

## ORIGINAL ARTICLE

# Effects of Methanol Extract of *Illicium verum* Hook.f on Coagulation Parameters in Rabbits

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### ABSTRACT

**Background:** Chinese Star anise is known as *Illicium verum* hook.f; a distinguished spice utilized in dishes, especially in the Asian region. However, it was not evaluated for its anticoagulant potential. The study was designed specifically to evaluate the effect of methanol extract of *Illicium verum* hook.f on various coagulation parameters in animal models.

**Methods:** In the study, effect of methanol extract of *I. verum* hook.f was evaluated on various coagulation parameters such as Prothrombin time (PT), activated partial thromboplastin time (aPTT) and thrombin time (TT) in rabbits at three different doses, i.e. 150, 250 and 350 mg/kg body weight after 60 days of continuous dosing. Comparison was also done with the standard anticoagulant drug i.e., Warfarin. Data was compared with control group's values by One-Way ANOVA trailed by post hoc Tukey's test.

**Results:** The current analysis revealed that administration of methanol extract of *I. verum* hook.f at 250 mg of dose for 30 days resulted in elevation of PT, aPTT and TT, whereas, highly significant increase was observed in PT and aPTT at the 350 mg/kg. However, administration of the extract for 60 days at 250 mg/ kg resulted in significant increase in PT while, significant increase in aPTT. Administration at 350 mg/kg of dose for same period resulted in significant rise in PT and aPTT in contrast to TT.

**Conclusion:** Methanol's extract of *I. verum* hook.f (star anise) showed significant anticoagulant effects, which is important in hypercoagulable conditions and cardiovascular diseases. However, isolation and identification of active ingredients is required to further validate the results.

**Keywords:** Star Anise; Thrombin Time; Prothrombin Time; Activated Partial Thromboplastin time.

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### INTRODUCTION

Coagulation is a diverse set of physical and biochemical consequences resulting in generation of thrombus<sup>1</sup>. Development of thrombus takes place when natural anticoagulant mechanisms genetically impairs or damaged by any injury<sup>2</sup>. Millions of people experience heart attacks every year in the Western countries, mostly associated with coronary thrombosis<sup>3</sup>. Thrombosis contributes pathogenic part in the evolution of atherosclerosis and heart disease in patients suffering from vascular diseases<sup>4</sup>. Management is needed at each level of

blood coagulation pathway, because of its deadly consequences<sup>5</sup>. Prevention and control of coagulation abnormalities is extensively studied both in animals and humans<sup>6</sup>.

In current medical practice, for the prevention of thromboembolic disorders use of an oral anticoagulant drug, warfarin is very common<sup>7</sup>. However, due to high rate of morbidity and mortality and severe adverse effects drug withdrawal is often needed. Modern medicine having many favorable effects is still unable to completely prevent the cardiovascular diseases, thus people are paying attention to

complementary and alternative medicines most importantly herbal medicines<sup>8</sup>.

Moreover, it is reported that the occurrence of cardiovascular diseases such as acute coronary disorders associated with thrombosis is considerably reduced by the ingestion of vegetation including herbs and spices<sup>9</sup>. Researchers validated many active constituents such as eugenol, piperine, pinene,  $\alpha$ -terpinol, borniol, geraniol, cinnamaldehyde, sinigrine, thymol present in numerous types of herbs and spices, accountable for anticoagulants action<sup>10</sup>.

*Illicium verum* hook.f recognized as a (Chinese star anise) belonging to the Schisandraceae family, is a well-known spice, and is the fruit of perennial arbor plant, collected from the star-shaped pericarp. It is cultivated in Indochina, Southern China, Northern Vietnam, Japan and various other tropical and subtropical regions of Asia<sup>11</sup>. Chemical constituents present in *I. verum* hook.f responsible for its various biological activities include anethole and others such as  $\alpha$ -pinene, limonene, linalool and shikmic acid<sup>12</sup>. Additionally, numerous other biochemical constituents such as butylated hydroxytoluene, carmine acid, caryophyllene, estragole, p-anisyl acetone resveratrol, trans-m-propenyl, guaiacol,  $\gamma$ -muurolene,  $\gamma$ -selinene, (+)- $\alpha$ -tocopherol, 2,3-dehydro-4-oxo- $\beta$ , 2,5-di-isopropylphenol are also present<sup>13</sup>.

Chinese star anise was affirmed safe as a "food and medicine both" by the Ministry of Health of the people Republic of China<sup>14</sup>. Researchers have explored many pharmacological activities of this spice such as antibacterial, anti-inflammatory, antifungal, anticancer and anti-HIV properties<sup>11,15-17</sup>. Moreover, numerous pharmaceutical preparations contain this as a supplement<sup>18</sup>. Coagulation tests such as thrombin time (TT), prothrombin time (PT) and activated partial thromboplastin time (aPTT) measure blood's capability to clot and can measure danger of extreme bleeding or evolving clots (thrombosis) in blood vessels. Increased thrombosis is directly linked with augmented hazard of developing coronary artery disease<sup>19,20</sup>. Keeping in mind the presence of rich bioactive components in the spice, present research was aimed specially to evaluate the effect of *I. verum* hook.f. (MEIV) methanol extract on following coagulation parameters thrombin time (TT), prothrombin time (PT) and activated partial thromboplastin time (aPTT).

## METHODS

This experimental research was performed at the Pharmacology Department of Bahria University Medical and Dental College, Karachi-Pakistan for a period of 60 days from March to May 2018; fruits of *I. verum* hook.f in the dried form were acquired from a local herbal shop of Karachi, Pakistan. After

obtaining, fruit was identified and validated by Prof. Dr. Iqbal Azhar, Dean, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Pakistan. Voucher specimen (IV-01-17) was deposited in Pharmacognosy Department, University of Karachi, Pakistan. The animal care and used protocols were assessed and approved by an ethical review committee (ERC) of the Bahria University Medical and Dental College, Karachi-Pakistan.

The desiccated fruits of *I. verum* hook.f were washed, air-dried and grounded. Methanol extract was prepared by adding approximately 100g of star anise dried sample (in the form of coarse powder) into 400 ml methanol for 24 hr at room temperature along with stirring at various time intervals. Straining of solvent was done with cotton cloth and for further filtration filter paper (Whatman No.1) was used. Afterward vaporization of methanolic extract was completed by reducing pressure at (40-45 °C) in a rotary evaporator and later on at -30 °C freeze-drying. The final extract collected was kept on -20 °C until used for other procedures<sup>21</sup>. The extract was found to produce 21.34% of dry weight and it was a dark semisolid residue of chocolate brown color.

Warfarin sodium tablets (5 mg) at 0.54 mg/kg<sup>22,23</sup> oral dose were used as a reference drug, prepared by crushing and diluting the drug with 1 ml distilled water. Star anise extract was dissolved in distilled water in a way that each 1 ml contained 1 mg of extract. The study was performed on thirty (30) healthy white rabbits of either sex, weighing from 1000-1500 grams. Animals placed at the animal house of Pharmacology department of Bahria University Medical and Dental College, were acclimatized under standard condition that is (22±2 °C), with humidity (55-65%) providing food and water ad libitum.

Animals were unevenly assigned into five (5) groups, each encompassing of six (6) animals. Group I served as control and was provided distilled water 1ml/day. Group II labeled as standard and was given warfarin (0.54 mg/kg) orally once a day. Three of the groups labeled as test groups and were given three different doses of MEIV i.e. 150, 250, and 350 mg/kg once a day orally<sup>24</sup>. Dosing was sustained for 60 days via oral route of administration once daily. Body weights of each animal were measured at baseline and then weekly. Base line readings of coagulation parameters were also recorded for comparison.

Coagulation tubes encompassing 3.2% sodium citrate were utilized for collecting blood samples after 30 and 60 days from all the animal groups taken through central ear vein of rabbits<sup>22</sup>. Plasma was instantly detached from blood samples by centrifuging it at 2000 RPM for 15 minutes on centrifuging machine Humax 14 K (Wiesbaden, Germa-

ny). Thrombin time (TT), Prothrombin time (PT) and activated partial thromboplastin time (aPTT) were measured by using a standard reagent kit Huma Clot duo (Wiesbaden, Germany).

Data was compared with control group's values and data were evaluated by computing the mean and SEM (standard error of mean) by One way ANOVA trailed by post hoc Tukey's Honest significant Difference (HSD) test, p-values less than <0.05 were taken as significant and p-values less than <0.005 as highly significant. All statistical procedures were done on SPSS software version 20.0.

**RESULTS**

Comparative outcome of various doses of MEIV and Warfarin on coagulation parameters after 30 days of continuous administration is shown in Table 1 (a, b) and Figure 1. Effect of MIEV in the test group

was seen at three different doses i.e. 150, 250 and 350 mg/kg as compared to the control group, which received normal saline 1ml/kg/body weight. Warfarin was used as the standard drug. Test group given MEIV at the dose of 150 mg/kg for 30 days did not exhibit changes on any coagulation parameter matched to control animals. However, a significant increase in PT 14.13±0.35 s and aPTT 27.06±0.81 s at 250 mg/kg dose was found as compared to the control animals. TT was not affected at this dose. Administration of MEIV for 30 days at 350 mg/kg also caused noteworthy elevation in PT 15.01±0.23 s and aPTT 32.38±0.21s as matched to control animals. TT was also not affected at this dose.

Similarly, administration of Warfarin at the dose of 0.54 mg/kg for continuous 30 days resulted in noteworthy upsurge in PT i.e, 15.64±0.72s, aPTT 33.13±0.71s and TT 14.98±0.37s as compared to control animals.

**Table 1a: Baseline coagulation parameters in rabbits.**

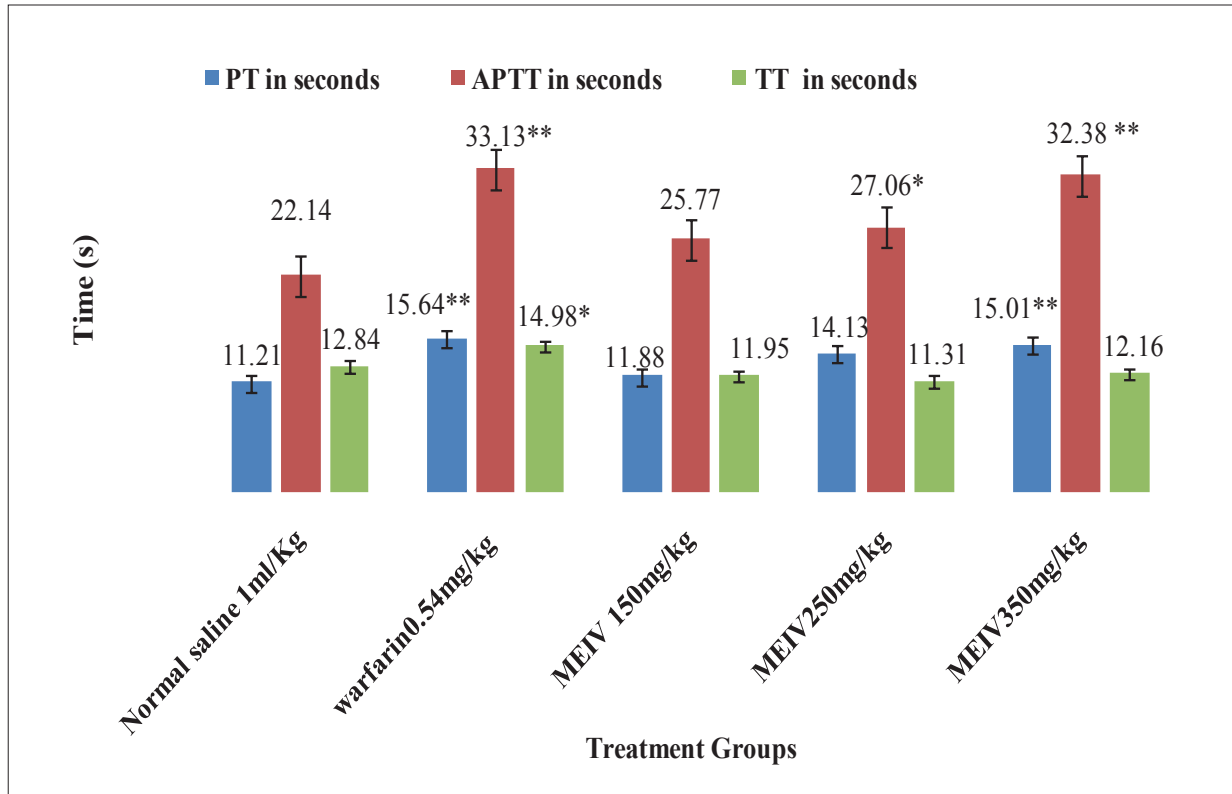
Parameters (in Seconds)	Control group Normal Saline 1ml/kg	Standard group Warfarin 0.54 mg/kg	Test groups Methanol Extract of <i>I. verum hook.f.</i> (MEIV) in mg/kg		
			150	250	350
PT	11.10±0.39	11.34±0.72	10.33±0.24	11.13±0.33	11.01±0.14
aPTT	21.03±0.93	21.10±0.1	22.12±0.67	22.08±0.34	19.28±0.59
TT	11.00±1.80	12.32±0.56	11.57±0.0	12.56±0.18	11.66±0.53

N = 6, Mean ± SEM.

**Table 1b: Coagulation parameters in rabbits after 30 days.**

Parameters (in Seconds)	Control group Normal Saline 1ml/kg	Standard group Warfarin 0.54 mg/kg	Test groups Methanol Extract of <i>I. verum hook.f.</i> (MEIV) in mg/kg		
			150	250	350
PT	11.21±0.39	15.64±0.72* (p=0.03)	11.88±0.48 (p=0.07)	14.13±0.35* (p=0.04)	15.01±0.23* (p=0.03)
aPTT	22.14±0.93	33.13±0.71** (p=0.00)	25.77±0.80 (p=0.09)	27.06±0.81* (p=0.03)	32.38±0.21** (p=0.00)
TT	12.84±0.59	14.98±0.37* (p=0.02)	11.95±0.37 (p=1.23)	11.31±1.80 (p=0.07)	12.16±0.55 (p=0.06)

N = 6, Mean ± SEM, \*p < 0.05, \*\*p < 0.005, as compared to control.



**Figure 1: Coagulation parameters in rabbits after 30 days.**

Table 2 and Figure 2 reveal the comparison of various doses of MEIV and Warfarin on coagulation parameters in rabbits after 60 days of continuous supply. Dosing was continued for a period of 60 days and again the assessment of coagulation parameters was made at three different doses as compared to control animals. Animals (n= 6) received extract at 150 mg/kg for 60 days presented a remarkable increase in PT i.e.  $14.9 \pm 0.56$  as compared to the control animals i.e.  $12.09 \pm 0.36$ .

Other parameters were not affected at this dose. Administration of the extract at 250 mg/kg resulted in highly significant increase in PT i.e.  $15.05 \pm 0.77$ , while significant increase in aPTT i.e.  $33.16 \pm 0.53$  was found compared to control animals. TT was not affected at this dose. Whereas, administration of the extract at 350 mg/kg resulted in highly significant rise in PT and aPTT i.e.  $16.00 \pm 1.06$  and  $38.5 \pm 1.56$  as compared to control animals. However, TT was not affected at this dose.

**Table 2: Coagulation parameters in rabbits after 60 days.**

Coagulation Parameters (in seconds)	Control group Normal Saline 1 ml/kg	Standard group Warfarin 0.54 mg/kg	Methanol Extract of <i>I. verum</i> hook.f. (MEIV) in (mg/kg)		
			150	250	350
PT	$12.09 \pm 0.36$	$17.25 \pm 0.69^{**}$	$14.9 \pm 0.56^*$	$15.05 \pm 0.77^{**}$	$16.00 \pm 1.06^{**}$
aPTT	$22.26 \pm 0.89$	$39.12 \pm 0.74^{**}$	$25.11 \pm 0.76$	$33.16 \pm 0.53^*$	$38.5 \pm 1.56^{**}$
TT	$13.00 \pm 0.47$	$16.37 \pm 0.644^*$	$13.21 \pm 0.36$	$14.01 \pm 0.52$	$14.28 \pm 0.58$

N = 6, Mean  $\pm$  SEM, \*p < 0.05, \*\*p < 0.005, as compared to control.

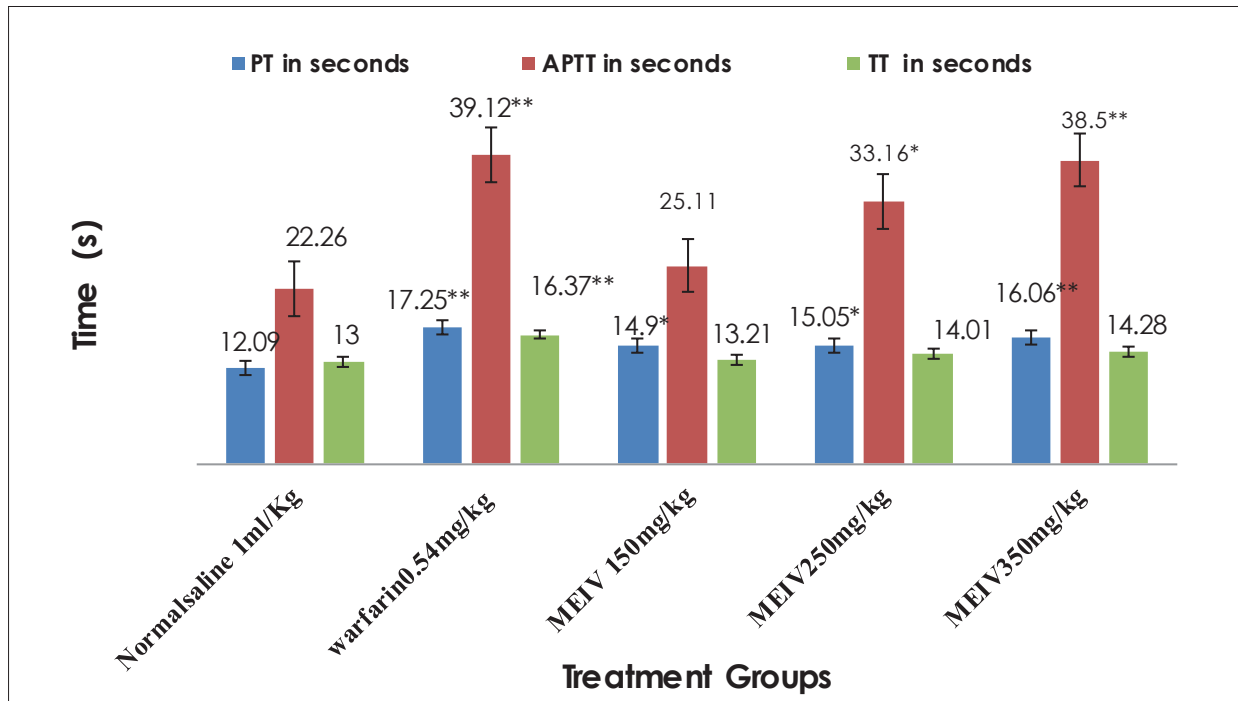


Figure 2: Coagulation parameters in rabbits after 60 days.

## DISCUSSION

Current investigation was aimed to examine the anticoagulant effects of methanol extract of *I. verum hook.f* by evaluating its effects on various coagulation parameters using three different doses. Present study revealed significant anticoagulant activity of the extract as the administration of MIEV at 250 mg for 30 days resulted in significant increase in PT and aPTT while at 350 mg/kg significant increase in PT and highly significant increase in aPTT was found. Administration of the extract at 150 mg/kg showed non-significant results in all parameters. Whereas, administration of same extract for 60 days at 150mg/kg resulted in significant increase in PT, significant increase in PT and aPTT at 250 mg/kg, and highly significant increase in PT and aPTT at 350mg/kg.

Literature review showed that Shikmic Acid which is an important bioactive component of *Illicium verum hook.f* can inhibit platelet aggregation induced by ADP (adenosine 5'-diphosphate) and collagen in vitro<sup>25</sup>. Moreover, Triacetyl Shikmic acid exhibited anticoagulant and antithrombotic activities in rats<sup>26</sup>. In addition to its antithrombotic effects, monopalmitoyloxy Shikmic acid increased the coagulation time. The 3,4-oxo-isopropylidene Shickimic also exhibited anticoagulant and antithrombotic activities<sup>27,28</sup>. It can be safely concluded that anticoagulant activity of *Illicium verum hook.f* can be due to presence of this important bioactive component.

PT effectively monitors oral anticoagulant therapy and in clinical tests of blood coagulation, it effects on extrinsic clotting pathway<sup>29</sup>. In extrinsic clotting pathway, coagulation factors like V, VII and X reduction are responsible for prolongation of PT<sup>26</sup>. It is considered that deficiency of clotting factors V, VII and Xa leads to prolonged PT<sup>27</sup>. However, intrinsic factors VII, IX, XII, XIII, and von Willebrand's factor generally represent extension of aPTT<sup>30</sup>. TT assay is a fundamental test in clinical laboratories to screen abnormalities in the alteration of fibrinogen to fibrin.

The methanol extract of *I. verum hook.f* led to significant increase in PT and aPTT almost at all the administered doses. Response of high dose was equivalent to the response of standard drug warfarin. The main mechanism by which warfarin acts is by inhibiting the enzyme vitamin K-epoxide reductase, which is essential for the stimulation of the vitamin K-dependent clotting factors II, VII, IX and X<sup>31</sup>. Accordingly, it may be suggested that *I. verum hook.f* produced an effect similar to warfarin. Current findings indicated considerable increase in aPTT, this typically signifies that it may be due to the lack of clotting factors, such as VII, VIII, IX, XI and von Willebrand's tangled in intrinsic pathway<sup>32</sup> for this reason reduction of these factors can lead to the extension of aPTT.

Currently various other authors have studies effects of *Illicium verum* and have found significant antioxidant potential of it<sup>33</sup>. Another author reported efficacy of *Illicium verum* essential oil

(IvEO) against food borne moulds and its nanoencapsulation for enhancing antifungal and antiflatogenic potency<sup>34</sup>. Administration of Warfarin resulted in significant increase in all the coagulation parameters i.e. PT, aPTT and TT while the *I. verum* hook.f extract mainly caused increased in PT and aPTT. Results of highest dose of *I. verum* hook.f extract i.e., at 350 mg/kg, the anticoagulant activity was comparable to Warfarin. Previously the effects of Shikmic acid (SA) were seen in rats induced by middle cerebral artery thrombosis (MCA) and it was found that shikmic significantly reduced the thrombosis in MCA with the pretreatment by SA 25 mg/kg<sup>26</sup>.

### CONCLUSION

In vivo administration of methanol extract of *I. verum* hook.f led to significant increase in PT and aPTT at almost all the administered doses in rabbits, strongly depicting its anticoagulant potential. This can be attributed to its bioactive components specifically shikmic acid.

### ACKNOWLEDGEMENTS

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### CONFLICT OF INTEREST

There is no conflict of interest to declare.

### ETHICS APPROVAL

The animal care and used protocols were assessed and approved by an ethical review committee (ERC) of the Bahria University Medical and Dental College, Karachi-Pakistan (Ref No: ERC 41/17).

### PATIENT CONSENT

Not applicable since, it is an animal study.

### AUTHORS' CONTRIBUTIONS

TS perceived the idea, conducted research, write-up of manuscript, LR supervised the whole project, examined the manuscript and TA critically examined the manuscript, helped in data analysis and bibliography.

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