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## Evaluation of methanolic extract of dragon's blood on platelet activation markers in streptozotocin-induced diabetic wistar rats

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### ABSTRACT

**Objectives:** Platelet activation plays a critical role in the development of diabetic complications, including thrombosis and cardiovascular diseases. Natural plant extracts are increasingly investigated for their potential therapeutic effects in diabetes management. This study evaluated the impact of *Dracaena cinnabari* (DC) extract on platelet activation markers and associated histopathological changes in streptozotocin-induced diabetic Wistar rats.

**Materials and Methods:** A total of 36 rats were divided into six groups: Group I (non-diabetic control), Group II (diabetic control), Groups III-V (diabetic rats treated with 150, 300, and 600 mg/kg of DC extract, respectively), and Group VI (diabetic rats treated with 12 mg/kg of aspirin as standard). Treatments were administered orally for 21 days. At the end of the experiment, rats were anesthetized, and blood samples were collected for molecular analysis of von Willebrand factor (vWF) and cluster of differentiation 41 (CD41) using reverse transcription-polymerase chain reaction. Liver and pancreatic tissues were collected for fibrinogen gene expression analysis and histological examination. Data were analyzed statistically using analysis of variance.

**Results:** Blood glucose levels were significantly higher ( $P < 0.05$ ) in diabetic controls compared with the non-diabetic group. Fibrinogen expression was significantly elevated in diabetic control rats ( $P = 0.00$ ) but markedly reduced in treatment groups and the non-diabetic control ( $P = 0.00$ ). No significant changes were observed in vWF and CD41 expression ( $P > 0.05$ ). Histological analysis of pancreatic tissue showed evidence of healing across treatment groups, whereas no such reversal was observed in liver tissue.

**Conclusion:** The findings suggest that DC extract exerts potential therapeutic effects on hyperglycemia and hyperfibrinogenemia in diabetic rats, indicating its promise in mitigating thrombotic risks associated with diabetes.

**Keywords:** Dragon's blood (*Dracaena cinnabari*), Fibrinogen, Platelets' activation, von Willebrand factor

## INTRODUCTION

Diabetes mellitus is a metabolic condition characterized by the presence of hyperglycemia resulting from a fault in insulin action, a shortfall in insulin secretion, or both.<sup>[1]</sup> Diabetes and uncontrolled hyperglycemia are well-known risk factors for the development of atherosclerotic cardiovascular diseases.<sup>[2]</sup> Insulin's anabolic features cause inconsistencies in micronutrient metabolism. These metabolic deformities are caused by insufficient insulin levels to produce an adequate response and/or insulin resistance in target tissues, primarily skeletal muscles and adipose tissue, and to a lesser extent in the liver, at the level of insulin receptors, signal transduction systems, and/or effector enzymes or genes.<sup>[1]</sup> Type 1 diabetes is the most common type of diabetes, characterized by a complete lack of insulin due to pancreatic beta-cell dysfunction. In contrast, hyperglycemia in type 2 diabetes is caused by insulin resistance.<sup>[3]</sup>

Blood platelets are essential for maintaining proper hemostasis, and poor platelet function can lead to hemostatic diseases and atherosclerosis.<sup>[4]</sup> Those with diabetes have defective coagulation due to hyperactive platelets that exhibit increased adhesion, aggregation, and thrombin production.<sup>[5]</sup> These platelet hyperactivities might contribute to the inconsistencies in metabolic activities. Patients with diabetes not only have a larger atheromatous plaque burden but also a thrombotic diathesis that is in part related to alterations in the coagulation system with increased levels of plasma fibrinogen, increased intravascular thrombin production, and decreased fibrinolytic capacity.<sup>[6,7]</sup> Then, platelets are activated, and they rapidly translocate and express P-selectin from their  $\alpha$ -granules to the cell surface.<sup>[8,9]</sup> Moreover, P-selectin plays an important function in hemostasis because it promotes the adhesion of activated platelets to neutrophils and monocytes to facilitate the innate immune response, as well as stimulating platelet-to-platelet binding and aggregation.<sup>[10]</sup> Thus, P-selectin proteins can be secreted into the bloodstream, now known as soluble P-selectin, as part of platelet-derived microparticles or as free spliced variants of the protein.<sup>[11]</sup>

*Dracaena cinnabari* (DC) is a perennial tree native to Socotra Island, located off the southern coast of Yemen. It belongs to the family Agavaceae and is locally known as Damm Alakhwain.<sup>[12,13]</sup> The species is characterized by a single, stout trunk that can reach up to 10 m in height, with smooth gray bark and distinctive umbrella-shaped crowns formed by dichotomously branching, sausage-shaped segments.<sup>[14]</sup> The tree produces a deep red resin, commonly referred to as "Dragon's Blood" or "Two Brothers' Blood," which exudes from fissures and wounds in the bark or branches.<sup>[14]</sup> Conventionally, the resin has been widely utilized in Socotra for diverse purposes, including dyeing wool, pottery decoration, as a breath freshener, lipstick,

and as a binding agent. Medicinally, it has long been valued across cultures for its astringent, hemostatic, antiseptic, and antiulcer properties, and for treating diarrhea and dysentery.<sup>[14]</sup> Phytochemical investigations of DC resin have identified several bioactive compounds, including flavonoids, homoisoflavonoids, chalcones, sterols, and terpenoids. Notably, homoisoflavonoids and chalcones exhibit potent antioxidant activity,<sup>[15]</sup> while flavonoids contribute to the anti-inflammatory effects of methanolic extracts.<sup>[14]</sup> The chemical constituents of the resin have been comprehensively reviewed.<sup>[14]</sup> Furthermore, methanolic extracts have demonstrated antiviral activity against Herpes simplex and Influenza viruses, as well as antimicrobial, antioxidant, and food-preservative properties due to inhibition of various foodborne pathogens.<sup>[14]</sup> However, its methanol extract has also been reported to exert non-specific antiparasitic effects associated with high cytotoxicity.<sup>[15]</sup> Importantly, the phytochemical profile of Dragon's Blood resin varies depending on the plant species from which it is derived.<sup>[14]</sup> Dragon's blood extract obtained from *Croton* species is traditionally used in the treatment of diabetes because it contains several bioactive compounds with diverse medicinal properties. These include phenolic substances with strong antioxidant activity, which may help reduce oxidative stress commonly associated with diabetes.<sup>[16]</sup> This extract has long been used for its therapeutic properties, and it is now gaining popularity for its ability to treat diabetes. Studies show that it enhances the levels of antioxidants such as superoxide dismutase and glutathione in tissues, potentially improving overall metabolic health.<sup>[16]</sup> The aim of this study was to explore the effect of Dragon's blood extract on platelet activation markers in streptozotocin (STZ)-induced diabetic Wistar rats.

## MATERIALS AND METHODS

### Study area

The study was conducted at the Department of Hematology, School of Medical Laboratory Sciences, and the Center for Advanced Medical Research and Training, Usmanu Danfodiyo University, Sokoto, Nigeria.

### Study design

Thirty-six (36) albino Wistar rats were randomly assigned into six groups, with six (6) rats in each group. Group I served as the non-diabetic control and was left untreated while receiving standard feed and clean water. Group II comprised diabetic control rats that were also untreated and maintained on standard feed and clean water. Group III consisted of diabetic rats treated with 150 mg/kg of Dragon's Blood extract, while Group IV included diabetic rats treated with 300 mg/kg of Dragon's Blood Extract. Group V was

made up of diabetic rats administered 600 mg/kg of Dragon's Blood Extract. Group VI served as the standard treatment group and consisted of diabetic rats treated with 10 mg/kg of aspirin.

### Plant material

Dragon's blood DC resin was procured from MoNatural International Limited, Abuja, Nigeria. Authentication was carried out by a taxonomist in the Botany Section, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, where a voucher specimen was deposited (Voucher No.: PCG/UDUS/ASPA/0001).

### Dragon's blood extract preparation

The resin was grinded into powder using an electric blender. A total of 50 g of powdered resin was macerated with 500 mL of methanol (1 g of resin to 10 mL of methanol) at room temperature for 3 days on a shaker at 100 rpm. The extract was filtered using a muslin cloth, followed by filtration through a Whatman Grade 1 filter paper. Methanol was separated using a rotary evaporator (Eyela) under reduced pressure at 40°C, yielding a gummy red resin. A freeze dryer was used to obtain 28 g of dry powdered extract, which was stored at 4°C, wrapped in aluminum foil to prevent photo-oxidation.<sup>[17]</sup>

### Sub-chronic oral toxicity study (28-day repeated dose)

For toxicity assessment, eight rats were divided into four groups of two rats each. Group 1 (control) received 10% dimethyl sulfoxide, while Groups 2, 3, and 4 received Dragon's blood resin extract at doses of 300, 1000, and 1500 mg/kg body weight, respectively. The extract was administered orally once daily for 28 days. Mortality, food and water intake, and general signs of toxicity were monitored. Body weights were recorded weekly. The extract was well tolerated up to 2000 mg/kg, classified as category 5.<sup>[18]</sup>

### Experimental animals

The study was conducted on albino Wistar rats weighing 120–150 g, obtained from the Animal House, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. The animals were acclimatized for 2 weeks in controlled environmental conditions, with ad libitum access to feed and water.

### Ethical approval

The Animal Ethics Committee of Usmanu Danfodiyo University in Sokoto, Nigeria, provided a letter of ethical approval (reference number PTAC/Pa/(Dr)/OT/63-23).

### Induction of diabetes (high-fat diet [HFD]/STZ model)

Insulin resistance was induced by feeding the rats an HFD for 2 weeks. Afterward, STZ was administered intravenously at a dose of 50 mg/kg. To prevent hypoglycemia, the rats were provided with 10% glucose for 24 h. Fasting blood glucose (FBG) was measured using on-call plus glucometer strips 72 h post-STZ injection, and rats with FBG >200 mg/dL were considered diabetic.<sup>[19]</sup>

### Sampling techniques

#### Measurement of body weight

The body weight of each rat was measured using a gravimetric method at the following time points: Before HFD administration, before and after STZ-induced diabetes, and after intervention. Weight was recorded in grams using a weighing balance.

#### blood sample collection and processing

Rats were anesthetized using chloroform, and blood samples (5 mL) were collected through cardiac puncture into ethylenediaminetetraacetic acid tubes for quantitative polymerase chain reaction analysis of von Willebrand factor (vWF) and integrin (*CD41*) gene expression. Liver and pancreas samples were collected for histological analysis and stored in 10% formalin.

### Test procedures

#### Plasma glucose estimation

Plasma glucose levels were estimated using On Call<sup>®</sup> Plus glucometer strips. Blood samples were obtained from the tail vein using a sterile lancet.

#### Real-time reverse transcription-polymerase chain reaction (RT-PCR) for fibrinogen

Fibrinogen expression was quantified using reverse transcription-polymerase chain reaction (RT-PCR) with an FC-96GE thermal cycler, and amplification was monitored based on the fluorescence emitted by SYBR Green I dye. Specific forward (5'-GGCAATGACTACCTCCACTTAC-3') and reverse (5'-GTACTCCGCATAAGCCTCTTT-3') primers were employed for the assay. The polymerase chain reaction (PCR) protocol consisted of 40 amplification cycles, each comprising denaturation at 94 °C, annealing at 59 °C, and extension at 74 °C.

#### RT-PCR for vWF

The expression of von Willebrand factor (vWF) was quantified using the same RT-PCR protocol, employing specific primers consisting of a forward primer

(5'-AAGCTGTCACTAGACGTTTCC-3') and a reverse primer (5'-CCTGGAAGATGTCCTGGTAAG-3').

### RT-PCR for integrin (CD41/CD61)

The expression of integrin (CD41/CD61) was quantified using reverse transcription-polymerase chain reaction with specific primers, comprising a forward primer (5'-TGCACTCAGAAGAAGGAGAATG-3') and a reverse primer (5'-ACCAACATCGTGATTCCTATCC-3'). The amplification was carried out under the same PCR conditions as previously described.

### Data analysis

Data generated from the present study were analyzed using the Statistical Package for the Social Sciences version 23. Results were expressed as mean  $\pm$  standard error of the mean for the rats in each group. Blood glucose and platelet activation markers were analyzed statistically using one-way analysis of variance (ANOVA), while the differences were considered significant when  $P \leq 0.05$ .

### RESULTS

The result from this study showed that the body weight, glucose level, fibrinogen, vWF, and CD41 parameters in Wistar rats orally administered with methanolic extract of Dragon's blood and aspirin for 21 days are presented in the tables and plates, as shown below. Table 1 shows the effect of methanolic extract of Dragon's blood on the FBG level of diabetic Wistar rats. Where there is statistically significant difference ( $P \leq 0.05$ ) between control group I of

the initial glucose and group VI, while there is no statistically significant difference ( $P > 0.05$ ) in the final blood glucose level after intervention between the various groups including the control Group I. Table 2 shows the effect of methanolic extract of Dragon's blood on fibrinogen level in diabetic Wistar rats. The result is expressed as mean  $\pm$  standard error of the mean for each group. The ANOVA and *post hoc* analysis of fibrinogen gene expression show a statistically significant difference ( $P = 0.00$ ) between diabetic control group II and the rest of the Groups (I, III, IV, V, and VI). Table 3 shows the effect of methanolic extract of dragon's blood on vWF level in diabetic Wistar rats. vWF factor gene expression result indicates no statistically significant differences between the various groups (I, II, III, IV, V, and VI) with  $P = 0.65$ . Table 4 shows the effect of methanolic extract of dragon's blood on *CD41* gene expression in diabetic Wistar rats. The result indicates no statistically significant differences between and within Groups (I, II, III, IV, V, and VI) on *CD41* gene expression, with  $P = 0.154$ .

### DISCUSSION

This study investigated the effects of Dragon's blood extract in STZ-induced diabetic Wistar rats. While our findings did not demonstrate a statistically significant reduction in FBG following treatment with Dragon's blood, earlier studies using other herbal extracts, such as *Strychnos spinosa*, reported significant decreases in blood glucose levels ( $P < 0.05$ ) in diabetic rats.<sup>[20]</sup> Such discrepancies may be due to variations in the severity of diabetes induction, differences in experimental protocols, or the duration of treatment. Nonetheless, in this study, a statistically significant reduction in blood glucose levels ( $P = 0.006$ ) was observed in diabetic-induced rats when

**Table 1:** The effect of methanolic extract of dragon's blood on the fasting blood glucose level of diabetic Wistar rats.

Groups	I	II	III	IV	V	VI	Total	f-value	P-value
<i>n</i>	6	6	6	6	6	6	36		
Initial glucose (mmol/L)	3.13 $\pm$ 0.47 <sup>a</sup>	7.43 $\pm$ 1.02	13.53 $\pm$ 1.99	12.83 $\pm$ 5.74	12.87 $\pm$ 1.47	24.37 $\pm$ 3.55 <sup>a</sup>	12.36 $\pm$ 1.88	5.861	0.006
Final glucose (mmol/L)	3.30 $\pm$ 0.21	10.97 $\pm$ 2.28	8.00 $\pm$ 0.81	13.97 $\pm$ 7.66	8.97 $\pm$ 1.47	18.60 $\pm$ 1.89	10.63 $\pm$ 1.89	1.461	0.272

Group I: Non-diabetic control 1, Group II: Diabetic control 2, Group III: Treatment with 150 mg/kg of dragon's blood extract, Group IV: Treatment with 300 mg/kg dragons blood extract, Group V: Treatment with 600 mg/kg of dragon's blood extract, Group VI: Treatment with 12 mg/kg of Aspirin (standard). Values bearing a superscript <sup>a</sup>at the same column varies significantly ( $P=0.006$ ),  $P = 0.006$  at fasting blood glucose level.

**Table 2:** The effect of methanolic extract of Dragon's blood on fibrinogen gene expression of diabetic Wistar rats.

Groups	I	II	III	IV	V	VI	Total	f-value	P-value
<i>n</i>	6	6	6	6	6	6	36		
FIB	1.00 $\pm$ 0.00 <sup>a</sup>	29.80 $\pm$ 0.34 <sup>abcde</sup>	1.04 $\pm$ 0.07 <sup>b</sup>	0.91 $\pm$ 0.35 <sup>c</sup>	2.54 $\pm$ 1.30 <sup>d</sup>	1.29 $\pm$ 0.46 <sup>e</sup>	6.10 $\pm$ 2.58	377.208	0.00

Values bearing superscript<sup>abcde</sup> in the same column varies significantly ( $P=0.00$ ). Values are expressed in mean $\pm$ standard error of the mean, *n* is the number of samples. Group I: Non-diabetic control 1, Group II: Diabetic control 2, Group III: Treatment with 150 mg/kg of dragon's blood extract, Group IV: Treatment with 300 mg/kg dragons blood extract, Group V: Treatment with 600 mg/kg of dragon's blood extract, Group VI: Treatment with 12 mg/kg of Aspirin (standard). FIB: Fibrinogen.

**Table 3:** The effect of methanolic extract of Dragon's blood on von-Willebrand factor gene expression of diabetic Wistar rats.

Groups	I	II	III	IV	V	VI	Total	f-value	P-value
n	6	6	6	6	6	6	36		
VWF	1.00±0.00	0.39±0.230	0.77±0.33	0.93±0.56	0.35±0.11	0.64±0.45	0.68±0.13	0.639	0.675

Group I: Non-diabetic control 1, Group II: Diabetic control 2, Group III: Treatment with 150 mg/kg of dragon's blood extract, Group IV: Treatment with 300 mg/kg dragons blood extract, Group V: Treatment with 600 mg/kg of dragon's blood extract, Group VI: Treatment with 12 mg/kg of aspirin (standard), VWF: von Willebrand Factor

**Table 4:** The effect of methanolic extract of Dragon's blood on CD41 gene expression of diabetic Wistar rats.

Groups	I	II	III	IV	V	VI	Total	f-value	P-value
n	6	6	6	6	6	6	36		
CD41	1.00±0.00	0.30±0.05	0.55±0.29	0.40±0.00	0.51±0.12	0.63±0.27	0.57±0.08	1.982	0.154

Group I: Non-diabetic control 1, Group II: Diabetic control 2, Group III: Treatment with 150 mg/kg of dragon's blood extract, Group IV: Treatment with 300 mg/kg dragons blood extract, Group V: Treatment with 600 mg/kg of dragon's blood extract, Group VI: Treatment with 12 mg/kg of aspirin (standard), CD41: Cluster of differentiation 41

compared with the non-diabetic control group. Dragon's blood, derived from *Daemonorops draco*, has been shown in experimental studies to enhance glucose uptake in muscle cells. However, *in vivo* investigations have largely indicated only a downward trend in FBG, without achieving statistical significance.<sup>[21]</sup> A significant decrease in fibrinogen gene expression was found in this study in treatment groups (III, IV, V, and VI) and control Group I compared to diabetic control group II. This suggests that Dragon's Blood may have a therapeutic effect in reducing hyperfibrinogenemia associated with diabetes.<sup>[22]</sup> The significant decrease in fibrinogen levels in treatment groups compared to diabetic controls indicates that dragon's blood may effectively mitigate thrombotic risks associated with diabetes. Dragon's blood exhibits clinical antithrombotic efficacy comparable to low-molecular-weight heparin, showing significant decreases in D-dimer and fibrinogen degradation products in patients undergoing surgery. Furthermore, Bembde (2012) investigated the fibrinogen level in type 2 diabetic patients and its relationship to glycemic control. The study discovered increased plasma fibrinogen concentration and pool in diabetic patients compared to non-diabetic controls. He then proposed that increased fibrinogen production may precede clinical cardiovascular complications in type 2 diabetes.<sup>[23]</sup> The observed changes in fibrinogen gene expression in diabetic rats treated with Dragon's blood in the rodent model of this study imply a potential therapeutic effect on diabetes-associated hyperfibrinogenemia. The ANOVA and *post hoc* analyses of vWF gene expression show no statistically significant changes ( $P > 0.05$ ) between control group I, diabetes control group II, and the treatment groups. Research reveals that gene expression profiles in diabetic animals frequently show diversity, but individual treatments may not significantly affect vWF levels.<sup>[24]</sup> This implies that Dragon's blood had no significant effect on vWF gene expression as compared to controls. The absence of variance in vWF expression could

indicate that this factor is stable under the present settings, or that dragon's blood has a minor effect on vWF expression. The lack of substantial variations in vWF gene expression between the control and diabetes groups, as well as the treatment groups, may be consistent with research showing that some herbal therapies have selective effects on specific biomarkers. Furthermore, this finding is in contrast to a study of type 2 diabetes patients, where vWF concentrations were raised but did not correlate with specific gene polymorphisms, implying that genetic factors may not influence vWF expression considerably in diabetic situations.<sup>[19]</sup> Acupuncture is frequently used to treat a variety of ailments.<sup>[25]</sup> This study has revealed no statistical differences in CD41 gene expression between the control group, the diabetic control group, and the treatment groups. This finding is consistent with previous research suggesting that some herbal extracts may not have a significant influence on platelet glycoprotein gene expression in diabetes.<sup>[26]</sup> And also in agreement with the findings of Gupta *et al.*, whose studies on CD41+ cells in lymph nodes also report stable expression levels, reinforcing the notion that CD41 does not fluctuate significantly in response to diabetic conditions.<sup>[27]</sup> This finding implies that Dragon's blood did not have a substantial effect on the gene expression of CD41, a platelet glycoprotein implicated in clot formation. This could also imply that the extract may not have a pronounced effect on the genetic regulation of CD41. The lack of statistical differences in CD41 expression suggests that it may not be a primary factor in the pathogenesis of diabetic complications, as indicated by the absence of variation in gene expression across different groups.<sup>[28]</sup>

## CONCLUSION

This study adds to the growing body of knowledge about herbal therapies for diabetes and thrombosis. The methanolic extract of Dragon's blood does not appear to have a

substantial effect on the body weight of diabetic Wistar rats. While Wistar rats' glucose levels increased dramatically following STZ induction, STZ-induced diabetic Wistar rats treated with the methanolic extract of Dragon's blood showed no significant decrease. The results indicate that Dragon's blood has a considerable influence on the downregulation of fibrinogen gene expression in diabetic Wistar rats. Diabetic Wistar rats treated with dragon's blood extract show no significant influence on *vWF* and *CD41* gene expression. The findings indicate that dragon's blood methanolic extract will help avoid thrombus formation/complications in diabetic patients.

**Ethical approval:** The research/study was approved by the Institutional Review Board at Usmanu Danfodiyo University Sokoto, number NHREC/UDU-HREC/25/06/2023, dated 16 May 2024.

**Declaration of patient consent:** Patient's consent was not required as there are no patients in this study.

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