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Preliminary investigation of antithrombotic activities of methanolic seed extracts of *Garcinia Combogia* in rats

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ABSTRACT

Garcinia combogia is a flowering plant with a wide range of medicinal uses which include antiparasitic, antimicrobial, antithrombotic, antidiabetic, antihepatotoxic and as purgative. However its antithrombotic properties have not been fully elucidated hence this present study. In this study, we investigated the antithrombotic activities of the methanolic seed extracts of *Garcinia combogia* in comparison with a known standard drug (aspirin) as the positive control and Normal saline as the negative control. The work is purely an experimental research on rats. The result showed that both the bleeding time and clotting time were prolonged and dose dependant but not superior to the standard drug (aspirin), while the platelet count was reduced showing that the methanolic seed extract of *Garcinia combogia* has antithrombotic properties. Also the extract tested positive to the following phytochemical compounds – alkaloids, glycosides, flavinoids, resins, proteins and tannins after phytochemical analysis following a standard procedure which confer on the plant other medicinal properties. Finally, we recommended that further work be done to establish the mechanism of action of its antithrombotic activities and to use this plant to develop better alternatives than the present drugs currently used in clinical medicine.

Keywords: antithrombotic, *Garcinia Combogia*, phytochemical, toxicity

INTRODUCTION

Records of ancient medical practice indicate that *Garcinia Combogia* was used as an antithrombotic agent. What is *Garcinia Combogia*? *Garcinia combogia* is a species of flowering plants in the *Guttiferae* or *Clusiaceae* family. It is found in Nigeria, Benin, Cameroon, Democratic Republic of Congo, Ivory Coast, Gabon, Ghana, Liberia, Senegal and Sierra Leone. Its natural habitat is subtropical or tropical moist lowland forests. It grows in the humid rain forest near the coast as a medium sized tree with an average height of about 12m and has a great ability to tolerate shade. It is also distributed in dense population in the natural rain forest reserves of south eastern and south western parts of Nigeria as well as in the secondary forests which spray up as a result of deforestation activities [1]. The *Garcinia Combogia seed* is culturally and socially significant in some parts of South Eastern Nigeria (West Africa) where the yellow nut is served for traditional hospitality in private, social and cultural functions. As a result of its wide spread consumption, especially among the Ibos of South Eastern Nigeria, some studies have been carried out on the extract of various components of the plant.

Garcinia combogia is known by the following common names in Nigeria:

- Bitter kola, (Pigin English)
- Naniji goro (Hausa)
- Orogbo (Yoruba)
- Akilu (Igbo)

It is commonly called bitter kola because of the bitter taste of the nut.

Phytochemical properties confer the wide range of Medicinal uses of *Garcinia combogia*.

The phytochemical studies shows that *Garcinia Combogia* contains phenolic compounds, steroids, xanthines, benzophenones, [2] tannins, guttiferins and saponins [3]. Animal and human studies revealed that the extracts of *Garcinia Combogia* exhibit aphrodisiac effects on male subjects [4, 5] for which reason they are sometimes called “male kola” in some parts of Nigeria. It is reported to suppress ovulation and delay fertility in female subjects [6]. *Garcinia Combogia* extracts have been shown to possess antipyretic, anti-inflammatory, analgesic [7], antiviral, hepatoprotective [8, 9], CNS stimulant [5], antidepressant, antioxidant [10], antidiabetic [11, 12] and antithrombotic [13] activities. The registration of *Garcinia Combogia* formulation as a hop substitute in the brewing of beer and wine was under consideration by the Food and Drug Administration in the United States of America [14]. It is used for treatment of parasitic skin diseases while the latex is internally used for the treatment of gonorrhoea and glucose lowering agent [15]. Also the plant is used for the treatment of liver disorders and as a chewing stick, [4] and is used for the treatment of catarrh and abdominal colicky pain [10]. Considerable experimental evidence has been adduced in support of the antihepatotoxic efficacy of kolaviron in animals against such hepatotoxicants as paracetamol [16], carbon tetrachloride, thioacetamide, galactosamine, phalloidine, and ethanol [10, 17]. The toxicity is very low, the oral 50% lethal dose being above 5000 mg/Kg b. w [18]. The antithrombotic activity of *Garcinia combogia* plant has not been fully studied hence this present work.

MATERIALS AND METHODS

Collection and preparation of plant materials:

Fresh *Garcinia combogia* (seed) was bought from Onitsha Main Market in Anambra State. The seeds were identified by Mr. Ozioko of Botany Department, University of Nigeria, Nsukka. The epicarp of the seed was neatly peeled off with a blade, exposing the inner milkfish-white fleshy parts. The seeds were sliced into very small pieces and dried under shade until their weight is the same. The dried *Garcinia combogia* seeds were further reduced in size into firmly fine but coarse sized particles by grinding using a miller.

Preparation of methanolic seed extracts

50g of ground sample was macerated in 250ml of methanol for 48hrs. The mixture was separated by sieving with a clean glass bottle. The extract was concentrated using a rotary evaporated and was further subjected to a temperature of 50⁰c. The dried sample was stored in a clean glass bottle.

Animal Used

Wister Albino rats weighing 90g – 138g and Wister albino mice weighing 30g – 32g were employed in this test. Both animals were of mixed sexes. They were housed in a clean gauzed cage and had free access to food and water.

Phytochemical Tests

Phytochemical tests were carried out on the methanolic seed extract of *Garcinia combogia* using the procedure outlined by Trease and Evans (1989).

In general, test for the presence or absence of phytochemical compounds using the above method involved the addition of an appropriate chemical agent to the methanolic seed extract of the plant in a test tubes and shaking vigorously or lightly as the case may be. Gentle heat may sometimes be required.

Dilution of extract & standard drugs

Method of Dilution of Extract:

1g (1000mg) of the extract was weighed using an electronic weighing balance. It was transferred into a beaker containing 2ml of normal saline with a spatula. The concentration of the stock solution was 100 mg/ml. it was then stored in a clean glass bottle with cover in a refrigerator.

Method of forming the stock solution of aspirin

Two tablets of aspirin containing 300mg of aspirin per tablet were transferred into a porcelain mortar. They were properly ground to obtain a fine powder. The powder was transferred into a small beaker (25ml). Then 3ml of normal saline was added to the drug. It was stirred properly and the volume was made up to 6ml. It was stored in a refrigerator inside a glass bottle with cover. A solution of 100mg/ml was formed.

Calculation of doses of extracts and standard drugs used

Group A:	Normal saline	0.5ml daily	X 14 days
Group B:	Aspirin Daily	250mg/kg	X 14 days

Group C: Extracts only 250mg/kg X 14 days
 Group D: Extracts only 500mg/kg X 14 days

For mice in acute toxicity test

Phase 1

Group A

Average weight of mice = 32g

Dose of extract to be administered = 10mg/kg

Concentration of stock solution of extracts = 50mg/ml

From the above data the volume of the extract to be given to the mice was calculated using the formula:

Normal dose x body weight (kg)

Concentration of stock solution

Normal dose = 10mg/kg

Weight = 32g

Concentration of stock = 50mg/ml

$$\frac{10\text{mg/kg} \times (32/1000) \text{ kg}}{50 \text{ mg/ml}} = 0.01\text{ml}$$

Therefore, 0.01ml of the extract was given to the mice in group A. The formula was used in calculating the volume of the extract to be given to other mice in other groups to meet their respective doses i.e. 100mg/kg, 1000mg/kg etc.

Test of antithrombotic activities

Amount of Aspirin to be administered to the rats in group B was calculated as follows:

The average weight of rats = 125g

Dose of Aspirin to be administered = 250mg/kg

Concentration of stock = 100mg/kg

From the above data, the volume of aspirin to be used was calculated using the formula:

Normal Dose X weight

Concentration of stock

Normal Dose = 250mg/kg

Weight of rats = 125g

Concentration of stock = 100mg/kg

$$\frac{250\text{mg/kg} \times (125/1000) \text{ kg}}{100\text{mg/ml}} = 0.31\text{ml}$$

Therefore 0.31ml of Aspirin will be administered to the rats

Amount of extract to be administered to the rats in group c

Average weight of rats = 138g
 Dose of extracts to be Administered = 250mg/ml
 Concentration of stock = 100mg/ml

The value to be administered was calculated using the formula

$$\frac{\text{Normal Dose} \times \text{weight}}{\text{Stock}}$$

Normal dose = 250mg/kg
 Weight of rats = 138g
 Stock = 100mg/ml

$$\frac{250\text{mg/ml} \times (138/100) \text{ kg}}{100\text{mg/ml}} = 0.38\text{ml}$$

Therefore, the amount of extracts to be administered to group C is = 0.38ml

Amount of extract to be administred to rat in group d

Average weight of rats = 92g
 Dose of extract to be administered = 500mg/ml
 Concentration of stock = 100mg/ml

The volume to be administered was determined by the formula:

$$\frac{\text{Normal Dose} \times \text{Weight}}{\text{Concentration of stock}}$$

Normal Dose = 500 mg/ml
 Weight of rats = 92g
 Concentration of stock = 100mg/ml

$$\frac{500\text{mg/kg} \times (92/1000) \text{ kg}}{100\text{mg/ml}} = 0.46\text{ml}$$

Amount of extract to be administered to the rats in group D is 0.46ml

Administration of extracts and drugs***Method used in administering the drugs***

The animals were divided into groups of A, B, C, and D with each group having a close range of weights. Thus, their weight range was used to calculate the volume of drugs to be administered to each animal. When administering the extract, aspirin and normal saline a canula was fitted onto the nozzle of a 1ml syringe thereby enhancing easy administration. Each animal was picked up with hand by using the thumb and index finger to hold the loose skin of the back of the neck and then lifting the animal allowing the back to rest on your palm then place the tail between the small finger and the ring finger. This is how to hold the animal in place. Then the appropriate

volume of each drug or extract was delivered directly into the oesophagus with the aid of a canula. The canula was used to prevent the influx of drug into the tracheal tube.

Pharmacological tests

Acute toxicity test (LD₅₀)

Lorke Dietrich [19] method of LD₅₀ determination was employed. It involved twelve mice. The experiment was carried out in two stages:

Stage I: Here nine mice were involved. They were divided into 3 groups of 3 mice in each group. The animals in group A received the extracts at a dose of 10mg/kg. Group B received 100mg/kg. Group C received 1000mg/kg. The animals were monitored for 24hrs. The number of deaths in each group was noted.

Stage II: This stage was carried out based on the results of the first stage. Here another three groups of animals, of which one animal was in each group, was employed.

Group A received 250mg/kg extract

Group B received 500mg/kg

Group C received 900mg/kg

The animals were monitored for another 24hrs and number of deaths noted

Tests for antithrombotic activity

In this test 20 Wister rats weighing 90g-138g of both sexes were employed. The animals were divided into 4 groups:

Group A received 0.5ml of normal saline

Group B received 250mg/kg of aspirin (standard drug)

Group C received 250mg/kg extracts

Group D received 500mg/kg extracts

All administrations were a single dose of oral route. The extract, standard drugs and normal saline were administered to the rats for 14 days, and on the 15th day they were taken for hematological analysis.

Haematological analysis

The following hematological analysis/tests were carried out

- Bleeding time test
- Clotting time test and
- Platelet count test

Bleeding time test:

Method – IVY'S METHOD [20]

Methodology:

The animal was placed in a restrainer. Its tail was passed through one end of the opening. The tail of the animal was massaged especially at the point where the tail joins the pelvis. The tail was disinfected with spirit solution, wiped and dried and pricked with a sterile lancet about 4mm deep. The stop clock was started. The oozing blood was wiped with a filter paper at 15sec intervals. The exercise was repeated every 15 seconds on fresh spots of the filter paper until the

bleeding stopped. The number of blood spots on the filter paper were added and divided into 4 to get the bleeding time in minutes.

$$\text{i.e. Bleeding Time} = \frac{\text{Number of blood spots on filter paper}}{4}$$

Whole blood clotting time

Method: The plane slide technique.

Methodology:

The animal was placed in a restrainer and the tail was passed through one of the holes. The tail was massaged especially at the point it joins the rat's pelvis, the tip of the rat tail was disinfected and wiped dry. A pair of sterile scissors was used to clip off the tip of the rat's tail. A drop of blood was placed in the middle of the slide. The blood was gradually lifted up at 15 second intervals using a pin. This was continued until coagulation was noticed. The time for the coagulation was noted.

Platelet count test

Method: Dilution/microscopy Technique

Methodology: Each animal in each group was anaesthetized by confining them in an enclosed container with cotton wool wetted with chloroform. The blood sample was collected through cardiac puncture from each animal. The blood sample was placed in an EDTA container. In the plastic tube, 0.38ml of filtered ammonium oxalate diluting fluid was dispensed; 0.02ml of well mixed EDTA anticoagulated blood was added to the measured diluting fluid in the plastic tube and mixed properly.

A cover slip was fixed on the counting chamber until the Newton Ring appeared on the edges of the chamber. The sample-diluents mixture was remixed properly. Using a Pasteur pipette one of the grids of the chamber was filled with the sample mixture. The charged chamber and its contents were placed in a Petri dish on dampened paper and covered with a lid. The chamber was left undisturbed for 20 minutes to allow the platelets to settle. The underside of the chamber was dried, and, using the 10 x objectives, the rulings of the grid were focused and the central square of the chamber. This was brought into view. The small platelets were focused using the 40 x objective and counted.

Calculation:

$$\text{Platelet counts (per litre)} = \text{No of cells counted} \times \text{dilution factor} \times 10^6$$

Note:

Features of platelets

They are smaller than red blood cells. They either appear as round, oval or elongated particles. They are retractile, stain light bluish colour and they also appear like dirt or debris. Blood is diluted 1 in 20 in a filtered solution of ammonium oxalate reagent which lyses the red blood cells.

Let:

Diluting factor = 20

Volume counted = total area of squares counted X depth

Depth = 0.1mm

Area of one smallest square = $1/25 \times 1/16 = 1/400 \text{ mm}^2$ Total of smallest squares counted = $5 \times 16 = 80$ smallest squares

Let the number of cells counted = N

$$\text{Count/liter} = \frac{N \times 20 \times 10^6}{\frac{1}{4} \times 80 \times 0.1} = \frac{N \times 400 \times 10^6}{80 \times 0.1}$$

$$= \frac{N \times 8000 \times 10^6}{8} = N \times 1000 \times 10^6/\text{L}$$
Platelet counts = $N \times 10^9/\text{L}$ **RESULTS/STATISTICAL ANALYSIS / EVALUATION****Table 1: Results of Phytochemical analysis of seed extract of *Garcinia combogia***

Constituents	Relative Abundance
Carbohydrates	-
Reducing sugar	++
Alkaloids	+
Glycosides	+++
Saponins	-
Tannins	+
Flavonoids	+++
Resins	+++
Protein	++
Oil	-

Key:

- Absent

+ Present

++ Significantly present

+++ Abundantly present

Phytochemical analysis of seed extract of *Garcinia combogia* showed in abundance the presence of glycosides, flavonoids and resins while reducing sugar, and protein are present significantly. Alkaloids and tannins are present while carbohydrate, saponins and oil were absent.

Table 2: RESULT OF ACUTE TOXICITY TEST (LD₅₀)

STAGE	DOSE	NO OF DEATHS
I	10mg/kg	0/3
	100mg/kg	0/3
	1000 mg/kg	3/3
II	250 mg/kg	0/1
	500mg/kg	0/1
	900 mg/kg	1/1

CALCULATION OF LD₅₀

$$LD_{50} = \sqrt{a \times b}$$

Where a = the maximum dose that did not cause death
 b = the minimum dose that caused death

$$LD_{50} = \sqrt{500 \times 900}$$

$$= 670.82\text{mg/kg}$$

Table 3: RESULT OF ANTITHROMBOTIC TESTS

Group	Dose	Bleeding time	Clotting time	Platelet
A	(0.5ml) normal saline	(mins)	(mins)	counts (/L)
		3.75	2.15	400
		4.75	2.00	276
		3.50	2.15	359
		4.25	2.00	390
		4.75	2.00	320
		x 4.20	x 2.06	x 349
B	250mg/kg	7.10	1.15	70
		6.30	2.30	65
		8.00	2.45	72
		7.35	2.00	61
		6.50	2.30	54
		x 7.05	x 2.04	x 64
C	250 mg/kg extract	5.30	2.30	85
		6.15	2.45	80
		6.80	2.00	91
		5.35	2.30	88
		7.00	2.30	96
		x 6.12	x 2.24	x 88
D	500 mg/kg extract	6.38	3.00	75
		7.50	2.30	81
		8.18	2.15	70
		7.30	2.00	84
		9.00	2.00	90
		x 7.67	x 2.29	x 80

$$\text{Variance } (S^2) = \frac{\sum (x_i)^2 - \frac{(\sum x)^2}{n}}{n - 1}$$

$$= \sqrt{S^2}$$

$$\text{S. E} = \sqrt{\frac{S^2}{n}}$$

Where n = no of animals in each group
 Xi = each variance (animal) in a group
 Calculating Mean $\pm$ S.E (Standard Error)

Table 4: RESULT OF ANTITHROMBOTIC TESTS IN M $\pm$ S.E

Group	Dose	Bleeding time (mins)	Clotting time (mins)	Platelet Count (/L)
A	0.5ml Normal saline	4.20 $\pm$ 0.11	2.06 $\pm$ 0.01	349 $\pm$ 10.25
B	250 mg/kg Aspirin	7.05 $\pm$ 0.14	2.04 $\pm$ 0.01	64 $\pm$ 1.44
C	250mg/kg Extract	6.12 $\pm$ 0.15	2.44 $\pm$ 0.03	88 $\pm$ 1.20
	500mg/kg Extract	7.67 $\pm$ 0.19	2.29 $\pm$ 0.08	80 $\pm$ 1.55

Table 5: COMPARISON OF NEGATIVE CONTROL (NORMAL SALINE) WITH THE POSITIVE CONTROL (ASPIRIN) USING STUDENTS T-TEST

Parameter	Group	X	N	SS	DF	T. calc.	T. critical	P – value
Bleeding time	A	4.14	5	1.31	4	3.18	2.306	P<0.05
	B	7.05	5	1.85	4	9.45		P<0.05
Clotting time	A	2.06	5	0.03	4			
	B	2.04	5	1.07	4			
Platelet Count	A	349	5	10552	4	997.2	2.306	P<0.05
	B	64	5	210	4			

Note: P < 0.05 means that there is a significant difference between the two parameters compared.

A= Negative control (normal saline)

B= Positive control (aspirin)

Table 6: Comparison of the Negative control (normal saline) with doses of extract (250mg/kg and 500 mg/kg) using students T –tests

Parameter	Group	X	N	Ss	df	t. cal	t. critical	p-value
Bleeding time	A	4.14	5	1.31	4	6.21	2.306	*
	C	6.12	5	2.49	4			P<0.05
	A	4.14	5	1.31	4	11.04	2.306	*
	D	7.67	5	3.85	4			P<0.05
Clotting time	A	2.06	5	0.03	4	2.00	2.306	+
	C	2.24	5	0.11	4			p> 0.05
	A	2.06	5	0.03	4	6.88	2.306	* p<0.05
	D	2.29	5	0.58	4			
Platelet count	A	349	5	10552	4	1015.7	2.306	*
	C	88	5	162	4			P<0.05
	A	349	5	10552	4	1003.8		*
	D	80	5	242	4			P< 0.05

Note:

* $p < 0.05$ = there is significant difference
+ $p > 0.05$ = There is no significant difference between the parameters being compared
A= Negative control (normal saline)
C= 250mg Extract
D= 500mg Extract

Table 7 comparison of the effects of different doses of the extracts (250mg/kg and 500mg/kg) with the positive control (aspirin) using the Student T-test

Parameter	Group	X	n	ss	df	t. cal	t.critical	p-value
Bleeding time	B	7.05	5	1.85	4	0.41	2.306	*
	C	6.12	5	2.49	4			P<0.05
	B	7.05	5	1.85	4	8.33	2.306	*
	D	7.67	5	3.85	4			P< 0.05
Clotting time	B	2.04	5	1.07	4	9.6	2.306	*
	C	2.24	5	0.11	4			P<0.05
	B	2.04	5	0.07	4	3.76	2.306	*
	D	2.29	5	0.58	4			P< 0.05
Platelet count	B	64	5	210	4	25.02	2.306	*
	C	88	5	162	4			P<0.05
	B	64	5	210	4	15.02	2.306	*
	D	80	5	422	4			P<0.05

Note:

* $P < 0.05$ = there is significant difference
+ $P > 0.05$ = There is no significant difference although there is a slight, difference between rear mean
B= Positive control (aspirin)
C= 250mg Extract
D= 500mg Extract

The result of the antithrombotic test as shown in tables 3 and 4 showed that the bleeding time and clotting time were prolonged with the 500mg extract having more effect on the bleeding time. The platelet counts of the extracts were reduced when compared with both controls with the 500mg/kg extract more reduced. When the negative control (normal saline) was compared with the positive control (aspirin), the bleeding time, clotting time and platelet count were found to be significantly reduced as shown in table 5. When the negative control was compared with

the 250mg/kg extract as shown in table 6, the bleeding time and clotting time were significantly reduced while the reduction in platelet count was not significant. Also when compared with the 500mg/kg extract, the bleeding time, clotting time and platelet count were significantly reduced as shown in table 6. When both extracts were compared with the positive control, the bleeding time, clotting time and platelet count were found to be significantly reduced as shown in table 7.

DISCUSSION

Based on the result obtained, as well as from further statistical evaluation the methanolic seed extract of *Garcinia combogia* demonstrated variability in its ability to influence the hematological indices – bleeding time, clotting time, as well as platelet counts, when compared with values obtained from rats treated with the controlled drugs-normal saline and aspirin-even though in most instances the extract proved to be a good antithrombotic agent.

On bleeding time, (with a normal range of 1 -6 minutes) at dose levels of 250mg/kg and 500mg/kg the extract of *Garcinia combogia* greatly and significantly increased the time it took for the rats to stop bleeding when compared with the values obtained from pretreatment with the negative control-normal saline (4.2 ± 0.11). The prolongation in the bleeding time proves to be dose dependent as a dose of 250mg/kg of the bleeding time for the rat is 7.05 ± 0.19 minutes.

Since bleeding time is an index used to indicate the amount of circulating platelet in blood, it then implies that the extract greatly reduced the number of circulating platelets thereby decreasing the tendency for blood to coagulate via formation of a platelet plug (one of the first processes involved in blood coagulation that is after vasoconstriction), making it less possible to occur and consequently increasing the time it takes for bleeding to stop after its initiation. Since the $p > 0.05$, this proves to be an insignificant statistical result.

Also when the bleeding time values of the effect of the extract is compared with those obtain from rats pretreated with the positive control- aspirin - it was seen that the rate of increment was dose dependent because 250mg/kg of aspirin being an antiplatelet drug produced a longer bleeding time of 7.05 ± 0.14 minutes when compared with that of the extract which produced 6.12 ± 0.15 minutes; while the extract at a dose of 500mg/kg has a longer bleeding time of 7.67 ± 0.19 minutes when compared with aspirin at a dose of 250mg/kg. This then implies that aspirin should reduce to a greater extent the number of circulating platelets when compared with the extract at a dose of both 250mg/kg and 500mg/kg. Thus, when compared statistically, there was a significant difference ($p < 0.05$ as there was a prolongation in the bleeding time at the dose of 500mg/kg (extract).

When comparing the result obtained from the extract, negative control (normal saline) and positive control (aspirin) on the level of platelet circulating in the blood, the results showed a highly inconsistent finding in several instances though not in all respects, as it does correlate with the result obtained from the effect of these drugs substances from the bleeding time of the rats used, which is an index that gives a rough estimate of the level of circulating platelets in the blood. Comparing the result as turned out by the administration of the extract to that due to the administration of normal saline, it can be observed that the extract at the different dose levels, reduced to an appreciable extent the number of circulating platelets, that is from $349 \pm 10.25 \times$

10^9 k to $80 \pm 1.55 \times 10^9$ k and $88 \pm 1.20 \times 10^9$ k for doses of 250mg/kg and 500mg/kg respectively ($P < 0.05$). This result even though consistent with the fact that the extract is capable of decreasing the platelet level in the blood, does not reflect the ability to do that at a greater rate with a higher dose as seen with 250mg/kg and 500mg/kg doses and hence we may at a significant level of 0.05 say that the effect of the extract is dose dependent as it concerns decreasing the platelet number.

Also, comparing the statistically evaluated platelet count values of extract to those of aspirin, it can be observed that aspirin has greater capacity to decrease the number of platelets in the blood, as 250mg/kg of it has $64 \pm 1.44 \times 10^9$ k value of platelets while extract has $88 \pm 1.20 \times 10^9$ k and $80 \pm 1.55 \times 10^9$ k at doses of 250mg/kg and 500mg/kg respectively. The observations on the antithrombotic properties of the extract with regards to the platelet number when compared with dose of aspirin and normal saline may be due to some possible experimental errors or the effect of biological variables in the rats used. Some of these errors are (i) The ability to manipulate appropriately the experimental procedures or achieve uniformity of experimental conditions related to platelet counting, errors arising from the counting fluid used for the dilution as well as the biological variables and hence the rats used for each group.

On a different note, comparing the result obtained from measurement of clotting time (which is an index used to evaluate a person's clotting mechanism, especially either the defectiveness of its prothrombin activator system or thromboplastin level in blood) due to the effect of the extract at the two dose levels used (i.e. 250 and 500mg/kg) to those of both normal saline and aspirin, it was observed that the extract is in all cases significantly better in its ability to prolong the clotting time of rats this was similar to what was observed by Olajide [12]. Hence the extract can be said to be superior or better in this respect. It then implied that the extract significantly decreases the level of circulating thromboplastin and the rate of formation of thrombin since $\text{Promthrobim} + \text{Thromboplastin} + \text{Calcium} = \text{Thrombin}$.

CONCLUSION

The result obtained from the pool of statistically evaluated data shows that the methanolic seed extract of *Garcinia combogia* possesses the antithrombotic properties as it positively influenced the hematological indices-bleeding time, clotting time and platelet number. Where this is true, the antithrombotic effect produced by the extract did not however prove to be superior to the antithrombotic effect produced by aspirin. It should be noted nevertheless that, in the case of clotting time where the extract had a superior effect to aspirin, implying that the extract has a significant ability to decrease the level of circulating thromboplastin in the blood. From the result obtained the methanolic seed extract of *Garcinia combogia* definitely has antithrombotic properties that are dose dependent but not superior to aspirin.

Recommendation:

Since the extract demonstrated a significant antithrombotic effect with regards to prolongation of clotting time in rats, further studies should be designed to:

- i. Delineate its mechanisms of action.
- ii. Extrapolate the result of the findings to human beings
- iii. Explore the possibility of developing new drugs with the seed extracts.

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