Research Article

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Effects of *Tetracarpiduim Conophorum* (African Walnut) on Haemostasis and Body Weight of Swiss Wistar Rats

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ABSTRACT

Haemostasis has been described as the process of preventing or stopping bleeding and as well the tendency to moderate blood in terms of forming clots. This study was designed to investigate haemostatic activity of *Tetracarpiduim conophorum*, (African Walnut) extracted lectin. Wistar Rats (n=35) with 6-8 weeks aged rats weighing from 78g-129g, were purchased and kept for the study in Federal University Lokoja animal house. The wistar rats were grouped into O, P, Q, R, and S. The basal level of all rats in the groups were collected and analyzed for Platelets, Prothrombin Time (PT) and Partial Thromboplastin Time with Kaolin (PTTK) and body weight at every interval. Group O (Control-received Swan water), group P (received 30% lectin), group Q (received 50% lectin), group R (received 70% lectin) and group S (received 90% of lectin) by intra-peritoneal injection. The analysis at intervals revealed Platelet count, Prothrombin Time (PT) and Partial Thromboplastin Time with Kaolin (PTTK) with body weights based on the lectin percentages viz: 30%, 50%, 70%, 90% respectively. The body weight of experimental animal showed significant increase among different concentration $P<0.005$, the platelet count was significantly increased on the basis of days of exposure of $P<0.05$, but Prothrombin Time (PT) and Partial Thromboplastin Time with Kaolin (PTTK) did not show any significant change within the intervals of the study at $P<0.05$. *Tetracarpidium conophorum*, (African walnut) Lectin is contributory to weight gains but do not have any significant effect on haemostatic functions and parameters in Swiss wistar rats.

KEYWORDS: *Tetracarpidium conophorum*: African walnut; Haemostasias; PTTK; PT; Platelets; Weight

INTRODUCTION

Tetracarpidium conophorum (African walnuts) are edible single secreted stone fruit whose plant is mostly cultivated for its nut, which is cooked and consumed as snack. Akpuaka [1] described African walnut seeds as source of Manganese, Zinc, Potassium, Phosphorus, Magnesium, Iron, Vitamin B6, Folate, Calcium, Vitamin K and Vitamin E, and others. Kabiri et al. [2] added several antioxidant activities and numerous biochemical present in the walnut. Some researchers have commented extensively on the nutritional and medicinal importance [3-5], with others dwelling on

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medical laboratory parameters. However, this study is contributing to the aspect of the effects of walnut on hemostasis. Bases on the effects of Lectins on Thrombin, in a bid to show the principles associated with it, Ezealisiji et al. [3] puts it that lectin activity of stimulated platelet was blocked by galactosamine, glucosamine, mannosamine, lysine, and arginine, but not N-acetylated sugar, other neutral sugar or other amino acid. Inhibitor of the thrombin-induced Lectins activity also blocked thrombin-induced platelet aggregation. It appears that a membrane surface complement that has lectin activity mediates platelet aggregation. This however, showed the wound healing activities of walnut.

Alexander et al. [4] shows that Nigerian walnut Lectin is nontoxic and has a positive effect in haematopoietic activity of Swiss Wistar rats. Pieters et al. [5] in their study concluded that high walnut diets consumption had no influence on haemostatic factors. Innih [6] stated that walnut reversed Iron Overload-Induced Cardiac Toxicity in Wistar Rats in addition to Oguwike [7] findings that walnut reduced hypertention among the subjects that used the walnut. No wonder Canales et al. [8] advised that the "Walnut meat diet should be considered a functional meat because it improves the thrombogenic status mainly in individuals with high-cholesterol levels or high BMI". If there is a remarkable increase up to 15% in the case of PTTK and 25% in the case of PT and decrease of up to 50% in Platelets in 24 hours interval, these haemostatic parameters should be said to be in a critical variation unless they subjects are administered with anticoagulant Lippi [9].

Rock [10] highlighted that walnut reduced weight and other parameters contreibutory to heart diseases based on the diet regimen studied. However, Bello [11] showed that walnut leaf residue is less expensive and promising dietary supplementation that would positively affect growth and water quality of *Clarias gariepinus* in aquaculture. Ezzat [12] also showed that walnut lectin increased "growth performance, feed utilization, immunity, and disease resistance of the Nile tilapia, *Oreochromis niloticus fingerlings*". Some researchers are of the opinion that walnut increased body weights of animals (rats). The aim of the study was to investigate the potential effects of *Tetracarpiduim conophorum* on some haemostatics and clotology parameters and body weight of wistar rats with specific objectives to evaluate the effects of *Tetracarpiduim conophorum* on body weights, PT and PTTK of Swiss wistar rats.

MATERIALS AND METHODS

Collection of Materials

Tetracarpiduim conophorum African walnut were bought from Obollo Afor Nsukka of Enugu States in June, 2019. Botany Department, University of Benin confirmed the walnuts (reference number UBHP401).

The Federal University Lokoja animal house was the venue of the study as the rats were housed there. Activated partial thromboplastin time (APTT), prothrombin time (PT) and platelets kits were bought from Apex surgical and Laboratory Equipment Nigeria Limited, Lokoja. Acetic acid and ammonium Sulphate salt were also procured from the same sale's agent in Lokoja, Kogi State. All reagents were of analytical grade and were used without purification. The manufacturer's name of the above reagent is Thame Diagnostic Reagent Limited US. The reagents name for the PTTK and PT, used is Oxon with lot number T, catalogue number CRBT000.

Preparation of *Tetracarpiduim Conophorum,* Lectin

The seeds were firmly crushed into power using a homogenizer and prepared inline with a modified version of Vijaya [13] methodology.

Animal's Study Population

35 numbered 6-8 weeks old wistar rats grouped into 5 (7 per 45X30X42cm cage) were used. The cages were labelled Group O-S for easy identification. The laboratory rats bought in Federal University, Lokoja were stationed at the study section and allowed to acclimatize under the room temperature (25ºC) and relative humidity.

Body Weight and Volume of Lectin Administration

Respective groups O-S were weighed with aid of spring balance and adequate volume/concentration of the Lectin that was administered; the administered dose was determined according to their body weight using the formular (1) below:

$$\text{Dose} = \text{Body weight} \times 5\text{mL} / 1000 \quad \ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Lectin Concentration

Group O – Swan bottled water

Group P - 30% lecithin prepared by dissolving 30g of dry grinded *Tetracarpiduim conophorum* Lectin in 100ml of Swan bottled water.

Group Q - 50% lecithin prepared by dissolving 50g of dry grinded *Tetracarpiduim conophorum* Lectin in 100ml of Swan bottled water.

Group R - 70% lecithin prepared by dissolving 70g of dry grinded *Tetracarpiduim conophorum* Lectin in 100ml of Swan bottled water.

Group S - 90% lecithin prepared by dissolving 90g of dry grinded *Tetracarpiduim conophorum* Lectin in 100ml of Swan bottled water.

Lectin Administration

Wistar rat groups, Group P-S were injected intraperitoneally with *Tetracarpiduim conophorum* Lectin according to their body weight while the control group, Group O were injected with Swan bottled water.

Sample Collection

3 set of sample collection were made from the animals. After acclimatization (Basal Collection-BL), the after first lectin administration (First Collection-FC) and another collection after the second lectin administration (Second Collection-SC).

About 5mL of blood were collected into sodium citrate solution sample bottle and 2ml into EDTA bottle via ocular puncture of the rats for PT and APTT and PLT analysis respectively.

Interval of Sample Collection

The samples for for PT, APTT, and PLT were collected after 11 days following BL, FC and SC respectively.

Sample analysis: Manual methods of analysis were carried out for PT, PTTK and PLT according to Laffan [14] as well as Ochei [15]. SPPS 20 was used to analyze the data.

Determination of Body Weight

With the aid of a spring balance, all Swiss rats were weighed same time prio to sample collections made on the animals.

RESULTS

The means, standard deviation (STD), and the P-Value of the parameters Prothrombin Time (PT), Partial Thromboplastin Time with Kaolin (PTTK) and weight of the animal groups O-S are presented in the tables.

(Table 1) presents the result of mean and standard deviation of the basal collection analysis at different concentrations for Wieghts, PLT, PT and PTTK. The mean weight for Group O (Control) is significant at $p<0.05$ lower than that of test groups. The PLT for the control is significantly ($p<0.05$) higher than other groups but there is no significant difference between control and test groups at $p>0.05$ in the means of PT and PTTK.

Table 1: Result of mean and standard deviation of the groups basal collection analysis.

Parameters	Group O	Group P	Group Q	Group R	Group S	F	*P*
	(Control)	30%	50%	70%	90%		
Weight(g)	77.60±17.90	78.80±14.23	89.40±14.64	88.60±12.22	119.01±19.24	39.02	<0.0001
PLTx10^9(/L)	365.00±9.90	272.00±8.60	314.10±3.11	352.00±24.15	307.30±4.11	309.3	<0.0001
PT(S)	14.40±0.77	14.60±0.74	14.54±0.78	15.15±1.03	14.30±0.61	5.996	0.0002
PTTK(S)	38.87±5.56	38.63±4.69	38.87±5.56	38.87±5.56	40.40±2.06	0.7596	0.553

Key: PLT: Platelets; PT: Prothrombin time; PTTK: Partial Thromboplastin Time with Kaolin.

(Table 2) Presents the result of mean and standard deviation of the Group P (30% concentration). There was no significant difference in the mean of weights but PT and PTTK showed lower values which is significant when compared with other collection days.

Table 2: Result of mean and standard deviation of the group *P* (30% Concentration).

Parameters	BC	FC	SC	F	*P*
Weight(g)	95.2±15.85	94.14±16.89	95.00±15.9	0.0422	0.9587
PLTx10^9(/L)	289.9±10.445	395±5.487	403±2.165	2907	<0.0001
PT(S)	14.38±0.356	14.70±0.6144	14.29±0.348	7.797	0.0007
PTTK(S)	40.5±1.199	41.24±0.18	43.3±1.749	48.81	<0.0001

Key: BC-Basal Collection, FC-First Collection and SC: Second Collection.

(Table 3) Presents mean and standard deviation of the Group Q (50% concentration of lectin) Mean weight and PTTK for basal collection is significantly lower than that of other collection days.

Table 3: Result of mean and standard deviation of the group Q (50% Concentration).

Parameters	BC	FC	SC	F	P
Weight (g)	104.9±1.90	112.38±0.86	113±0.66	446	<0.0001
PLTx10^9(/L)	350.1±1.17	371.8±41.12	363.9±6.17	7.32	0.0011
PTTK(S)	43.09±0.64	43.5±2.87	45.8±3.67	10.13	<0.0001
PT(S)	14.6±1.32	15.64±0.86	16.38±0.99	24.25	<0.0001

(Table 4) Presents the result of mean and standard deviation of the Group R (70% concentration) The mean weight shows statistically significant lower mean values in the basal collection compared to other collection days.

Table 4: Result of mean and standard deviation of the group R (70% Concentration).

Parameters	BC	FC	SC	F	P
Weight (g)	105.13±1.90	113.10±0.93	121.00±1.00	1208	<0.0001
PLTx10^9(/L)	412.60±2.12	364.00±2.29	525.63±4.09	27283	<0.0001
PT(S)	16.50±1.12	15.64±0.86	16.09±0.57	2639	<0.0001
PTTK(S)	49.90±2.85	43.50±2.87	50.63±3.67	1880	<0.0001

(Table 5) shows the result of mean and standard deviation of the Group S (90% concentration) The table also shows the mean weight, PLT, PT shows statistically significant lower mean values in the basal collection compared to other collection days.

Table 5: Result of mean and standard deviation of the group S (90% Concentration).

Parameters	BC	FC	SC	F	P
Weight(g)	120.1±2.278	128.13±2.803	128.13±2.803	108	<0.0001
PLTx10^9(/L)	357.8±4.763	420.3±4.465	465.0±10.689	1941	<0.0001
PT(S)	15.60±0.150	16.55±0.479	16.88±1.166	28.78	<0.0001
PTTK(S)	51.0±1.118	50.0±1.00	51.25±0.968	14.41	<0.0001

(Table 6) presents the result of mean and standard deviation of the 30% concentration of lectin administered to the animals. There was no significant difference in the mean weight of the three groups. The table also shows that the mean PTTK for baseline is significantly lower than that of other collection days.

Table 6: Result of group P (30% concentration) exposure days.

Parameters	BC	FC	SC	F	P
Weight (g)	95.2±15.85	94.14±16.89	95.0±15.91	0.04218	0.9587
PLTx10^9(/L)	289±0.445	395±5.487	403±2.165	12151	<0.0001
PT(S)	14.38±0.356	14.7±0.6144	14.29±0.348	7.797	0.0007
PTTK(S)	40.5±1.199	41.24±0.187	43.3±1.749	48.78	<0.0001

(Table 7) presents the result of group Q (30% concentration) exposure days.

Table 7: Result of group Q (30% concentration) exposure days.

Parameters	BC	FC	SC	F	P
Weight (g)	104.9±1.899	112.38±0.857	113.0±0.660	446.8	<0.0001
PLTx10^9(/L)	350.1±1.166	371.8±4.116	363.9±6.173	224.5	<0.0001
PT(S)	14.6±1.317	15.64±0.857	16.38±0.992	24.31	<0.0001
PTTK(S)	43.09±0.635	43.5±2.872	45.8±3.614	10.32	<0.0001

(Table 8) shows the result of mean and standard deviation of the 70% concentration of lectin administered to rats. There was no significant difference in the mean PTTK over the different collection days. The table also shows that the mean PT for basal collection is significantly higher than that of other collection days. While for the mean weight, PTTK shows statistically significant lower mean values in the basal collection compared to other collection days.

Table 8: Result of group R (70% concentration) exposure days.

Parameters	BC	FC	SC	F	P
Weight (g)	105.1±1.899	113.1±0.927	121±1.00	1214	<0.0001
PLTx10^9(/L)	412.6±2.118	364.0±2.291	525.6±4.091	27268	<0.0001
PT(S)	16.5±1.118	15.6±1.495	16.09±0.572	5.592	0.005
PTTK(S)	49.9±2.848	50.25±3.152	50.63±3.672	0.4439	0.6428

(Table 9) presents the result of mean and standard deviation of the 90% concentration administered to the animals. The mean weight, PT and PTTK shows statistically significant lower mean values in the basal collection when compared to other collection days.

Table 9: Result of group S (90% concentration) exposure days.

Parameters	BC	FC	SC	F	P
Weight (g)	120±2.278	128±2.803	129.3±3.799	96.92	<0.0001
PLTx10^9(/L)	358±4.763	420.3±4.465	465±10.689	1933	<0.0001
PT(Sec)	15.6±0.150	16.6±0.479	16.88±1.166	29.5	<0.0001
PTTK(Sec)	51.0±1.118	51.0±1.118	51.25±0.968	0.6365	0.5312

DISCUSSION

There was body weight gain in the animals based on the concentrations of the lectin administered to them, even at high volume of blood collected from the rats- and this support the earlier report of Sabaté [16] in humans, Adeyomo [17] on broilers. Kim [18] and Luo et al. [19] in mice. The weight showed significant increased value of 0.02215 (P<0.05) when the harvest results were compared with the baseline results, this is an indication that lectinic content of Tetracarpidium conophorum in capable of improving weight and growth, and this is in agreement with the work of Ojobor [20] and Kim [18]. The platelet count were significantly increased at value of 0.0026(P<0.05) from the basal collection result, when compared with first and second collection, this is a clear evidence that *Tetracarpidium conophorium* lectin can induce platelet in the rats but does not enhance or promote coagulation test procedure

in rats serum samples. Likewise, this confirmed Kirichuk [21] that some plant lectins specifically bind to different carbohydrate determinant glycoprotein.

Prothrombin Time (PT) and partial thromboplastin Time with Kaolin (PTTK) only showed significant difference at the basal collection as compared to the first and second collection. That makes it clear that the first and the second collection showed no significant difference which agreed with the study of Pieters etal. [5] and Rojer etal. [22]..

Basal collection result in the light of the various concentration of administration ranging from 30%, 50%, 70% and 90%. This is actually for the baseline sample analysis before the administration of lectin. The result recorded for the basal collection were of low values as compared to after lectin administration particularly for weight a while the increase after lectin administration had no much effect on PT and PTTK respectively. Weight increased significantly during the study and this agreed with the studies conducted in the past [23,24]. This growth could be due to cellular trapping of glucose [25] no wonder in a study [26]. It was adequately highlighted that walnut helps in weight management.

CONCLUSION

The lectin increased platelet count and reduces PT and PTTK based on how often it is being consumed, while on the note, there was a body gain of the experimental animals when the lectin was administered at higher concentration despite the fact the *Tetracarpidium conophorum* seed has been processed. The study gave a clear indication that *Tetracarpidium conophorum* does not enhance coagulation or clotting profile procedures and do not affect the clotting or fluidity of blood. This study also support that African walnut can be used in weight control and management.

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