24% of the total variation of the coagulation time.

To verify the assumptions of normality and independence of the residuals were made the Shapiro-Wilk test and Durbin-Watson respectively. The test results are shown in Table IX.

Table IX. Statistics associated with the residual analysis of exponential asymptotic models with 2 and 3 parameters for *Jathropha curcas* extract.

Models	Durbin-Watson		Shapiro-Wilk		Levene	
	D(5)	p-value	W(6)	p-value	F(7)	p-value
Model B	1.4828	0.0610	0.8574	0.0030	1.2803	0.3202
Model C	1.6321	0.1236	0.9306	0.1006	1.2804	0.3202

From Table IX it has that the p-value of the Shapiro-Wilk statistic is greater than 1%, only to the model C, which indicates the non rejection *H*0, that is, the normality assumption is permissible of the residuals to this model. As for the pattern B the normality assumption of the residuals is not fulfilled, which is also used as a criterion for the rejection of such model. The assumptions of independence and uniformity of residuals have been met by Levene test and Durbin-Watson, respectively, for the two models (ROYSTON, 1983).

Table X.
Estimates of parameters (in brackets the-standard errors) of the exponential asymptotic models with 2 and 3 parameters to the concentration data of the *Jathropha curcas* extract and coagulation time.

Models	Parameters	Estimates	99% de confidence	
			LI	LS
Model B	**a**	120.8347 (1.4142)	116.8484	124.8211
	b	1.7029 (0.0864)	1.4592	1.9465
Model C	**c**	121.2033 (1.2654)	117.6206	124.7860
	d	115.9683 (2.1243)	109.9536	121.9831
	e	1.5986 (0.08256)	1.3648	1.8324

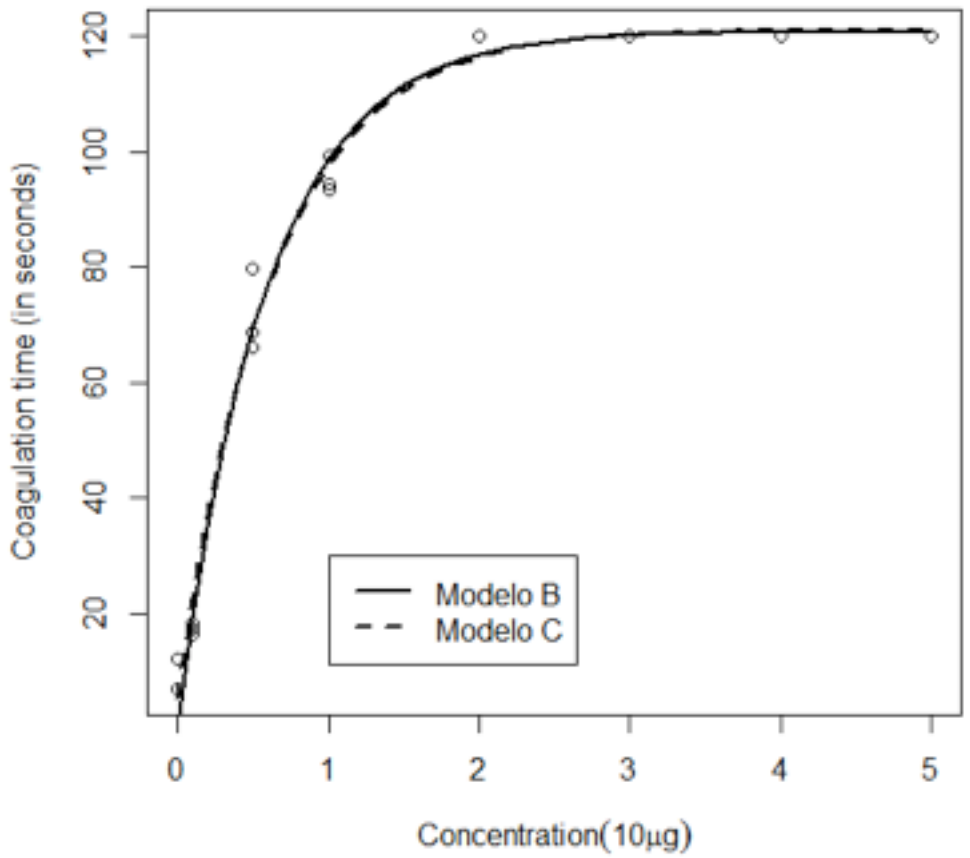

Figure 2. Values observed and adjust of the exponential asymptotic models with 2 and 3 parameters to the data of concentration levels of *Jathropha curcas* plant extract versus coagulation time (in seconds).

Note that all analyses were implemented in the freeware R Development Core Team (2015).

5. Conclusions

The linear model provided a good adjust to the data of concentration levels of *Hedychium coronarium* plant extract versus coagulation time (in seconds) that according to Anderson et al. (2007), models that can capture 60% of the total variation can be useful. Therefore, the fitted model was **Time = 1.8812 Dose + 11.923.** As noted it is suggested that the use of this extract should be done without scraping the leaves, because if done the plant loses its main feature which is to inhibit the snake venom.

The asymptotic exponential model with three parameters (model C) showed a best fitted to the data of concentration levels of *Jathropha curcas* plant extract versus coagulation time (in seconds) according to the evaluators of the quality of adjust. The model in question has captured 99.24% of the total variation of the coagulation time. Therefore, from the model **Time = 121.2033 - 115.9683 exp (-1.5986 Dose)** can be obtained good results for predicted values within the range studied.

References

BÉRNILS, R.S; COSTA, H. E. (Org.). **Répteis brasileiros: lista de espécies**. São Paulo: Sociedade Brasileira de Herpetologia, 2012. Disponível em: <http://www.sbherpetologia.org.br/lista_repteis/ListaRepteis12Dezembro2012-PORTUGUES.pdf>. Acesso em: 19 nov. 2015.

BERNARDE, P. S. Mudanças na classificação de serpentes peçonhentas brasileiras e suas implicações na literatura médica. **Gaz Med da Bahia**, Salvador, v.81, n.1, p.55-63, jan./jun. 2011.

WORLD HEALTH ORGANIZATION (OMS). **The global burden of snakebite: A literature analysis and modelling based on regional estimates of envenoming and deaths**. 2008. Disponível em: <

http://www.who.int/neglected¬_diseases/diseases/snakebites/en/index.html>. Acesso em: 20 nov. 2015.

BRASIL. Ministério da Saúde. **Animais peçonhentos: serpentes**. Disponível em: <http://portalsaude.saude.gov.br/index.php/o-ministerio/principal/secretarias/svs/animais-peconhentos-serpentes>. Acesso em: 19 junho de 2015.

LUNA, K. P. O; SILVA, M. B; PEREIRA, V. R. A. Clinical and immunological aspects of envenomations by *Bothrops* snakes. **J Venom Anim Toxins Incl Trop Dis**, Botucatu, v. 17, n. 2, p. 130-141, 2011.

IZIDORO, L. F. M.; RODRIGUES, V. M.; RODRIGUES, R. S.; FERRO, E. V.; HAMAGUCHI, A.; GIGLIO, J. R.; HOMSI-BRANDEBURGO, M. I. Neutralization of some hematological and hemostatic alterations induced by neuwiedase, a metalloproteinase isolated from *Bothrops neuwiedi pauloensis* snake venom, by the aqueous extract from *Casearia mariquitensis* (Flacourtiaceae) **Biochimie**, Paris, v. 85, n. 7, p. 669-675, july 2003.

MATSUDA, H.; MORIKAWA, T.; SAKAMORO, Y.; TOGUCHIDA, I.; YOSHIKAWA, M. Labdane-type diterpenes with inhibitory effects on increase in vascular permeability and nitric oxide production from *Hedychium coronarium*. **Bioorg & Med Chem**, Oxford, v. 10, n. 8, p. 2527-2534, Aug. 2002.

BORGES, M. H.; ALVES, D. L. F.; RASLAN, D. S.; PILÓ-VELOSO, D.; RODRIGUES, V. M.; HOMSI-BRANDEBURGO, M. I.; LIMA, M. E. Neutralizing properties of *Musa paradisiaca* L. (Musaceae) juice on phospholipase A2, myotoxic, hemorrhagic and lethal activities of crotalidae venoms. **J Ethnopharmacol**, Iausanne, v. 28, n. 1-2, p. 21-29, Apr. 2005.

MAGALHÃES, A.; SANTOS, G. B.; VERDAM, M. C. S.; FRAPORTI, L.; MALHEIRO, A.; SILVA, E. S.; SANTOS, M. C. Inhibition of the inflammatory and coagulant action of *Bothrops atrox* venom by the plant species *Marsypianthes chamaedrys*. **J Ethnopharmacol**, Lousane, v. 134, n. 1, p. 82-88, Mar. 2011.

FARRAPO, N. M.; SILVA, G. A. A.; COSTA, K. N.; SILVA, M. G.; COGO, J. C.; DAL BELO, C. A.; SANTOS, M. G.; GROPPO, F. C.; OSHIMA-FRANCO, Y. Inhibition of *Bothrops jararacussu* venom activities by *Plathymenia reticulata* Benth extracts. **J Venom Res**, Bethesda MD, v. 2, p. 52-58, Dec. 2011.

ASSAFIN, M.; CORIOLANO, E. C.; BENEDITO, S. E.; FERNADES, C. P.; LOBO, J. F. R.; SANCHEZ, S. F.; ROCHA, L. M.; FULY, A. L. *Hypericum brasiliense* plant extract neutralizes some biological effects of *Bothrops jararaca* snake venom. **J Venom Res**, Bethesda, v.2, p. 11-16, May 2011.

CARDELLINO, R. A.; SIEWERDT, F. Utilização correta e incorreta dos testes de comparação de médias. **Rev Soc Bras Zootec**, Viçosa, MG, v. 21, n. 6, p. 985-995, 1992.

PEREIRA, J.M.; NOMELINI, Q. S. S.; IZIDORO, L. F.M.; OLIVEIRA, S. X.; RODRIGUES, V. M.; HOMSI-BRANDEBURGO, M.I.; HAMAGUCHI, A.; MENDES, M. M.; ALVES, L. M. Predição da neutralização do efeito coagulante da peçonha de *Bothrops pauloensis* pelo extrato aquoso de *Hedychium coronarium* (zingerberaceae) através de modelos de regressão. **Bioscience Journal**, Uberlândia, v. 26, n. 6, p. 973-980, Nov./Dec. 2010.

DRAPER, N. R.; SMITH, H. A. **Applied regression analysis**. 3, ed. New York: John Wiley, 1998.

ANDERSON, D. R.; SWEENEY, D. J.; WILLIAMS, T. A. **Estatística aplicada à administração e economia**. 2. ed. São Paulo: Thomson Learning, 2007.

ROYSTON, J. B. Some techniques for assessing multivariate based on the Shapiro- Wilk W. **J R Stat Soc Ser Appl Stat**, London, v.32, n.2, p. 121-133, 1983.

R DEVELOPMENT CORE TEAM. **R: a language and environment for statistical computing**. Vienna: R Foundation for Statistical Computing, 2015. Disponível em: <http://www.R-project.org>. Acesso em: 15 out. 2015.

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