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Deteriorating Hemostatic Functions of Adult Female Wistar Rats Mediated by Activities of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) – Piroxicam and Vitamin E

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Summary: The status of hemostatic parameters, are useful physiological markers of organ and tissue damage and dysfunction. This study investigated the effect of Piroxicam on some hemostatic parameters of albino Wistar rats. Twenty-four (24) female albino Wistar rats were used for this study, they were randomly divided into four (4) groups of six (6) rats each. Group A served as control, Group B and C were rats treated with 0.1 mg/kg and 0.2 mg/kg piroxicam while Group D served as 0.2 mg/kg piroxicam treated rats administered with Vitamin E. The experiment lasted for a period of 4 weeks, after which the rats were euthanized. Blood sample was collected for measurement of bleeding time, clotting time, fibrinogen level and platelets count. One-way ANOVA was used to compare, means and a $p<0.05$ was considered significant. **Result:** Data generated showed that Piroxicam significantly ($p<0.05$) decreased the clotting time, platelets count and fibrinogen level. Piroxicam also significantly ($p<0.05$) increased the bleeding time level of the rats. Co-administration of Vitamin E significantly ($p<0.05$) increased the bleeding time, it also significantly decreased the clotting time, fibrinogen level and platelets counts. **Conclusion:** This study therefore shows that Piroxicam impairs hemostasis while Vitamin E administration further enhances the activities of Piroxicam on hemostatic parameters.

Keywords*:* Hemostasis, NSAIDs, Piroxicam, Bleeding Time, Clotting Time, Fibrinogen.

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INTRODUCTION

Some synthetic agents including non-steroidal anti-inflammatory drugs have been found valuable in management of heart attacks and other complications of cardiovascular disorders (Handoll, Farrar, McBirnie et al., 2002). Non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen, indomethacin, aspirin, and naproxen are used as analgesics and anti-inflammatory agents and produce their therapeutic effects through the inhibition of prostaglandin synthesis (Klaassen, 2001). The drugs (NSAIDs) provide effective relief of pain and inflammation caused by a variety of clinical disorders, including arthritis, nonarthritic musculoskeletal conditions, dysmenorrhea, and headache (Taubert, 2008; Simmons, Botting, and Hla, 2004). Among the NSAIDS used in dentistry, piroxicam, a non-selective COX inhibitor, has been extensively studied (Barroso, Lima, Guzzo et al., 2006; Graziani, Corsi, Fornai et al., 2005). Piroxicam is a strong inhibitor of cyclooxygenase-2 (COX-2). It is known as a non-steroidal anti-inflammatory drug (NSAID) that has an analgesic effect. The effectiveness of piroxicam as an anti-inflammatory agent is likely due to the inhibition of PGE synthesis (Starek and Krzek, 2009).

Haematological parameters are a very important diagnostic tool and used as a routine clinical evaluation of health as well as an essential factor for surgeons and anaesthetist before initiating any surgical procedure (Saliu et al., 2012). The status of haemostatic parameters, are useful physiological markers of organ and tissue damage and dysfunction, but blood clotting, bleeding time, platelet level and prothrombin level has been used in the past to assess both the intrinsic and extrinsic pathways of coagulation cascade (Laffan and Bradshaw, 2000). Preoperative haemostasis evaluation has always been a significant factor in the minds of surgeons and anaesthetists before taking any case for surgery (Bharadwaj, 2001).

Evaluation of blood groups, bleeding and clotting time are the most important and initial haematological parameters investigated. Bleeding Time (BT) and Clotting Time (CT), are significant to study clinically. Bleeding time is mainly a test to assess the platelet adhesion and aggregating. It is found to be prolonged significantly in platelet defects, either congenital or

acquired (Issitt, 1985). While the time interval between the blood vessels puncture and fibrin threads formation is called clotting time (Franchini, Capra, Targher et al., 2007). These two parameters, although considered by many as obsolete provides enough information about platelet activation and function and may serve as a means of accessing clinical conditions such as disseminated intravascular coagulation (DIC), von Willebrand Disease (vWD) and thrombocytopenia. With increasing prescription of NSAIDs by physicians, and recent documentation of high haemostatic disorders prevalence, this study therefore aim to assess the effect of Piroxicam on some haemostasis parameters.

MATERIALS AND METHODS

Piroxicam was sold under the trade name Dolonex DT® and Vitamin E (α-tocopherol) tablets were purchased from the local chemist shop in Abraka, Delta State, Nigeria.

Ethical Consideration

Prior to the commencement of the study, ethical approval was sought and obtained (FBS/PHS/RBC/013) from the Research and Bio-ethical Committee of the Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka, Nigeria.

Study Design

The study is experimental in nature. A total of twenty-four (24), 12±2 weeks old female albino Wistar adult rats weighing between 180 ± 20 g from the breeding colony in the Animal House of the Department of Physiology, Delta State University, Abraka were used for the study. The animals were housed in wooden cages with wood shavings as bedding and allowed two (2) weeks for acclimatization. Subsequently, the rats were weighed and randomly divided into four groups (n = 6). Group A (Control) were fed with normal rat chow and water, Group B treated with 0.1mg/kg Piroxicam, Group C treated with 0.2 mg/kg Piroxicam, and Group D treated with 0.2 mg/kg Piroxicam + 150 mg/k Vit E. Freshly dissolved tablets of Piroxicam and Vitamin E were administered through oro-gastric cannula once daily for twenty-eight (28) days.

Sample Collection

At the end of 28 days of treatment, the bleeding and clotting time were assessed in rats restrained manually, after which the animals were euthanized via cervical dislocation. Two (2) mls of blood samples were collected via the common carotid into anti-coagulant free containers. collected from the retro-bulbar plexus of the medial canthus and amputating 5 mm of the tail tip with a scalpel blade.

Bleeding Time determination

Bleeding Time was determined using a modified Duke method (Ochei and Kolhatkar, 2000). A skin puncture was made quickly using disposable lancet and the stopwatch was started as soon as bleeding started. The puncture was dabbed with blotting paper every 15 seconds until there was absence of blood stains on the blotting paper. Bleeding time was recorded as the time when there was stoppage of blood flow from the puncture.

Clotting time estimation

Clotting time was determined using capillary glass tube method. A standard incision was made in the skin of the ear and the blood was taken into a capillary glass tube and the time of collection was noted. Pieces of capillary glass were broken from one end at every thirty seconds and the appearance of fibrin threads was used as the end point and the time was noted in seconds (Harris et al., 1956).

Platelet Count Determination

Platelet concentration was assessed under 40x magnification, scan to ensure even distribution. Platelets were counted in all twenty-five small squares within the large center square. Platelets appear greenish, not refractile. Count cells starting in the upper left of the large middle square. Continue counting to the right hand square, drop down to the next row; continue counting in this fashion until the total area in that middle squares) have been counted. Count all cells that touch any of the upper and left lines, do not count any cell that touches a lower or right line. Count both sides of the hemacytometer and take the average.

Fibrinogen analysis

Plasma fibrinogen concentration was determined as defined by the clot weight method of Ingram (1961). Blood was first collected into sample vials containing 3.2% sodium citrate in the ratio 1:9 with blood. Blood plasma was obtained by centrifuging blood in a stopped vial at 1000 g for 10 min. 0.2 ml of the test plasma was put into a test tube and incubated in a water bath for 3 min at 37°C. 0.2 ml of thrombin time-reagent was added to test plasma, mixed and the clot formed harvested with a wooden applicator stick. The resulting clot was transferred into a tube containing acetone to dry and harden for about 10 min; the acetone was decanted and the clot placed on a filter paper for the remaining acetone to evaporate. The clot was then recovered and weighed. The process of fibrinogen concentration determination was completed within 3 h of blood collection. Thus, fibrinogen concentration of citrated plasma in mg/dl equals clot weight (mg) divided by plasma volume (dl).

Statistical Analysis

Data were analyzed for mean, and standard deviation. Comparison for significance between the control and experimental group were analysed using One-Way Analysis of Variance (ANOVA) with Scheffe's Post

Hoc test. The level of significance for all experiment was $p< 0.05$.

RESULTS

Effect of Piroxicam on Bleeding Time

It was observed that bleeding time increased in a dose dependent manner following administration of graded doses of piroxicam. The mean bleeding time in control group was 7.50 ± 1.11 mins while those of group B and C were 8.33 ± 1.11 mins and 9.17 ± 1.00 mins respectively. The mean bleeding time of rats in Group D was 10.00 ± 1.16 mins. These changes in bleeding time due to the effect of Piroxicam were not significant in this experiment.

Effect of Piroxicam on Clotting Time

The mean clotting time in the control group was 127.00 ± 23.76 s while those of group B, C and D were 90.00 ± 13.40, 71.01 ± 6.32 and 60.00 ± 5.22 s respectively as shown in Fig 2. Piroxicam administration caused a dose dependent decrease in the clotting time of the albino Wistar rats with significance ($p<0.05$) recorded in a higher dose 0.2mg/Kg of Piroxicam. Further decrease in clotting time was

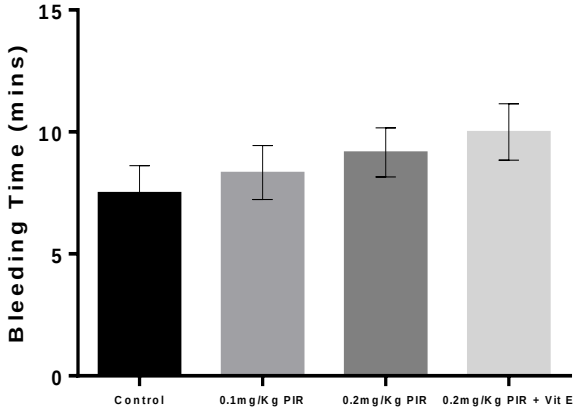

Fig 1 Effect of Piroxicam on Bleeding Time

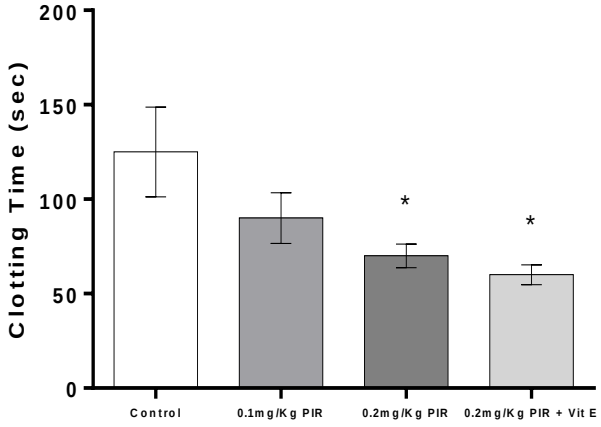

Fig 2 Effect of Piroxicam on Clotting time. *:significance ($p<0.05$) when compared to control;

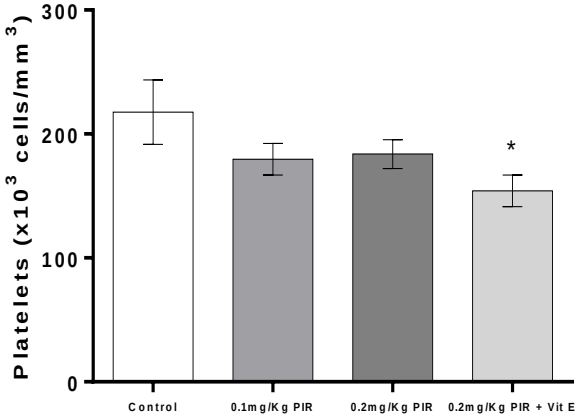

Fig 3 Effect of Piroxicam on Platelet count
*:significance ($p<0.05$) when compared to control;

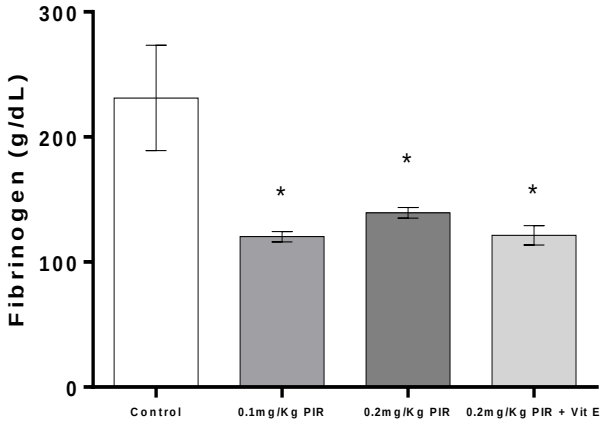

Fig 4 Effect of Piroxicam on Fibrinogen level. (n=6)
*: significance ($p<0.05$) when compared to control;

observed following administration of Vitamin E to the 0.2mg/Kg Piroxicam treated rats. This Vitamin E effect on the clotting time was also significant ($p<0.05$) when compared to control.

Effect of Piroxicam on Platelet count

The mean platelet concentration in the control group was 217.67 ± 25.90 $x10^3/mm^3$ while those of group B, C and D were 179.67 ± 12.82, 183.83 ± 11.69 and 154.16 ± 12.74 $x10^3/mm^3$ respectively as shown in Fig 3. Piroxicam decreased the platelets count, and the co-administration of Vitamin E enhanced the effect of piroxicam by showing significant ($p<0.05$) decrease in platelets count.

Effect of Piroxicam on Fibrinogen level.

The mean plasma fibrinogen in control group was 231.17 ± 42.40 g/dl while those of group B and C were 120.33 ± 4.15 and 139.33 ± 4.32 g/dl respectively. The mean fibrinogen of rats in Group D was 121.33 ± 7.67 g/dl. It was observed that piroxicam caused significant ($p<0.05$) decrease in fibrinogen level when compared to control. It was observed that co-administration of Vitamin E did little in changing the effect of 0.2 mg/kg of piroxicam on fibrinogen level as fibrinogen

concentration remained at the range of fibrinogen level of piroxicam treated rats, significance was also recorded when compared to control.

DISCUSSION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the most commonly prescribed and self-administered drugs (Kaur, Singh, Bassi et al., 2015). However, up to 50% of patients taking these medications experience some variety of alteration in hemostasis (Wolfe 1999). This study showed the effect of piroxicam on the bleeding time, clotting time, platelets count and fibrinogen level. It was observed that piroxicam increased the bleeding time especially at higher doses. The increase in bleeding time may likely be as a result of decrease in platelets count reported in this study.

Cronberg et al. (2004) showed the effect of other NSAIDs on platelets function show similarities with piroxicam induced effect in this study. Ibuprofen inhibits platelet aggregation and thromboxane A2 synthesis. Ha et al (1999) showed that Ibuprofen inhibited platelet aggregation at 1.5, 3, and 6 hours after a single 800-mg oral dose. A single dose of ibuprofen between 300 mg and 900 mg blocked platelet aggregation 2 hours after administration; however, the effect was lost within twenty-four (24) hours (Mcintyre et al., 2008). Effect of Indomethacin, which is as potent inhibitor of platelet thromboxane production occurred within 2 hours of drug ingestion and persist for up to 8 hours. (Cronberg et al., 2004). Since most NSAIDs have similar properties, it is therefore understandable that decrease in the platelets count in this study could be as result of piroxicam inhibiting effect of platelets production and thus reducing its aggregating strength.

This was further confirmed by the increase in bleeding time following administration of graded doses of piroxicam. The normal BT by Duke's filter paper method is usually in the range of 1–5 min (Pal and Pal, 2010). These values were well exceeded due to the anti-thrombocytes activities of piroxicam. Data from this study was also in accordance with previous studies of other NSAIDs effect on bleeding time. Ibuprofen, Diflunisal (Green et al., 2001), Indomethacin (Taivainen, et al., 2009), and Ketorolac (Conrad, Fagan, Mackie et al., 2008) diclofenac (Taivainen, et al., 2009) all caused prolongation of bleeding time.

It might be expected that simultaneous inactivation of platelet and endothelial cyclooxygenase by Piroxicam would have deleterious effects on haemostasis by blocking thromboxane A2 and prostacyclin formation. The significant ($p<0.05$) decrease in fibrinogen following piroxicam administration, shows that piroxicam influence over haemostasis is not only platelet dependent. In support to the current finding, Danesh et al. (2005) showed other NSAIDs have a decreasing effect on fibrinogen. In their study, fibrinogen levels were lower in patients taking celecoxib and non-selective NSAIDs but not significantly different in those taking rofecoxib compared to controls.

It was observed that Vitamin E acted as a permissive agent for piroxicam as it enhanced the effect of piroxicam on the various haemostatic parameters of this study. Vitamin E further reduced the platelet count and fibrinogen level with significance ($p<0.05$). The possible explanation for the platelet incorporation of vitamin E both in vitro and in vivo leads to dose-dependent inhibition of platelet aggregation (Freedman et al., 1996). Another possible mechanism of Vitamin E inhibition on haemostatic function is the antagonistic interaction with Vitamin K, a known contributor to coagulation (Dowd and Zheng, 1995).

Piroxicam had similar effect to other NSAIDs on haemostatic parameters by decreasing the clotting time, platelets count and fibrinogen level whilst increasing bleeding time. With the widespread use of NSAIDs (Piroxicam) for a variety of clinical indications, physicians must be aware of their potential to cause bleeding complications. The chronic use of even very low dose Piroxicam can produce maximal inhibition of platelet function and primary haemostasis.

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