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# Anticoagulant Activity of Ginger (*Zingiber Officinale* Rosc., Zingiberaceae) Rizhome Extract

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

**Abstract**
**Background:** Herbal medicines with anticoagulant therapeutic claims could serve as veritable sources of new oral anticoagulant drugs with possible wider safety margins than the currently available ones
**Objectives:** This work was aimed at evaluating a Ginger Rhizome Methanolic Extract *in vivo* in rats for its potential anti-coagulant activity.
**Materials and Methods:** Thirty-six albino rats were randomized into 6 equal groups, comprising four extract-treated, one standard reference and one control groups. Each of the four extract-treated groups received one of 50 mg/kg, 100 mg/kg, 200 mg/kg and 400 mg/kg ginger rhizome extract prepared in 2% Tween 20. The standard reference group received 1mg/kg warfarin while the control group received 10 ml/kg 2% Tween 20. These dosing were carried out daily concurrently for 14 consecutive days after which blood samples were collected from the animals and evaluated to obtain mean Prothrombin Time (PT), activated Partial Thromboplastin Time (aPTT) and Thrombin Time (TT), comparing each test value with the control and standard reference using one way ANOVA followed by turkey comparisons.
**Results:** The Ginger Rhizome Methanolic Extract significantly prolonged PT, aPTT and TT, compared to the control ($p < 0.001$). Warfarin did not significantly prolong TT and the extract's TT prolongation effect was significantly greater than that of warfarin ($p < 0.001$).
**Conclusion:**
This work shows that Ginger Rhizome Methanolic Extract has anticoagulant activity and could therefore be a veritable source of new oral anticoagulants with possible better safety margins.
**Keywords:** Anticoagulants, Ginger rhizome, Thrombosis, Ischaemic diseases

## INTRODUCTION

Thrombosis (or pathological haemostasis) is intravascular clotting of blood without a bleeding trigger. It is the direct cause of ischaemic strokes and cardiac arrests, two deadly medical conditions that are now rampant in virtually all societies (Mackman, 2008). Given that haemostasis (or blood loss arrest) is a composite of the rather complementary platelet aggregation and blood coagulation mechanisms (Spronk et. al., 2003; Tanaka et. al., 2009), ideal antithrombotic therapy offering maximum protection against the afore-mentioned diseases can only be possible with routine prophylactic use of combinations of safe and tolerable antiplatelet and anticoagulant therapies. However, while current antiplatelet therapies are largely safe and routinely applied (Merritt and Bhatt, 2004; Best et. al., 2008), the same cannot be said of their anticoagulant counterparts, whose inter- and intra-subject

variability and narrow therapeutic indices are necessities for administration under expert supervision and routine therapeutic monitoring (Hirsh et. al., 1998; Palareti and Cosmi, 2009). There is therefore a high and urgent need for the discovery of new and safer anticoagulant drugs to complement routine antiplatelet therapies for effective prophylactic control of thromboembolic diseases.

Ginger has been extensively applied in agriculture to protect harvested food crops without any history of poisoning when such ginger-protected food items are consumed (Zhang et. al., 2004; Thakore, 2006; Ukeh et. al., 2009). Rather than poisoning, ginger itself is actually freely consumed as food, and as spice and condiment in foods, beverages and folkloric medicines (Mansour, 2012). Medicinal applications of ginger are diverse, including its use in the treatment of emesis, inflammation, hyperlipidaemia, viral infections, inflammation, gastric ulcer and male infertility, to mention a few (Tanabe et. al., 1993; Denyer et. al., 1994; al-Yahya et. al., 1998). In addition, there are verbal claims by local herb sellers in Lagos, Western Nigeria, that alcoholic tincture of Ginger Rhizome is used as a "blood thinner". Though this traditional use is yet-to-be-documented, there are a number of scientific reports on ginger's interference with platelet aggregation and/or coagulation that support it (Dorso et. al., 1980; Srivastava, 1984; Srivastava, 1986; Weidner and Sigwart, 2000; Lesho et. al., 2004). While there are speculations linking most of the therapeutic and other uses of ginger to its essential oils and the paradol and shogaol phenolic constituents (Shukla and Singh, 2007; Ali et. al., 2008), there is a paucity of information on the linkage of these activities to specific secondary metabolites.

In the light of the above, therefore, we evaluated a Ginger Rhizome Methanolic Extract (GRME) *in vivo* in mice for its potential anticoagulant activity using clinical coagulation parameters, Prothrombin Time, (PT), activated Partial Thromboplastin Time (aPTT) and Thrombin Time (TT), with the aim of confirming the potential anticoagulant activity of ginger and its possible use as an explorable natural resource for the discovery of new oral anticoagulants.

## Material and Methods

*Materials, animals and reagents*

Ginger rhizomes were purchased from a local market in Lagos and identified in the Botany Department of the University of Lagos. PT, aPTT and TT reagents were obtained from Thermo Fisher Scientific, citrated bottles were obtained from Becton Dickinson and Company, New Jersey, USA. Clotting time measurements were obtained from a two-channel Coatron M2 coagulation analyzer (TECO, Niederbayern, Germany). All solvents and other chemicals used were obtained from Sigma Aldrich and were of at least analytical standards. Wistar albino rats (of both sexes) aged 5-8weeks weighing 122g-178g were used for this study, they were obtained from the animal house of the College of Medicine of the University of Lagos, acclimatized for 7days to the experimental environment, sustained on standard feed (Pfizer) and water *ad libitum*.

All animal procedures as stipulated by the animal and ethics committee of the College of Medicine, University of Lagos were followed, and were in accordance with the UK animal (scientific procedure) act, 1986.

*Extraction*

Ginger Rhizomes were dried under shade for 14 days and then ground to powder. The powder was (2kg) was extracted by cold maceration in methanol (12 Litres) for 36 hours. The extract was decanted and the marc subjected to further maceration with fresh methanol (12 Litres) for another 36 hours, after which the extract was also decanted. The extracts were bulked, filtered and evaporated to dryness at 45$^{o}$C *in vacuo*.

*Animal experiment*

The animals were randomly distributed into six groups (groups 1-6) of six animals each. Group 1 (negative control) was treated with 10 ml/kg of the extract-suspending medium, 2% Tween 20. Groups 2, 3, 4 and 5 were treated with 50 mg/kg, 100 mg/kg, 200 mg/kg and 400 mg/kg ginger rhizome extract respectively, the choice of these graded doses having been guided by previous studies that ascertained ginger extracts as safe in rats at doses as high as 1000mg/Kg (Weidner and Sigwart, 2000; Weidner and Sigwart, 2001). Group 6 (standard reference) was treated with 1 mg/kg warfarin. These dosing were carried out for fourteen consecutive days after which the animals were fasted overnight, their blood collected and then sacrificed.

*Blood collection and platelet poor plasma (PPP) preparation*

Blood sample (2 mL) was drawn via the retro-orbital sinus plexus of each rat, under mild ether anaesthesia, into a citrated bottle (Becton Dickinson and Co., New Jersey, U. S. A.) and centrifuged at 2000 *g* for 10 min at room temperature (25$^{o}$C) to obtain platelet poor plasma (PPP) stored at -20$^{o}$C until use. Each animal was subsequently sacrificed by cervical dislocation..

*Clotting times (PT, aPTT, TT) analyses of Platelet Poor Plasma (PPP) samples*

Platelet Poor Plasma (PPP) samples collected from animals in each group were subjected to PT, aPTT and TT coagulation assays following the reagents kits procedures with little modifications as follows. For the aPTT tests, 25µl of thawed citrated PPP was pipetted into the two-channel Coatron M2 coagulation analyzer and incubated for 3 min at 37$^{o}$C. This was followed by the addition of 25µl of the aPTT reagent (preincubated for 5 min at 37$^{o}$C) and the mixture incubated for another 2min at 37$^{o}$C. Clotting was subsequently induced by the addition of 25 µl of 0.025M calcium chloride and the clotting time recorded. For the PT assays, 50 µl of thawed PPP was pipetted into the two-channel Coatron M2 coagulation analyzer and incubated for 3 min at 37$^{o}$C, followed by the addition of 100 µl of the PT reagent (pre-incubated at 37$^{o}$C for 5 min) and the clotting time recorded. For the TT assays, 50 µl of thawed PPP was transferred into the two-channel Coatron M2 coagulation analyzer and incubated for 3minutes at 37$^{o}$C. 100 µl of the TT reagent (pre-incubated at 37$^{o}$C for 5 min) was then added and the clotting time recorded.

*Data analysis*

Results were expressed as Mean ± Standard error of the mean (SEM). One-way Analysis of Variance (ANOVA) followed by turkey comparison was used to compare each test group with both the control and the reference standard.

**Results**

GRME showed prolongation of the three clotting times investigated to varying degrees. While the 50mg/kg extract did not have a significant prolongation effect on TT, all the doses tested, including the 50mg/kg dose, showed very highly significant prolongation of aPTT ($p < 0.001$) compared to the control. In the same vein, all the doses of the GRME showed significant prolongation on the TT ($p < 0.05$) compared to the control. Tables 1- 3 respectively show the mean PT, aPTT and TT for each experimental animal group, and their separate comparisons with the control and standard groups.

**Table 1: Mean Prothrombin Time (PT) of the test groups compared to those of the control and standard.**

| Group | PT(Sec.) Mean ± SEM | Pairwise comparison with control (p - value) | Pairwise comparison with standard (p - value) |
|---|---|---|---|
| Control | 9.77 ± 0.37 | - | < 0.0001 |
| 50 mg/Kg GRME | 12.43 ± 0.34 | 0.0705 | 0.0012 |
| 100 mg/Kg GRME | 16.52 ± 0.83 | 0.0035 | 0.0575 |
| 200 mg/Kg GRME | 21.17 ± 1.13 | < 0.0001 | 0.0882 |
| 400 mg/Kg GRME | 25.38 ± 1.47 | < 0.0001 | 0.6763 |
| 1mg/Kg Warfarin | 30.13 ± 1.23 | < 0.0001 | - |

**Table 2: Mean activated Partial Thromboplastin Time (aPTT) of the test groups compared with those of the control and standard.**

| Group | aPTT(sec) Mean ±SEM | Pairwise comparison with control (p-value) | Pairwise comparison with standard (p-value) |
|---|---|---|---|
| Control | 24.52 ± 1.24 | - | < 0.0001 |
| 50 mg/kg GRME | 65.45 ± 1.32 | < 0.0001 | 0.0187 |
| 100 mg/kg GRME | 68.68 ± 3.02 | < 0.0001 | 0.2365 |
| 200 mg/kg GRME | 86.65 ± 2.72 | < 0.0001 | 0.9816 |
| 400 mg/kg GRME | 89.85 ± 2.84 | < 0.0001 | > 0.9999 |
| 1mg/kg Warfarin | 90.22 ± 4.25 | < 0.0001 | - |

**Table 3: Mean Thrombin Time (TT) of the test groups compared with those of the control and standard.**

| Group | TT(sec) Mean ± SEM | Pairwise comparison with control (p-value) | Pairwise comparison with standard (p-value) |
|---|---|---|---|
| Control | 21.25 ± 1.50 | - | 0.8186 |
| 50 mg/Kg GRME | 30.38 ± 0.90 | 0.0392 | 0.2869 |
| 100 mg/Kg GRME | 35.12 ± 3.47 | 0.0088 | 0.5350 |
| 200 mg/Kg GRME | 36.18 ± 1.31 | 0.0069 | 0.0097 |
| 400 mg/Kg GRME | 46.93 ± 3.12 | < 0.0001 | 0.0036 |
| 1mg/Kg Warfarin | 25.27 ± 1.47 | 0.8186 | - |

## DISCUSSION

The extrinsic and intrinsic pathways of the coagulation cascade merge into a common pathway that ultimately leads to the key coagulation reaction, i.e., the conversion of fibrinogen (by the enzyme thrombin) into fibrin clots (Broze Jr, 1995; Triplett, 2000; Gailani and Renné, 2007). Clotting time tests are designed to evaluate the functional effects and/or availability of the factors in one or more of these coagulation pathways by measuring time for fibrin clot formation after certain factors have been made available. They are thus suitable for routine diagnosis of coagulopathies, therapeutic monitoring and in the screening of potential anticoagulant agents (Osoniyi and Onajobi, 2003; Li and Wang, 2010; Sievert, 2011). Three of the most commonly run clotting time tests are Prothrombin Time (TT), activated Partial Thromboplastin Time (aPTT) and Thrombin Time (TT). The use of an automated coagulometer as opposed to visual observation of clots formation in clotting times measurement is more accurate as it is devoid of experimenter-related errors. Nevertheless, its high sensitivity could be disadvantageous as

haemolysis- and/or lipaemia-induced plasma turbidity could lead to artefacted prolongation of clotting times read off coagulometers (Kamal et. al., 2007). Particular precautions were thus taken to minimize haemolysis during blood collection and chances of lipaemia-induced plasma turbidity reduced to the barest minimum by ensuring blood collection from fasted animals.

Prothrombin time is a one-stage test based on how long it takes fibrin clots to form after the addition of Tissue Factor (Thromboplastin), phospholipid and calcium to citrated (i.e., decalcified) platelet poor plasma (PPP). Though the nomenclature still reflects its original design to measure Prothrombin (factor II), it is actually sensitive to the functional activities of all the factors in the extrinsic and common pathways, comprising factors VII, X and V and II (Quick, 1940). Table 1 shows that the GRME at doses as low as 100 mg/Kg significantly prolonged TT compared to the control ($p < 0.01$). It also shows that there was no significant difference between the TT prolongation activities of the standard reference and the GRME extract at 100 mg/Kg-400 mg/Kg doses.

aPTT, in contrast to PT, involves incubating a platelet poor plasma (PPP) sample with a phospholipid and a contact factor (kaolin, in this case) at 37ºC. This set up simulates the activation of the intrinsic pathway of the coagulation cascade with a line-up of enzymatic activities ultimately leading to thrombin formation without the Tissue factor (Thromboplastin), and hence the name activated partial thromboplastin (Nurmohamed et. al., 1994). aPTT is therefore a measure of the wholesomeness of the factors in the intrinsic and common pathways including factors XII, XI, IX and VIII (Nurmohamed et. al., 1994; Kamal et. al., 2007). The aPTT results (Table 2) show a very highly significant prolongation ($p < 0.0001$) of aPTT by all the tested concentrations of ginger extract compared to the negative control. This portends that the GRME had direct inhibition and/or inhibited biosynthesis effects on the factors in the intrinsic and/or common pathways of the coagulation cascade. Moreover, a pairwise comparison of the mean aPTT values of the GRME extract doses with that of the standard (Table 2) shows that there is no significant difference between the aPTT prolongation effect of warfarin and that of GRME at 100 mg/Kg or more ($p > 0.05$).

The TT test reagent is essentially thrombin. Its prolongation is therefore indicative of defective plasma fibrinogen availability and/or direct thrombin inhibition. Table 3 shows that there is a significant prolongation of TT by the GRME especially at the 400 mg/kg dose compared to the control. In addition, Table 3 shows that the GRME showed greater TT prolongation effects compared to warfarin, the reference standard, especially at the 200 mg/Kg and 400 mg/Kg extract doses ($p > 0.05$). The lack of significant TT prolongation effect shown by warfarin compared to the control ($p > 0.05$) is in agreement with the inhibition of the biosynthesis of factors involved in vitamin K biosynthesis. The additional TT prolongation by the GRME is an indication that ginger may contain principles directly inhibiting thrombin or at least interfering with fibrinogen availability (Flanders et. al., 2003). These results are in agreement with various reports indicating ginger's possible interference with hemostasis (Dorso *et. al.*, 1980; Kruth et. al., 2004; Lesho *et. al.*, 2004).

**Conclusion**

This work shows that Ginger Rhizome Methanolic Extract has anticoagulant activity and could therefore be explored for the discovery of new oral anticoagulants with possible better safety margins.

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