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RESEARCH ARTICLE

Hemostatic Parameters in Administration of Graded Doses of Hydrocortisone in Wistar Rats

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Abstract

Clinical trials have shown that an even newer compound, hydrocortisone, might be a more potent hemostatic agent and less likely to cause side-effects than any other known corticosteroid. This study investigated the changes in selected hemostatic parameters; clotting time, bleeding time, platelets count and serum fibrinogen levels in albino wistar rats, following administration of graded doses of hydrocortisone. Forty-two (42) adult male albino rats of the wistar strain were procured, then, randomly grouped into seven (7) of six (6) rats each. With Group A receiving normal diets and rat water *ad libitum* (Control), Groups B – G were respectively fed with standard diets and graded doses of hydrocortisone (for two weeks) as; 2 mg/kg, 4 mg/kg, 6 mg/kg, 8 mg/kg, 10 mg/kg and 12 mg/kg respectively. Following two weeks administration period, rats were then euthanized, blood samples obtained (by cardiac puncture) and passed under diethyl ether to determine hemostatic parameters. Using the one-way analysis of variance (ANOVA), obtained serum was also compared for total platelet count and fibrinogen concentration. Careful observation revealed that hydrocortisone in higher doses decreased bleeding and clotting time, with noticeable significant increase ($p < 0.05$) in serum platelet and fibrinogen counts. However, treatments with vitamin E reversed this effect in high dosed hydrocortisone groups by increasing bleeding and clotting time, while decreasing fibrinogen levels and platelet count in turn. Thus, demonstrating hydrocortisone as potent in hemostasis, possibly by its role in significantly decreasing bleeding and clotting times, and also promoting hemostatic factors with significant increase in fibrinogen and platelet counts.

Keywords: Hydrocortisone, Hemostasis, Vitamin E

Introduction

Hemostasis is a dynamic relationship between a mixture of cellular and biochemical events that function together to keep blood in the liquid state of the veins and arteries, avoiding blood loss after injury [1]. Various systems, including the vascular system, coagulation system, fibrinolytic system, etc., have been involved in haemostasis [2]. When blood vessel endothelial linings are compromised by physical, mechanical or chemical damage to create clots, these mechanisms work together. Via the fibrinolytic process, these clots stop bleeding and are removed.

The hemostasis (blood flow stoppage) mechanism is a combination of cellular and biochemical events that function together to maintain blood in the liquid state within the veins and arteries and prevent blood loss after injury by forming a blood clot [1, 3]. It consists of a complex controlled mechanism that relies on a delicate multi-system balance. The mechanisms involved in the hemostatic mechanism are the vascular system, the coagulation system, the fibrinolytic system, the platelets, the kinin system, serine protease inhibitors and the complement system [2, 4].

Hematological parameters [coagulation time, bleeding time, platelet count and fibrinogen level] are correlated with health indices in clinical practice and are of diagnostic importance in regular assessment of an individual's health status [3]. Experimental studies have shown that the use of glucocorticoids can substantially increase the level of fibrinogen and clotting factors [4], with a risk of venous thromboembolism [5]. As suggested by the rise in fibrinogen levels [6], glucocorticoids have been found to decrease fibrinolytic activity. Increased PAI-I formation is due to decreased fibrinolysis, which inhibits plasmin synthesis and thus prevents fibrin and fibrinogen breakdown.

Hydrocortisone, a synthetic glucocorticoid, has been shown to be 50 times more likely than cortisol [7] to interact with a glucocorticoid receptor. It is used to treat a range of disorders, including rheumatic problems, a variety of skin diseases, extreme allergies, asthma, chronic obstructive pulmonary disorder, croup, swelling of the brain, and tuberculosis antibiotic.

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Patients receiving exogenous glucocorticoids have been documented to have altered levels of hemostatic factors [8].

Long-term glucocorticoid exposure has also been studied to induce drastic changes in hemostatic parameters [9]. Bleeding and coagulation time parameters tend to provide ample information on platelet aggregation, adhesion, activation, work to this stage and serve as a means of accessing many conditions such as thrombocytopenia, von Willebrand disease, liver failure, etc. [10]. The objective of this research was to determine the effect of hydrocortisone, using albino wistar rats as experimental models, on certain hemostatic parameters. In particular, the study examined the impact of hydrocortisone on total body weight, bleeding and coagulation time, fibrinogen and platelet levels.

Materials and Methods

Scope of Study

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

Study Design

Experimental in nature, with forty-two (42) rats of average body weight of 100-150g were randomly divided into seven (7) groups of six rats each (n=6);

GROUP A: Control group fed with normal rat chow and clean water ad libitum

Group B: received 2 mg/kg body weight of hydrocortisone

Group C: received 4 mg/kg body weight of hydrocortisone

Group D: received 6 mg/kg body weight of hydrocortisone

Group E: received 8 mg/kg body weight of hydrocortisone

Group F: received 10 mg/kg body weight of hydrocortisone

Group G: received 12 mg/kg body weight of hydrocortisone

Procedure

Animals were treated with varying doses of synthetic glucocorticoid (hydrocortisone) for a period of fourteen (14) days, using orogastric cannula. After period of administration of test substance, animals were then euthanized, blood samples obtained (by cardiac puncture) and assessed for hemostatic parameters [clotting time, bleeding time, and platelets count and serum fibrinogen levels]. Weekly, body weight changes were also obtained and compared between groups using the weighing balance.

Hydrocortisone Administration

Hydrocortisone injection 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, and then placed in heparinized capillary tubes. Hemostatic parameters were subsequently measured.

Measuring the Clotting time

By putting them in a water bath of 37 ° C, four separate test tubes were prepared. Blood was collected into these test tubes via syringes and a stop clock began immediately when the test tubes were put in the water bath. In each of the four (4) rat test tubes, blood was collected and analyzed for an interval of thirty seconds.

The observation was conducted by gentle tilting of the test tubes prior to clotting. The time of coagulation was then recorded as the average time provided 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

About 0.28ml 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 lens 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.

Assessment Fibrinogen Levels

Plasma, 0.05 ml, was diluted in the test tube with 5.5 ml of barbitone saline buffer and 3.0 ml of the mixture was carefully transferred for examination to a 1 cm cuvette. The remainder of the mixture has been decanted as a blank into a similar cuvette. Both cuvettes were put in the spectrophotometer and zero absorbance was calibrated for the instrument. 0-0.15ml of the calcium-thrombin reagent was applied to the contents of the test cuvette and mixed quickly and carefully to reduce air bubble production. The spectrophotometer was replaced by the cuvette and the program began. The trace was drawn for 10 minutes at least.

Ethical Approval

Ethical approval was obtained from the Bioresearch and ethics committee of the College of Medicine, Ambrose Alli University, Ekpoma, Edo state.

Analytical Approach

Obtained data was represented as mean ± standard error of mean. One-way analysis of variance was used to compare

differences in means between data, performing post-hoc test where differences exist (tukey test) to ascertain the responsive variable. Statistical analysis was performed with the graph pad prism (version 8.1), while considering p-level < 0.05 as statistically significant.

Results

(Figure 1-5)

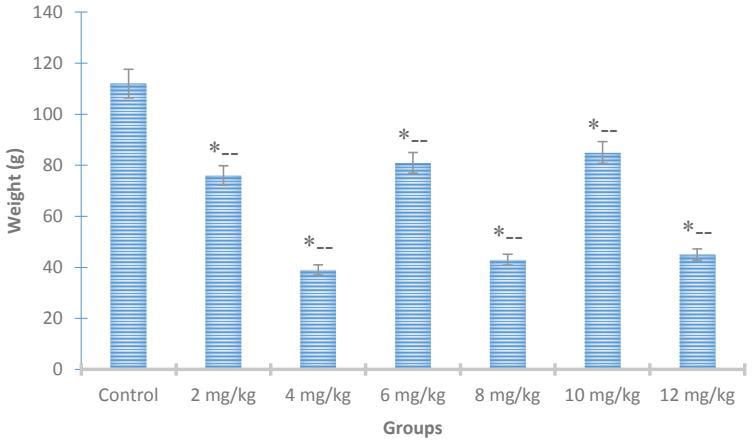

Figure 1: Showing Body Weight Changes in Hydrocortisone Treated Rats.

*p < 0.05 compared with control group. From the figure, a statistically significant decrease was observed for body weight of rats irrespective of administered hydrocortisone dosage, compared to control group. This decrease was however inconsistent upon comparison between groups, proving highest in 10 mg/kg and 6 mg/kg administration.

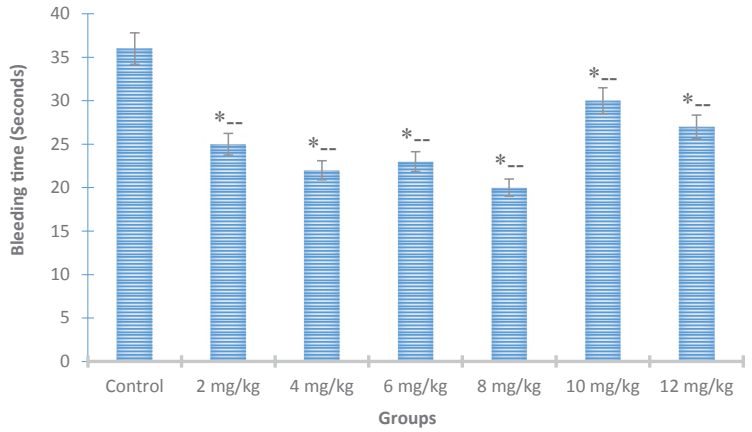

Figure 2: Effect of Hydrocortisone on Bleeding Time.

*-- = significant decrease (p < .05) when compared to control.

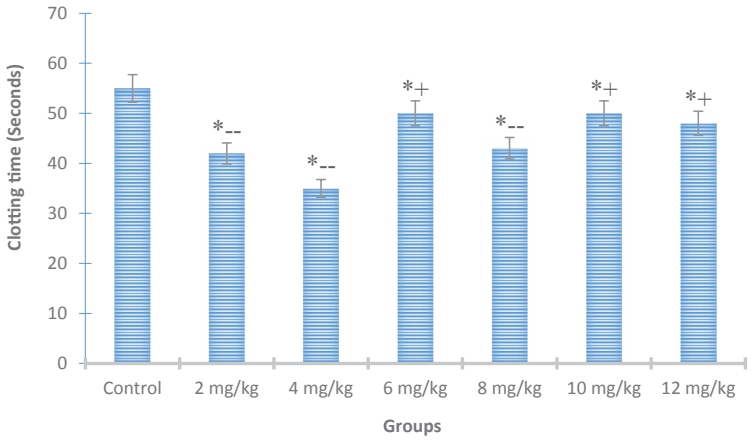

Figure 3: Effect of Hydrocortisone on Clotting Time.

*-- significant decrease (p > 0.05) compared to control; *+ = significant decrease (p < 0.05) compared to 2 mg/kg hydrocortisone fed rats.

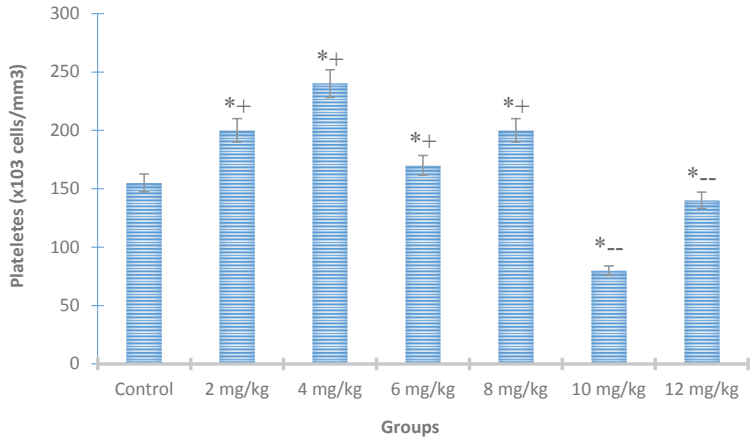

Figure 4: Effect of Hydrocortisone on Platelet Count.

*-- significant decrease ($p > 0.05$) compared to control; *+ = significant increase ($p < 0.05$) compared to control hydrocortisone fed rats.

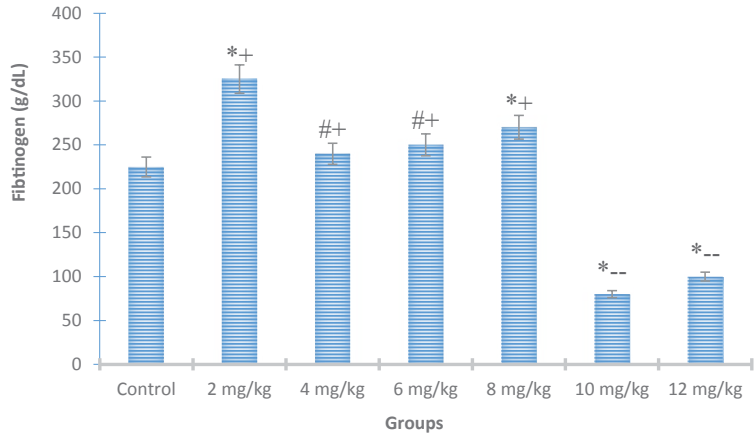

Figure 5: Effect of Hydrocortisone on Fibrinogen Level.

*+ = significant ($p < 0.05$) increase when compared to control.

*-- = significant ($p < 0.05$) decrease when compared to control rats.

#+ = insignificant ($p > 0.05$) increase when compared to control rats.

Discussion

This research investigated the effect (s) on certain hemostatic parameters of Hydrocortisone. Data suggest that Hydrocortisone reduced bleeding and coagulation times, thus increasing the number of platelets and fibrinogen levels. These changes were significant ($p < 0.05$), particularly in rats treated with higher than control doses of hydrocortisone. After treatment with Dexamethasone, another glucocorticoid, Shashidhara et al. (2013) showed an insignificant increase in the platelet count of children infected with dengue fever [11]. Kularatne (2005) has observed a similar observation of the recovery of the platelet count after a maximum decline, with increasing platelet counts steadily over three days without any intervention [12].

The findings of this study also support previous studies that showed that high dose hydrocortisone in previously untreated patients with Primary Immune Thrombocytopenia (ITP) previously referred to as idiopathic thrombocytopenic purpura induced long-term responses. A single dose of the same test substance in a study by Cheng et al. (2003) produced a 50 percent sustained platelet count response of 50,000/μL at 6 months after initial treatment [13, 14]. A multi-center study by Borst et al. (2004) found that 59% of previously untreated adult patients, after 1-6 cycles of Hydrocortisone treatment, had a sustained response of 2-31 months.

In this research, the increase in coagulation time following treatment with Hydrocortisone indicates that it may have an effect on increasing the synthesis of some coagulation factors, including fibrinogen. Fibrinogen, on the other hand, increased significantly after administration of hydrocortisone and increased significantly ($p < 0.05$) above control levels. Although these findings conform to those observed by several investigators [15], the study in which autoimmune and malignant patients undergoing similar corticosteroid

therapy were treated was not confirmed [16]. This suggests that the underlying nature of the disease may interfere with the findings and that it may be better for an animal model to study the blood coagulation effect of glucocorticoids per se. The results of Morange et al. (1999) showed that synthetic hydrocortisone decreased the amount of fibrinogen [17] in a contrasting report.

Rats treated with hydrocortisone co-administered with vitamin E showed increased bleeding and coagulation time and reduced levels of fibrinogen and platelet count. The explanation for these observed changes may be due to the hydrocortisone receptor activity of vitamin E (drug-drug interaction), reversing its effects on bleeding and coagulation time, platelet count and level of fibrinogen. The potential reason for this platelet incorporation, both in vitro and in vivo, of vitamin E contributes to dose-dependent platelet aggregation inhibition. The antagonistic association with Vitamin K, a recognized contributor to coagulation, is another potential mechanism of Vitamin E inhibition of hemostatic activity.

From Figure 1, a substantial ($p<0.05$) and dose-dependent decrease in the percentage of body weight gain of rats was caused by hydrocortisone. The percentage weight of the animals was improved by subsequent treatment with vitamin E, although the significance ($p < 0.05$) was still observed when compared with control. In contrast to the percentage body weight gain of rats treated with their corresponding doses despite the increase in the percentage body weight gain, the reversal effect and percentage weight gain elicited by vitamin E were negligible ($p < .05$). Data from this research also reported improvements in the bleeding time of Hydrocortisone-treated rats. In rats treated with Hydrocortisone, a dose-dependent decrease ($p < .05$) was observed, causing a small increase in rat bleeding time. In contrast with the bleeding time of the control rats, this increase was significant ($p < 0.05$). Vitamin E induced a more potent reversal/antagonist effect on bleeding time of the Hydrocortisone acts. The increase observed was not important when compared to control and bleeding time in rats treated with Hydrocortisone at separate doses of 2 mg/kg and 4 mg/kg.

The effects of Hydrocortisone on platelet count are shown in Figure 4. Hydrocortisone increased the number of platelets with substantial increases ($p < 0.05$) observed in rats treated with a higher dose of Hydrocortisone in a dose dependent manner. Following vitamin E administration, this rise in platelet counts was reversed. In contrast to their respective doses of 2 mg/Kg and 4 mg/Kg of Hydrocortisone, vitamin E demonstrated a potent reversal shift in the action of Hydrocortisone with a substantial ($p<0.05$) decrease in platelet count.

Conclusion

This study has shown Hydrocortisone to improve hemostasis by significantly decreasing bleeding and clotting times. Hydrocortisone also enhanced hemostatic functions with significant increase in fibrinogen and platelet counts. We recommend that this study be ascertained in humans, considering diseases associated with coagulation. Patients prone to coagulation disorders should be sensitized on Vitamin E intake, considering its activities.

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