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Evaluating Effectiveness of Select Natural Remedies for their Anti-coagulating Properties on Bovine Plasma: An *In Vitro* Study

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Abstract

The primary objective of this *in vitro* study was to evaluate if select natural remedies bromelain, nattokinase, and serrapeptase, have an effect on the clotting time of bovine plasma. The formation of blood clots within the body can lead to fatal diseases such as stroke, heart attack, pulmonary embolism, and deep vein thrombosis [1]. Although various drugs with anticoagulant properties are commercially available for treatment of such conditions, these drugs come with various side-effects which could pose significant health risk, when used for a prolonged period [2]. My hypothesis was select natural remedies (bromelain, nattokinase, and serrapeptase) have anticlotting abilities; in addition, when two of the natural remedies were used simultaneously the clotting time of the bovine plasma would have a more significant increase. In this study, activated Partial Thromboplastin Time (aPTT) [3], was used as a measure of clotting time, with higher aPTT values indicating greater anticlotting properties. First, three different concentrations of select natural remedies were evaluated for aPTT in comparison with bovine plasma samples with no treatment. Next, a combination of two selected natural remedies at a time, were evaluated for their anticlotting abilities against samples with no treatment. Experimental results indicated that the three selected natural remedies were able to extend the average clotting time between 4% to 28% depending upon concentration dosage. Higher concentrations of these remedies showed better anticlotting abilities that lower concentrations. When two of the remedies were combined, they yielded better results than the remedies tested alone. Statistical evaluation of the data showed that the aPTT results obtained from the samples treated with select natural remedies were indeed different from the untreated samples. The experimental results fully supported the hypothesis and can act as a basis for further studies. Although many research papers and scientific resources commented on anti-coagulating effects of bromelain, nattokinase, and serrapeptase; this study established a systematic baseline of anti-coagulating effects of these natural remedies.

Keywords: Natural remedies; *In Vitro*; Blood clot; Bovine plasma

Introduction

Formation of blood clots within the body is one of the leading causes of human death all over the world. Blood clots within arteries leads to stroke or heart attack, blood clots in lungs leads to pulmonary embolism, and formation of blood clots within deep veins of the body leads to deep vein thrombosis-all of which, if not treated in time, can cause human mortality [1]. In order to prevent blood clot within the body various drugs with anti-coagulating abilities are regularly prescribed by medical professionals, which affect different parts of the clotting cascade [4]. Therefore, understanding the biochemical pathways of how these drugs work are critical. When the blood vessels break or rupture in an area within the body, platelets, a kind of blood cells, and rush to the spot and try to stop the bleeding. After that collagen which borders the outside of the cell chemically reacts with the platelets to form a thicker bond. Next fibrin which automatically sticks together goes and makes the bond such that no more blood could get out of the rupture, known as polymerization. These steps are taken to help fix the ruptured part of the blood vessel. Fibrin also helps produce fibrinogen. Fibrinogen has a special chemical that prevents the fibrins to stick to one another inside of the blood vessels. In the absence of fibrinogen, fibrin will form blood clots. The fibrin has a special chemical attached to it called tissue factor. The tissue factor breaks off from the fibrin when the fibrin is fixing the blood vessel damage. After the damage is repaired the blood flow returns to normal [5]. If there is trauma to the body's vascular system, then the intrinsic pathway of the clotting cascade begins. It can start if there is any exposed endothelium, chemicals, or collagen leaking into the body. On the other hand, blood may also form clots internally if there is any external trauma resulting in damage to the vascular system, which may result in internal haemorrhage [6]. Over time, different anticoagulants such as, aspirin, warfarin, heparin, etc. have been discovered which are known to prolong the amount of time the blood takes to clot. Stronger dosage of anticoagulants is used in surgeries so that the blood does not clot during surgeries [7]. Prescription dosage of anticoagulants such as heparin is used to prevent harmful clots to form in the blood vessels [8]. Heparin is one of the anticoagulants used during open heart surgery, kidney dialysis, bypass surgery, and blood transfusions. In the body heparin stops coagulating factors thrombin and fibrin in delaying to form blood clots [9]. Another known anticoagulant is aspirin. Aspirin is an over the counter medication which is used as both pain killer and to reduce one's chances of a heart attack. In the body aspirin prevents the platelets from clumping and therefore clotting in the arteries [10]. Although many of these drugs are available over the counter, however, most of these drugs come with various side effects, which by themselves could be fatal, especially when used for a long term [2]. One of the risks is that if you take anti-coagulating drugs frequently then it may increase the risk of bleeding or having internal bleeding. While taking an anti-coagulating drug one's diet needs to be steady. If one does not have a

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steady diet while taking anticoagulants, then some of the food may interfere with the medications [11]. Another risk is that one may get

exhausted while playing an intense sport which in turn can exhaust one's organs. These risks can ultimately lead to death if they go untreated. On the other hand, remedies derived from natural food sources (i.e., natural remedies) do not have all these risks. Since they are naturally available in various foods they have better potential to be absorbed by the body, whereas the anti-coagulating drugs can take longer time. However, due to their presence within the blood stream for a longer period, drugs are typically more potent [12] than natural remedies and hence has better capability in treating various conditions. To avoid the harmful side effects of the commercially available drugs with anti-coagulating properties, three natural remedies: bromelain, nattokinase, and serrapeptase–were chosen for this study. Bromelain is found in pineapple, nattokinase is found in soybean, and serrapeptase is found in silkworm extracts [13]. So, when one is eating one of these foods, they get a dose of these natural chemicals and any beneficial effects that come along with them. Many people eat pineapple on a regular basis without knowing the presence of the enzyme, bromelain, which has many positive effects [14,15] including its effect as an anti-inflammatory. Since many of the anti-inflammatory drugs (e.g., warfarin, heparin, to name a few) are also known to have anti-coagulating properties, I thought to investigate bromelain for its anti-coagulating potential in this study. The second chosen natural remedy, nattokinase, is an enzyme that is extracted from natto, which is a folk medicine from Japan, is said to treat heart and blood diseases [16]. It is claimed to treat high blood pressure, high cholesterol, heart diseases, strokes, and DVT. Nattokinase is also claimed to act as blood thinner, which can prevent and/or prolong clot formation. The third chosen natural remedy, serrapeptase is a chemical taken from the silkworm, which is classified as a dietary supplement [17]. Serrapeptase is known to delay the blood clotting time. It is claimed to treat many diseases including atherosclerosis, a buildup of fat and cholesterol on the walls of the arteries, and various inflammatory diseases.

Although the three remedies selected for this study were claimed to have anti-inflammatory and blood thinning abilities [18], during my reference search we could not find any *in vitro* studies on any of these selected chemicals that systematically evaluated their anti-coagulating properties. The main question that we wanted to answer through this study was to evaluate if select natural remedies-bromelain, nattokinase, and serrapeptase, possess anti-coagulating properties and how they compare with untreated samples in an *in vitro* study. The hypothesis for the study was that select natural remedies (bromelain, nattokinase, and serrapeptase) would have anticlotting abilities [19]; in addition, when two of the natural remedies were used simultaneously the clotting time of the bovine plasma would have a more significant increase.

Materials

Following materials were used in this study:

- 125 mL citrated bovine plasma
- One bromelain 600 gelatin digesting unit (GDU) per gram tablet from Source Natural
- One nattokinase 2,000 fibrin degradation (or fibrinolytic) units (FUs) per capsule from Amazing Nutrition
- One serrapeptase 40,000 serrapeptase units (SPU) capsule from Doctor's Best
- Anhydrous laboratory grade calcium chloride
- Main Stays Quick-Response thermometer
- Professional digital jewellry scale
- 50 ACTT tubes by Haematologic
- Two plastic containers
- 50 Vacu tubes
- Ten 3-mL plastic synergies
- Fine point sharpie
- Mortar and pestle
- Hot tap water
- Plastic cups
- Wax paper
- 10 disposable plastic spoons

Methods

In this study in lieu of human blood [20,21], bovine plasma was used as the media to avoid logistical complexities related to approval process for such projects, precautions to be taken for potential blood borne diseases, and access to approved facilities where this project could be conducted. Additionally, bovine plasma has many similarities with human blood [22] and has been used in many studies in lieu of human blood [23]. Since plasma is part of the blood that contains blood cells and necessary enzymes minus the liquid part (i.e., serum), it is a preferred media for *in vitro* experimental studies [24,25]. The bovine plasma was obtained from Hemostat Laboratories, in Dixon, California which is a commercial source and the project was carried out entirely at Canyon Animal Hospital, a veterinary hospital, which agreed to provide access to their facility and supervise this work. The primary ingredient for this study were three selected natural remedies-bromelain, nattokinase, and serrapeptase; and naproxen sodium, the positive control. These materials were obtained in tablet or capsule form from local health shops and Amazon.com. Additionally, 500 grams of anhydrous calcium chloride was bought from a local scientific supply store. Haematologic Technologies, Inc. of Essex Junction, Vermont, donated 50 Activated Clotting Time Test (ACTT) [26] tubes. Canyon Animal Hospital in Laguna Beach, California provided 50 Vacuette White Top tubes (Vacu tubes) [27,28]. First the tablets and contents of capsules of naproxen sodium, bromelain, nattokinase, and serrapeptase were separately crushed and grinded. Then 2 milligrams (mg) of naproxen sodium was measured and placed in a Vacu tube. Additionally, 2 mg, 3 mg, and 4 mg of bromelain, nattokinase, and serrapeptase were separately measured and placed in separate Vacu tubes. Once the Vacu tubes were ready the frozen citrated bovine plasma was placed in a water bath for 30 minutes at a temperature between 92°-96° Fahrenheit (°F). After 30 minutes, using a 3 mL syringe 2 mL of citrated bovine plasma was extracted and placed in 14 separate Vacu tubes. The control contained only 2 mL of citrated bovine plasma. The positive control contained 2 mL citrated bovine plasma and 2 mg naproxen sodium. After that three separate Vacu tubes each containing 2 mL of citrated bovine plasma, and 2 mg, 3 mg, and 4 mg of bromelain were prepared separately. The same was done for both nattokinase and serrapeptase. For the combinations three combinations: bromelain and nattokinase, bromelain and serrapeptase, and nattokinase and serrapeptase were used. In separate Vacu tubes 2 mL of citrated bovine plasma along with 2 mg of each tested chemical (example: 2 mg of bromelain and 2 mg of nattokinase) were placed. The Vacu tubes were then placed in a water bath for 15 minutes at a

temperature between 92°-96°F, additionally, 11 ACTT tubes were also placed in another water bath for 10 minutes at a temperature between 92°-96°F. After 15 minutes one granule (approximately 2 mg in weight) of anhydrous calcium chloride was added in each of the Vacu tubes and was then shaken with the lid closed for 30 to 60 seconds until the calcium chloride granule completely dissolved. Then the content of each Vacu tube was carefully transferred into separate ACTT tubes, was shaken for five times, and then were placed into the water bath at a temperature between 92° to 96°F. The timer was set when each ACTT tube was placed in the water bath. The contents of the ACTT tubes were tilted slightly after 60 seconds, 120 seconds, and every 10 seconds thereafter until the plasma transformed from the liquid to a 'gello like' semi-solid state. The time was noted when the content of the tube was first noted to have transformed into a semisolid state. The same procedure was then repeated for two additional times.

Results

Experimental results of bovine plasma samples treated with one chemical were presented in Table 1 and summarized in Table 2. Results of plasma samples treated with a combination of two chemicals were presented in Table 3 and summarized in Table 4. Additionally, the average aPTT results of treated and untreated samples were shown in Figure 1 titled "Comparative Analysis of Anti-coagulating Activity of Select Natural Remedies". Experimental results indicated that the three selected natural remedies were able to extend the average clotting time was between 4% to 28% depending upon dosage. Higher dosage of these remedies showed better anti-coagulating abilities than lower dosage. When two of the remedies were combined, they yielded better results than the remedies tested alone. Student 't' test was conducted on the treated and untreated (i.e., control) samples, results of which are summarized under Appendix A. Statistical evaluation of the data showed that the aPTT results obtained from the samples treated with select natural remedies were indeed different from the untreated samples.

$$\%\ \text{Change} = \left(\frac{average\ clotting\ time\ of\ sample - average\ clotting\ time\ of\ control}{average\ clotting\ time\ of\ control} \right) \times 100$$

Sample ID	Composition	Sample explanation	Clotting time as aPTT (seconds)	Average clotting time as aPTT (seconds)	% Change (compared to control)
C1	2 mL plasma	Control	165		
C2	2 mL plasma	Control	159	170.3	
C3	2 mL plasma	Control	187		
Brom2Serra2-1	2 mL plasma+2 mg bromelain+2 mg serrapeptase	Bromelain+serrapeptase	184		
Brom2Serra2-2	2 mL plasma+2 mg bromelain+2 mg serrapeptase	Bromelain+serrapeptase	201	193	0.13
Brom2Serra2-3	2 mL plasma+2 mg bromelain+2 mg serrapeptase	Bromelain+serrapeptase	194		
Brom2Natto2-1	2 mL plasma+2 mg bromelain+2 mg nattokinase	Bromelain+nattokinase	203		
Brom2Natto2-2	2 mL plasma+2 mg bromelain+2 mg nattokinase	Bromelain+nattokinase	192	199	0.17
Brom2Natto2-3	2 mL plasma+2 mg bromelain+2 mg nattokinase	Bromelain+nattokinase	202		
Serra2Natto2-1	2 mL plasma+2 mg serrapeptase+2 mg nattokinase	Serrapeptase+nattokinase	203		
Serra2Natto2-2	2 mL plasma+2 mg serrapeptase+2 mg nattokinase	Serrapeptase+nattokinase	204	210.5	0.24
Serra2Natto2-3	2 mL plasma+2 mg serrapeptase+2 mg nattokinase	Serrapeptase+nattokinase	217		
Natto3-1	2 mL plasma+2 mg nattokinase	3 mg nattokinase	203		
Natto3-2	2 mL plasma+2 mg nattokinase	3 mg nattokinase	204	198	16%
Natto3-3	2 mL plasma+2 mg nattokinase	3 mg nattokinase	188		
Nato4-1	2 mL plasma+4 mg nattokinase	4 mg nattokinase	243		
Natto4-2	2 mL plasma+4 mg nattokinase	4 mg nattokinase	184	210	23%
Natto4-3	2 mL plasma+4 mg nattokinase	4 mg nattokinase	203		
Serra2-1	2mL plasma+2 mg serrapeptase	2 mg serrapeptase	176		
Serra2-2	2mL plasma+2 mg serrapeptase	2 mg serrapeptase	182	179	5%
Serra2-3	2mL plasma+2 mg serrapeptase	2 mg serrapeptase	179		
Serra3-1	2mL plasma+3 mg serrapeptase	3 mg serrapeptase	182		
Serra3-2	2mL plasma+3 mg serrapeptase	3 mg serrapeptase	183	184	8%
Serra3-3	2mL plasma+3 mg serrapeptase	3 mg serrapeptase	187		
Serra4-1	2 mL plasma+4 mg serrapepase	4 mg serrapeptase	204		
Serra4-2	2 mL plasma+4 mg serrapepase	4 mg serrapeptase	203	204	20%
Serra4-3	2 mL plasma+4 mg serrapepase	4 mg serrapeptase	204		
Note: Plasma: Citrated Bovine Plasma; aPTT: Activated Partial Thromboplastin Time					

Table 1: Clotting time measurements of bovine plasma samples treated with single natural remedy

Test			aPTT (seconds)							
Treatment			Control	Naproxen	Bromelain			Nattokinase		
			0 mg	2 mg	2 mg	3 mg	4 mg	2 mg	3 mg	4 mg
Matrix	Volume	Statistic								
Plasma 2 mL		N	3	3	3	3	3	3	3	3
		Mean	170.3	191	189.3	180.3	219.7	176.3	198.3	210
		SD	14.74	11.53	8.39	12.5	6.66	10.02	8.96	30.12
		CV%	8.65	6.04	4.43	6.93	3.03	5.68	4.52	14.34
		Geometric mean	169.9	190.8	189.2	180	219.6	176.1	198.2	208.6
Note: N: Number of samples; SD: Standard Deviation; CV: Coefficient of Variation										

Table 2: Summary statistics of bovine plasma samples treated with single natural remedy.

Sample ID	Composition	Sample explanation	Clotting time as aPTT (seconds)	Average clotting time as aPTT (seconds)	% Change (compared to control)
C1	2 mL plasma	Control	165		
C2	2 mL plasma	Control	159	170.3	
C3	2 mL plasma	Control	187		
Brom2Serra2-1	2 mL plasma+2 mg bromelain+2 mg serrapeptase	Bromelain+serrapeptase	184		
Brom2Serra2-2	2 mL plasma+2 mg bromelain+2 mg serrapeptase	Bromelain+serrapeptase	201	193	0.13
Brom2Serra2-3	2 mL plasma+2 mg bromelain+2 mg serrapeptase	Bromelain+serrapeptase	194		
Brom2Natto2-1	2 mL plasma+2 mg bromelain+2 mg nattokinase	Bromelain+nattokinase	203		
Brom2Natto2-2	2 mL plasma+2 mg bromelain+2 mg nattokinase	Bromelain+nattokinase	192	199	0.17
Brom2Natto2-3	2 mL plasma+2 mg bromelain+2 mg nattokinase	Bromelain+nattokinase	202		
Serra2Natto2-1	2 mL plasma+2 mg serrapeptase+2 mg nattokinase	Serrapeptase+nattokinase	203		
Serra2Natto2-2	2 mL plasma+2 mg serrapeptase+2 mg nattokinase	Serrapeptase+nattokinase	204	210.5	0.24
Serra2Natto2-3	2 mL plasma+2 mg serrapeptase+2 mg nattokinase	Serrapeptase+nattokinase	217		
Note: Plasma: Citrated Bovine Plasma; aPTT: Activated Partial Thromboplastin Time					

Table 3: Clotting time measurements of bovine plasma samples treated with two natural remedies.

Test			aPTT (seconds)				
Treatment			Control	Naproxen	Bromelain+Nattokinase	Bromelain+Nattokinase	Nattokinase+Serrapeptase
			0 mg	2 mg	2 mg each chemical	2 mg each chemical	2 mg each chemical
Matrix	Volume	Statistic					
Plasma 2 mL		N	3	3	3	3	3
		Mean	170.3	191	193	199	210.5
		SD	14.74	11.53	8.54	6.08	7.81
		CV%	8.65	6.04	4.43	3.06	3.71
		Geometric mean	169.9	190.8	192.9	198.9	207.9
Note: N: Number of samples; SD: Standard Deviation; CV: Coefficient of Variation							

Table 4: Summary statistics of bovine plasma samples treated with two natural remedies.

Discussion

The primary reason this study was conducted were to see if select natural remedies possess anti-coagulating properties and if they could increase the *in vitro* clotting time. Based on literature review, three natural remedies-bromelain, nattokinase, and serrapeptase, were claimed to have possessed anti-coagulating abilities. There are many ways to measure the blood clotting time. One of the most common ways is the Prothrombin Time (PT) test. Prothrombin is a protein in the plasma which turns into thrombin when the coagulation or clotting cascade starts [29,30]. Another commonly used method is called Activated Partial Thromboplastin Time (aPTT) test [3]. The aPTT can be measured using the ACTT tubes. Once the plasma or blood is poured into the ACTT tube a chemical called kaolin, which is present in the tube, activates the clotting cascade. Three different amounts (2 mg, 3 mg, and 4 mg) of selected natural remedies bromelain, nattokinase, and serrapeptase-were measured separately and were then placed them in separate Vacu tubes with 2 mL of citrated bovine plasma. Then the tubes were placed in a water bath (temperature between 92° F to 96°F) for 15 minutes so that chemicals could react with the plasma proteins. Then 2 mg of anhydrous calcium chloride was added in each tube and shook them until it was completely dissolved to neutralize the effect of trisodium citrate, which otherwise prevented the clotting cascade to initiate. Contents of the tube were transferred to separate ACTT tubes,

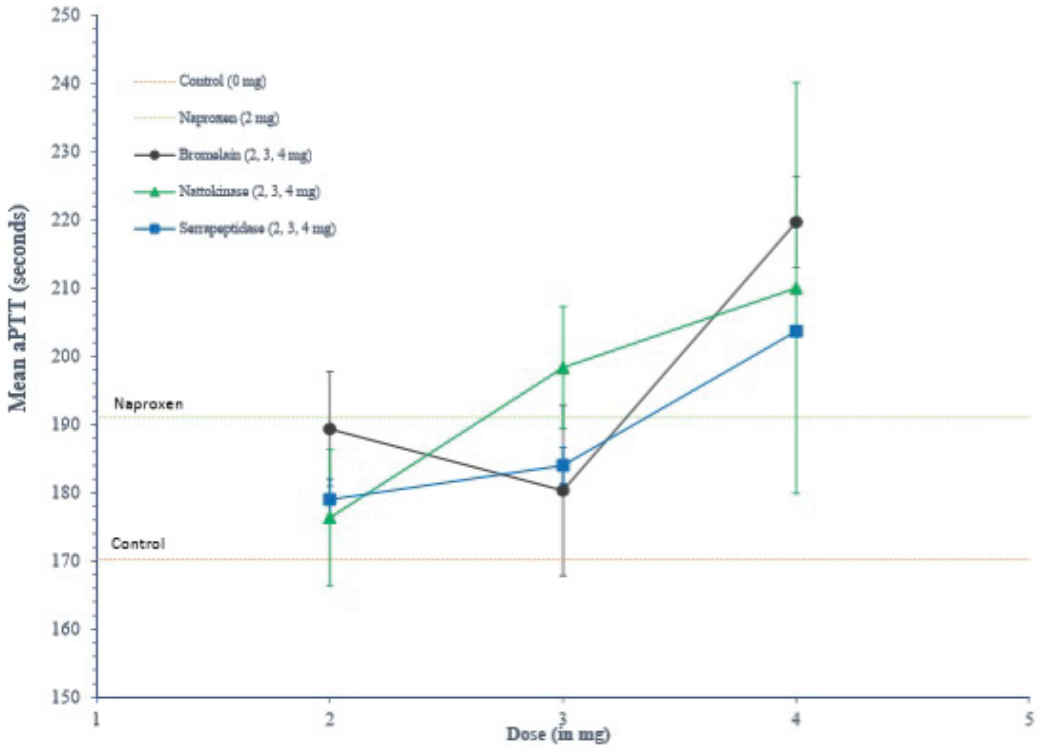

Figure 1: Comparative analysis of anti-coagulating activity of select natural remedies.

placed them in water bath, and started the timer. The first time the timer was stopped when the plasma was turning into a semi-solid state. This process was done for all the samples in this experiment. The experiments were repeated three times to gain better confidence. Naproxen sodium was used as a positive control because it is an anti-inflammatory drug, and also is known to delay the clotting time. Since, warfarin or heparin could not be obtained; naproxen sodium was used for positive control. The primary objective of this *in vitro* study was to evaluate if select natural remedies–bromelain, nattokinase, and serrapeptase, have an effect on the clotting time of bovine plasma. My hypothesis was select natural remedies (bromelain, nattokinase, and serrapeptase) have anticlotting abilities; in addition, when two of the natural remedies were used simultaneously the clotting time of the bovine plasma would have a more significant increase. The experimental results fully supported the hypothesis. As it had been hypothesized, the clotting time of bovine plasma increased when it was treated with one of the selected natural remedies (i.e., bromelain, nattokinase, or serrapeptase). When the clotting time data was compared with the control (i.e., untreated bovine plasma sample), average clotting time of treated samples were always higher than the average clotting time of control samples. Since the clotting time increased when samples were treated with one of the three natural remedies in comparison with untreated samples the hypothesis was proven to be correct. In this study, aPTT was used as a measure of clotting time, with higher aPTT values indicating greater anti-coagulating properties. The unit of measurement used in this study was in seconds. As presented in Table 1 and summarized in Table 2, among the samples that were treated with bromelain, the highest average aPTT of 218 seconds was noted for samples treated with 4 mg of bromalin. For samples treated with nattokinase, the highest average aPTT of 210 seconds was noted for samples treated with 4 mg of nattokinase. For samples treated with serrapeptase, the highest average aPTT of 204 seconds was noted for samples treated with 4 mg of serrapeptase. Samples treated with 2 mg of nattokinase exhibited the least average aPTT of 176 seconds among treated samples. As presented in Table 3 and summarized in Table 4, among the sample that were treated with a combination of two remedies, samples treated with a combination of 2 mg of serrapeptase and 2 mg of nattokinase showed the highest average aPTT of 211 seconds, which was a 24% increase in comparison with untreated samples. The average aPTT of samples treated with a combination of serrapeptase and nattokinase was higher than the samples treated with 2 mg of serrapeptase and 2 mg of nattokinase separately. For samples treated with 2 mg of bromelain and 2 mg of serrapeptase, the average aPTT of 193 seconds was noted which was 13% higher than the untreated samples and was also higher than the samples treated with 2 mg of bromelain and 2 mg of serrapeptase separately. For samples treated with 2 mg of bromelain and 2 mg of nattokinase, the average aPTT of 199 seconds was noted which was 17% higher than the untreated samples and was also higher than the samples treated with 2 mg of bromelain and 2 mg of nattokinase separately. Student 't' tests were performed on three occasions: (a) aPTT results obtained from bovine plasma samples treated with one or two of the select natural remedies and untreated (i.e., control) samples; (b) aPTT results obtained from bovine plasma samples treated with one of the select natural remedies and untreated samples; and (c) aPTT results obtained from bovine plasma samples treated with a combination of two of the select natural remedies and untreated samples. In all three occasions Student 't' result indicated such that the null hypothesis was rejected, which proved that the aPTT results obtained from the samples treated with select natural remedies were indeed different from the untreated samples. The primary manipulated variables in my project were the

selected natural remedies: bromelain, nattokinase, and serrapeptase. Another manipulated variable was different concentrations of the natural remedies that were evaluated. The responding variable was the aPTT. For each bovine plasma sample, aPTT was measured to see how it varied from one sample to another as a measure of effectiveness of select remedies as anti-coagulating agents. The control in this study was the untreated sample of bovine plasma. In addition, bovine plasma sample treated with naproxen sodium, a commercially available drug with known anti-coagulating effects was used as positive control for the experiment. In this project there was one uncertainty: sensitivity of the measuring scale. In this study a jewelry measuring scale was used with a precision of 0.001 gram or 1 mg. Since it was incredibly sensitive any slight disturbance (such as breathing, vibration etc.) could disrupt the accuracy of the scale. Although care had been exercised to measure the chemicals accurately, however, unintentional inaccuracy could have been introduced by the measuring scale.

Conclusion

The primary objective of this *in vitro* study was to evaluate if select natural remedies –bromelain, nattokinase, and serrapeptase, have an effect on the clotting time of bovine plasma. The results demonstrated that the natural remedies were effective anti-coagulating agents when data was compared with control samples and samples treated with naproxen sodium. Since selected remedies were derived from natural foods, they were not expected to possess harmful side effects. So, if they are taken over a longer period, they should not cause any significant health risks. Although many research papers and scientific resources previously commented on anti-coagulating effects of bromelain, nattokinase, and serrapeptase; this study established a systematic baseline of anti-coagulating effects of these natural remedies. This study could be extended on a larger scale using human blood or plasma using a more precise clotting time measuring instrument and a better weighing scale for measuring small quantities. For future directions, this project will be able to see a comparison between the selected natural remedies with the commercially available anti-coagulating drugs, such as heparin, warfarin, using human blood or plasma.

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