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Mitigations in Selected Hemostatic Parameters in Administration of Graded Doses of Dexamethasone and Its Blockers on Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author EKN designed the study, Author EIE performed the statistical analysis and wrote the protocol, author ENE performed the literature searched, and Author MOO managed the bench work. All authors read and approved the final manuscript.

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ABSTRACT

Clinical trials have shown that an even newer compound, Dexamethasone (Dex.), might be more potent and less likely to cause side-effects than any other known corticosteroid medication. This study investigated the possible changes in selected hemostatic parameters [clotting time, bleeding time, platelets count and fibrinogen level] in albino wistar rats, following administration of graded doses of Dex. Forty-two (42) male albino Wistar rats were randomly grouped into seven (7) of six (6) rats each. With Group A receiving normal diets (Control), Groups B – G were respectively given 0.1 mg/Kg of Dex, 0.3 mg/Kg of Dex, 0.1 mg/Kg of Dex + 33 mg/Kg of Ketokonazol (Keto), 0.3 mg/Kg of Dex + 33 mg/Kg of Keto, 0.1 mg/Kg of Dex + Vitamin (Vit.) E and 0.1 mg/Kg of Dex. + Vit E. following two-weeks administration period, rats were euthanized and blood samples obtained by

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cardiac puncture under diethyl ether to determine hemostatic parameters. One-way Analysis of Variance (ANOVA) revealed that Dex, in higher doses decreased bleeding and clotting time, with significant increase ($p < .05$) in platelet and fibrinogen counts. However, Vitamin E and Ketoconazole treatment reversed this effect by increasing bleeding and clotting time withdecreased fibrinogen levels and platelet count. Thus, study demonstrates that Dexamethasone affect hemostasis by significantly decreasing bleeding and clotting times, and also enhances hemostatic function with significant increase in fibrinogen and platelet count which may predispose to thrombotic disorders.

Keywords: Dexamethasone; hemostasis; ketoconazole; vitamin E.

1. INTRODUCTION

Hemostasis is an intricate interplay of a combination of cellular and biochemical events that act together to keep blood in the liquid state within veins and arteries, preventing blood loss following injury [1]. Various systems have been implicated in hemostasis, including the vascular system, coagulation system, fibrinolytic system, etc [2]. These systems work together when blood vessel endothelial linings are affected by physical, mechanical, or chemical trauma to produce clots. These clots stop bleeding and are separated through the fibrinolytic process.

In clinical practice, Hematological parameters [clotting time, bleeding time, platelets count and fibrinogen level] are linked with health indices, and are of diagnostic significance in routine evaluation of an individual's state of health [3]. Experimental studies have shown that glucocorticoid use can significantly increase the levels of clotting factors and fibrinogen [4], with a risk of venous thromboembolism [5]. Glucocorticoids have been found to decrease fibrinolytic activity as indicated by increase in fibrinogen levels [6]. The decreased fibrinolysis is attributed to increased PAI-I formation which inhibits plasmin synthesis and thus prevents the breakdown of fibrin and fibrinogen.

Dexamethasone, a synthetic glucocorticoid has been shown to have 50 times more tendency to conjunct with glucocorticoid receptor than cortisol[7]. It is used in the treatment of many conditions, including rheumatic problems, a number of skin diseases, severe allergies, asthma, chronic obstructive lung disease, croup, brain swelling, and along with antibiotics in tuberculosis.

Altered levels of hemostatic factors have been reported in patients receiving exogenous glucocorticoids [8]. Long term exposure to glucocorticoids has also been investigated to cause dramatic changes in hemostatic parameters [9]. To this point, bleeding and clotting time parameters tend to provide enough information about platelet aggregation, adhesion, activation, function, and serve as a means of accessing several conditions such as thrombocytopenia, von Willebrand disease, liver failure, etc [10].

The aim of this study was to determine the effect of Dexamethasone (Dex.) on some hemostatic parameters, using albino Wistar rats as experimental models. Specifically, study assessed the effect of Dex. on total body weight, investigated the effect of Dex. and glucocorticoid receptor blockers on bleeding and clotting times, and on fibrinogen levels. Study also ascertained the effect(s) of Dex. and glucocorticoid receptor blockers on platelets counts

2. MATERIALS AND METHODS

2.1 Scope of Study

Animal models, specifically the albino wistar rats, instead of human subjects were used for the study. These rats are biologically similar to humans and are susceptible to many of the same health problems in humans.

2.2 Study Design

Experimental in nature, with forty-two (42) rats randomly divided into seven (7) groups of six rats each (n=6);

GROUP A: Control rats fed with chow and clean drinking water
GROUP B: Treated with 0.1 mg/kg/day of Dex
GROUP C: Treated with 0.3 mg/kg/day of Dex.
GROUP D: Treated with 0.1 mg/kg/day of Dex and 33 mg/kg of Ketoconazole
GROUP E: Treated with 0.3 mg/kg/day of Dex and 33 mg/kg of Ketoconazole
GROUP F: Treated with 0.1 mg/kg/day of Dex and 150 mg/kg of Vitamin E

GROUP G: Treated with 0.3 mg/kg/day of Dex and 150 mg/kg body weight of Vitamin E

2.3 Procedure

Rats were treated with a synthetic glucocorticoid (Dexamethasone), Vitamin E and Ketoconazole for a period of fourteen (14) days

Weight measurement: The body weight change of the albino Wistar rats was measured using an electronic weighing balance.

Vitamin E. administration: Vitamin E (α-tocopherol) tablet manufactured by Roche Nigeria Ltd was purchased from an accredited dealer GPS pharmacy. The tablets were administered orally via an oro-gastric cannula daily and at a dose of 150mg/kg body weight.

Ketoconazole Administration: Ketoconazole tablet (anti-blocker) was dissolved in distilled water and administered orally via oro-gastric cannula daily at a dose of 3.3 mg/kg body weight

Dexamethasone administration: Dexamethasone injection was administered at separate doses of 2 mg/kg and 4 mg/kg body weight. It was administered subcutaneously daily for the experimental period of two (2) weeks.

Sample collection: Following period of administration of test substances, rats were anaesthetized with diethyl ether and sacrificed by cervical dislocation. Blood samples were collected via cardiac puncture into heparinized capillary tubes. Hemostatic parameters were subsequently measured.

Measuring the clotting time: Four different test tubes were prepared by placing them in a 37ºC water bath. Blood was collected via syringes into these test tubes and a stop clock was started immediately the test tubes were placed in the water bath. Blood was collected in each of the four (4) test tube of a rat and examined in an interval of thirty seconds. Observation was done before clotting by gentle tilting of the test tubes. The clotting time was then reported as the average of times given by the four test tubes

Measuring the bleeding time: Each rat was pricked at two different spots on the tail with a lancet. Immediately a stopwatch starts recording time. The filter paper was used to wipe blood every 15 seconds; this was repeated every 15 seconds until bleeding stopped completely.

Platelet count: 0.28 ml of filtered ammonium oxalate diluting fluid was measured and dispensed into a small test tube. 0.02ml of well mixed anti coagulated blood was added into the test tube and then mixed thoroughly. The counting chamber was assembled and filled with a well-mixed sample. Counting chamber was then left undisturbed for 20 minutes and was covered with a cover lid to prevent drying of the fluid. The underside of the chamber was dried and placed on the microscope stage. The 10x objective lens was then used to focus the rulings of the grid and bring the central square of the chamber into view. The objective was changed to 40x and then focused on the small platelet, the platelets were seen as small bright fragments and platelets were counted in the small squares

Fibrinogen: Plasma, 0.05ml, was diluted with 5.5 ml of barbitone saline buffer in a test tube and 3.0 ml of the mixture was carefully transferred to a 1 cm cuvette for the test. The remainder of the mixture was decanted into a similar cuvette as a blank. Both cuvettes were placed in the spectrophotometer and the instrument was adjusted to zero absorbance. Of the calcium- thrombin reagent, 0-0.15 ml was added to the contents of the test cuvette and mixed rapidly and carefully to minimize the production of air bubbles. The cuvette was replaced in the spectrophotometer and the programme commenced. The trace was drawn for at least 10 minutes.

2.4 Analytical Approach

Obtained data were represented as Mean ± Standard Error of Mean. One Way Analysis of Variance was used to compare means. Statistical analysis was done using SPSS 21 Software. A p-level < .05 was considered statistically significant.

3. RESULTS

This study was undertaken to show how well some hemostatic parameters can be mitigated in administration of graded doses of Dexamethasone in wistar rats. The studied parameters were age, bleeding and clotting times, as well as platelets and fibrinogencounts.

4. DISCUSSION

This study examined the effect(s) of Dexamethasone on some parameters of hemostasis. Data show that Dexamethasone

decreased bleeding and clotting times, while increasing platelet count and fibrinogen levels. These changes were significant ($p < .05$), especially with rats treated with higher doses of Dexamethasone as compared to control. Shashidhara et al. showed an insignificant increase in platelets count of children infected with dengue fever, following treatment with Dexamethasone [11]. A similar observation of the recovery of the platelet count after a maximum drop, with increasing platelet counts gradually over three days without any intervention, was observed by Kularatne [12].

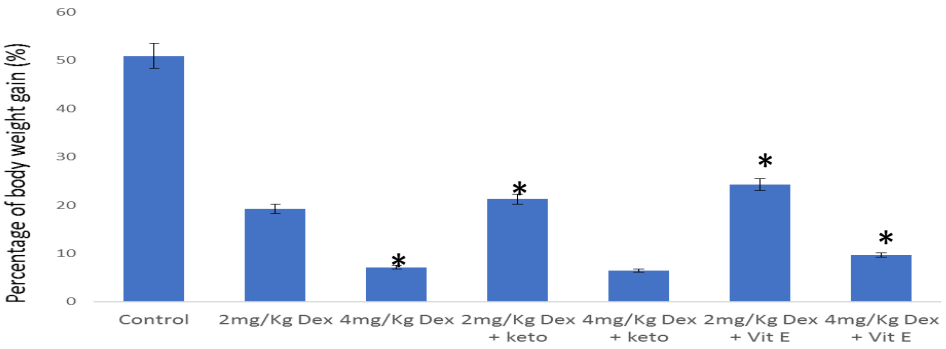

Chart 1. Showing percentage body weight in Dexamethasone treated rats

Dexamethasone caused a significant ($p < .05$) and dose dependent decrease in percentage body weight gain of rats. Here,$p < 0.05$ compared with control group; +$p < 0.05$ compared with 0.2 mg/Kg Piroxicam + Vit E.*

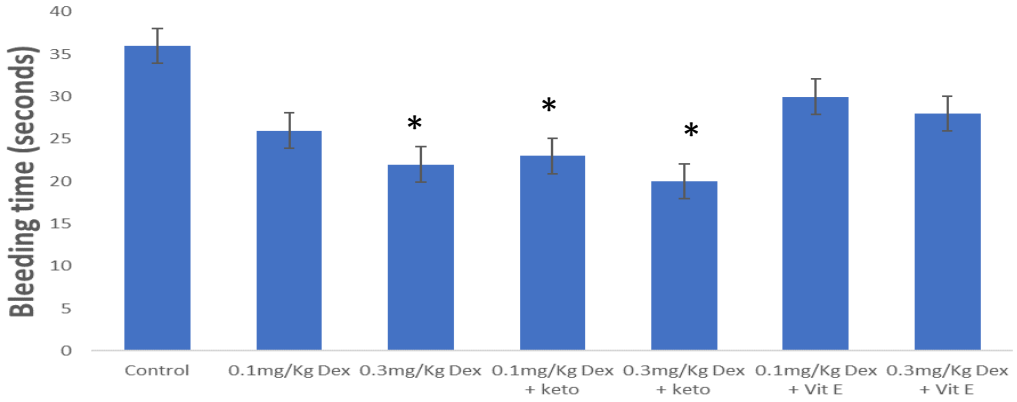

Chart 2. Showing effect of Dexamethasone on bleeding time

**: significance ($p < .05$) when compared to control*

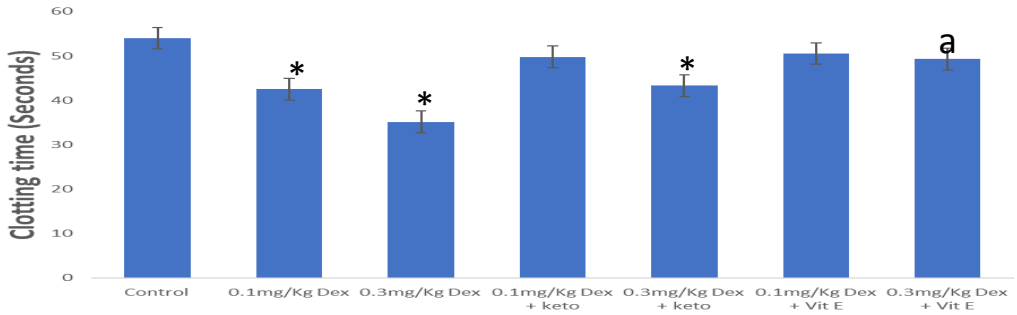

Chart 3. Showing effect of Dexamethasone on clotting time

**:significance ($p<0.05$) when compared to control; a:significance ($p<0.05$) when compared to 0.3 mg/Kg Dexamethasone rat*

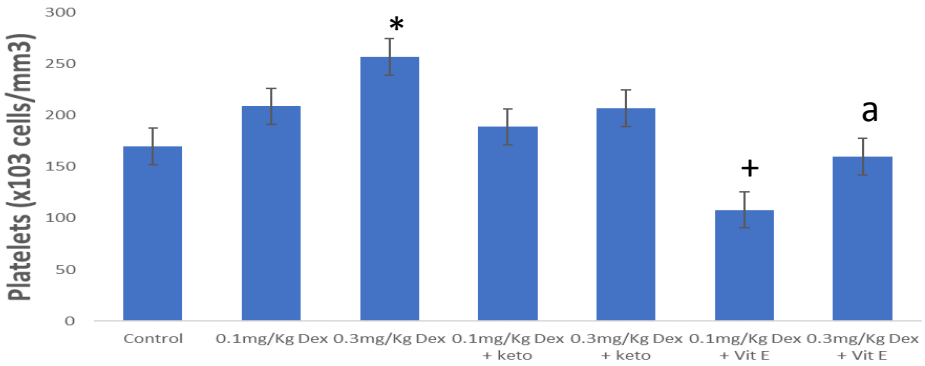

Chart 4. Showing effect of Dexamethasone on platelet count

*In a dose dependent manner, Dexamethasone increased platelets count with significance (p<0.05) recorded in rats treated with higher dose of 0.3 mg/Kg of Dexamethasone. *: significance (p<0.05) when compared to control;*

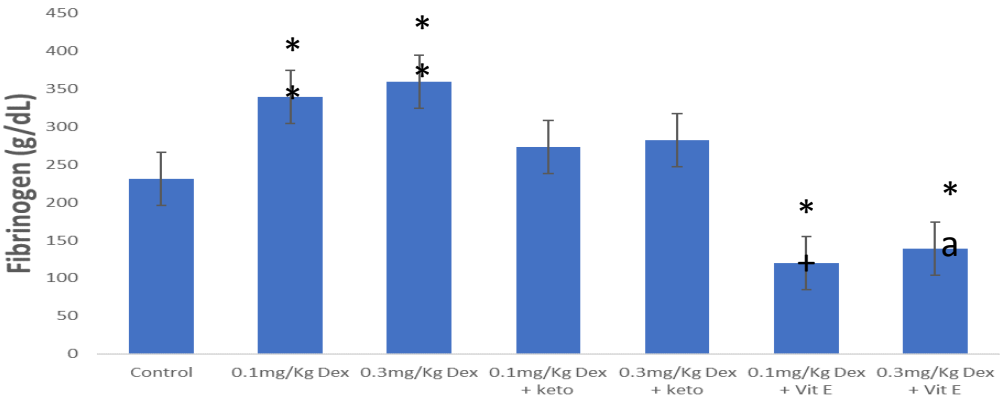

Chart 5. Showing effect of Dexamethasone on fibrinogen level

**: significance (p<0.05) when compared to control;*
+: significance (p<0.05) when compared to 1.3 mg/Kg Dexamethasone rats
a: significance (p<0.05) when compared to 0.3 mg/Kg Dexamethasone rats

The results of this study also support previous studies that found High Dose Dexamethasone (HD-DXM) to produce long-term responses in previously untreated patients suffering from primary immune thrombocytopenia (ITP) previously called idiopathic thrombocytopenic purpura. In a study by Cheng et al. a single course of HD-DXM for adult ITP produced a 50% sustained response of platelet count ≥ 50,000/μL at 6 months after the initial treatment [13-14]. A multi-centre study by Borst et al. found that 59% of previously untreated adult ITP patients obtained a sustained response 2 - 31 months after 1 - 6 cycles of HD-DXM therapy.

Increase in clotting time following Dexamethasone treatment suggests that Dexamethasone could have influence in increasing the synthesis of certain clotting factors including fibrinogen. Fibrinogen, on the other hand, increased markedly after Dexamethasone administration, and significantly ($p<0.05$) higher than control level. Although these results correspond to those found by several investigators (Hogevold et al. [15], they were not confirmed by a study, in which patients with autoimmune and malignant diseases, receiving corticosteroid therapy, were used (Jorgensen et al. [16]. This indicates that the underlying disease condition may interfere with the results and that an animal model may be preferable to study the effect of glucocorticoids per se on blood coagulation. Findings of Morange et al. showed contrasting report that synthetic glucocorticoids decreased fibrinogen level [17].

Dexamethasone treated rats co-administered with Ketoconazole showed increased bleeding and clotting time and decreased fibrinogen level and platelet count. The reason for these

observed changes could be attributed to the antagonist activities of Ketoconazole on Dexamethasone receptors. Vitamin E had a similar effect to Ketoconazole by reversing the effect of Dexamethasone in on bleeding and clotting time, platelet count and fibrinogen level. The possible explanation for this platelet incorporation of vitamin E both in vitro and in vivo leads to dose-dependent inhibition of platelet aggregation. Another possible mechanism of Vitamin E inhibition of hemostatic function is the antagonistic interaction with Vitamin K, a known contributor to coagulation.

From Chart 1, Dexamethasone caused a significant ($p < .05$) and dose-dependent decrease in percentage body weight gain of rats. Subsequent treatment with Ketoconazole and Vitamin E increased the percentage weight of Wistar rats, even so, the significance ($p < .05$) was still observed when compared to control. The reversal effect and percentage weight gain elicited by Ketoconazole and Vitamin E was insignificant ($p < .05$) when compared to the percentage body weight gain of rats treated with their corresponding doses despite the increase in percentage body weight gain. Data from this study showed the changes in bleeding time of rats treated with Dexamethasone. A dose-dependent decrease ($p < .05$) was observed in rats treated with Dexamethasone. Ketoconazole antagonized the effect of the respective doses of Dexamethasone, causing a minimal increase in bleeding time of rats. This increase was significant ($p < .05$) when compared to the bleeding time of control rats. 150 mg/Kg of Vitamin E caused a more potent reversal/antagonist effect to Dexamethasone actions on bleeding time. The increase observed was not significant when compared to control, and bleeding time rats treated with separate doses of 0.1 mg/Kg and 0.3 mg/Kg of Dexamethasone.

Chart 4 shows the effect of Dexamethasone on platelets count. In a dose-dependent manner, Dexamethasone increased platelets count with significance ($p<0.05$) recorded in rats treated with higher dose of 0.3 mg/Kg of Dexamethasone. This increase in platelet counts were reversed following administration of Ketoconazole and Vitamin E. Vitamin E provided a potent reversal change to the activities of Dexamethasone with significant ($p < .05$) decrease in platelets count when compared to their respective dose of 0.1 mg/Kg and 0.3 mg/Kg of Dexamethasone.

4.1 Relevance of Study

The study will provide an explanation on the mechanisms behind the effect of glucocorticoid specifically (dexamethasone) on hemostatic functions. Data from this study will add to the already existing body of knowledge on hemostasis. The study will be beneficial to health practitioners.

5. CONCLUSION

This study has shown Dexamethasone to improve hemostasis by significantly decreasing bleeding and clotting times. Dexamethasone also enhanced hemostatic functions with a significant increase in fibrinogen and platelet counts. Vitamin E and Ketoconazole antagonized the effect of Dexamethasone

6. RECOMMENDATIONS

We recommend that this study be extended to humans, considering diseases associated with coagulation. Patients prone to coagulation disorders should be sensitized on Vitamin E intake, considering its anti-coagulation activities.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval was obtained from the Bioresearch and ethics committee of the College of Health Sciences, Delta State University, Abraka, Delta state, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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