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Original Article

Evaluation of Anticoagulant Effect of Alum in Rats

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ABSTRACT

Potassium aluminium sulfate, $KAl(SO4)_2$, also called Alum, is an acidic white chemical substance. The role of alum in bleeding and clotting is not fully understood. **Objective:** To determine the effects of alum on clotting time, D-dimer levels, fasting blood glucose level, and lipid profile. **Methods**: The study was conducted on 24 male Wistar rats, which were randomly divided into six groups. Four groups were given different concentrations of alum solutions. The remaining two groups received warfarin, and distilled water, which are control, and placebo groups, respectively. Blood tests such as fasting blood sugar (FBS), D-dimers, clotting time, and lipid profile were performed. **Results:** The study found that the administration of alum prolonged the time it took for blood to coagulate. Alum showed a dose dependent increase in clotting time when compared to the warfarin-control group and group 4 (100 mg/kg alum dose) showed the most significant effect. Similarly, in the case of D-dimers, a dose dependent decrease in the level of D-dimers was seen and the most significant effect was found for high concentration. The plasma blood glucose and lipid level of animals treated with alum did not show any significant effect as compared to placebo. **Conclusions:** The efficacy of alum as an anticoagulant drug was investigated, and it was found to significantly prolong clotting time while simultaneously reducing the level of D-dimers. Furthermore, it was deemed safe and showed no effects on fasting plasma glucose and lipid profile. The safety profile of alum was assessed to be favorable, thus highlighting its potential as an anticoagulant drug of the future.

INTRODUCTION

Hemostasis is the physiological process that causes blood vessels to stop leaking blood. It requires the coexistence of coagulant and anticoagulant actions in a balanced manner. It involves three stages: vasoconstriction, platelet plug formation and blood coagulation. When a blood vessel is injured, it tightens endothelium to decrease blood and expose collagen, which attracts platelets and activate them [1]. Platelets adhere to collagen with the help of the von Willebrand factor. Adenosine diphosphate (ADP) and thromboxane A2 are secreted by platelets, which stimulate and draw increasing numbers of platelets, forming temporary platelet plugs. Coagulating factors lead to fibrin, binds to platelet plug, prevents blood loss [2]. The endothelium regulates vascular relaxation/constriction, solute extravasation, and platelets/blood cells, which is crucial for maintaining vascular homeostasis [3,4]. Von Willebrand factor (vWF), a plasma glycoprotein, binds to exposed collagen of disrupted vessels to serve as the site for platelet adhesion. The GPIb receptor on platelets binds to vWF, leading to a conformational change and platelet activation. Hemostatic mediators, including additional vWF from alpha granules, are then released to increase platelet accumulation [5,6]. Many proteins help in platelet aggregation and clot stabilization, facilitating the rapid formation of a microthrombus [3]. Formation of blood clot in the blood vessels, causes serious cardiovascular complications, including myocardial infarction, acute ischemia, stroke, and venous thromboembolism [7]. The parenteral anticoagulants include heparin and low molecular weight heparins, argatroban, bivalirudin,

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desirudin, and fondaparinux. The vitamin K antagonists include warfarin. Similarly, the direct oral anticoagulants include dabigatran and direct oral factor X_a inhibitors (apixaban, betrixaban, edoxaban, and Rivaroxaban [8]. $KAl(SO4)_2$, also called Alum, is an acidic white chemical substance. It is an efficient hemostatic agent due to its chemical interaction with blood proteins [9, 10]. Alum prevents minor cuts and abrasions from bleeding [11], esophageal varices bleeding in advanced rectal cancer, and is used as a treatment for persistent vesical haematuria because it is secure and efficient [12]. Drugs like heparin [13] and warfarin [14] are associated with substantial side effects such as excessive bleeding and hemorrhagic consequences. In addition, these medications are expensive and have a limited half-life. The effect of alum i.e., potassium aluminium sulfate $KAl(SO4)_2$ on bleeding is controversial as no conclusive or definite evidence exists in this regard. Some studies have found it to be an anticoagulant [9,15], while others have found it to be a coagulant [10,16]. The role of alum in bleeding and clotting is not fully understood. Moreover, its safety profile has not been determined, and its toxic effects should be investigated to evaluate its safety.

The aim of the study was to determine the effects of alum on clotting time, D-dimer levels, fasting blood glucose level, and lipid profile.

METHODS

The study was undertaken in the Animal House of Khyber Medical University, Peshawar. Rats were acquired from the animal house of the Institute of Basic Medical Sciences, Khyber Medical University, Peshawar. The study was conducted on 24 male Wistar rats, which were randomly divided into six groups. Four groups were given different concentrations of alum solutions. The remaining two groups received warfarin, and distilled water, which are control, and placebo groups, respectively. The experimental drugs were administered orally. The study was approved by the KMU committee on animals' ethics and the study registration number is KMU/IBMS/IRBE/4th meeting/2023/9821-16. Animals were grouped as;

Group 1.....alum 25 mg/kg
Group 2.....alum 50 mg/kg
Group 3.....alum 75 mg/kg
Group 4.....alum 100 mg/kg
Control .....Warfarin 2.5 mg/kg
Placebo ..... Distilled Water

Clotting Time (CT) was evaluated via capillary tube method. Blood was withdrawn via cardiac puncture from the individual rat. A few drops of that blood were then placed on a glass slide. From there, blood was taken into the capillary tube. After every twenty seconds, a part of the capillary tube was broken to check the formation of threads (that in turn indicated clot formation). This procedure was repeated after every twenty seconds until the clot formation was observed. A digital stopwatch was used to note the time it took for the clot to form [17]. D-dimer is a protein fragment that is created in our body when a blood clot dissolves. D dimer level was measured in the blood by a test known as the D-dimer test with the help of ELISA [18]. For the determination of lipid profile, the blood samples were collected and stored in an Ethylene Diamine Tetra-acetic Acid (EDTA) tube. Subsequently, the sample underwent centrifugation at room temperature with 3000 rpm for 15 minutes to separate the serum. The serum was then utilized to measure cholesterol, triglycerides, HDL, and LDL levels. Blood triglyceride levels were determined using the glycerol-3-phosphate-oxidase (GPO) colorimetric enzymatic method [19]. The examination of cholesterol was carried out directly via the Cholesterol Oxidase-Peroxidase Aminoantipyrine test (CHOD-PAP) [20]. The levels of high-density lipoprotein (HDL) were ascertained using precipitation reagents to isolate chylomicrons, very low-density lipoprotein (VLDL), and low-density lipoprotein (LDL). This was achieved by the addition of phosphotungstic acid and magnesium ions to the sample, which facilitated the formation of precipitates. The levels of HDL in the supernatant layer were then determined using an enzymatic CHOD-PAP test [21]. LDL cholesterol analysis was conducted using the Friedewaldetal formula, which calculates LDL cholesterol levels indirectly. This formula involves subtracting HDL and one-fifth of triglycerides from total cholesterol. The glucose level of blood was measured using a digital glucometer. A small amount of blood was placed on a test strip and inserted into the device. Within a span of 20 seconds, the glucose level was displayed on the screen [22]. A one-way ANOVA test followed Tukey post hoc test was performed for multiple comparisons in the study. For properly distributed variables, the resulting data were shown as a mean standard deviation (SD) in graphical and tabular formats. The data analysis was conducted using a p-value of 0.05 or below.

RESULTS

Alum, in all doses, has shown a significant effect ($p<0.05$) on clotting time except 25 mg/kg when compared to the placebo. However, a more significant effect was observed on at 100 mg/kg dose. Furthermore, in comparison with warfarin, the effect of even a large dose of alum did not cause any significant effect on clotting time (Figure 1).

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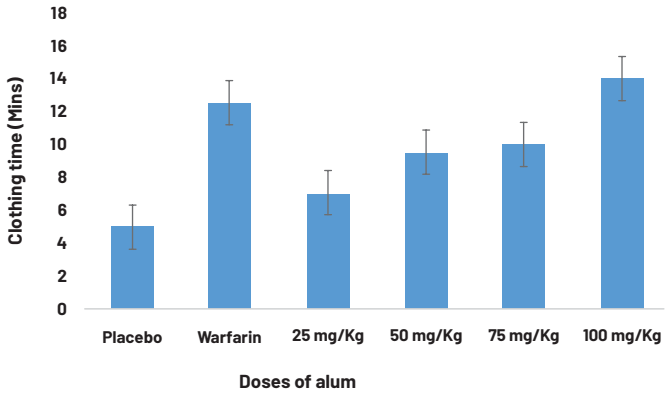

Figure 1: The Effect of Alum on Clotting Time

In comparison to the placebo, alum in all doses has caused a decrease in the level of D-Dimers except 25 mg/kg, which shows similar levels in comparison to placebo. However, more pronounced effects ($p<0.05$) were observed at higher doses of 75 mg/kg and 100 mg/kg (Figure 2).

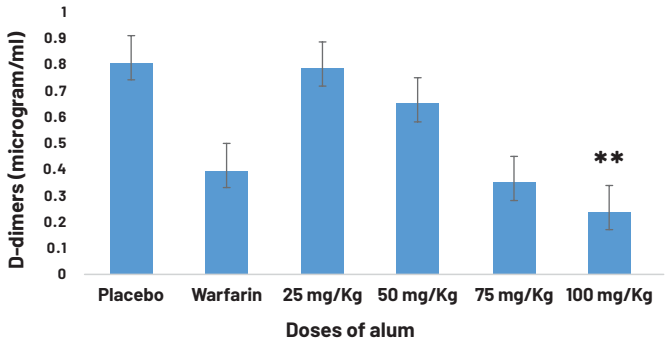

Figure 2: The Effect of Alum on D-Dimers Level

Alum in all selected doses did not cause any significant changes in the level of High-Density Lipoproteins (HDL), Low-Density Lipoproteins (LDL), Cholesterol, and Triglyceride (Table 1).

Table 1: The Effect of Alum on HDL, LDL, Cholesterol and Triglyceride

Groups	HDL (mg/dL)	LDL (mg/dL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)
Placebo	32.3 ± 4	26.3 ± 1	60.6 ± 1	200 ± 21
Group 1	32.5 ± 6	25.5 ± 3	30.2 ± 3	130 ± 12
Group 2	31.8 ± 6	23.8 ± 2	28.5 ± 3	123.2 ± 32
Group 3	32.4 ± 1	27.4 ± 3	28.4 ± 2	114.1 ± 22
Group 4	38.3 ± 4	20 ± 2	25.9 ± 2	50 ± 11

Furthermore, alum in all doses caused no significant effect on Fasting Plasma Glucose (FPG) in comparison to placebo (Table 2).

Table 2: Effect of Alum on Fasting Blood Glucose (FBG) Level.

Groups	Placebo	Group 1	Group 2	Group 3	Group 4
FBG (mg/dL)	111 ± 08	92 ± 3	100 ± 5	91 ± 10	123 ± 12

DISCUSSION

Alum is therapeutically used in diseases like bleeding, hematuria, bleeding piles, bleeding gums, and mucous surface bleeding [23]. Studies reported high prothrombin and partial thromboplastin times with increased serum aluminium levels [24, 25]. A previous study showed that in vitro treatment with alum inhibits human platelet aggregation induced by collagen, epinephrine, adenosine diphosphate (ADP), and thrombin [15]. In a study conducted at the University of Jordan, the effect of alum on bleeding time in rabbits was checked. The study found that the bleeding time had significantly prolonged in 8 rabbits, from a mean of 2.36 minutes to 3.79 minutes [9]. Another study found the antiplatelet effect of alum in humans [15]. In contrast, some studies show a cessation of bleeding by irrigation of alum in severe hemorrhage from the bladder [12,16]. Our study demonstrated the effect of alum with different doses on clotting time in rats. The results showed that the low dose of alum has almost the same clotting time as the placebo. As the dose of alum increased, the clotting time also increased, and the 100 mg/kg showed the highest clotting time (15 minutes). The highest dose of the alum showed a similar result as warfarin. These results suggest the anticoagulant effect of alum. D-dimer is a protein fragment created in the body when a blood clot dissolves. It is detected in the blood by a test known as the D-dimers test. It is also known as fibrin degradation fragment [26]. In our study, the warfarin control group showed a significant difference from the placebo group in D-dimer levels. Significant high D-dimer levels were found when comparing the 25 mg/kg alum dose to the warfarin-control group, as indicated by a p-value less than 0.05. However, D-dimer levels were found the same when compared to the placebo. As compared to all other groups, 100 mg of alum per kilogram showed significantly lowest D-dimer levels. Placebo and warfarin results correlated to clotting time. As D-dimer level decreases, clotting time increased. The 75 mg/kg and 100 mg/kg alum doses showed a significant decrease in D-dimer levels and an increase in clotting time. In our study, a dose-dependent decrease in the D-dimer levels was recorded. As the dose of alum was increased, the levels of D-dimers decreased accordingly. It showed that since alum acts as an anticoagulant, the clot is prevented from forming, which causes a decrease in D-dimers. Previously, no studies have been done on the effect of alum on the D-dimer levels. Therefore, looking into this aspect of alum is a novel parameter. It would provide a baseline for future studies in this regard. For a drug to be used in the human population, it is necessary to determine its safety profile since adverse effects always accompany it [27]. To fulfill this requirement, the fasting glucose levels and lipid profile were assessed. Various parameters, such as high-

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density lipoproteins (HDL), low-density lipoproteins (LDL), triglycerides, and blood glucose levels were checked. It was found that alum did not have toxic effects on the glucose levels and lipid profile. In summary, our study suggested the anticoagulant effect of the alum in rats by increasing clotting time and decreasing D-dimer levels. In addition, the lipid profile and blood glucose test confirmed that the alum has no adverse effects on these parameters. Although our study suggested the anticoagulant effect of alum, it has certain limitations. Our study evaluated the safety profile of the alum by checking its effects on glucose levels and lipid profiles. The safety of alum should be further explored by evaluating its effects on other parameters such as liver, kidney, and heart function.

CONCLUSIONS

The efficacy of alum as an anticoagulant drug was investigated, and it was found to significantly prolong clotting time while simultaneously reducing the level of D-dimers. Furthermore, it was deemed safe and showed no effects on fasting plasma glucose and lipid profile. The safety profile of alum was assessed to be favorable, thus highlighting its potential as an anticoagulant drug of the future.

Authors Contribution

Conceptualization: MHAK
Methodology: MHAK
Formal analysis: HB
Writing-review and editing: GB, HS

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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