

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

Abdelrhman AH*, Abdelgadir AA and Abdelaziz ZS

Department of Hematology and Immunohematology, Omdurman Islamic University, Sudan

*Corresponding author: Abdelrhman AH, Department of Hematology and Immunohematology, Omdurman Islamic University, Sudan, Tel: 00249929485305, E-mail: amgedhussen66@gmail.com

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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

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; Chrysin have 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 on 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.value:0.00) (Table 3). Also there is a significant prolongation in aPTT of 25% of honey when compared with 10% (72±9.3,

Tests			P value
	PT NS	PT 10% honey	
Mean ±SD	21 ±1.48	32 ±6	0.000
	PT NS	PT 25% honey	
	21 ±1.48	43±6.8	0.000
	PT 10% honey	PT 25% honey	
	32±6	43±6.8	0.000

Table 1: Compare means of different concentration of PT with control and 10% with 25% honey

Tests			P value
	INR NS	INR 10% honey	
Mean ±SD	1.56±0.11	2.36±0.47	0.000
	INR NS	INR 25% honey	
	1.56±0.11	3±0.6	0.000
	INR 10% honey	INR 25% honey	
	2.36±0.47	3±0.6	0.000

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).

Tests			P value
	aPTT NS	PTT 10% honey	
Mean ±SD	32±3.9	56±11.9	0.000
	aPTT NS	PTT 25% honey	
	a32±3.9	72±9.3	0.000
	aPTT 10% honey	PTT 25% honey	
	56±11.9	72±9.3	0.000

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

Age		
Test	R value	P value
PT NS	0.19	0.1
PT 10% honey	-0.1	0.4
PT 25% honey	-0.02	0.8

Table 4: Correlation of PT with age

Age		
Test	R value	P value
PT NS	0.06	0.6
PT 10% honey	0.3	0.01
PT 25% honey	0.1	0.3

Table 5: Correlation of PTT with age

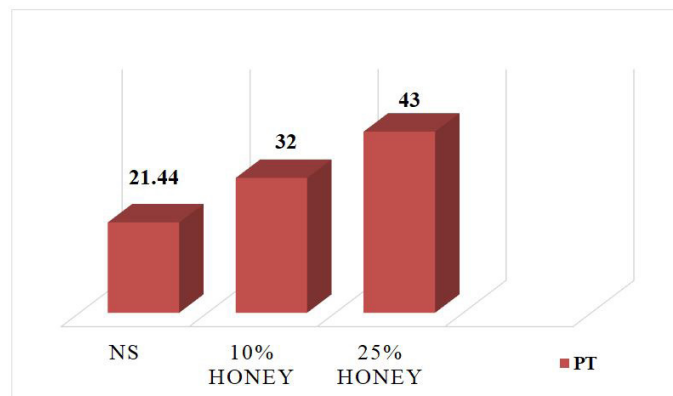


Figure 1: Show mean of control of PT and different conc of honey

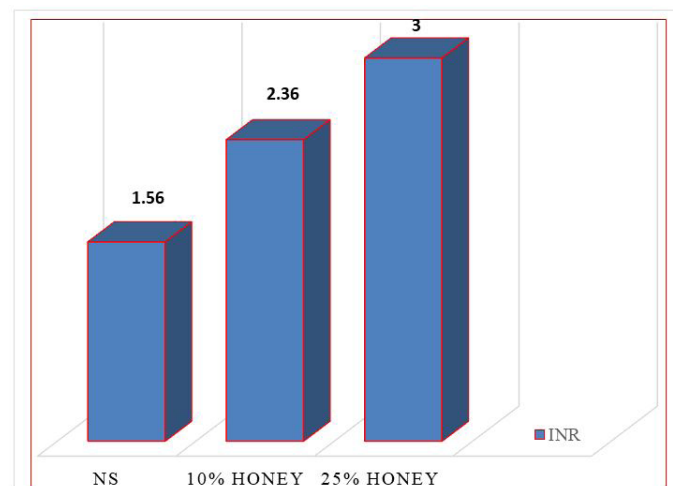
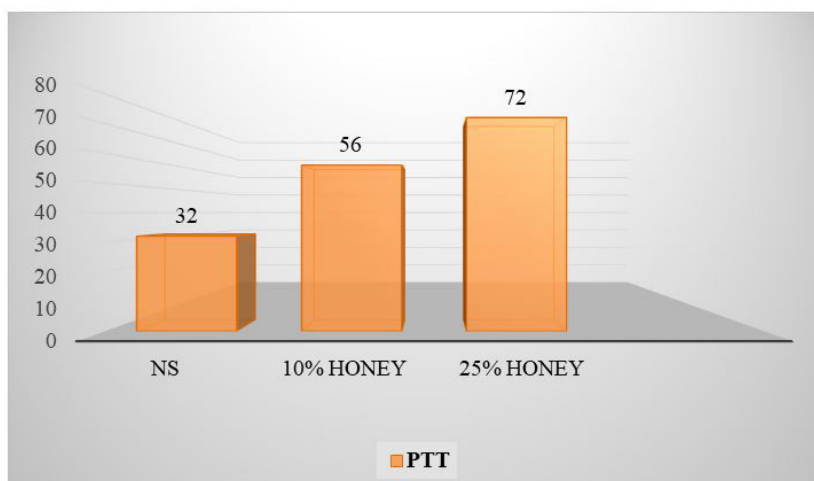


Figure 2: Show mean aPTT of N.S and different conc of honey



Discussion

All around world continuous research is conducting in physiochemicals of anticoagulant prosperities, these study 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 prolong 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 agree with study done by ASIF AHMED *et al* in *Pakistan* it was concluded that samples treated with honey show significantly prolonged aPTT and PT in compare with control sample that not treated increasing concentration with honey. And there is a correlation between prolongation of PT, aPTT and of honey [7]. Results of present study demonstrate that honey inhibited the coagulation proteins of all three coagulation pathways i.e., intrinsic pathway (assayed by aPTT); extrinsic pathway (assayed by PT) and final common pathway (assayed by TT). Moreover, during this study honeyinduced decrease in fibrinogen levels that is 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 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 level of glucose interferes with coagulation through different mechanisms such as, non-enzymatic glycation, the development of increased oxidative stress, and a decrease in the levels of sub endothelial 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 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 assumed that honey might interferes 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].

Conclusion

Based on experimental study done we observed that, samples treated with honey significantly prolonged APTT and PT in compare 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.

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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

1. Muhammad A, Odunola OA, Farooq AD, Mesaik AM, Choudhary MI, et al. (2012) Fractionation of acacia honey affects its antioxidant potential in vitro. *J Acute Dis* 1: 115-9.
2. Grodstein F, Kang JH, Glynn RJ, Cook NR, Gaziano JM (2007) A randomized trial of beta carotene supplementation and cognitive function in men: the Physicians' Health Study II. *Arch Intern Med* 167: 2184-90.
3. Ahmed A, Khan RA, Azim MK, Saeed SA, Mesaik MA, et al. (2011) Effect of natural honey on human platelets and blood coagulation proteins. *Pak J Pharm Sci* 24: 389-97.
4. AL-Bukhaari, M (1994) Holy Hadith [Sahih Al-Bukhari, Arabic] (3rd Edn) Kazi Publications Chicago, USA.
5. Mobarok Ali AT (1995) Natural honey exerts its protective effects against ethanolinduced gastric lesions in rats by preventing depletion of glandular nonproteinsulphhydryls. *Trop Gastr* 16: 18-26.
6. Aliyu M, Odunola OA, Farooq AD, Mesaik AM, Choudhary MI, et al. (2012) Acacia honey modulates cell cycle progression, pro inflammatory cytokines and calcium ions secretion in PC-3 cell line. *J Cancer Sci Ther* 4: 401-7.
7. Muhammad A, Odunola OA, Ibrahim MA, Sallau AB, Erukainure OL, et al. (2016) Potential biological activity of acacia honey. *Circulation* 105: 1135-43.
8. Briggs WH, Folts JD, Osman HE, Goldman IL (2001) Administration of raw onion inhibits platelet-mediated thrombosis in dogs. *J Nutr* 131: 2619-22.
9. Cazenave AJP (1988). The effect of flavonoids on bloodvessel
10. Cohen AT, Hamilton M, Mitchell SA, Phatak H, Liu X, et al. (2015) Comparison of the Novel Oral Anticoagulants Apixaban, Dabigatran, Edoxaban, and Rivaroxaban in the Initial and Long-Term Treatment and Prevention of Venous Thromboembolism: Systematic Review and Network Meta-Analysis. *PLoS One* 10: e0144856.
11. Pichichero E, Cicconi R, Mattei M, Muzi MG, Canini A (2010) Acacia honey and chrysin reduce proliferation of melanoma cells through alterations in cell cycle progression. *Int J Oncol* 37: 973-81.
12. Ferreira ICFR, Aires E, Barreira JCM, Estevinho LM (2009) Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. *Food Chem* 114: 1438-43.
13. Hoffbrand AV, Paul AH (201) Platelets, blood coagulation and haemostasis In: Hoffbrand's Essential Haematology (7th Edn) John Wiley and Sons Ltd, USA.
14. Mandal MD, Mandal S (2011) Honey: its medicinal property and antibacterial activity. *Asian Pac J Trop Biomed* 1: 154-60.
15. Lazarević KB, Andrić F, Trifković J, Tešić Z, Milojković-Opsenica D (2012) Characterisation of Serbian unifloral honeys according to their physicochemical parameters. *Food Chem* 132: 2060-4.
16. Ahmad A, Azim MK, Mesaik MA, Khan RA (2008) Natural honey modulates physiological glycemic response compared to simulated honey and D-Glucose. *J Food Sci* 73: 165-7.
17. Ahmad A, Khan RA, Mesaik MA (2009) Antiinflammatory effect of natural honey on bovine thrombin induced oxidative burst in phagocytes. *Phyther Res* 23: 801-8.
18. Ahmad SS, London FS, Walsh PN (2003) The assembly of the factor X-activating complex on activated human platelets. *J Thromb Haemost* 1: 48-59.

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