Assessment of an *in vitro* Effect of natural Acacia Honey on Some Coagulation Profile (PT and APTT) in Sudanese Healthy Individuals

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**Citation:** Abdelrhman AH, Abdelgadir AA, Abdelaziz ZS (2021) Assessment of an *in vitro* Effect of natural Acacia Honey on Some Coagulation Profile (PT and APTT) in Sudanese Healthy Individuals. J Hematol Blood Disord 7(1): 101

**Received Date:** December 19, 2020, **Accepted Date:** January 8, 2021, **Published Date:** January 11, 2021

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**Abstract**

**Background:** The most serious and common adverse side effect associated with anticoagulant is increased risk of bleeding, both non-major and major bleeding events. Risk of bleeding is dependent on the class of anticoagulant agent used, patient's age, and pre-existing health conditions. Drugs derived from plants as green medicine is believed to be safe and dependable, compared with costly synthetic drugs that have adverse effects. Honey has been used since ancient times for its nutritional and therapeutic value. Acacia honey is available worldwide and it is inexpensive in compare to anti-thrombotic and thrombolytic agent. And has anticoagulant activity for treatment these problems.

**Materials and Methods:** In this study 100 normal blood samples from normal individuals with age range (18-30) years, 50% male and 50% female. PT and APTT tests were done before adding acacia honey by mixture patient plasma with normal plasma (as controls) and after adding acacia honey with different concentrations (10% and 25%).

**Results:** The results were analyzed by using SPSS and showed that the acacia honey has a strong statistically significant (p= 0.000) in all concentrations in both PT and APTT tests.

**Conclusion:** This study approved that acacia honey has a strong anticoagulant effect; so acacia honey can be used as a supplementary anticoagulant agent to improve and/or prevent thrombosis and cardiovascular diseases.

**Keywords:** Natural Acacia Honey; Coagulation; PT and APTT; Sudanese healthy individuals

**List of abbreviations:** PT: Prothrombin Time; APTT: Activated Partial Thromboplastin Time; DVT: Deep Vein Thrombosis; PPP: Platelet poor plasma; BV: Blood Vessel; VWD: Von willebrand disease

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**Introduction**

Functional foods are well characterized by their natural properties of health promotion, and disease prevention in addition to numerous nutritional values [1]. One of the most consumed functional foods is honey which has been defined as a natural product produced by honeybees via a regurgitation mechanism of the plant parts [2]. There are different types of honey depend on plant from which bees collect nectar, acacia Honey is rich in phenolic compounds, which act as natural antioxidants and have promising effect in the treatment of cardiovascular diseases. Many epidemiological studies have shown that regular intake of phenolic compounds is associated with reduced risk of heart disease. Many such phenolic and flavonoid compounds e.g; Chrysinhave antioxidant and anti-platelet potential, and hence may ameliorate cardiovascular diseases (CVDs) through various mechanisms, such as by decreasing oxidative stress and inhibiting blood platelet activation [3]. Studies have found flavonoids to exert beneficial action son the cardiovascular system via inhibition of blood platelet activation, reduction of LDL cholesterol level, honey phenolic compounds such as apigenin, quercetin, catechin, and luteolin inhibit blood platelet aggregation though binding to the thromboxane A2 receptor in an *in vitro* model [4]. It also modulated vascular function by increasing the bioavailability of nitric oxide, inhibited the development of atherosclerosis by reducing vascular inflammation, and prevented vascular smooth muscle cell proliferation and thrombogenesis [5]. Chrysin (flavonoid compound) potentially serve as a promising anti-thrombotic agents, it inhibited blood platelet functions and thrombus formation in vitro, and did not exert cytotoxic effects on blood platelets [6].
Materials and Methods

Study Participants

This is Experimental study design, 100 healthy Sudanese individuals both sexes with age (18-30) years was enrolled in this study.

Sample preparation and methods

Suitable area for venipuncture was selected; venipuncture area was cleared with alcohol, then venipuncture was performed. Blood was collected under aseptic condition. 2.5 ml of Blood was collected in 3.2 % tri sodium citrate container and centrifuged immediately at 4000 - 4500 rpm for 15 to obtain PPP, that separated in plain container and stored at – 20 °C.

Preparation of different concentrations of honey

Different concentration of honey was prepared with 0.9% normal saline (9gm of sodium chloride + litter D.W).

Preparation of 10% concentration of Acacia honey [V/V]

10% honey concentration was prepared by mixing 1 ml of honey with 9 ml of normal saline.

Preparation of 25% Acacia honey [V/V]

Was prepared by mixing 1 ml of honey with 3 ml of normal saline.

Mixing study using prothrombin time and activated partial thromboplastin time

Each PPP sample was divided into 3 parts, one mixed with N.S [50:50] second with 10% conc honey [50:50] and third with 25% honey [50:50] then PT and aPTT for each were measured. Automated coagulometer used to evaluate the PT and APTT results.

Statistical Analysis

Statistical assessment was carried out with statistical package for social sciences (SPSS) version 17.0 for windows statistical software.

Results

PT and APTT tests were done before adding acacia honey by mixing patient plasma with normal plasma (as controls) and after adding acacia honey with different concentrations (10% and 25%), the results were analyzed by using SPSS. The mean of PT in control group was found to be 21±1.48, 10% concentration honey 32 ±6, 25% concentration honey 43 ±6.8. There is a significant prolongation in PT of 10% and 25% conc of honey when compared with control (32±6, 43 ±6.8, 21±1.48), respectively (P value:0.00) (Table 1). Also there is a significant prolongation in PT of 25% conc of honey when compared with 10% (43±6.8, 32±6.), respectively (P value:0.00) (Table 1). There is a significant increase in INR of 10% and 25% conc of honey when compared with control (2.36±0.47, 3±0.6, 1.56±0.11), respectively (P:0.00) (Tables 1,2 and 3). Also there is a significant increase in INR of 25% conc of honey when compared with 10% (3±0.6, 2.36±0.47), respectively (P:0.00) (Table 2). Regarding aPTT there is a significant prolongation in aPTT of 10% and 25% of honey when compared with control (56±11.9, 72±9.3, 32±3.9), respectively (P:0.00) (Table 3). Also there is a significant prolongation in aPTT of 25% of honey when compared with 10% (72±9.3,

<table>
<thead>
<tr>
<th>Tests</th>
<th>Mean ±SD</th>
<th>P value</th>
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<tbody>
<tr>
<td>PT NS</td>
<td>21 ±1.48</td>
<td>32 ±6</td>
</tr>
<tr>
<td>PT 10% honey</td>
<td>43±6.8</td>
<td>0.000</td>
</tr>
<tr>
<td>PT 25% honey</td>
<td>32±6</td>
<td>43±6.8</td>
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Table 1: Compare means of different concentration of PT with control and 10% with 25% honey

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<tr>
<td>INR NS</td>
<td>1.56±0.11</td>
<td>2.36±0.47</td>
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<tr>
<td>INR 10% honey</td>
<td>1.56±0.11</td>
<td>3±0.6</td>
</tr>
<tr>
<td>INR 25% honey</td>
<td>2.36±0.47</td>
<td>3±0.6</td>
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Table 2: Compare means of INR of different concentration of PT with control and 10% with 25% honey
56±11.9), respectively (P value:0.00) (Table 3). There is no correlation between age PT and aPTT of control 10% and 25% (P value:0.1, 0.4, 0.8)(0.6, 0.01,0.3) respectively (Tables 3,4 and 5) (Figures 1 and 2).

<table>
<thead>
<tr>
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<th>P value</th>
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<tr>
<td>aPTT NS</td>
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<td>0.1</td>
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<td>PT 10% honey</td>
<td>-0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>PT 25% honey</td>
<td>-0.02</td>
<td>0.8</td>
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<table>
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<tr>
<th>Age</th>
<th>R value</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>PT NS</td>
<td>0.06</td>
<td>0.6</td>
</tr>
<tr>
<td>PT 10% honey</td>
<td>0.3</td>
<td>0.01</td>
</tr>
<tr>
<td>PT 25% honey</td>
<td>0.1</td>
<td>0.3</td>
</tr>
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</table>

Table 3: Compare means of different concentration of PTT with control and 10% with 25% honey

Table 4: Correlation of PT with age

Table 5: Correlation of PTT with age

Figure 1: Show mean of control of PT and different conc of honey

Figure 2: Show mean aPTT of N.S and different conc of honey
All around the world, continuous research is being conducted in physiochemicals of anticoagulant properties. These studies are well thought out to therapeutically better anticoagulant, multiple targets, and safer. This study demonstrates that honey in different concentrations (10%, 25%) inhibits clot formation and prolongs PT and aPTT. It also shows that increasing concentrations of honey strongly delay the coagulation process and increases PT and aPTT. Thus, honey 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. Our study agrees with the study done by ASIF AHMED et al. in Pakistan; it was concluded that samples treated with honey show significantly prolonged aPTT and PT in comparison with control samples that were not treated. Increasing concentration with honey and there is a correlation between prolongation of PT, aPTT, and hemostasis [7]. Results of the present study demonstrate that honey inhibited the coagulation proteins of all three coagulation pathways, i.e., intrinsic pathway (asessed by aPTT); extrinsic pathway (assayed by PT) and final common pathway (assayed by TT). Moreover, during this study, honey-induced decrease in fibrinogen levels was in agreement with the prolongation of aPTT, PT, and TT observed. There could be several reasons for natural honey to have anticoagulant attributes such as, honey contains a variety of flavonoids that may affect the activity of coagulation factors like fibrinogen and factor VII [8,9]. Similarly, different types of sugars affect the process of blood coagulation. Honey contains glucose (28-36%) and it has been suggested that high levels of glucose interfere with coagulation through different mechanisms such as, non-enzymatic glycation, the development of increased oxidative stress, and a decrease in the levels of subendothelial heparin sulphate [10,11]. Moreover, honey contains maltose (1.7-11.8%) that also reported to interfere with blood coagulation [12,13]. Medicinal properties of honey encompass various mechanisms that might play a role in the prevention of atherosclerotic vascular disorders, e.g., cardiovascular and cerebrovascular disorders. Honey has been reported to inhibit thrombin (main enzyme of blood coagulation) induce formation of reactive oxygen species from phagocytes; as free oxygen radicals particularly superoxide and hypochlorous acid provides the nidus for the development of atherosclerotic plaque, thus honey might interrupts the nidus formation of atherosclerotic plaque (Ahmad et al., 2009). Honey independently inhibits LDL oxidation that also prevents the development of primary atherosclerotic lesion [14]. It is reported that fasting blood sugar is an independent predictor of platelet dependent thrombosis in patients with coronary artery [15]. Honey has reported to have physiological euglycemia in fasted human subjects [16]. This effect also possibly interferes with the process of thrombosis. On the basis of present and previous results, it can be assumed that honey might interfere at several steps in the formation of atherosclerotic disease; this effect finally translates into the prevention of vascular disorders such as cardiovascular and cerebrovascular disorders [17].

**Discussion**

Based on experimental studies done, we observed that samples treated with honey significantly prolonged APTT and PT in comparison with control samples that were not treated with indicate that acacia honey has a strong anticoagulant effect and can be used as a supplementary anticoagulant agent to improve and/or prevent thrombosis and cardiovascular diseases.

**Conclusion**

Based on experimental study done, we observed that, samples treated with honey significantly prolonged APTT and PT in comparison with control sample that not treated with indicate that acacia honey has a strong anticoagulant effect and can be used as a supplementary anticoagulant agent to improve and/or prevent thrombosis and cardiovascular diseases.

**Declarations**

**Ethical approval and consent to participant**

Approval of this study was obtained from hematology department of medical laboratory science (MLS), Omdurman Islamic University, and ministry of health issued by the local ethical committee, Khartoum State, Sudan. Written consent was taken from each member of the study.
Consent for publication

Not applicable.

Availability of data and materials

The datasets generated during and/or analyzed in this study are not publicly available due to Bahri hospital center ethical policy in order to protect participant confidentiality.

Competing interest

The authors declare that they have no competing interests.

Funding

No funding was obtained for this study

Authors contributions

AH, ZS and AA contributed in literature search and manuscript writing. ZS had the main idea of the study and contributed in manuscript writing, AA contributed to clinic work, AH contributed in statistical analysis. AA supervised the study and critically reviewed the manuscript. All authors read and approved the final draft of the manuscript.

References

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