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**Haemostatic Potentials of Ethanolic Leaf Extract of *Ocimum Gratissimum* on Coagulation Profiles with Oral Anticoagulant Therapy in Albino Rats**Olalere Fatai Dayo<sup>1,3</sup>, Olatunbosun Luqman Olayinka<sup>2,3</sup>

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<https://dx.doi.org/10.4314/sokjmls.v10i2.25>**Abstract**

Haemostasis is a remarkably complex mechanism which maintains blood in a fluid state intravascularly but very quickly changes blood to a jellylike mass upon disruption of the vasculature. This study evaluated the haemostatic potentials of ethanolic extract of *Ocimum gratissimum* (OG) on coagulation profiles with oral anticoagulant therapy in albino rats. Thirty rats weighing between 120-160 g were used. The rats were divided into five groups; with group A and B as the normal and positive control respectively. Increasing doses of 500 mg/kg, 800 mg/kg and 1000 mg/kg of the extract were administered orally to the tested groups for a period of 14 days. Significant differences between the controls and tested group C, D and E were observed. There were no significant differences in all the weights ( $p = 0.997$ ). There was no statistically significant differences between the value of PT ( $p = 0.103$ ) and PTTK ( $p = 0.903$ ) in tested group C (500 mg/kg) of OG with respect to the normal control. There was no significant difference ( $p > 0.05$ ) in the PT ( $p = 1.000$ ), PTTK ( $p = 1.000$ ) and INR ( $p = 0.237$ ) between the Positive control and Group C (500 mg/kg) respectively and also when compared with normal, positive and tested group OG (800mg/kg). Statistically significant differences ( $p < 0.05$ ) were observed between the mean value of PT ( $p = 0.008$ ) and PTTK ( $p = 0.007$ ) respectively between the Normal control and tested group of OG (1000mg/kg). There was no significant difference ( $p > 0.05$ ) in the PT and PTTK between the Positive control and of OG (1000mg/kg). This study has demonstrated a dose-dependent haemostatic potentials as revealed from different dosages in the oral administration of

ethanolic leaf extract of *Ocimum gratissimum* following anticoagulant therapy.

**Keywords:** Haemostasis, Coagulation Profiles, *Ocimum gratissimum*, Oral anticoagulant

**Introduction**

Haemostasis is the physiological process that stops bleeding at the site of an injury while maintaining normal blood flow elsewhere in the circulation. Blood loss is stopped by formation of a haemostatic plug. The endothelium in blood vessels maintains an anticoagulant surface that serves to maintain blood in its fluid state, but if the blood vessel is damaged, components of the subendothelial matrix are exposed to the blood (Achneck *et al.*, 2010). Several of these components activate the two main processes of haemostasis to initiate formation of a blood clot, composed primarily of platelets and fibrin. This process is tightly regulated such that it is activated within seconds of an injury but must remain localized to the site of injury (Zucker-Franklin, 2000).

There are two main components of haemostasis. Primary haemostasis refers to platelet aggregation and platelet plug formation. Platelets are activated in a multifaceted process (see below), and as a result they adhere to the site of injury and to each other, plugging the injury. Secondary haemostasis refers to the deposition of insoluble fibrin, which is generated by the proteolytic coagulation cascade. This insoluble fibrin forms a mesh that is incorporated into and around the platelet plug. This mesh serves to strengthen and stabilize the blood clot. These two

processes happen simultaneously and are mechanistically intertwined. The fibrinolysis pathway also plays a significant role in haemostasis. Pathological thrombus formation, called thrombosis or pathological bleeding can occur whenever this process is disregulated. The complexity of these systems has been increasingly appreciated in the past few decades.

Multiple anticoagulant mechanisms regulate and control these systems to maintain blood fluidity in the absence of injury and generate a clot that is proportional to the injury. The proper balance between procoagulant systems and anticoagulant systems is critical for proper hemostasis and the avoidance of pathological bleeding or thrombosis (Falati *et al.*, 2002; Furie, 2009).

## Materials and Methods

### Plant collection and authentication

The leaves of *Ocimum gratissimum* (OG) were collected from Malete, Kwara State, Nigeria. The plant was subsequently identified and authenticated by a Botanist, (Mr Bolu Ajayi) of the department of Botany, University of Ilorin, Ilorin, Kwara state, Nigeria. Voucher number (UILH / 001/2020 / 984) was assigned and deposited at the herbarium unit of the department.

### Preparation of extract

Fresh leaves of *Ocimum gratissimum* (OG) plant were first separated from the stalk, rinsed with water to remove dirt, air dried at room temperature and milled to fine powder. The extract was prepared by the use of a substrate extractor at a temperature of 350<sup>c</sup> – 400<sup>c</sup>. The rotary evaporator was then used to concentrate the extract to 10% of its original volume at a temperature of 400<sup>c</sup>. The concentration was put in an oven for complete dryness. The extract yielded was 75g, and this was dissolved in freshly prepared distilled water (i.e. 1g of extract in 25ml of distilled water) daily.

### Acute toxicity study (Determination of LD50)

The median lethal dose (LD50) of the plant extract was determined by method of (Lorke, 1983), using twelve rats weighing between 100 – 190g. In the first phase, four rats were divided into two groups of two rats each and were treated with the aqueous leaf extract of the plant at

dosages of 1600 and 2000mg/kg body weight intra-peritoneally. They were observed for 24 hours for signs of toxicity. In the second phase, eight rats were again divided into four groups of two rats each and were also treated with aqueous extract of *Ocimum gratissimum* at dosages of 3000mg/kg, 3200mg/kg, 4000mg/kg and 4500 mg/kg body weight intra-peritoneally. The median lethal dose (LD50) was calculated using the second phase.

### Experimental animals

The experimental animals that were used for this study were locally bred female and male albino rats from University of Ilorin Central laboratory animal house. The animals were acclimatized for two weeks and maintained under the standard environmental conditions and were allowed to have free access to animal feeds (super starter feeds) and water at libitum

### Experimental designs

A total of 30 rats with body weight ranging from 120g to 160g were used. The rats were divided into five groups of six rats each group and were distributed in cages. The rats were grouped as follows:

- Group A:** Negative control (NC)
- Group B:** Positive control treated with an oral anticoagulant (PC)
- Group C:** Extract treated Group (500 mg/kg) of OG with anticoagulant
- Group D:** Extract treated Group (800 mg/kg) of OG with anticoagulant
- Group E:** Extract treated Group (1000 mg/kg) of OG with anticoagulant

### Animal treatment

Group A and B are the control groups receiving only animal feeds (Super starter feed) and water only and equal ml of oral anticoagulant respectively. Group C, D and E were treated with the prepared extract at different concentrations on daily basis as single dose administration of extract with equal concentration of oral anticoagulant was carried out using the oral route by means of 1ml and 5mls calibrated syringe with attached oral cannula so that actual dose could be determined and introduced into the animal. The animals received their doses every day for a period of fourteen days (14) days. The rats in group C were treated orally with 500

mg/kg of extract of OG per body weight of rats, the rats in group D were treated orally with 800 mg/kg of extract of OG per body weight of rats, and rats in group E were treated orally with 1000 mg/kg of extract of OG per body weight of rats.

**Sample collection**

The animals were fasted overnight and sacrificed. They were anaesthetized using chloroform after fourteen (14) days of oral anticoagulant and extract administration; blood sample was collected from the animal by cardiac puncture. 4.5 ml of blood samples were collected into tri-sodium citrate anticoagulant bottle of 0.5ml of 3.2/3.8% (9 volume of blood to 1 volume of the anticoagulant). Blood is mixed properly and thereafter

centrifuged at 3000 rpm for 15 minutes to obtain platelet poor plasma (PPP).

**Results**

The basic characteristics of different groups are presented on Table 1. A total of 30 Albino rats were categorized into five (5) different groups, i.e. group A; Normal control (n = 6), group B; positive control (n = 6), group C; 500 mg/kg of OG extract (n = 6), group D; 800 mg/kg of OG extract (n = 6) and group E; 1000 mg/kg of OG extract (n = 6).

The mean weight for the five groups were compared in all groups and there was no significant difference (P>0.05) in the weight of all the groups (p = 0.997).

**Table 1: Mean PT, PTTK, INR and weight of Wistar rat in different groups**

Groups	Weight (g)	PT (secs)	PTTK (secs)	INR
<b>A (Negative control)</b>	151.50 ± 2.12	14.00 ± 2.12	29.00 ± 1.41	-
<b>B (Positive control)</b>	157.50 ± 3.54	17.00 ± 1.41	39.00 ± 1.41	1.22 ± 0.11
<b>C OG (500 mg/kg)</b>	151.50 ± 1.30	35.75 ± 10.78	49.00 ± 4.97	2.55 ± 0.77
<b>D OG (800 mg/kg)</b>	152.00 ± 1.63	27.01 ± 4.55	39.00 ± 6.58	1.85 ± 0.38
<b>E OG (1000 mg/kg)</b>	155.25 ± 1.26	46.25 ± 10.21	58.75 ± 11.33	3.31 ± 0.73

The values are mean ± SD in different group.

PT- prothrombin time, PTTK- Partial thromboplastin time with kaolin, INR- International normalized ratio

**Table 2 Comparison of PT, PTTK and INR between Normal and Positive control and OG (500 mg/kg).**

The mean value of PT, PTTK and INR in controls and group C which the rats were administered with dose of 500 mg/kg of OG were compared, and the results were presented as shown on the table 2 below; there was no significant difference between the mean value of PT (p = 0.103) and PTTK (p = 0.903) respectively in tested group C (500 mg/kg) of OG with respect to the Normal control and no significant difference (p > 0.05) on PT (p = 1.000), PTTK (p = 1.000) and INR (P = 0.237) between the Positive control and Group C respectively.

**Table 2: Comparison of PT, PTTK and INR between the Normal, Positive control and of tested group OG (500 mg/kg)**

Parameters	Normal control	OG (500 mg/kg)	P-value
<b>PT (secs)</b>	14.00 ± 2.12	35.75 ± 10.78	0.103
<b>PTTK (secs)</b>	29.00 ± 1.41	49.00 ± 4.97	0.093
<b>INR</b>	NA	2.55 ± 0.77	ND
	Positive control	OG (500 mg/kg)	P-value
<b>PT (secs)</b>	17.00 ± 1.41	35.75 ± 10.78	1.000
<b>PTTK (secs)</b>	39.00 ± 1.41	49.00 ± 4.97	1.000
<b>INR</b>	1.22 ± 0.11	2.55 ± 0.77	0.237

The values are mean ± SD, p-value were determined by Student's t test as appropriate p < 0.05 was considered significantly different, \*when there is intergroup significant difference. NA= not applicable, ND = not determined.

PT- prothrombin time, PTTK- Partial thromboplastin time with kaolin, INR- International normalized ratio

**Table 3 Comparison of PT, PTTK and INR between the controls and at OG (800 mg/kg)**

The mean value of PT, PTTK and INR in controls and group D which the rats were administered with dose of 800 mg/kg of OG were compared, and the results were presented as shown on the table 3; there were no significant differences between the mean value of PT (p = 0.920) and PTTK (p = 1.000) between the normal control and the group D respectively, also there was no significant difference (p>0.05) in mean values of PT (1.000), PTTK (1.000) and INR (1.000) between the Positive control and the group D respectively. The PT and PTTK values of tested group OG (800 mg/kg) is significantly high compared to the Normal control. Therefore, there was no significant difference on PT and PTTK (P > 0.05) mean values between the Normal control, positive control and that of tested group D of OG (800mg/kg), thereby the coagulation effect is lower compared to the normal and positive control.

**Table 3: Comparison of PT, PTTK and INR between the controls and OG (800 mg/kg)**

Parameters	Normal control	OG (800 mg/kg)	P-value
PT (secs)	14.00 ± 2.12	27.00 ± 4.55	0.920
PTTK (secs)	29.00 ± 1.41	39.00 ± 6.58	1.000
INR	--	1.85 ± 0.38	ND
	Positive control	OG (800 mg/kg)	P-value
PT (secs)	17.00 ± 1.41	27.00 ± 4.55	1.000
PTTK (secs)	39.00 ± 1.41	39.00 ± 6.58	1.000
INR	1.22 ± 0.11	1.85 ± 0.38	1.000

The values are mean ± SD, p-value were determined by Student's t test as appropriate p < 0.05 was considered significantly different, \*when there is intergroup significant difference. NA= not applicable, ND = not determined. PT- prothrombin time, PTTK- Partial thromboplastin time with kaolin, INR- International normalized ratio.

**Table 4: Comparison of PT, PTTK and INR between the Normal control and OG (1000 mg/kg)**

The mean value of PT, PTTK and INR in controls and group E which the rats were administered with dose of 1000 mg/kg of OG were compared and the results were presented as shown on table 4. Significant differences (p<0.05) were observed between the mean value of PT (p = 0.008) and PTTK (p = 0.007) respectively between the Normal control and tested group of OG (1000 mg/kg). There was a high significant difference (P<0.05) in PT (p=0.008\*) and PTTK (p=0.007\*) of tested group OG (1000mg/kg) to the Normal control. But there was no significant difference (P> 0.05) on PT and PTTK between the Positive control and of OG (1000mg/kg), the PT and PTTK mean values were significantly increased at OG (1000mg/kg) to the positive control thereby indicating that its coagulation effect is lower.

**Table 4: Comparison of PT, PTTK and INR between the controls and at OG (1000mg/kg)**

Parameters	Normal control	OG (1000mg/kg)	P-value
PT (secs)	14.00 ± 2.12	46.25 ± 10.21	0.008*
PTTK (secs)	29.00 ± 1.41	58.75 ± 11.33	0.007*
INR	--NA	3.31 ± 0.73	ND
	Positive control	OG (1000mg/kg)	P-value
PT (secs)	17.00 ± 1.41	46.25 ± 10.21	0.016
PTTK (secs)	29.00 ± 1.41	58.75 ± 11.33	0.100
INR	1.22 ± 0.11	3.31 ± 0.73	0.018

The values are mean ± SD, p-value were determined by Student's t test as appropriate p < 0.05 was considered significantly different, \*when there is intergroup significant difference. NA= not applicable, ND = not determined.

PT- Prothrombin time, PTTK- Partial thrombin time with kaolin, INR- International normalized ratio

### Discussion

The purpose of this study was to evaluate the possible haemostatic effect of ethanolic leaf extract of *Ocimum gratissimum* (OG) *in vitro* and in order to discover the potential of *Ocimum gratissimum* leaf as a coagulant agent. The haemostatic effect of *Ocimum gratissimum* was determined by Prothrombin time (PT) and Activated partial thromboplastin time (APTT) tests using normal coagulation control plasma. An abnormally prolonged PT triggered by anticoagulant agents is associated with the interference of the integrity of coagulation proteins in the extrinsic coagulation pathway, especially factor VII (Manicam *et al.*, 2010). APTT test measures the 'intrinsic' pathway and 'common' pathways of coagulation, where it assessed the action of factors XII, XI, IX, VIII, X, II and fibrinogen (Thornton and Douglas, 2010). Besides, to check the other function of blood clotting factors involved in the extrinsic and common pathway, such as factors II, VII, X and fibrinogen (Sahu and Saxena, 2013). Generally, the PT results suggests that *Ocimum gratissimum* ethanolic leaf extract significantly interfere with the extrinsic coagulation pathway and the ethanolic extraction showed better extraction for PT as a coagulant. The higher value in the PT can be attributed to the reduction in the production of these clotting factors. Our findings are in line with the result of an earlier study (Sahu and Saxena, 2013). The prolonged mean (S.D) PT values might also be suggestive of impaired liver function which could directly

or indirectly affect the synthesis of coagulation factors. This delay in coagulation could further be attributed to a reduction in the function of platelet in circulation.

The plasma coagulation time for all different concentration of *Ocimum gratissimum* ethanolic leaf extract (500 mg/kg, 800 mg/kg, and 1000 mg/kg) were compared to the plasma coagulation time of normal control (Those that receive feeds and water only). The Activated partial thromboplastin time (APTT) test revealed a significant prolongation at the highest concentration of ethanolic leaf extract. The result *in vitro* study of *Ocimum gratissimum* in rats showed that there was no significant differences between the mean value of APTT in test groups C and D when compared with the control group (p<0.920) and (p<1.000) respectively (table 2 and 3). However, a significant difference was observed between the value of PTTK in group E with normal control (p=0.007) (table 4). Prothrombin time (PT) test was performed to know the effects of *Ocimum gratissimum* ethanolic leaf extract on coagulation factors involved in the extrinsic pathway. The PT results in tested groups C, D and E were compared with the control group. There were no significant differences between the value of PT in tested groups C and D in respect with the control groups (p<0.103) and (p<0.093) respectively (table 2 and 3). However, a significant difference was observed between the PT in tested group E and normal control (p=0.008) as shown on table 4.

Inability of the lower concentration studied to confer coagulation may be attributed to the concentration of the phytochemical contents or the solvent of extraction (Sahu and Saxena, 2013).

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