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ROLE OF VITAMINK ON THE MANAGEMENT OF STROKE DUE TO ANTITHROMBOSIS

Abirame S.*

Intern Medical Officer, Ayurvedic Central Dispensary, Vavuniya.

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***Corresponding Author**
Abirame S.
Intern Medical Officer,
Ayurvedic Central
Dispensary, Vavuniya.

ABSTARCT

This study was a comparative in vivo study, conducted in Wistar albino rats at Trincomalee Campus, Eastern University of Sri Lanka. Thrombosis is the formation of a blood clot inside a blood vessel, obstructing the flow of blood through the circulatory system. When a blood vessel is injured, the body uses platelets (thrombocytes) and fibrin to form a blood clot to prevent blood loss. Thrombosis is a common pathology underlying ischemic heart disease, ischemic stroke and VTE. Major burden of disease across low-income, middle-income and high-income countries. Adult female Wister albino rats of weighing around 200-250gm were obtained. The animals housed in cages under standard laboratory condition. The Wister albino rats were divided into 2 groups 6 animals in each. Mean values of baseline bleeding time 84.50 ± 14.761sec in standard group and 88.50 ± 9.813 sec in test group after overnight fasting for 12 hours. After induced by intraperitoneal injection of the thromboplastin (#0203) - 0.48ml injection the mean values of bleeding time were subsequently decreased 69.50 ± 12.787 sec in standard group and 76.33± 9.688 sec in test group. Then the treatment was continued. Standard group administered with Phytomenadione (Vitamin K) and test group administered with test drug. After that rat's tail bleeding time was noted at 0 min, 30min and 60min after 3h and 24h. Study was tested for (using assessment (Time-Dependence Pattern)) anti-thrombotic activity. Mean value results in Standard group (0 min: 76.50 ± 10.083s, 30 min: 74.20 ± 9.757s and 60 min: 75.80 ± 8.786s) after 3h. Mean value results in Standard group (0 min: 77.00 ± 8.485s, 30 min: 81.80 ± 7.396s and 60 min: 89.33± 6.377s) after 24h. In comparison of bleeding time, both the standard and test groups indicate significance of bleeding time (P value less than 0.05). Phytomenadione (Vitamin K) possess source of antithrombotic activity compounds for the management of various thrombogenic disorders.

KEYWORDS: Antithrombotic activity, Phytomenadione (Vitamin K), Thrombosis, Thromboplastin, Bleeding time.

1. INTRODUCTION

This study is a comparative in vivo study. Haemostasis is the process that retains the blood within the vascular system during periods of injury. The coagulation mechanism may be thought of as a complex series of cascading reactions involving development of enzymes from their precursor (zymogens). Most of the substances which are necessary for coagulation are present in an inert form and must be converted to an activated state. Most adult cardiovascular disorders involving hypertension, cerebral hemorrhage, coronary thrombosis, arteriosclerosis and CHD are caused by problems in the blood circulatory system as blood clotting disorders which constitute a serious medical problem (Sliver et al, 1974). Anti-thrombotic include anticoagulants, anti-platelets and thrombolytic that decrease the rate of blood clotting in the body by dissolving already formed ones or prevent clot formation (Webster, 2001). Oral anticoagulants have been used in the management of atherothrombotic stroke treatment (Donnan et al, 2008) which accounts for 61% of all strokes and have been relied upon for prevention and treatment for several decades. In order the research has been plan to assess the antithrombotic activity of test drug.

2. OBJECTIVE

2.1. General objective

To evaluate the antithrombotic activity on test drug.

2.2. Specific objective

To assess the antithrombotic activity of Vitamin K.

To assess the antithrombotic activity (Time-Dependence Pattern.

3.LITERATURE REVIEW

3.1 Review of phytomenadione (vitamin k)

Phytomenadione, also known as vitamin K1 or phylloquinone, is a vitamin found in food and used as a dietary supplement. Phytomenadione has both anticoagulation & coagulation properties.

3.2. Pharmacology and monitoring of VKAs

The VKAs produce their anticoagulant effect by interfering with the cyclic interconversion of vitamin K and its 2,3 epoxide (vitamin K epoxide), thereby modulating the -carboxylation of glutamate residues (Gla) on the N terminal regions of vitamin K-dependent proteins. Vitamin K epoxide reductase, is sensitive to coumarins, whereas vitamin K reductase is less sensitive. The anticoagulant effect of the coumarins can be overcome by low doses of vitamin K1 (phytonadione). Patients treated with large doses of vitamin K1 can become resistant to warfarin for up to 1 week or more because the vitamin K1 accumulating in the liver is available to the coumarin-insensitive reductase. The coumarins also interfere with the carboxylation of Gla proteins that are synthesized in bone.

3.3. The antithrombotic effect of VKAs

The antithrombotic effect of VKAs has conventionally been attributed to their anticoagulant effect, which in turn is mediated by the reduction of four vitamin K-dependent coagulation factors. More recent evidence, however, suggests that the anticoagulant and antithrombotic effects can be dissociated, and that the reduction of prothrombin and possibly factor X are more important than the reduction of factors VII and IX for the antithrombotic effect. This evidence is indirect and has been derived from the observations. First, the experiments of Wessler and Gite l70 over 40 years ago using a stasis model of thrombosis in rabbits showed that the antithrombotic effect of warfarin requires 6 days of treatment, whereas an anticoagulant effect develops in 2 days.

3.4. Clinical applications of VKA therapy

The clinical effectiveness of VKAs in the treatment of a variety of disease conditions has been established by well-designed clinical trials. VKAs are effective for the primary and secondary prevention of venous thromboembolism, for the prevention of systemic embolism in patients with prosthetic heart valves or atrial fibrillation, for the prevention of acute myocardial infarction in patients with peripheral arterial disease and in men who otherwise are at high risk, and for the prevention of stroke, recurrent infarction, or death in patients with acute myocardial infarction. Although effectiveness has not been proven by a randomized trial, VKAs are also indicated for the prevention of systemic embolism in high-risk patients with mitral stenosis (Jack Ansell et al, 2004).

4. MATERIALS AND METHOD

4.1 Study design: Comparative in vivo study

4.2 Study area: Trincomalee Campus, Eastern University.

4.3 Study population: Wistar albino rats

4.4 Inclusion criteria: Well-being rats weight 200-250g

4.5 Exclusion criteria: Diseased rats weight below 200g

4.6. Chemical Substance

Phytomenadione (Vitamin K) and Thromboplastin (#0203) were obtained from the General Hospital, Vavuniya.

4.7. Anti- thrombotic studies

Wister albino adult female rats weighing 200-250gm were obtained from animal house of Medical Research Institute, Colombo. The animals were grouped and housed in cages with under standard laboratory conditions and the rats were given 12h light and 12 h dark cycles. The animals were allowed to acclimatize to the environment for 7 days. They were fed with standard pellet diet and water. The rats were divided into two groups of six rats each. Then rat's tail baseline bleeding time was noted overnight fasting (12H) female Wister albino rats. After that thrombus was induced in Wister albino rats by single intraperitoneal injection thromboplastin (#0203) (0.48ml/225g) anesthetized by Ether. Thromboplastin (#0203) is thrombogenic agent. After 10min again bleeding time was noted.

Figure 4: 1 Induction of thrombus.

Figure 4: 2 Thromboplastin.

The standard group of each 6 rats (R1,R2,R3,R4,R5,R6) administered with Phytomenadione (Vitamin K) 0.43mg/225g twice a day and the test group of each 6 rats (R7,R8,R9,R10,R11,R12) administered test drug twice a day. Rat's tail bleeding time was noted at 0 min, 30min and 60min after 3H and 24H of treat with test and standard doses.

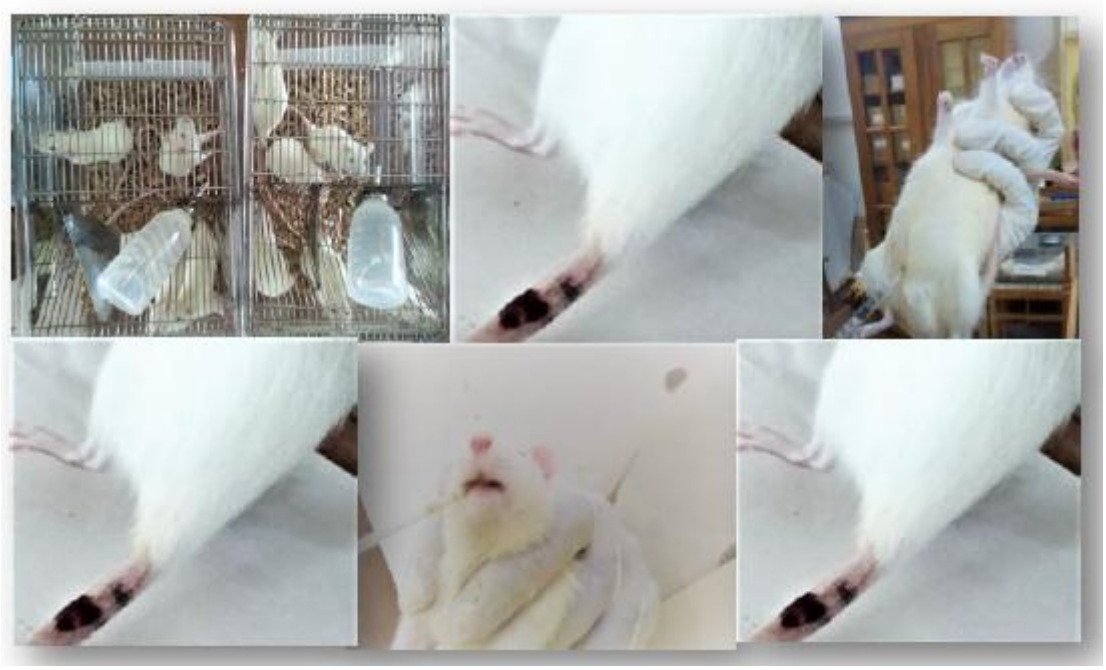

Figure 4: 3 Procedure of antithrombotic study.

4.8. Statistical analysis

All data obtained were analysed by compare means test using the Statistical Package for the Social Sciences (SPSS) version 21 at a statistical significance level of P.

5. RESULTS AND DISCUSSION

5.1 Changes of bleeding time with Vitamin K

Table 5.1 shows the Wister albino rat's tail baseline bleeding time was noted and thrombus was induced by intraperitoneal injection of thromboplastin(#0203) after overnight fasting female Wister albino rats were anesthetized by Ether.

Again Wister albino rat's tail bleeding time was noted then Wister albino rat's tail bleeding time was noted at 0 min, 30min and 60min after 3h and 24 h of treat with test and standard doses.

Table 5.1 Changes of bleeding time with Vitamin K.

Standard	R1	R2	R3	R4	R5	R6
Baseline	1 min 18sec	1 min 30sec	1min 03sec	1 min 25sec	1 min 48sec	1 min 23sec
Thrombogenic Agent (Thromboplasin)	1min 02sec	1min 15sec	51sec	1min 12sec	1min 29sec	1min 08sec
1st Dose Is Given						
After 3H	**0min**	1 min 15sec	**ND**	1min 12sec	1min 31sec	1min 08sec
30 min	1min 05sec	1 min 16sec	**ND**	1min 12sec	1min 30sec	1min 08sec
60 min	1min 08sec	1 min 18sec	**ND**	1min 13sec	1min 30ec	1min 10sec
2nd Dose Is Given						
After 24H	**0min**	1min 08sec	**ND**	1 min 15sec	1min 30sec	1 min 12sec
30 min	1 min 16sec	1 min 25sec	**ND**	1 min 20sec	1min 33sec	1 min 15sec
60 min	1 min 26sec	1 min 32sec	1 min 31sec	1 min 28sec	1 min 39sec	1 min 20sec

*__R1, R2, R3, R4, R5, R6__ - Wister albino rats

*__ND__ - Not Defined

5.2. Mean value of bleeding time with Vitamin K

Table 5.2 indicates mean value of bleeding time in standard group. Mean value of bleeding time before treatment was 69.50. Mean value of bleeding time after 3h treatment in 0min was 76.50. Mean value of bleeding time after 3h treatment in 30min was 74.20.Mean value of bleeding time after 3h treatment in 60min was 75.80. Mean value of bleeding time after 24h treatment in 0min was 77.00. Mean value of bleeding time after 24h treatment in 30min was 81.80. Mean value of bleeding time after 24h treatment in 60min was 89.33 respectively.

Standard deviation of bleeding time before treatment was ±12.787. Standard deviation of bleeding time after 3h treatment in 0min was ±10.083. Standard deviation of bleeding time after 3h treatment in 30min was ±9.757. Standard deviation of bleeding time after 3h treatment in 60min was ±8.786. Standard deviation of bleeding time after 24h treatment in 0min was ±8.485. Standard deviation of bleeding time after 24h treatment in 30min was ±7.396. Standard deviation of bleeding time after 24h treatment in 60min was ±6.377 respectively.

Standard error of mean of bleeding time before treatment was 5.220. Standard error of mean of bleeding time after 3h treatment in 0min was 5.041. Standard error of mean of bleeding time after 3h treatment in 30min was 4.363. Standard error of mean of bleeding time after 3h treatment in 60min was 3.929. Standard error of mean of bleeding time after 24h treatment in 0min was 3.795. Standard error of mean of bleeding time after 24h treatment in 30min was 3.308. Standard error of mean of bleeding time after 24h treatment in 60min was 2.603 respectively.

Table 5.2 Mean value of bleeding time with Vitamin K.

Standard Group	Mean	Std. Deviation	Std. Error of Mean
Before Treatment	69.50	12.787	5.220
After 3h Treatment	0min	76.50	10.083 / 5.041
30min	74.20	9.757	4.363
60min	75.80	8.786	3.929
After 24h Treatment	0min	77.00	8.485 / 3.795
30min	81.80	7.396	3.308
60min	89.33	6.377	2.603

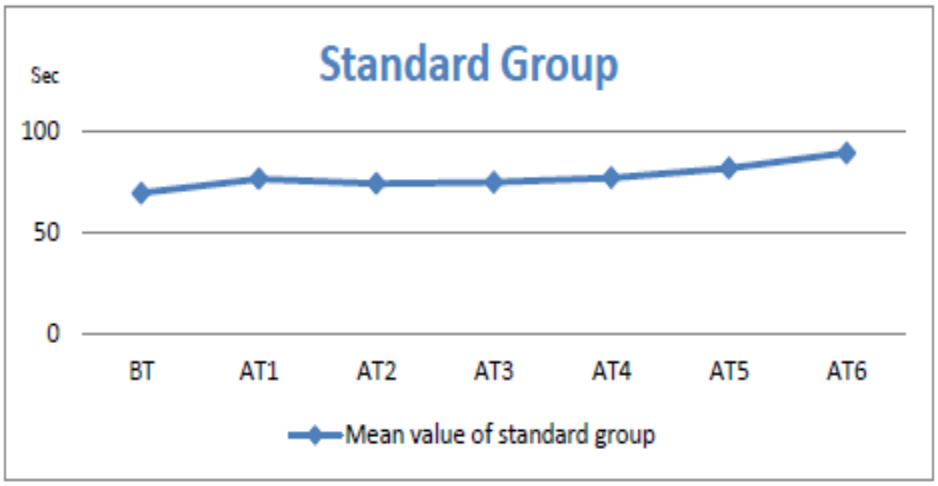

Figure 5: 1 Mean value of bleeding time with Vitamin K.

BT:-Before Treatment

AT1:-After 3h treatment in 0min

AT2:-After 3h treatment in 30min

AT3:-After 3h treatment in 60min

AT4:-After 24h treatment in 0min

AT5:-After 24h treatment in 30min

AT6:-After 24h treatment in 60min

5.3. Result of significance of bleeding time in standard group

Paired "t" value of bleeding time after 3h treatment in 0min was (-1.000). Paired "t" value of bleeding time after 3h treatment in 30min was (-1.826). Paired "t" value of bleeding time after 3h treatment in 60min was (-2.804). Paired "t" value of bleeding time after 24h treatment in 0min was (-4.417). Paired "t" value of bleeding time after 24h treatment in 30min was (-5.177). Paired "t" value of bleeding time after 24h treatment in 60min was (-4.417) respectively.

P value of bleeding time after 3h treatment in 0min was 0.391. P value of bleeding time after 3h treatment in 30min was 0.142. P value of bleeding time after 3h treatment in 60min was 0.049. P value of bleeding time after 24h treatment in 0min was 0.012. P value of bleeding time after 24h treatment in 30min was 0.007. P value of bleeding time after 24h treatment in 60min was 0.007 respectively.

Table 5.3: Result of significance of bleeding time with Vitamin K.

Standard Group	Paired 't'	Sig. (2-tailed)	
Before Treatment	-	-	
After 3H Treatment	**0min**	-1.000	0.391
30min	-1.826	0.142	
60min	-2.804	0.049	
After 24H Treatment	**0min**	-4.417	0.012
30min	-5.177	0.007	
60min	-4.417	0.007	

Thrombosis is a common pathology underlying ischemic heart disease, ischemic stroke, and VTE. The GBD, Injuries, and Risk Factors GBD Study 2010 documented that ischemic heart disease and stroke collectively caused one in four deaths worldwide. In order the research was plan to assess the antithrombotic activity.

Blood coagulation is a host defense mechanism that assists in maintaining the integrity of the closed, high-pressure mammalian circulatory system after blood vessel injury. In the abnormal conditions, it is also involved in the thrombosis, atherosclerosis, inflammation and metastasis by the activation of enzymes in the coagulation cascade and the platelets. The key enzyme, thrombin, and platelets, play an important role in the initiation of the coagulation process and involve in the formation of the fibrin clot and platelet plug in the vascular system. Thus, safe and effective inhibitors of thrombin and platelets should be useful tools in the treatment of venous thrombosis, arterial fibrillation, restenosis arterial thrombosis, and in the prevention of myocardial infarction. Because of this, the modulation of thrombin by direct inhibitors and antiplatelet agents are widely sought goals in the development of anticoagulant agents. Bleeding time is affected by many factors including vasoconstrictive effect of blood vessels, the formation of hemostatic plug and platelet activity. In general, fatty acids, palm oil and aspirin have been reported to increase BT in animals and humans, whereas saturated fatty acids and cholesterol decrease BT (Juan H, 1989).

6. CONCLUSION

As per this experimental study, the data reveals Vitamin K possessing the antithrombotic activity.

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