THE SUB – ACUTE EFFECTS OF RAW HONEY ON PROTHROMBIN TIME, ACTIVATED PARTIAL THROMBOPLASTIN TIME AND PLATELET VALUES IN ALBINO WISTAR RATS

ABSTRACT

For centuries honey has been regarded as wonderful gift of nature in which the properties of an excellent food, beneficial alike to adults and children, are combined with medicinal properties. Surprisingly, its sub-acute effect on coagulation is unknown. Hence; this present study aims at evaluating the effects of raw honey on coagulation in albino wistar rats. Thirty (30), 3-4 months old albino wistar rats both males and females were used for the study. The experimental animals were divided into five (A, B, C, D, E) groups with six rats per group. The test groups (B-E) were gavaged with graded doses (625, 1250, 2500, 5000mg/kg body weight) respectively of the raw honey once daily for nine days. Group A served as control. Two (2) animals were bled from each group after 3, 6 and 9 days through the ocular plexus. Four (4) ml of venous blood was collected. Two (2) ml was delivered into 0.25ml trisodium citrate anticoagulant bottle for determination of Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT). The remaining two (2) ml was delivered into K_3EDTA anticoagulant bottle for platelet value determination. There was no statistical significant difference ($P > 0.05$) recorded in all the parameters investigated among the test groups when compared with the control group on Day 3. However, group B revealed a statistical significant decrease ($P < 0.05$) in PT when compared with the control group on Day 6. In addition, no statistical significant difference ($P>0.05$) was recorded on Day 9 when all the parameters investigated among the test groups were compared with the control group. Furthermore, there was no exposure related statistical significant difference ($P>0.05$) in the test groups in PT and APTT in the ANOVA. However, there was a time related significant difference ($P<0.05$) in platelet value of group C when Days 3, 6 and 9 were compared. It can be concluded that raw honey possesses a sub-acute coagulation stimulatory potentials which is likely to be dose and duration related.

Keywords: *raw honey, prothrombin time, activated partial thromboplastin time, platelet values, albino wistar rats*

INTRODUCTION

Honey is an ancient remedy for treatment of infected wounds, which has recently been 'rediscovered' by the medical profession, particularly where conventional modern therapeutic agents are failing. There are now many published reports describing the effectiveness of honey in rapidly clearing infection from wounds, with no adverse effects to show the healing process; there is also some evidence to suggest that honey may actively promote healing. In laboratory studies, it has been shown to have an antimicrobial action against a broad spectrum of bacteria and fungi.

Honey was used to treat infected wounds as long ago as 2000 years before bacteria were discovered to be the cause of infection. In c.50 AD, Dioscorides described honey as being "good for all rotten and hollow ulcers" (Gunther, 1959). More recently, honey has been reported to have an inhibitory effect to around 60 species of bacteria including aerobes and anaerobes, gram-positives and gram negatives (Molan,1992).An antifungal action has also been observed for some yeasts and species of *Aspergillus* and *penicillium* (Molan,1992),as well as all the common dermatophytes (Brandy *et al.*, 1997). The current prevalence of antibiotic-resistant microbial species has led to a re-evaluation of the therapeutic use of ancient remedies, including honey (Select Committee on Science and Technology, 1998).Coagulation is a complex process by which blood forms clots. It is an important part of hemostasis, the cessation of blood loss from a damaged vessel, wherein a damaged blood vessel wall is covered by a platelet and fibrin-containing clot to stop bleeding and begin repair of the damaged vessel. Disorders of coagulation can lead to an increased risk of bleeding(hemorrhage) or obstructive clotting(thrombosis).coagulation is highly conservative throughout biology; in all mammals, coagulation involves both a cellular(platelet) and a protein (coagulation factor)component. The system in humans has been the most extensively researched and is therefore the best understood. Coagulation begins almost instantly after an injury to the blood vessel has damaged the endothelium lining the vessel. Exposure of the blood to proteins such as tissue factor initiates changes to blood platelets and plasma protein fibrinogen, a clotting factor .Platelets immediately form a plug at the site of injury; this is called primary hemostasis. Secondary hemostasis occurs simultaneously: proteins in the blood plasma, called coagulation factors or clotting factors,

respond in a complex cascade to form fibrin strands, which strengthen the platelet plug (Furie and Furie, 2005)

AIMS

To determine the sub-acute effect of raw honey on Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT) and Platelet values in albino wistar rat.

MATERIALS AND METHODS

HONEY COLLECTION

Two (2) litres of raw honey was purchased from a local honey farmer in Enugu – Ezike, Enugu State.

Five hundred grams (500g) of the honey was dissolved in 100mls of water from which, graded concentrations (625, 1250, 2500 and 5000mg/kg) were constituted. This was stored at room temperature.

EXPERIMENTAL ANIMALS

Thirty (30) 3-4 months old albino Wistar rats both males and females were purchased from an animal farm in Enugu State and left to acclimatize for one week in the Animal Research Unit of University of Nigeria, Enugu Campus under standard condition and fed with clean tap water and super starter feed. Good hygiene was maintained by constant cleaning and removal of faeces and spilled feed from the cages daily.

EXPERIMENTAL DESIGN

The experimental animals were divided into five (5) experimental groups (A, B, C, D and E) with six (6) rats per group. The test groups (B-E) were orally fed with graded doses (625, 1250, 2500, 5000mg/kg) of the constituted honey once daily for nine (9) days. Group A served as control and received no treatment. Two (2) animals were bled from each group after 3, 6 and 9 days through the retro bulbar plexus of the median cantus of the eye.

Four (4) ml of venous blood was aseptically collected. Two (2) ml delivered into 0.25ml trisodium citrate anticoagulant bottle for determination of Prothrombin Time (PT) and Activated

Partial Thromboplastin Time (APTT) and mixed by gentle inversion. The remaining two (2) ml of was delivered into tripotassium ethylene diamine tetraacetic acid (K_3EDTA) anticoagulant bottle for platelet value determination.

All samples were investigated within two (2) hours of collection. Prothrombin time (PT) and activated partial thromboplastin time (APTT) was dertermined as described by Dacie and Lewis (2008) while platelet count was analyzed using haemotology auto-analyzer (Sysmex KX-21N) following the manufacturer's operational guidelines.

DETERMINATION OF PROTHROMBIN TIME (PT)

METHOD: Quick one - stage

PROCEDURE

- 0.1ml of plasma was delivered into a clean test tube.
- 0.1ml of thromboplastin was added and allowed to warm for two minutes in a water bath at 37^0C
- 0.1ml of pre-warmed calcium chloride ($Cacl_2$) at the same temperature was added and the stop watch started immediately .
- The time for clot formation was recorded.

DETERMINATION OF ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT)

METHOD: Proctor and Rapa port

PROCEDURE

- 0.025M $cacl_2$ was prewarmed at 37^oC
- 0.1ml of plasma was dispensed into a clean test tube
- 0.1ml of hemoscann reagent was added and mixed with the plasma
- The mixture was incubated for 3 minutes at 37^oC
- 0.1ml of the $cacl_2$ was added and a stop watch started.
- The time of clot formation was recorded.

DETERMINATION OF PLATELET VALUES

METHOD: Automated Method

Normal range: 150 – 400 $x10^9$ / L

STATISTICAL ANALYSIS

Statistical analysis was carried out using statistical software Graph – Pad-prism. Students''t' – test and one way analysis of variance (ANOVA) were adopted for comparison. The data were expressed as mean $\pm$ standard error (S.E).

$P < 0.05$ was considered significant.

RESULTS

Table 1 shows a comparism of the mean $\pm$ S.E of the Prothrombin Time (PT), Activated Partial Thromboplastin time (APTT) and Platelet values after 3 days of oral raw honey administration with the control group.

There was no statistical significant difference ($P > 0.05$) in the mean $\pm$ S.E in all the parameters investigated when the test groups were compared with the control on Day 3.

Table 2 shows a comparism of the mean $\pm$ S.E of the Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT) and platelet values after 6 days of oral raw honey administration with the control group.

There was a statistical significant decrease ($P < 0.05$) in Prothrombin Time $\pm$ S.E mean values in group B when compared with the control group on Day 6.

Table 3 shows a comparism of the mean $\pm$ S.E of the Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT) and Platelet values after 9 days of oral raw honey administration with the control group.

There was no statistical significant difference ($P >0.05$) in the mean $\pm$ S.E of the Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT) and Platelet values when compared with the control group on Day 9.

Table 4 shows a one-way analysis variance (ANOVA) and comparism of Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT) and Platelet count mean (SEM) values between the different duration of exposure (days) in the test groups.

There was no exposure related statistical significant difference (P>0.05) in test groups B,D,E in Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) when Days 3,6,9 were compared. However, there was a time related significant difference (P<0.05) in platelet value of Group C when Days 3, 6 and 9 were compared.

Table 1: Comparison of the mean ±S.E of the PT, APTT and Platelet values after 3 days of oral raw honey administration with the control group

Parameter	Group A (0mg/kg)	Group B (625mg/kg)	Group C (1250mg/kg)	Group D (2500mg/kg)	Group E (5000mg/kg)
PT (sec)	14.70±2.6	18.50±5.50	16.65±3.35	22.90±8.90	13.50±0.50
APTT (Sec)	36.15±6.15	45.00±5.00	35.00±5.00	30.00±2.00	28.50±3.50
PLATELET ($x10^9$)	761.50±74.50	799.00±195.00	752.00±21.00	640.00±162.00	738.00±23.00

Table 2: Comparison of the mean ±S.E of the PT, APTT and Platelet values after 6 days of oral raw honey administration with the control group

Parameter	Group A (0mg/kg)	Group B (625mg/kg)	Group C (1250mg/kg)	Group D (2500mg/kg)	Group E (5000mg/kg)
PT (Sec)	14.00±5.00	**7.00±1.00***	9.50±3.50	9.00±4.00	9.50±2.50
APTT (Sec)	40.00±5.00	50.50±1.50	36.50±9.50	31.50±0.50	24.00±1.00
PLATELET ($x10^9$)	984.50±90.50	1088.00±460.50	823.50±21.50	690.00±113.00	763.00±28.00

Key:

*compared with the control group * P<0.05

Table 3: Comparison of the mean ±S.E of the PT, APTT and Platelet values after 9 days of oral raw honey administration with the control group

Parameter	Group A (0mg/kg)	Group B (625mg/kg)	Group C (1250mg/kg)	Group D (2500mg/kg)	Group E (5000mg/kg)
PT (sec)	17.50±7.50	17.00±1.00	14.50±2.50	16.50±3.50	14.50±2.50
APTT (sec)	40.00±3.00	45.00±5.00	40.00±4.00	44.50±15.00	38.00±3.00
PLATELET ($x10^9$/L)	1347.00±770.00	678.50±22.50	516.50±40.50	668.50±60.50	706.00±149.00

TABLE 4: Shows one-way analysis of variance (ANOVA) and comparison of Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT) and Platelet Count mean (S.E.M) values between the different duration of exposure (days) in the test groups

PT(Sec)				
Group	D 3	D 6	D 9	P-value
B	18.50±5.50	7.00±1.00	17.00±1.00	p>0.05
C	16.65±3.35	9.50±3.50	14.50±2.50	p>0.05
D	22.90±8.90	9.00±4.00	16.50±3.50	p>0.05
E	13.50±0.50	9.50±2.50	14.50±2.50	p>0.05
p-value	p>0.05	p>0.05	p>0.05	p>0.05
APTT(Sec)				
B	45.00±5.00	50.50±1.50	45.00±5.00	p>0.05
C	35.00±5.00	36.50±9.50	40.00±4.00	P>0.05
D	30.00±2.00	31.50±0.50	44.50±15.00	p>0.05
E	28.50±3.50	24.00±1.00	38.00±3.00	p>0.05
p-value	p>0.05	p>0.05	p>0.05	p>0.05
PLATELT ($x10^9$/L)				
B	799.00±195.00	1088.00±460.50	678.50±22.50	p>0.05
C	752.00±21.00	823.50±21.50	516.50±40.50	**P<0.05**
D	640.00±162.00	690.00±113.00	668.50±60.50	p>0.05
E	738.00±23.00	763.00±28.00	706.00±149.00	p>0.05
p-value	p>0.05	p>0.05	p>0.05	p>0.05

DISCUSSION

As with all nutritive sweeteners, honey is mostly sugars and contains only traces of vitamins or minerals. Honey also contains tiny amounts of several compounds thought to function as

antioxidants, including chrysin, pinobanksin, vitamin C, catalase and pinocembrin (Martos *et al.*, 2000; Gheldof, 2002).

There was no statistical significant difference ($P > 0.05$) recorded in all the parameters investigated among the test groups when compared with the control group on Day 3 of oral raw honey administration. The most probable explanation might be that the raw honey might not have any coagulative potentials on the parameters investigated in the experimental groups in the peripheral system directly, or neither did the raw honey have any stimulating effect on the liver where most of the coagulation factors or co-factors are synthesized. This was further elucidated by a no statistical significant difference ($P > 0.05$) in the platelet count which play a major role in the initiation of coagulation process in particular and haemostasis in general.Hence, one might be tempted to suggest that raw honey does not have any thrombopoietic stimulatory effect, though this is subject to further investigation.

However, group B (625mg/kg) revealed a statistical significant decrease ($P < 0.05$) in Prothrombin Time (PT) when compared with the control group on Day 6. The most probable explanation could be that the raw honey, at lower concentration is likely to have either a stimulatory effect on the coagulation factors / proteins or an inhibitory effect on the synthesis of coagulation inhibitory factors at lower doses as was elucidated by a marginal non statistical significant increase ($P>0.05$) with increase in dosage. There was no previous literature to the best of our knowledge for comparison. Hence one might be tempted to suggest that raw honey could be indicated for the treatment of bleeding disorders and other related haemostatic abnormalities.

In addition, no statistical significant difference ($P>0.05$) was recorded on day 9 when all the parameters investigated among the test groups were compared with the control group. This could be attributable to a negative feedback mechanism by the coagulation system either by inhibiting the clotting inhibitory factors or stimulating the coagulation mechanism as earlier mentioned. This was further supported by a none duration – dependent statistical significant difference ($P>0.05$) in the Prothrombin Time (PT) and Activated Partial Thromboplastin Time mean values in the one way analysis of variance (ANOVA). Even though platelet revealed an exposure related statistical difference ($P<0.05$) in group C.

Furthermore, from the above findings it can be suggested that raw honey has a high safety margin and could be used to remedy coagulation and other bleeding disorders. Though, was not evaluated in the present study.

CONCLUSION

It can be concluded that raw honey possesses a sub-acute coagulation stimulatory potentials this is likely to be dose and duration related. However, a chronic study to prove or disprove this is hereby advocated.

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