

Original Research Article

In vitro Measurement of Anticoagulant Effect of Acacia Nilotica Aqueous Extract on Normal Human Plasma

ABSTRACT:

Aims: To determine the anticoagulant effect of Acacia Nilotica aqueous extract on normal human plasma.

Study design: This is an experimental study.

Place and duration of the study: Department of Haematology, Faculty of Medical Laboratory sciences, Alzaiem Alazhari, Khartoum – Sudan, during the period from July – August 2021.

Methodology: A total of 20 human blood samples were collected for this study from apparently healthy subjects following ethical considerations. Samples were collected in tri-sodium citrate and platelets poor plasma (PPP) were immediately prepared by centrifugation. Aqueous solution of Acacia nilotica was prepared with distilled water (D.W), three concentrations of the solution (50%, 75% and 100%) were prepared. Prothrombin Time (PT) and Activated partial thromboplastin time (APTT) were tested by manual method before and after adding different concentrations of Acacia Nilotica solution. Statistical tests were performed by using SPSS version 16.

Results: The results of study showed that Acacia Nilotica significantly prolonged the PT and APTT results of human plasma (P values for 50%, 75% and 100% solutions were 0.000, 0.000 and 0.000 while for APTT 0.036, 0.000 and 0.000 for PT respectively). Also the study showed no significant correlation between control and A. nilotica concentrations among coagulation profile, except a significant positive correlation between control and 75% Acacia nilotica on APPT.

Conclusion: Acacia Nilotica solutions had anticoagulant effect in human plasma, and could be nominated as a potential preventive agent for thromboembolic diseases.

Keywords: Acacia nilotica, aqueous extract, Activated Partial Thromboplastin Time, Prothrombin Time.

1. INTRODUCTION

The World Health Organization (WHO) has listed more than 21,000 plants, which are used for many medicinal purposes around the world [1]. They observed that about 74% of 119 plant-derived

pharmaceutical medicines are used in modern medicine. It also estimates that 4 billion people (80 percent of the world population) presently use herbal medicine for health care [2]. Over hundreds of years, herbal medicines derived from medicinal plants minerals and organic matter is still the mainstay of about 75–80% of the world's population for health care marketed and gaining popularity in developed and developing countries [3]. Herbs have medicinal property due to presence of different active principles like alkaloids, volatile essential oils, glycosides, resins, oleoresins, steroids, tannins, terpenes and phenols [4]. In the last few years there is an exponential growth in the field of herbal medicine because of their natural origin, easy availability, efficacy, safety and less side effects with efficient to cure age-related disorders like memory loss, osteoporosis, immune disorders, etc. for which no modern medicine is available [5,6].

Medicinal plant researchers pursued with several goals like the development of low cost therapeutic compounds and the discovery of prototypic drugs [7]. *Acacia nilotica* is also known as Gum Arabic tree, Babul, Egyptian thorn, or Prickly Acacia is multipurpose nitrogen fixing tree legume. It occurs from sea level to over 2000 m and withstand at extreme temperature (>50 °C) and air dryness but sensitive to frost when it is young [8]. It is widely spread in subtropical and tropical Africa from Egypt to Mauritania southwards to South Africa, and in Asia eastwards to Pakistan and India [9, 10].

Coagulation studies are of great importance considering the role of blood in life [11]. These studies are important to investigate the arrest bleeding from small blood vessels by drugs[12] and also to study the anti-thrombotic effect of drug which it is applied to prevent atherosclerotic diseases, including myocardial and cerebral infarction. This therapy includes the suppression of primary hemostasis using anti-platelet drugs such as aspirin and ticlopidine hydrochloride, and suppression of the coagulation system using anticoagulants such as warfarin and heparin[13]. The present study examined the effect of *A. nilotica* on the coagulation system of normal human plasma by measuring Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT).

2. Material & Methods

This was an experimental study carried out in Al-Zaiem Al-Azhari University, Khartoum State-central Sudan, during the period from July – August 2021. The study included normal blood samples from apparently healthy individuals. Hemolyzed, jaundiced and contaminated samples were excluded from the study. The study received ethical approval from Research committee at faculty of medical laboratory sciences in the university of Al-Zaiem Al-Azhari. Informed consent was taken from all participants before enrolment in the study.

2.1. Preparation of the aqueous extract

Extraction was carried out according to method described by Umaru et,al [14]. The plant of *A.nilotica* had been collected from Al-khartoum market. The crushing of the pods was done by using pestle and mortar, after which it was ground into powder. Two hundred grams (200gm) of the powdered pod was weighed and introduced into a conical flask and 1 litre of distilled water was added thereafter . The mixture was then shaken and allowed to stand for 30 minutes, after which it was boiled (at 100°C) for one hour, cooled and shaken vigorously, before filtration using whatman No.1 filter paper. The filtrate was concentrated by placing in water bath at 95°C for three days until complete water evaporation

and stored at 4°C until used. (50%, 75%, 100%) of the aqueous pod extract (crud) was prepared by using D.W.

2.2. Coagulation testing

Determination of PT and APTT were performed by manual method. Briefly 10 ul of each aqueous extract solution were added to 90 ul of the plasma; then the clotting time was determined for the 100 ul mixture after adding 100 ul Thromboplastin reagent (for PT), 100 ul Koalin and 100 ul Calcium Chloride reagents (for APTT).

2.3. Data analysis

Descriptive statistics were used to summarize data. Numerical variables were expressed as mean \pm standard deviation (SD). Comparisons of mean values were conducted by one-way analysis of variance (ANOVA) and Pearson's correlation coefficient was applied to test the correlation between clotting time and the concentrations of *A. nilotica* solution. For all tests, P-values < 0.05 were considered statistically significant. All statistical analysis were conducted by SPSS version 16.

3. RESULTS

In this study, *Acacia nilotica* were tested for blood anti-coagulation effects in normal human plasma at three different concentrations (50%, 70% and 100%). Results showed significant prolongation of Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) of the human plasma (Tables 1 and 2). Results of diluent control revealed that PT and APTT were not affected by the addition of DW.

Also the results of study showed no significant correlation between control and *A. nilotica* concentrations among the coagulation profile (table 3), except a significant positive correlation between control and 75% *Acacia nilotica* on APPT (figure 1).

Table 1: Comparisons of mean PT in difference concentration of *A. nilotica*

Variables (I)	Variables (II)	Mean of PT (I)	Mean of PT (II)	P value
Control	50 %		18.1 \pm 2.3	0.036*
(Plasma without extract addition)	75 %	14.1 \pm 0.9	29.1 \pm 5.0	0.000*
	100 %		61.4 \pm 10.6	0.000*
	50 %	75 %	18.1 \pm 2.3	29.1 \pm 5.0
	100 %		61.4 \pm 10.6	0.000*
75 %	100 %	29.1 \pm 5.0	61.4 \pm 10.6	0.000*

*P value < 0.05 is significant

Table 2: Comparisons of mean APTT in difference concentration of *A. nilotica*

Variables (I)	Variables (II)	Mean of APTT (I)	Mean of APTT (II)	P value
Control	50 %		66.1 \pm 11.2	0.000*
(Plasma without extract addition)	75 %	31.8 \pm 2.6	83.5 \pm 17.9	0.000*
	100 %		115.0 \pm 35.8	0.000*
	50 %	75 %	66.1 \pm 11.2	83.5 \pm 17.9
	100 %		115.0 \pm 35.8	0.000*
75 %	100 %	83.5 \pm 17.9	115.0 \pm 35.8	0.000*

*P value < 0.05 is significant

Table 3: Correlations between control and difference concentration of *A. nilotica* among PT and APTT

			50 %	75 %	100 %
PT	Control	Pearson Correlation	-.049	-.242	-.171
		Sig. (2-tailed)	.838	.304	.472
		N	20	20	20
APTT	Control	Pearson Correlation	.402	.576	.396
		Sig. (2-tailed)	.079	.008	.084
		N	20	20	20

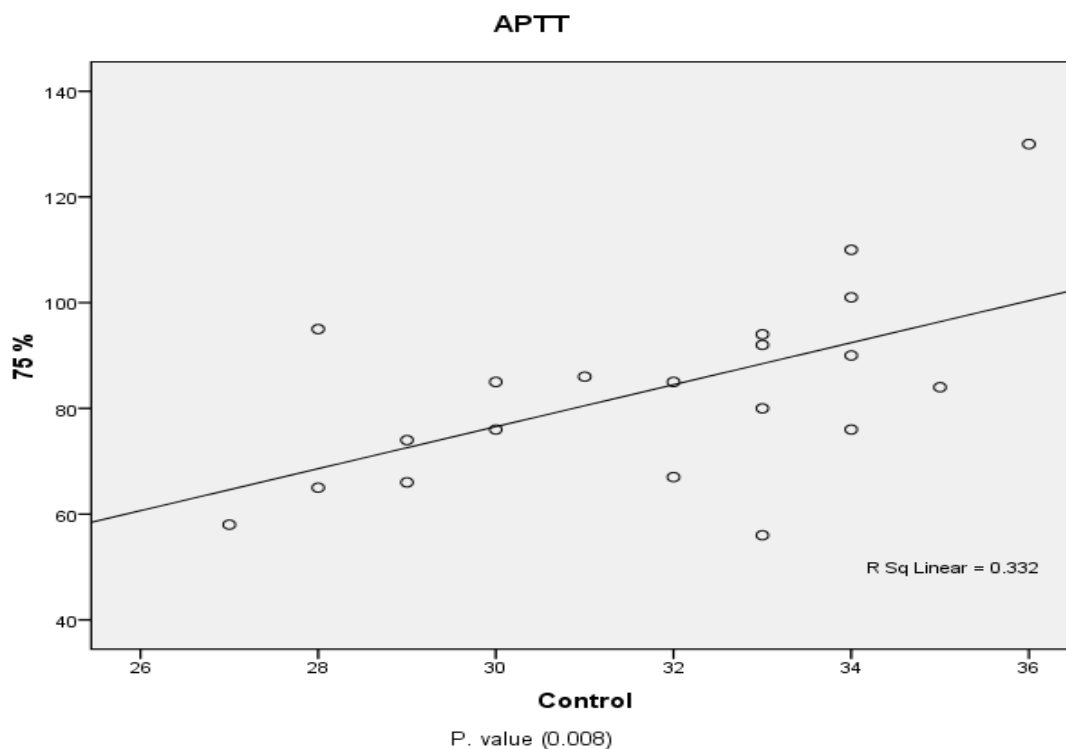


Figure 1: Correlations between control and 75 % of *A. nilotica* among APTT

4. DISCUSSION

Thrombosis is one of the main causes of cerebral infraction, acute myocardial infraction and others cardiovascular diseases [13]. Anticoagulants are types of drugs widely used in prevention and treatment of thrombotic disorders [15]. In this content, many epidemiological studies have indicated that, diet rich in polyphenol compounds may prevent the occurrence of cardiovascular disease [16]. On the others hand, the search for anticoagulant agents from natural herbal medicine is an active research area, because inhibition of blood coagulation through anticoagulant drugs is an important mean of preventing thrombotic diseases [17].

In This study, different concentrations of *Acacia nilotica* are investigated for their in-vitro anticoagulant activity in 20 samples of human plasma through the general tests of

coagulation: activated partial thromboplastin time (aPTT) and prothrombin time (PT). the aPTT explore the endogenous pathway of coagulation, while PT explore the exogenous pathway of coagulation [18]. Results of this study showed that, *Acacia nilotica* caused significant prolongation of PT and aPTT of plasma in concentration dependent manner (P values < 0.05) at all tested concentration compared to control. Data of present study clearly showed that *A. nilotica* had an important inhibition effect on both intrinsic and extrinsic coagulation pathways. Hence, these effects could be attributed to their fermented metabolite (acetate, propionate and butyrate). This can support the theoretical mechanism of action of *A. Nilotica* and indicted that the influence of fermentation products is on multiple target with in the coagulation cascade.

On the other hand, another finding of this study is that is moderate significant correlation between control and 75% concentration of *A. Nilotica*, while no correlation between the remaining concentrations among APTT and PT. In the literature, to the best of our knowledge no studies till now had demonstrated systematically correlation of coagulation parameters with the concentration of *Acacia Nilotica*.

The limitation of this study is that few concentrations of the extract were used. A wider view of the effect of *Acacia nilotica* could be obtained by using more concentrations of the extract for the coagulation testing. Moreover additional tests needs to be conducted in order to assess the extent of anticoagulation and any negative impact.

5. CONCLUSION

The concentrations of *A. nilotica* used in this study showed marked anticoagulant effect as indicated by the prolongation of PT and APTT.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the Faculty of Medical Laboratory Sciences at Alzaeim Alazhari university and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.”

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