

## EFFECT OF *Cucurbita Pepo* ON PROTHROMBIN TIME AND PLATELET COUNT IN HEPARIN-INDUCED THROMBOCYTOPENIC ALBINO WISTAR RATS

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### ABSTRACT

We studied the effect of methanolic extract of *Cucurbita pepo* seed and shell on prothrombin time and platelet count in heparin-induced thrombocytopenia in twenty albino wistar rats and in four control rats. After administration of extract of *Cucurbita pepo* seed, results showed a statistically significant difference in PT of Group A ( $38.25 \pm 4.97$  s) compared to CONT ( $34.0 \pm 7.29$  s), Group B ( $32.75 \pm 4.55$  s), and Group C ( $36.50 \pm 3.01$  s) at  $P < 0.05$ . There was statistically significant difference in PC between Group A ( $31.25 \pm 5.10 \times 10^9/L$ ) compared to CONT ( $159.0 \pm 4.21 \times 10^9/L$ ), group B ( $141.75 \pm 4.13 \times 10^9/L$ ) and Group C ( $154.75 \pm 3.43 \times 10^9/L$ ) at  $P < 0.05$ . The result of extract of *Cucurbita pepo* shell showed a statistically significant difference in PT of CONT ( $34.0 \pm 7.29$  s) compared to Group A ( $38.25 \pm 4.97$  s) compared to Group B ( $32.75 \pm 4.55$  s), Group C ( $39.00 \pm 4.08$  s) and Group D ( $46.50 \pm 5.07$ ) at  $P < 0.05$ . There was also statistically significant increase in Group D ( $46.50 \pm 5.07$ ) at  $P < 0.05$  compared to Group B and C respectively. Results showed statistically significant difference in platelet count (PC) between CONT ( $159.0 \pm 4.21 \times 10^9/L$ ) compared with Group B, C and D respectively at  $P > 0.05$ , ( $31.25 \pm 5.10 \times 10^9/L$ ,  $66.50 \pm 8.59 \times 10^9/L$  and  $75.75 \pm 13.55 \times 10^9/L$ ). These results suggest that methanolic extract of *Cucurbita pepo* seed shortens the PT after prolonged by heparin and increased PC after heparin-induced thrombocytopenia, whereas the extract of *cucurbita pepo* seed did not have any positive effect on PT and PC.

**Keywords:** *Curcubita pepo*, Prothrombin Time, Platelet Count, Heparin and Thrombocytopenia.

### INTRODUCTION

*Cucurbita pepo*, family *Cucurbitaceae*, (English name: Pumpkin; Ibo: Anyu; Yoruba: Elegede) is known to be used as human food in Nigeria. It has been used

locally in Eritrea to treat tapeworm and has also been used in other regions of the world to treat the early stages of prostate disorders (FAO, 1995; Matus *et al.*, 1993; Sicilia *et al.*, 2003). The seed has been used in traditional medicine as an antihelmintic agent and supportive treatment in functional disorders of the bladder and for difficulties in urination (Srivastava *et al.*, 1967). Heparin is a potent antithrombotic agent that has been used to prevent and treat venous thrombosis for more than 50 years. Prothrombin Time (PT) is a screening test for the extrinsic clotting system. It detects deficiency or inhibition of clotting factors in the extrinsic system and is the first test in screening for coagulation disorders. It has been demonstrated a strong relationship between heparin concentration in plasma and a percentage change in PT (Schultz *et al.*, 1991). Thus in this present study heparin was used as an anticoagulant, prolonged PT and induced thrombocytopenia. Work by Adepoju and Adebajo (2011) has shown that *Cucurbita pepo* increased the concentration of platelets in wistar albino rats; thus, the aim of this work is to study the effect of *Cucurbita pepo* on PT in relation to platelet count after administration of heparin in albino wistar rats.

## MATERIALS AND METHOD

Twenty-four albino wistar rats weighing between 150-250g were obtained from University of Nigeria, Nsukka, and used in this present study after two weeks of acclimatization. During this experiment, animals were housed in animal cages (Animal House Madonna University, Elele) with a 12-hour light: dark cycle, and allowed free access to tap water and standard rat chow. The experiment was in compliance with our institutional guideline for animal care. Control rats (n=4) received normal rat chow and tap water. Twenty experimental rats received oral administration of 200 mg/kg body weight of heparin 10 minutes before administration of methanolic extract of *Cucurbita pepo*. Experimental animals were divided into 3 groups for methanolic extract of *Cucurbita pepo* seed and 3 groups for methanolic extract of *Cucurbita pepo* shell.

## PREPARATION OF EXTRACT

*Cucurbita pepo* pumpkins were purchased from a local market in Elele, Rivers State, and were peeled to collect the bark and cut open to collect the seeds. The seeds and barks were sundried. The dried samples were grinded respectively into coarse form with an electric grinder. 100 mg of each grounded sample was separately mixed with 500 ml of methanol, and was placed in a

mechanical checker for 24 hours, after which it was sieved using a clean handkerchief. The filtrate was concentrated using a rotator evaporator and then concentrated in an oven. It was stored in a refrigerator at 4°C. Experimental animals were divided into 5 groups and received oral administration of methanolic extract of *Cucurbita pepo* seed and bark using gavage. Group A received 200 mg/kg of heparin only. Group B received 200 mg/kg of heparin plus 500 mg/kg of methanolic extract of *Cucurbita pepo* seed. Group C received 200 mg/kg of heparin plus 1000mg/kg of methanolic extract of *Cucurbita pepo* seed. Test was conducted to determine LD<sub>50</sub> before administration; animals were administered 2000 mg/kg of methanolic extract of *Cucurbita pepo* of both seed and bark with recording death. In the administration of methanolic extract of *Cucurbita pepo* bark, Group D and E received 200 mg/kg of heparin plus 500 mg/kg and 1000 mg/kg of methanolic extract of *Cucurbita pepo* bark respectively.

At the end of 48 hours experiment, the 24 animals were sacrificed under chloroform anesthesia, and 5 ml of blood was collected via cardiac puncture, and were kept in labeled EDTA bottle.

## **ANALYTICAL METHOD**

### **Determination of Prothrombin Time**

Blood was drawn into a test tube containing liquid sodium citrate, which acts as an anticoagulant by binding the calcium in a sample. The blood was mixed, and then centrifuged to separate blood cells from the plasma. The plasma was analyzed using an automated analyzing machine at 37°C. An excess of calcium was added to reverse the effect of the citrate, which enables the blood sample to clot. For accurate measurement the proportion of blood to citrate needs to be fixed. For prothrombin time test, the appropriate sample is sodium citrate tube, which is a liquid anticoagulant. Tissue factor (factor III) is added, and the time the sample takes to clot is measured optically.

### **Determination of Platelet Count**

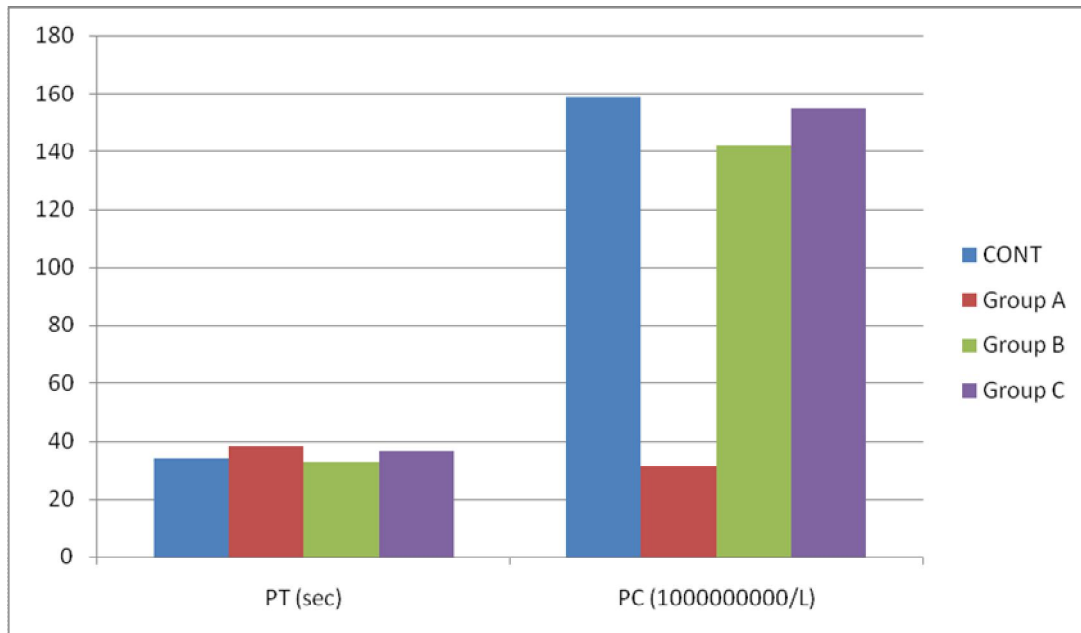
Platelet count was estimated by automated counter by counting the average number of platelets seen per 100x oil immersion field in the monolayer. In general, 10 oil immersion fields are counted and the results averaged (this accounts for uneven dispersal of platelets in the smear). Then the following formula is applied: Estimated platelet count/ $\mu$ L = average count in 10 fields x 15,000.

## STATISTICAL ANALYSIS

The results obtained from this study were analyzed using the Statistical Package for Social Sciences (SPSS) version 18.0 for windows and expressed as mean  $\pm$  SEM. Analysis of Variance (ANOVA) with Tukey test for multiple comparisons was used to analyze prothrombin time and platelet count data. Statistical significance was defined as  $P < 0.05$ .

## RESULTS

In figure 1. showed results of the groups that received methanolic extract of *Cucurbita pepo* seed extract. Results showed a statistically significant difference in PT of Group A ( $38.25 \pm 4.97$  s) compared to CONT ( $34.0 \pm 7.29$  s), Group B ( $32.75 \pm 4.55$  s), and Group C ( $36.50 \pm 3.01$  s) at  $P < 0.05$ . There was no statistically significant difference between the CONT ( $34.0 \pm 7.29$  s) compared with Group B ( $32.75 \pm 4.55$  s), and Group C ( $36.50 \pm 3.01$  s) at  $P > 0.05$ . Results showed no statistically significant difference in platelet count (PC) between CONT ( $159.0 \pm 4.21 \times 10^9/L$ ) compared with Group B and C respectively at  $P > 0.05$  ( $141.75 \pm 4.13 \times 10^9/L$  and  $154.75 \pm 3.43 \times 10^9/L$ ). However, there was statistically significant difference in PC between Group A ( $31.25 \pm 5.10 \times 10^9/L$ ) compared to CONT ( $159.0 \pm 4.21 \times 10^9/L$ ), Group B ( $141.75 \pm 4.13 \times 10^9/L$ ) and Group C ( $154.75 \pm 3.43 \times 10^9/L$ ) at  $P < 0.05$ .



**Figure 1: Showed the Effect of Methanolic Extract of *Cucurbita p.* Seed on Prothrombin Time and Platelet Count of Heparin-induced Thrombocytopenic Rats**

Figure 2 shows results of the groups that received methanolic extract *Cucurbita pepo* shell. Results showed a statistically significant difference in PT of CONT ( $34.0 \pm 7.29$  s) compared to Group A ( $38.25 \pm 4.97$  s) compared to Group B ( $32.75 \pm 4.55$  s), Group C ( $39.00 \pm 4.08$  s) and Group D ( $46.50 \pm 5.07$ ) at  $P < 0.05$ . There was also statistically significant increase in Group D ( $46.50 \pm 5.07$ ) at  $P < 0.05$  compared to Group B and C respectively. Results showed statistically significant difference in platelet count (PC) between CONT ( $159.0 \pm 4.21 \times 10^9/L$ ) compared with Group B, C and D respectively at  $P > 0.05$ , ( $31.25 \pm 5.10 \times 10^9/L$ ,  $66.50 \pm 8.59 \times 10^9/L$  and  $75.75 \pm 13.55 \times 10^9/L$ ).

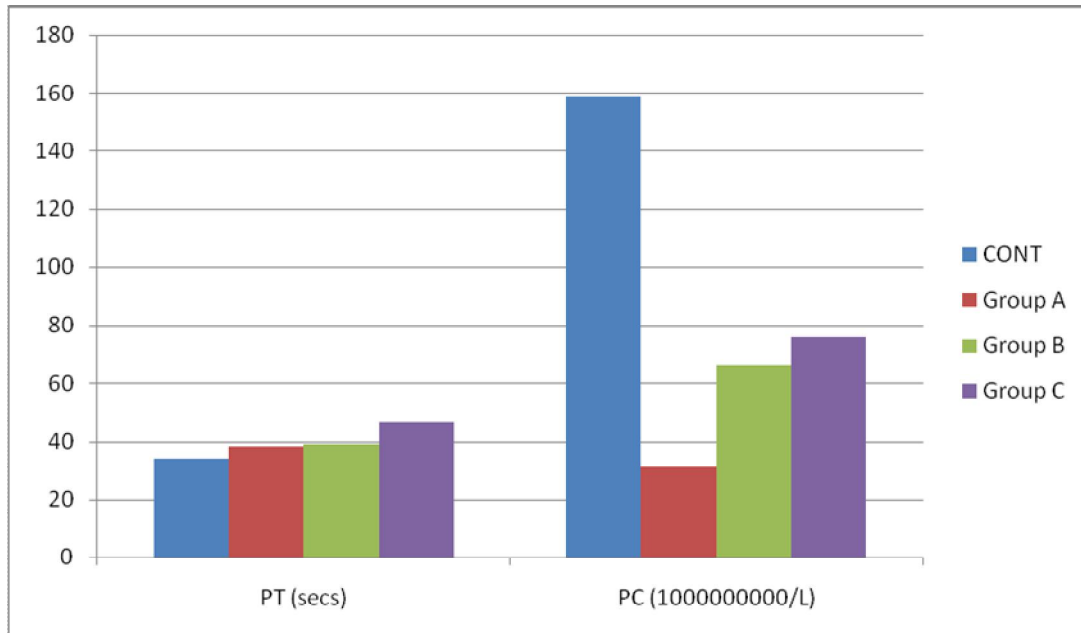


Figure 2: Showed the Effect of Methanolic Extract of *Cucurbita p. Shell* on Prothrombin Time and Platelet Count of Heparin-Induced Thrombocytopenic Rats

## DISCUSSION

Heparin-induced thrombocytopenia is defined as a decrease in platelet count during or shortly following exposure to heparin (Warkentin, 2004). The mechanism underlying heparin-induced thrombocytopenia is an immune response (Arepally and Cines, 2002; Reilly, 2003). Patients who develop heparin-induced thrombocytopenia produce immunoglobulin G antibody (Ig G) against the heparin-platelet factor 4 complex, which binds to complex on platelet surface through the Fab region (Kelton *et al.*, 1994). A strong relationship has been demonstrated between heparin concentration in plasma and a percentage change in PT (Schultz *et al.*, 1991). Since Prothrombin Time (PT) is the first test in screening for coagulation disorders; thus this study investigates the effect of *Cucurbita pepo* effect on PT and PC after 2 hours administration 20mg/kg of unfractionated heparin.

Results in figure 1 showed that methanolic extract of *Cucurbita pepo* seed did not show significant prolongation of PT, whilst the shell in a dose-dependent manner prolonged PT. PT is reported to be associated with extrinsic pathway (Joshua and Charles, 2008). Reports have shown that prolonged PT was

associated with vitamin K deficiency and can be corrected by administration of the vitamin (Bay *et al.*, 2005).

PC functions in blood coagulation/is very important in blood coagulation and heparin in this present study significantly reduced PC as expected. Gastrointestinal bleeding in Sudan is indicated by prolongation of PT and reduced PC (Munsoor *et al.*, 2011). The result in figure 1 showed that a relationship exists between PT and PC as indicated by the effect of seed extract on PT (shortened PT and increased PC after *Cucurbita pepo* seed extract similar with the control) in contrast to heparin-induced rats which showed a significant prolongation of PT and a significant reduction of PC.

The *cucurbita pepo* seed may have modulated the production of Ig G to increase the PC, since it had been reported that *Cucurbita pepo* has immunomodulatory property (Jafarian *et al.*, 2012) and enhanced the production of vitamin K to shorten the PT. Conversely, the *Cucurbita pepo* shell may have a slow effect or time- dependent effect on PT and PC as observed in the study. It can be concluded that *Cucurbita pepo* seed extract restored PT and PC in a dose-dependent manner after heparin-induced thrombocytopenia.

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