

# Effect of Methanol Extract of Ajwain (*Trachyspermum Ammi L*) on Blood Coagulation in Rats

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## ABSTRACT

**OBJECTIVE:** To assess the effect of methanol extract of Ajwain on coagulation parameters PT and aPTT and to determine usefulness.

**DESIGN:** This comparative study was conducted in the Department of Pharmacology, Faculty of Pharmacy, University of Karachi, Pakistan.

**METHODS:** Antithrombotic tests PT and aPTT were conducted after multiple dosing of Ajwain on rats using standard kits and reagents, which were performed on Humacloot duo Germany. The data was subjected to analysis by taking mean and standard error to the mean using student T- test.

**RESULTS:** The results revealed that Ajwain extract did not show any significant effect on aPTT whereas increase in PT was highly significant ( $P < 0.001$ ).

**CONCLUSION:** The results suggested that the methanol extract of Ajwain prolonged PT, similar to that of Warfarin suggesting its possible effects on the extrinsic pathway, while aPTT was not altered suggesting that it may have no effects on the intrinsic pathway.

**KEY WORDS:** *Trachyspermum Ammi (L)*, Warfarin, Prothrombin time, Activated Partial thromboplastin time.

## INTRODUCTION

Blood coagulation is a complex set of physical, cellular and biochemical events leading to thrombus formation.<sup>1</sup> Thrombus formation has an important role in the pathogenesis and progression of ischemic heart diseases and ischemic brain diseases.<sup>2,3</sup> Possible mechanisms in humans or animals regarding anticoagulation have been widely studied.<sup>4</sup>

Thrombosis is an intravascular event, often causing significant interruption of blood flow. Many factors predisposes it, including venous stasis, usually from immobilization, congestive heart failure, polycythemia, sickle cell disease, visceral malignancies and the use of oral contraceptives specially in association with cigarette smoking. The ideal antithrombotic drug would prevent pathologic thrombosis and limit reperfusion injury, yet allow normal response to vascular injury.<sup>5</sup>

Coagulation occurs by transformation of soluble fibrinogen into insoluble fibrin. Circulating proteins interact in a cascade, where clotting factors undergo limited proteolysis to become active serine proteases. Platelets also have a vital role in hemostasis, both for the formation of clot and activation of coagulation proteins. Anticoagulant agents (also known as antithrombotic agents) like heparin and warfarin, lowers the formation of fibrin clots and prevents the extension of already formed clots, however they are associated

with some serious complications like thrombocytopenia.<sup>6</sup> Hence there is a considerable need to explore traditional medicines for rational use in blood disorders.

According to world health organization around 80% of the world's population is dependent on traditional medicine to fulfill their primary healthcare needs and 85% of the traditional medicine involves the use of plant products. This means that around four billion people on the globe rely on plants as source of drugs. Herbs from prehistoric time till today have been used as a cure for many diseases. Today active research has been underway to discover the safe and pharmacologically active herbs.<sup>7-10</sup>

Ajwain, also known as *Trachyspermum Ammi. L*, is a small grayish egg shaped seed like fruit that belongs to the family Umbelliferae. Ajwain was originated in Egypt, but is now primarily grown and used in south Asian countries. Pakistan, India and Saudi-Arabia are the leading users of Ajwain.<sup>11</sup>

The seeds contain essential oil (2-3%), which contains about 40-50% Thymol.<sup>12-14</sup> Thymol can be crystallized easily from the seeds of Ajwain essential oil extract and remainder consist of rho-cymene, beta pinene, dipentene, beta terpinene and carvacrol.<sup>15</sup>

The seeds of Ajwain are used commonly as a household remedy for colic, diarrhea, dyspepsia and asthma<sup>16</sup> while it is known to have antibacterial,<sup>17</sup> antifun-

gal,<sup>18</sup> antihelminthic,<sup>19</sup> hypocholesterolemic,<sup>20</sup> bronchodilator<sup>21</sup> and antioxidant<sup>22</sup> effects as well.

Huge population in the world is now using herbal products for preventive and therapeutic purposes, still further research and studies are needed to be done to evaluate their other pharmacological effects. For this reason, present study was designed as no research work had been done before to evaluate the effects of methanol extract of Ajwain on blood coagulation.

## MATERIALS AND METHODS

This study was conducted in the Department of Pharmacology, Faculty of Pharmacy, University of Karachi, Karachi, Pakistan, after getting approval from University's Board of Advance Study and Research.

### Animals

The study was carried out on twenty one locally bred male rats weighing between 180- 220 gms. All animals were divided into three groups, control (Vehicle), standard (Warfarin) and test group (Ajwain extract) each comprising of 7 rats. Animals were kept in plastic cages under controlled conditions of temperature  $23\pm 2$  °C and were given free access to food and water. The animals were transferred to the laboratory at least one hour before the start of the experiment. All experiments were performed during day time.

### Plant Material and Preparation of Extract

The seeds of Ajwain were purchased from a local herbal store in Karachi, identified and authenticated by the Center for Plant Conservation Herbarium and Botanic Garden, University of Karachi and voucher specimen number TA-10-12 was deposited in the Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Karachi.

The seeds were rendered free from all impurities manually and were soaked in methanol for 30 days. Soaked material was then filtered with the help of filter paper and filtrate was collected separately. The filtrate was passed through a rotary evaporator and then was freeze dried in Hussain Ibrahim Jamal (HEJ) research institute of Chemistry. Extract of Ajwain was given in a dose of 50mg/kg orally.<sup>23</sup>

### Drugs

**Warfarin** sodium 5mg tablets were crushed, diluted in distilled water and administered to animals in a dose of 0.54mg/kg orally.<sup>24</sup>

### Gum Tragacanth

powder was used as suspending agent to prepare suspension of the test drug (Ajwain) and was adminis-

tered to control animals as placebo in the dose of 10ml/kg orally - 100ml of warm distilled water was added in 2 gms Gum Tragacanth powder to form 2% suspension. Suspensions were prepared freshly at the time of administration.<sup>25</sup>

### Study schedule

Drugs were given orally to overnight fasted animals as single dose every day in the morning for fourteen consecutive days and food was provided after one hour of drug administration so that food could not interfere with drug absorption.

Antithrombotic tests were done after fourteen doses and on the day of experiment i.e. on fourteenth day, the drugs were given forty minutes before the start of experiment.

### Measurement for coagulation parameters

Blood samples were collected in coagulation tubes; plasma was suspended by centrifugation at 1500 rpm for 15 minutes in 14k Humax centrifuge. PT and aPTT were measured by Humaclot duo, using standard reagent kits of Merck.<sup>26</sup>

### Statistical analysis

The data were subjected to analysis by taking mean and standard error to the mean using student T-test, P-values of < 0.01 were considered as significant and P < 0.001 as highly significant. All statistical methods were performed using SPSS software version 16.5.

## RESULTS

Table I, Figure I and II elaborate the comparative effect of Ajwain extract (50mg/kg) and warfarin (0.54mg/kg) on coagulation parameters after 14 days continuous administration of drugs to rats. The antithrombotic effect was assessed by determining aPTT and PT. Ajwain extract did not show any significant effect on aPTT as compared to control; however there was highly significant increase in PT i.e.  $28.00\pm 1.2$  seconds as compared to control i.e.  $13.57\pm 0.30$  seconds, whereas Warfarin altered both parameters i.e. aPTT and PT highly significantly as compared to controls.

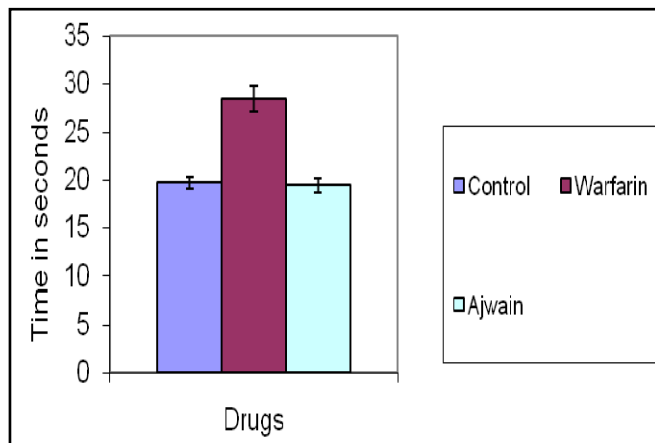
**TABLE I: EFFECT OF MULTIPLE DOSING OF AJWAIN AND WARFARIN ON APTT AND PT**

Parameters	Control (n=7)	Warfarin (n=7)	Ajwain (n=7)
aPTT	19.71±0.57	28.43±1.3**	19.43±0.78
PT	13.571±0.30	26.29±0.87**	28.00±1.2**

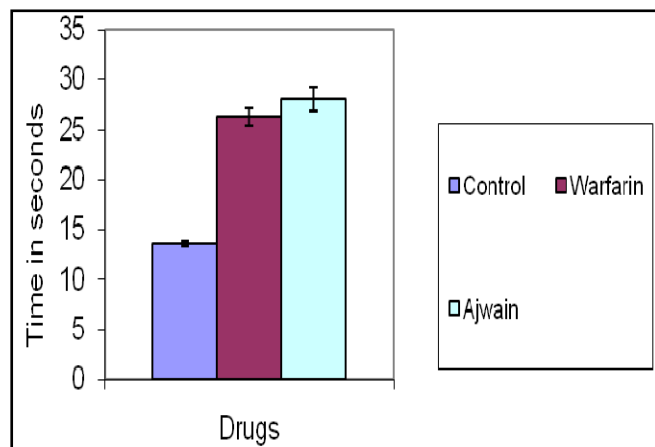
mean ± S.E.M

\*\*p < 0.001, highly significant as compared to control

**FIGURE I: COMPARATIVE EFFECT OF MULTIPLE DOSING OF AJWAIN AND WARFARIN ON APTT**



**FIGURE II: COMPARATIVE EFFECT OF MULTIPLE DOSING OF AJWAIN AND WARFARIN ON PT**



## DISCUSSION

The process of blood coagulation has a vital role in an organism's respond to vascular injury on one hand and in thrombosis and cardiovascular diseases (CVD) on the other hand<sup>27</sup>. Oral anticoagulant therapy must be monitored to ensure that the dose is providing the required response. Activated partial thromboplastin time (aPTT) and Prothrombin time (PT) are generally used to determine variations in coagulation factors.<sup>28</sup>

PT is an effective method of monitoring oral anticoagulant therapy and it reflects the overall efficiency of extrinsic clotting pathway in clinical test of blood coagulation.<sup>29</sup> A prolonged Prothrombin time indicates a deficiency in clotting factors V, VII and X. Where as activated aPTT is a test of intrinsic clotting activity. A prolonged activated partial thromboplastin time usually represents a deficiency in factors VII, IX, XII, XIII and von willebrand's factor<sup>30</sup>.

Present study revealed significant increase in PT with Ajwain extract, similar to that of Warfarin suggesting its possible effects on the extrinsic pathway, while

aPTT was not altered suggesting that it might have no effects on the intrinsic pathway but further studies are required on different species and large number of animal to investigate the exact mechanism of action.

## CONCLUSION

It may be concluded that Methanol extract of Ajwain has mild antithrombotic effect which may be of value in thrombotic states and cardiovascular diseases, however, nothing can be said definitely; hence further studies are needed to reach at final conclusion.

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