In vitro Anticoagulant Effect of Grape Seed Extract (Vitis vinifera) on Human Normal Blood Sample

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Authors’ contributions

This work was carried out in collaboration among all authors. Author SAH designed the study, performed the statistical analysis, author EIAA wrote the protocol and wrote the first draft of the manuscript. Authors MME and WEO managed the analyses of the study. Author ZSA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Scientists have used herbs nowadays for curing many diseases because they are safer and to overcome the side effect of the chemical drugs. Grape is one of the ancient herbs that used for diseases of the heart and blood vessels, high blood pressure, high cholesterol, skin care and many other conditions. Grape seeds have significantly higher concentrations of polyphenols which has anticoagulant and antithrombotic effect.

Materials and Methods: In this study 20 normal blood samples from healthy individuals with age range (19-38) years were enrolled in this study. Prothrombin time (PT) and activated partial thromboplastin time (APTT) tests were performed before adding grape seed extraction (GSE) (as control) and after adding GSE with different concentrations (25%, 50% and 75%).

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1. INTRODUCTION

Hemostasis is derived from a Greek word, which means a stop of bleeding. The process is a combination of biochemical and cellular events that collaborate to keep blood in the fluid state within the arteries and veins prevent blood loss after injury through the formation of a blood clot [1,2]. Thus, it's a system that represents a sensitive balance between pro-coagulant and anticoagulant mechanisms allied to a process for fibrinolysis. Hemostatic components Involved blood vessels, platelets, coagulation factors, coagulation inhibitors, and fibrinolysis [3].

Primary hemostasis is an aggregation of platelets (platelet plug) which forming at the damaged site of injured endothelial cells. Secondary hemostasis includes two pathways for coagulation; extrinsic and intrinsic pathway, that meet up at a point to activate the common pathway. The function of the coagulation pathway is to keep hemostasis or stop the bleeding. Finally the common pathway activates fibrinogen into fibrin. These fibrin subunits attach to each other and combine into fibrin strands that bind platelets together thus; stabilizing the platelet plug [4,5,6,7].

Practical intrinsic pathway measured by the partial thromboplastin time (PTT) that detects a deficiency in factors V, VIII, IX, X, XI, XII, prothrombin, and fibrinogen (all integral parts of the "intrinsic" and "common" pathway). It is usually prolonged if a patient has deficiency less than normal activity approximately 30% [8].

Factors in the extrinsic pathway can be measured by the prothrombin time (PT) which detects a deficiency of factors such as I, II, V, VII and X [9].

Herbal medicines are being used by about 80% of the world’s people mainly in the unindustrialized countries for primary health care. They have stood with time for their efficacy, safety, cultural acceptability and lesser side effect. Grape is one of the ancient herbs used for the treatment of diseases [10].

Grapes are a fruit that grows in clusters and have different color can be crimson, yellow, green, orange, black, and pink [11].

Each berry consists of skin, pulp with the juice and seed that represent 2%-5% of the berry, grape seeds have significantly higher concentrations of polyphenols (especially proanthocyanidine), flavonoids vitamin E, and linoleic acid than those for pulp and skin [12].

Grape seed proanthocyanidins, a combination of biologically active polyphenolic flavonoids including oligomeric proanthocyanidins, have been demonstrated to exert a novel spectrum of biological, pharmacological, therapeutic, and chemoprotective properties against oxygen free radicals and oxidative stress [13,14].

A lot of researches on grape seeds have been shown to display favorable health effects, such as antioxidative [15], cardioprotective [16] immunomodulatory, antitumor [17], and antithrombotic activity [18].

Anticoagulant drugs [warfarin, heparin] are used widely in the treatment of venous thromboembolic disease which can cause bleeding and reduced platelet count [19]: While Drugs are derived from plants is believed to be safer and dependable, compared with costly synthetic drugs that have adverse effects.

2. MATERIALS AND METHODS

2.1 Study Design and Population

This experimental comparative study included 20 blood samples collected from healthy individuals (11 females and 9 males, with age range (19-38) years. Any subject with a history of (smoking, diseases, regulatory supplement of drug or herbs) that have affect coagulation were excluded from the study.

**Results:** The results revealed that grape seed extract has an anticoagulant effect as proven by the increase of Prothrombin time and Activated partial thromboplastin time results of the blood samples in different concentrations of the extract. The GSE showed a high statistical significant (P= 0.000) in all concentrations of both PT and APTT tests.

**Conclusion:** This study suggests that GSE has a strong anticoagulant effect; so it can potentially be used as a supplementary anticoagulant agent to prevent thrombosis and cardiovascular diseases.

**Keywords:** In vitro; anti-coagulant effect; grape seed (Vitis vinifera); normal blood sample; Sudan.
2.2 Sample Collection

A suitable area for venipuncture was selected; the venipuncture area cleared with alcohol, then venipuncture was performed. Blood was collected under aseptic conditions. 2.5 ml of blood was collected in a 3.2% trisodium citrate container and was centrifuged immediately at 4000 - 4500 rpm for 15 minutes to obtain platelet poor plasma (PPP), that was separated in a plain container and stored at -20°C.

2.3 Preparation of Grape Seed Extraction (GSE)

The extract of grape seeds (V. vinifera), was purchased from LAVENDER COMPANY (Ebidkhatim street, Khartoum, Sudan). The extract was prepared as follow:

Grape seeds were collected from Syria and washed with excess water to remove adhering materials and then dried at room temperature. The seeds were homogenized with a mortar and pestle. The Powder form of the grape seeds was extracted overnight with a 50% (v/v) ethanol solution. The resulting solution was passed through a filter paper and dried by oven at 37°C. Then 20 gm was weighted and dissolved in 100 ml distilled water to obtain concentration of 20% and stored at 4°C for further use.

2.5 Coagulation Assay

The collected human platelet poor plasma (PPP) was assayed for coagulation using the following kits and equipment: prothrombin time (PT) and activated partial thromboplastin time (APTT) assays were performed according to the diagnostic kit’s manufacturer's instructions, from BIOMED COMPANY. Coagulation assays were performed manually by using water path.

Grape seed extraction of different concentration (25%, 50%, 75%) that prepared from the stock solutions was added to the normal blood samples (10 µL of extract + 90 µL of PPP). Then coagulation tests were done to assess the in vitro anticoagulant effect of the extraction.

All control and test samples were incubated 3-10 mints in water path and then assayed according to the test used procedures (100 µL of sample+200 µL of the reagent for PT test) and for APTT (100 µL of sample + 100 µL of reagent + 100 µL of calcium chloride).

2.6 Data Management and Analysis

The Questionnaire paper was prepared to collect important data from healthy individuals; Data were expressed as mean ± standard deviation (SD). Statistical analysis involved a one-way analysis of variance (ANOVA) and pair-T test. A value of P less than 0.05 (p < 0.05) was considered statistically significant, by using SPSS (Statistical Package for the Social Sciences) version 16.

3. RESULTS

3.1 Impact of GSE on the Prothrombin Time (PT) Test

When PT was performed using different concentrations of GSE (25, 50, and 75%) showed a statistically significant increased when compared with control (P= 0.000).

Also, GSE at concentrations 25% showed statistically significant (P=0.000) when compared with concentrations 50 and 75% Table 1.

3.2 Impact of GSE on the Activated Partial Thromboplastin Time (APTT) Test

When APTT was performed with incubation of GSE with different concentrations (25, 50, and 75%) showed a statistically significant increased when compared with control (P = 0.000).

Also, GSE at concentrations 25% showed statistically significant (P=0.000) when compared with concentrations 50 and 75% Table 2.

4. DISCUSSION

Hemostasis representing a delicate balance between pro-coagulant and anticoagulant mechanisms when there is an imbalance with this physiologic process, there can be an increased risk of developing a thrombosis. Thrombosis is the formation of an abnormal blood clot inside blood vessels [20], anticoagulant drugs (warfarin, heparin) are used widely in the treatment of venous
Table 1. Comparison of prothrombin time between different concentrations of GSE and control

| Extraction and control | Mean± SD   | P. value |
|------------------------|------------|----------|
| C 25%                  | 21.8 ± 1.4 | 0.00     |
| Control                | 16.2 ± 1.6 |          |
| C 50%                  | 24.8 ± 1.7 | 0.00     |
| Control                | 16.2 ± 1.6 |          |
| C 75%                  | 28.1 ± 2.0 | 0.00     |
| Control                | 16.2 ± 1.6 |          |

*The mean difference is significant at the 0.05 level

Table 2. Comparison of Activated partial thromboplastin time between different concentrations of GSE and control

| Extraction and control | Mean± SD   | P. value |
|------------------------|------------|----------|
| C 25%                  | 39.8 ± 3.7 | 0.00     |
| Control                | 31.4 ± 1.8 |          |
| C 50%                  | 48.1 ± 4.4 | 0.00     |
| Control                | 31.4 ± 1.8 |          |
| C 75%                  | 54.9 ± 5.9 | 0.00     |
| Control                | 31.4 ± 1.8 |          |

*The mean difference is significant at the 0.05 level.

thromboembolic disease [cardiovascular diseases (CVD) and cerebro-vascular accident (CVA) that accounts for high mortality and morbidity], which can cause bleeding and reduced platelet count [1,3,19]. Thus; continuous research is conducting in phytochemicals of anticoagulant properties, these are well-thought-out to be therapeutically better, have more effective anticoagulants and safe [21]. So this study was conducted to demonstrate anticoagulant activity of Grape seed.

The study showed a significant increased of PT an APTT in different test groups (25, 50 and 75% of GSE) with a mean (21.8 ± 1.4, 24.8 ± 1.7, 28.1 ± 2.0) (39.8 ± 3.7, 48.1 ± 4.4, 54.9 ± 5.9) respectively in comparison with PT and APTT of control group with a mean (16.2 ± 1.6) (31.4 ± 1.8) respectively (P value =0.00).

This result was agreed with study done by Bijak, Michał, et al, 2019 titled Dual Anticoagulant/Antiplatelet Activity of Polyphenolic Grape Seeds Extract in which GSE was added to sample with different concentrations (7.5 µg/mL, 15 µg/mL) and the anticoagulant activity of the tested compounds was evaluated using PT and APTT assays. Incubation with GSE (15µg/mL) showed statistically significant (p = 0.03) prolonged APTT of (33.6 Sec) compared to the control of (31.8), as well as PT (p = 0.03) of (16.2) versus (17.0) [21]. The study also was agreed with previous study done by Michelle Margarretta, Cuyos 2Catrina Mae C. Esplanada, and et al. 2015. titled Anticoagulant effect of grape seed extract (Vitis vinifera) on blood samples, In which grape seed extract was added to the blood samples with different concentrations (0.5, 5, 50 µg/ml) and Prothrombin time was performed, result showed that the blood samples with 50 µg/mL of extract had the most prolonged Prothrombin time compared to the blood samples with only 0.5 and 5 µg/mL, the control group gave significantly shorter Prothrombin time than the 5 µg/mL group by 12 seconds [22].

Michał Bijak, 2011 titled Anticoagulant effect of polyphenols-rich extracts from black chokeberry and grape seeds in which human plasma were incubated with grape seed extracts (5;50 µg/ml) result shown that significantly prolonged APTT and PT (38.8±0.8, 42.6±1.2) (18.1±0.6, 21.8±1.7) in compared with control (33.4±0.7/16.1±0.6) respectively and (P=0.001). All tested concentrations (0.5; 5; 50 µg/ml) resulted in a dose-dependent, significant (P=0.001) also concluded that result was significantly increased from the control to the highest concentration of grape seed extract [23]. Thus; GSE has anticoagulant properties through the prolongation of clot formation. This may be attributed to several polyphenols’ compounds especially proanthocyanin that has been noted in the seed [24]; or mechanism of this phenomenon is related to the inhibition of thrombin activity. The GSE inhibited the proteolytic activity of thrombin, observed as inhibition of thrombin-induced
fibrinogen polymerization, stabilized fibrin formation and platelet aggregation [23].

5. CONCLUSION

The current study suggests that GSE has a strong anticoagulant effect, so GSE can be used as a supplementary anticoagulant agent to prevent thrombosis and cardiovascular diseases. Furthermore, in vivo and or ex vivo studies with different type of extractions (aqueous, acetic acid and methanol extractions) are recommended to evaluate this effect and to determine the exact mode of action.

CONSENT AND ETHICAL APPROVAL

This study was approved by the ethical committee of the faculty of medical laboratory sciences, AlzaemAlazhari University. The purpose of the study was clarified and discussed with the participants, samples were taken after verbal consent from them.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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