

# IN VIVO EXAMINATION OF THE ANTICOAGULANT EFFECT OF THE *BRASSICA OLERACEA* METHANOL EXTRACT

Rafeeq Alam Khan<sup>1,\*</sup>, Tahira Asad<sup>2</sup>, Zeeshan Feroz<sup>3</sup> and Mansoor Ahmed<sup>4</sup>

<sup>1</sup> Department of Basic Medical Sciences, College of Medicine- Jeddah, King Saud bin Abdulaziz University for Health Sciences, KSA

<sup>2</sup> Bahria University Medical and Dental College, Karachi, Pakistan

<sup>3</sup> College of Science and Health Professions, Department of Basic Sciences, King Saud Bin Abdulaziz University for Health Sciences, Riyadh, Kingdom of Saudi Arabia

<sup>4</sup> Department of Pharmaceutical Chemistry, University of Karachi, Karachi 75270 Pakistan

\*Corresponding author: rkhan1959@gmail.com

**Abstract:** The anticoagulant effect of the methanol extract of *Brassica oleracea var. capitata* (MEB) was examined in rabbits. The animals were divided into five groups, each comprising seven animals. Three groups were administered increasing doses of MEB (200, 300, and 500 mg/kg, respectively); one group received warfarin (0.54 mg/kg); animals in the control group received saline (1 ml/day equivalent to the volume of doses applied to the treated and standard animals). Biochemical tests were performed on the 16<sup>th</sup> and 31<sup>st</sup> days of dosing. Animals that were administered MEB (500 mg MEB/kg) 30 days displayed increases of 24.07 s, 28.79 s and 4.08 s in activated partial thromboplastin (aPTT), fibrinogen (Fg) and thrombin time (TT). Compared to the control, the increase in aPTT and Fg was highly significant and the increase in TT was significant. The anticoagulant effect exhibited by MEB in rabbits may be due to inactivation or inhibition of factors affecting coagulation.

**Key words:** herbs; anticoagulant; rabbits; activated partial thromboplastin time; thrombin time and fibrinogen

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## INTRODUCTION

Cardiovascular disease and cerebrovascular accident (CCVD) are the causes of high mortality and morbidity all over the world (Beaglehole and Yach, 2003). According to the World Health Organiza-

tion, approximately 17.3 million people died from CVD and it is estimated that the number of deaths will dramatically increase to reach 23.3 million by 2030 (World Health Organization, 2013).

Thrombosis is strongly associated with the pathogenesis of CCVD as the formation of un-

wanted thrombus on the pre-existing atherosclerotic plaque causes the occlusion of blood vessels leading to acute myocardial infarction or cerebrovascular accident (Dzau et al., 2002).

Blood coagulation is a defense mechanism that assists in maintaining the integrity of the circulatory system following vascular injury, but in abnormal conditions, due to the activation of enzymes in the coagulation cascade and platelets, it leads to thrombosis, atherosclerosis, inflammation and metastasis (Gou et al., 2003).

Studies have shown the favorable impact of a vegetarian diet on coagulation (Pan et al., 1993; Li et al., 1999; Mezzano et al., 1999). The incidence of acute coronary disease associated with thrombosis was significantly decreased by the consumption of plants (Ozben, 2006). Many plants are identified with potent anticoagulant activities, e.g. organosulfur compounds found in the *Allium* family, polyphenols in cocoa drink, anthocyanins in red wine grape juice and isoflavones in brassicas were found to have potent antithrombotic effects (Rajaram, 2003).

*Brassica oleracea* L. var. *capitata* (Cruciferae), commonly called cabbage, is species of *Brassica* native to coastal southern and western Europe. It is naturally confined to limestone sea cliffs. The chemical composition of *Brassica oleracea* is similar to other *Brassica* vegetables (Gross, 1994). It is available in various shades of green, red or purple. The most popular varieties are green, red, Savoy and Chinese (Byers et al., 2002).

For the present study, we used cabbage that belongs to the capitata group of *Brassica oleracea*. It is widely used as a vegetable and remedy for different diseases all around the world (Kris-Etherton et al., 2002). Many of its activities

are established in literature, such as anticancer (Ang-Lee et al., 2001), antioxidant, antiplatelet and hypocholesterolemic activities (Waqar and Mohmood, 2010). It has been reported to attenuate bronchoconstriction and inflammation by virtue of its anti-anaphylactic activity (Ambrosone et al., 2004). It increases the production of antioxidant enzymes in the body and inhibits oxidant-induced activation of transcription factor and nuclear factor kappa. *Brassica oleracea* not only inhibits the production of free radicals but also the oxidation of LDL protein by them (Borek, 2001). Furthermore, red cabbage was found to improve the symptoms of diabetic nephropathy in rats (Kataya, 2008).

The compounds responsible for these activities of *Brassica oleracea* include isothiocyanates, glucosinolates and phenolics, including flavonoids and other non-nutrients (Jeffery and Araya, 2009). Furthermore, it contains  $\beta$ -carotene, lutein, and zeaxanthin (Kane et al., 2005). Flavonoids have common C6-C3-C6 phenylchromane structure. *Brassica* contain complex types of flavonoids, having up to five sugar residues, which may additionally contain hydroxycinnamic residues (Vallejo et al., 2004).

Compounds derived from natural products play a key role in the expansion of new drugs. Many medicines have been developed from plant source (Harvey, 1999), like quinine (Kremsner et al., 1994), morphine (Benyhe et al., 1990) and paclitaxel (Waniet al., 1997). Indole-3-carbinol, a dietary compound found in cruciferous vegetables, has antiplatelet and antithrombotic activities both *in vitro* and *in vivo* (Park et al., 2008). Some flavonoids, such as quercetin, kaempferol, and myricetin, inhibited platelet aggregation in dogs and monkeys (Devries et al., 1997). Kaempferol and quercetin glycosides are present in cabbage leaves (Nielsen et al., 1993). One study

showed that flavonoids are powerful antithrombotic agents both *in vitro* and *in vivo* because of their capability to inhibit cyclooxygenase and lipoxygenase pathways (Alcaraz and Ferrandiz, 1987). In another study, flavonoids exhibited antithrombotic effect by directly hunting free radicals (Gryglewski et al., 1987). The presence of flavonoids in *Brassica oleracea* species is reported by several authors (Rashed et al., 2010; Asadujjaman et al., 2011).

These results show that *Brassica oleracea* may have an effect on coagulation parameters. We performed an *in vivo* study that was specifically designed to examine the effect of *Brassica oleracea* L. var. *capitata* on various coagulation parameters, i.e. prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT) and fibrinogen (Fg), as these tests are often used to assess variation in coagulation factors and can better monitor the influence of *Brassica oleracea* extract on blood coagulation process.

## MATERIALS AND METHODS

The study was conducted in the Department of Pharmacology, Faculty of Pharmacy, after approval from Board of Advance Study and Research, University of Karachi.

### Plant material and preparation of extract

Fresh cabbage was purchased from a local market in Karachi and identified by Prof. Dr. Anjum Parveen, Director of the Center for Plant Conservation Herbarium and Botanic Garden, University of Karachi. The voucher specimen (H.No. BO-09-12) was deposited in the Department of Pharmacognosy, University of Karachi.

The crude extract was prepared in a cold extraction process (Hossain et al., 2010). After thorough washing, 5 kg of *Brassica oleracea* leaves were chopped into small pieces and dried under shade for about a week. The dried material was ground to a coarse powder. This powder was soaked in 80% methanol for 10 days with occasional shaking and stirring. The solvent was filtered through cotton and then through filter paper (Whatman No.1). After filtration, the methanol extract was evaporated under reduced pressure in a rotary evaporator at 40°C-45°C and then freeze-dried at -30°C; the extract thus obtained was kept at -20°C until further use. The resultant yield of extract obtained was 19.3% of dry weight.

### Animals

The study was conducted on 30 healthy white rabbits of both sexes (1100-1600 g), housed at the animal house of the Department of Pharmacology, University of Karachi, under controlled conditions of temperature (22±2°C) and humidity (50to60%) in an alternating 12-h light/dark cycle. The animals were kept in separate cages and given standard diet and water regularly. The research committee of the Department of Pharmacology permitted the use of animals in this experiment in accordance with the guidelines of NAELAR (National Advisory Committee for Laboratory Animal Research, 2004) and National Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

### Preparation of dosage of active drug and plant extract

Warfarin sodium tablets (5 mg) were used to prepare a solution of 0.54 mg active drug/ml. The *Brassica oleracea* extract was dissolved in

sterilized water so that each 1 ml contained 200, 300 and 500 mg of extract.

### Dosing

All animals were divided into five groups, consisting of 6 animals each. Three groups were treated as test animals and given *Brassica Oleracea* extract in three different doses: 200, 300 and 500 mg/kg body weight. Animals treated as standard were given warfarin in a dose of 0.54 mg/kg body weight (Zacchiga et al., 2004). Extract and test drug were administered continuously for 30 days through oral route on a once daily basis. The animals of the control group received normal saline 1 ml/day equivalent to the volume of doses given to test and standard animals.

### Sample collection

Blood samples were collected in coagulation tubes containing 3.2% sodium citrate after 15 and 30 days from all animal groups through the ear vein. Plasma was immediately separated out by centrifuging blood samples on a Humax 14 K (Germany) centrifuge at 3000 rpm for 15 min.

### Estimation of coagulation parameters:

PT, aPTT, TT and Fg were measured by Huma-Clot duo, using standard reagent kits supplied by Human (Chan et al., 2007).

### Statistical analysis

All values were compared with the control by taking mean and standard error to the mean using one sample *t*-test. Values of  $P < 0.05$  were considered as significant and  $P < 0.01$  as highly significant. All statistical methods were performed using SPSS version 17.0.

## RESULTS

Table 1 reveals the comparison of PT, aPTT, TT and Fg after 15 days administration of normal saline, *Brassica oleracea* extract and warfarin to respective groups of animals while a similar comparison between the same groups after 30 days is shown in Table 2. Animals receiving *Brassica oleracea* extract at doses of 200 mg/kg and 300 mg/kg body weight for 15 days showed insignificant changes in all parameters in comparison to the control. However, animals receiving *Brassica oleracea* at the dose of 500 mg/kg for a period of 15 days showed a significant increase in aPTT and TT, i.e.  $12.69 \pm 1.40$  s and  $12.43 \pm 0.90$  s in comparison to the control i.e.  $8.05 \pm 0.69$  s and  $9.35 \pm 0.93$  s, respectively. However, the increase in PT and Fg was insignificant in comparison to control.

Animals receiving warfarin in the dose of 0.54 mg/kg body weight for 15 days showed significant increase in all the parameters i.e. PT, aPTT, TT and Fg, i.e.  $15.33 \pm 0.23$  s,  $14.45 \pm 9.8$  s,  $16.77 \pm 3.2$  s and  $47.64 \pm 1.3$  s in comparison to the control, i.e.  $11.29 \pm 0.52$  s,  $8.05 \pm 0.69$  s,  $9.35 \pm 0.93$  s and  $23.02 \pm 2.7$  s, respectively.

The animals receiving *Brassica oleracea* extract in the dose of 200 mg/kg and 300 mg/kg body weight for 30 days also showed insignificant changes in all parameters, i.e. PT, TT, aPTT and Fg in comparison to control.

Animals receiving *Brassica oleracea* in the dose of 500 mg/kg for 30 days showed highly significant increase in aPTT and Fg, i.e.  $32.13 \pm 0.63$  s and  $52.2 \pm 8.4$  s in comparison to control, i.e.  $8.06 \pm 0.71$  s and  $23.41 \pm 2.3$  s, respectively, while the increase in TT was significant, i.e.  $13.41 \pm 0.90$  s in comparison to control  $9.33 \pm 0.81$  s. However, there was insignificant increase in PT.

Animals receiving warfarin in the dose of 0.54 mg/kg body weight for 30 days showed highly significant increase in PT and aPTT, i.e.  $22.34 \pm 8.9$  s and  $56.34 \pm 7.6$  s in comparison to the control,  $11.86 \pm 1.3$  s and  $8.06 \pm 0.71$  s, but the increase in TT and Fg was significant, i.e.  $19.67 \pm 8.6$  s and  $40.21 \pm 3.3$  s in comparison to the control,  $9.33 \pm 0.81$  s and  $23.41 \pm 2.3$  s, respectively.

## DISCUSSION

Thromboembolic disorders are a major cause of death and disability worldwide (Gross and Weitz, 2009). Thus, there is great medical need for new anticoagulant agents to conveniently replace warfarin since its long-term use has many limitations. Doses of warfarin differ between patients due to changes in genetic polymorphism as well as dietary intake of vitamin K

and drug-drug interactions. Therefore, patients require frequent dose adjustments and monitoring of treatment to ensure therapeutic response, which is inconvenient for patients and physicians and increases the cost of health care (Go et al., 1999).

Plants have always been an important source for drug development, since various plant constituents have been used directly as therapeutic agents or for the synthesis of new drugs as sources of pharmacologically active compounds (Banjare and Paul, 2014).

The present study was designed to investigate the anticoagulant effect of methanol extract of *Brassica oleracea* by assessing its effect on various coagulation parameters, i.e. prothrombin time, activated partial thromboplastin time, thrombin time and fibrinogen time.

**Table 1.** Effect of *Brassica oleracea* on blood coagulation parameters after 15 days of administration

Coagulation Parameters	Control Normal Saline 1ml/kg	Standard Warfarin 0.54mg/kg	Methanol Extract of Brassica Oleracea (mg/Kg)		
			200	300	500
PT	11.29±0.52	15.33±0.23*	11.10±0.78	11.37±0.0	12.86±0.70
aPTT	8.05±0.69	14.45±9.8*	8.00±0.42	9.23±9.99	12.69±1.40*
TT	9.35±0.93	16.77±3.2*	8.99±0.41	10.01±7.71	12.43±0.90*
Fg	23.02±2.7	47.64±1.3*	26.00±0.91	26.93±7.81	30.01±1.2

n=6; Average value  $\pm$  S.E.M; \*P <0.05 significant as compared to control; \*\*P < 0.01 highly significant as compared to control

**Table 2.** Effect of *Brassica oleracea* on blood coagulation parameters after 30 days administration

DOI:10.2298/ABS140610022K	Control Normal Saline 1ml/kg	Standard Warfarin 0.54mg/kg	Methanol extract of Brassica Oleracea (mg/Kg)		
			200	300	500
PT	11.86±1.3	22.34±8.9**	12.99±8.3	13.00±6.70	16.14±1.4
aPTT	8.06± 0.71	56.34±7.6**	15.23±7.50	14.12±3.21	32.13±0.63**
TT	9.33±0.81	19.67±8.6*	9.99±0.44	12.75±0.0	13.41±0.90*
Fg	23.41±2.3	40.21±3.3*	31.45±0.1	33.35±0.14	52.2±8.4**

n=6; Average value  $\pm$  S.E.M; \*P <0.05 significant as compared to control; \*\*P < 0.01 highly significant as compared to control

*Brassica oleracea* revealed significant increase in aPTT and TT in rabbits after a 15-day administration of the extract of 500 mg/kg body weight; there was highly significant increase in aPTT and Fg after 30 days at the same dose, but TT was increased significantly as compares to the control animals.

The increase in aPTT is usually an indicator of reduced activity of factors VIII, IX, XI, XII and vWF involved in the intrinsic pathway of coagulation (Chan et al., 2007). Heparin produces its effect by forming a complex with antithrombin III and hence removes many of the activated coagulation factors that could be measured by prolonged aPTT (Allford et al., 2007). Thus, it may be suggested that *Brassica oleracea* produced an effect similar to heparin.

The increase in Fg is an indicator for decreased plasma levels of fibrinogen, whereas an increased fibrinogen level has been recognized as an independent risk factor for thrombosis (Chan et al., 2007). *Brassica oleracea* reduces the risk of thrombosis by decreasing fibrinogen levels indicated by the highly significant increase in Fg. Inflammation and platelet aggregation increases fibrinogen concentration in the liver, while *Brassica oleracea* has known anti-inflammatory and antiplatelet effects (Lin et al., 2008). It can be assumed that the decrease in fibrinogens levels was due to the anti-inflammatory and antiplatelet effects of *Brassica oleracea*.

The present study also revealed a significant increase in thrombin time after 30 days administration of *Brassica oleracea* extract, which indicates a deficiency of fibrinogen or inhibition of thrombin (Lane et al., 2005). This may be due to the reduced activity of some coagulation factors, such as IX, X, XI and XII, which are essential for thrombin generation (Di Cera, 2008, Gailani and Renne, 2007).

There is a strong correlation between blood lipids and coagulation parameters. Alterations in lipid levels influence thrombosis by modifying the activity of coagulation proteins, platelets and fibrinolytic factors (Eitzman et al., 2000). Moreover, a high-cholesterol diet has been reported to significantly increase the plasma concentration of clotting factors II, VII and X in rabbits (Mitropoulos et al., 1987). *Brassica oleracea* has hypolipidemic activity (Komatsu et al., 1998, Tahira et al., 2013). Hence, it can be postulated that the anticoagulant effect of *Brassica oleracea* may be due to its hypolipidemic effect as a decrease in the concentration of cholesterol may decrease the concentration of coagulation factors.

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