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Effects of *Gnetum africanum* (Welw) methanol leaf extract on weight and haematological profile of wistar rats following chronic oral administration

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Abstract

Gnetum africanum (Welw) leaves have been in use as herbal remedy for treatment of pile, high blood pressure, diabetes and fungal infections especially in West Africa. There is a dearth of information on their effects on body weights, relative organ weights and haematology of rats following chronic administration. The leaves of *G. africanum* were collected and air dried, pulverized and soaked in 80 % methanol for 48 hours. The extract was concentrated *in vacuo* using a rotary evaporator and stored as *G. africanum* extract (GAE) at 4^{o}C. Fifty-six rats randomly divided into four groups (n=fourteen) were used in this study. The rats were fed growers ration (Vital® Feed, Nigeria) into which the extract was incorporated at various doses for ninety days. Rats in group 1 received feed without GAE, while rats in groups 2, 3 and 4 were given feed with GAE at 10, 20 and 40 mg/kg, respectively. Four rats from each group were weighed and humanely sacrificed at days 30, 60 and 90 for measurement of liver, kidney, lungs and spleen weights. Treated rats had significantly ($p < 0.05$) higher body weights and lower relative organ weights than the control. Also, rats given 20 mg/kg (group 3) and 40 mg/kg (group 4) of extract in feed at days 60 and 90, had significantly ($p < 0.05$) higher red blood cell count than the control. There were no significant effects on the white blood cells.

Keywords: Body weights, chronic toxicity test, *Gnetum africanum, relative organ weights*, wistar rats

Introduction

Gnetum africanum Welw (African jointifr) is an edible plant widely used as food. In Nigeria, it is called *afang* (Efik/ Ibibio); *ukazi, ukazi* (Igbo); *yala* (Ogoja); *ajaabaje; ajakotale* (Yoruba), in Cameroon it is known as *eru, okok, mfumbua or fumbua* and *koko* in Angola, Gabon and Central African Republic (Burkill, 1994). *Gnetum africanum* has been used in Nigeria in the treatment of piles and high blood pressure (Okafor, 1997), sore throats and as a cathartic (Burkill, 1994). In Ubangi (DR Congo), it is used in the treatment of nausea and considered as an antidote to arrow poison made from *Periploca nigrescens* Afzel (Burkill, 1994; Iwu 2010). In Cameroon, the leaves are chewed to mitigate the effects of drunkenness and also taken as an enema against constipation and to ease child birth (Burkill, 1994). They are also used to treat diabetes, boils and fungal infection in the fingers (Iwu, 2010). *Gnetum africanum* leaves have great culinary value in West Africa. They are eaten raw as local salad, shredded and used in preparing soups and stews (Burkill, 1994). It is a good source of protein and has been

noted as an anti-inflammatory, anti-carcinogenic and antioxidant (Ali *et al.*, 2011*)*. However, there is paucity of data on the effect of chronic administration of the crude extract in feed. The organs, especially the liver and kidneys, are most often affected by intake of herbs and natural medicines for long periods of time, leading to increases in liver and kidney sizes which sometimes lead to liver and kidney failures. Sub-chronic test is a 90-day repeated daily treatment of rodents through feed, gavage or drinking water from which the no-observed-adverse-effect-level (NOAEL) is often deduced (OECD, 1998).

This study was therefore conducted to determine the effect of *Gnetum africanum* methanol leaf extract on body weights, relative organ weights and haematology of Wistar rats using chronic toxicity methods.

Materials and Methods

Plant collection and extraction

Gnetum africanum (Welw.) leaves were collected between October and November 2014 from its natural habitat in Orba, Nsukka, Enugu State ($6^{o}5^{'}$ $7^{o}31^{'}$E) and identified by a Taxonomist Bioresources Development and Conservation Programme (BDCP), Aku Road, Nsukka, Enugu State. The leaves were dried under mild sunlight and pulverized into coarse powder of about 1 mm in diameter. Plant extraction was by soaking 2 kg of the dried leaves in 80 % methanol for 48 h with intermittent shaking at 2 h intervals after which they were filtered with Whatman® No. 1 filter paper. The filtrate was then concentrated *in vacuo* using rotary evaporator (Rotovap) connected to a cold water circulator with pressure pump at 210 milibar. The extract was stored in a refrigerator as *Gnetum africanum* extract (GAE). Percentage yield was calculated using the formula below:

$$\%\, YIELD = {}^{b}/_{a}\ x\ {}^{100}/_{1}$$

Where a = Weight of original plant material used for extraction and b = weight of the recovered extract.

Experimental animals

Mature Wistar rats of both sexes procured from the Laboratory Animal Units of the Faculties of Veterinary Medicine and Pharmaceutical Sciences, University of Nigeria, Nsukka, were used for the experiments. They were housed in an environment of normal ambient temperature and the lighting period was about 12 h daily and relative humidity of 40-60 %. The weight of the rats varied between 97 and 150 g. The rats were kept in stainless steel cages, supplied with clean drinking water and fed *ad libitum* with standard commercial pelleted feed (Vital® Feed, Nigeria).

Chronic toxicity test

Preparation of experimental feed containing different concentrations of G. africanum extract: *Gnetum africanum* (10, 20 and 40 mg/kg) representing low, medium and high doses was incorporated in feed (Grower's mash, Vital Feed®, Jos, Nigeria). The extract of each dose was first dissolved in 20 ml of water and then uniformly made up to 2.5 L per 3 kg feed. The feed and water containing extract were mixed thoroughly and the mixture was manually pelleted using a standard pelleting machine. The pelletized feed was dried for 5 days under mild sunlight. The feed was then stored in a dry environment with intermittent drying to prevent fungal growth.

Experimental procedure: The chronic toxicity study was done according to the OECD guideline for 90-day testing (OECD, 1998), with some modifications. Fifty six (56) albino rats of both sexes were randomly grouped into four groups (n = fourteen). Males and females were separated in different cages to avoid breeding. Group 1 rats received feed without extract (control) while rats in groups 2, 3 and 4 were given 10, 20 and 40 mg/kg GAE in feed respectively while clean drinking water was given *ad libitum.* On days 30, 60 and 90, four (4) rats from each group were randomly selected for the determination of changes in body weights and haematological studies after which they were humanely sacrificed using chloroform inhalation and internal organs (liver, kidney and lungs) were weighed.

Body weights and relative organ weights (ROW)

The changes in body weights of the rats were recorded. The organs were weighed using sensitive weighing balance. The relative organ weights for different groups on days 30, 60 and 90 were calculated using the following formula:

Relative organ weight = ${}^{weight\ of\ organ}/_{weight\ of\ rat}$

Haematology

Blood samples collected on days 30, 60 and 90 were used for haematology. Red Blood Cell (RBC) and White Blood Cell (WBC) counts were determined using haemocytometer methods as described by Schalm *et al.* (1975). Packed cell volume (PCV) and haemoglobin (Hb) concentrations were determined using haematocrit and cyanomethaemoglobin

methods, respectively. Red Blood Cell indices including Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated (Estridge *et al*., 2000).

Statistical analysis

Data generated were presented as mean ± SEM and subjected to one-way analysis of variance (ANOVA) using the statistical package for social scientists (SPSS) software. The variant means were separated using the least significant difference (LSD) method. The differences in means were considered significant at $P < 0.05$.

Results

Extraction of Gnetum africanum

The methanol leaf extract of *Gnetum africanum* had a very dark green colour and an oily consistency. The percentage yield was 10.30 % w/w.

Mean Body weights

Treated rats had a mean weight of 173.5±11.22 g at day 60 in the low dose group. This was significantly ($p < 0.05$) higher than that of the control (147.70±15.40 g). Also at day 90, rats in the high dose group weighed 175.66±18.70 g and was significantly ($p < 0.05$) higher than the control which had a mean weight of 156.16±21.08 g. (Table 1).

Relative organ weights.

The relative weights of the liver, lungs and kidney of the rats given GAE in feed for 90 days are presented in Table 2. Rats in the high dose group had significantly ($p < 0.05$) higher mean relative lung weights than the control on day 60. However, there were no significant ($p > 0.05$) differences between relative weights of the lungs of the rats in the control and treated groups on days 30 and 90. GAE did not cause significant ($p > 0.05$) increases in the weights of the kidney when compared to the control rats, however rats treated with the extract at 40 mg/kg had significantly ($p < 0.05$) lower kidney weights (6.42±0.44) than the control (7.71±0.58).

Table 1: Mean body weights of rats given GAE in feed for 90 days

		Mean body weights of rats ±SEM (g)			
Group	Treatment	Day 1	Day 30	Day 60	Day 90
1	Control	122.1±10.80	143.64±10.32	147.70±15.40	156.16±21.08
2	GAE 10 mg/kg in feed	129.21±10.90	148.21±9.80	173.5±11.22*	168.50±1.98
3	GAE 20 mg/kg in feed	125.21±16.96	142.14±18.08	160.20±16.66	172.16±17.68
4	GAE 40 mg/kg in feed	133.92±14.54	153.42±13.40	162.80±12.82	175.66±18.70*

*p <0.05 when compared to the control group. Data presented as mean ± SD; GAE= *Gnetum africanum* extract

Table 2: Relative organ weights of rats given GAE in feed ($x10^{-3}$ ±SEM g)

Group	Treatment	Day 30	Day 60	Day 90
	Liver			
1	Control	36.62±2.16	39.78±4.18	36.29±4.76
2	GAE 10 mg/kg in feed	34.02±4.30	40.22±1.88	36.78±4.92
3	GAE 20 mg/kg in feed	34.81±4.20	34.69±1.44**	31.89±1.20
4	GAE 40 mg/kg in feed	31.71±1.76	33.35±1.98***	32.23±1.94**
	Lungs			
1	Control	6.72±0.78	5.18±1.06	6.50±0.68
2	GAE 10 mg/kg in feed	7.41±1.12	6.78±9.22*	8.28±1.22
3	GAE 20 mg/kg in feed	8.40±2.86	6.07±0.20	7.68±6.72
4	GAE 40 mg/kg in feed	7.86±1.20	6.44±1.06	6.34±0.92
	Kidney			
1	Control	7.55±0.42	6.90±0.26	7.11±0.58
2	GAE 10 mg/kg in feed	7.44±0.76	7.15±0.98	6.71±0.40
3	GAE 20 mg/kg in feed	7.33±0.72	7.50±4.80	7.63±1.14
4	GAE 40 mg/kg in feed	6.77±0.42	7.92±1.32	6.42±0.44*

Data are presented as mean ± SD; GAE= *Gnetum africanum* extract

*p<0.05. **p< 0.01,***p< 0.001 when compared to the control group

Haematology

Rats that received 20 and 40 mg/kg of extract had significantly ($p < 0.05$) higher RBC count, MCV and MCH than the control on days 60 and 90. There were no significant ($p > 0.05$) differences between the WBC count, PCV, (Table 3), Hb concentration of treated rats and the control (Figure 1).

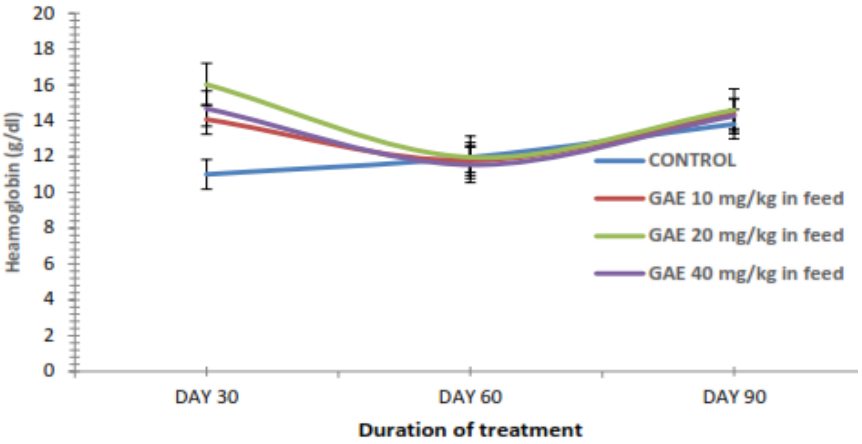

Figure: One Haemoglobin content of rats given GAE in feed for 90 days

Table 3: Haematological parameters of rats fed GAE daily for 90 days

Group	Treatment	Day 30	Day 60	Day 90
	RBC($x10^6$/ µl ± SEM)			
1	Control (feed without extract)	6.33±4.37	5.35±0.41	8.37±1.01
2	GAE 10 mg/kg	8.90±0.26	5.46±0.53	8.27±0.52
3	GAE 20 mg/kg	9.53±1.29	7.53±1.24**	12.47±3.81
4	GAE 40 mg/kg	8.41±0.84	5.40±0.38	13.20±3.90*
	WBC($x10^3$/ µl ± SEM)			
1	Control (feed without extract)	10.26±7.11	9.28±2.52	14.26±5.00
2	GAE 10 mg/kg	10.43±0.07	9.78±0.79	11.10±2.41
3	GAE 20 mg/kg	12.48±12.48	10.80±1.85	10.96±0.69
4	GAE 40 mg/kg	10.60±10.06	11.98±1.61	10.16±1.14
	PCV (% ± SEM)			
1	Control (feed without extract)	41.87±2.83	38.50±5.80	36.75±2.06
2	GAE 10 mg/kg	39.00±3.74	41.00±2.16	36.75±1.50
3	GAE 20 mg/kg	43.50±2.41	42.12±2.17	38.00±2.16
4	GAE 40 mg/kg	41.75±2.36	38.25±2.95	37.25±0.95
	MCV (fl ± SEM)			
1	Control (feed without extract)	13.12±8.81	72.92±15.48	44.24±4.70
2	GAE 10 mg/kg	15.84±1.46	75.75±9.80	44.57±3.79
3	GAE 20 mg/kg	17.04±2.64	56.92±8.89*	32.28±8.13*
4	GAE 40 mg/kg	17.52±1.47	70.87±3.64	29.90±7.80*
	MCH (pg ± SEM)			
1	Control (feed without extract)	25.85±17.25	22.57±4.02	16.63±2.45
2	GAE 10 mg/kg	36.58±2.74	20.87±4.36	17.39±0.97
3	GAE 20 mg/kg	36.64±2.28	16.28±3.57*	12.39±3.00*
4	GAE 40 mg/kg	34.68±0.39	21.4±1.63	11.39±2.68*
	MCHC (% ± SEM)			
1	Control (feed without extract)	38.12±25.73	33.53±6.62	37.51±2.35
2	GAE 10 mg/kg	43.56±6.26	28.79±2.36	39.15±2.60
3	GAE 20 mg/kg	46.36±4.64	28.40±1.74	38.47±1.62
4	GAE 40 mg/kg	50.58±5.11	30.22±2.22	38.30±1.44

Data are presented as mean ± SD; GAE= *Gnetum africanum* extract
*$p < 0.05$ and**$p < 0.01$, when compared to the control group

Discussion

In the chronic toxicity study, rats given GAE in feed had significantly ($p < 0.05$) higher body weights on days 60 and 90 than the control (Table 1). This could also suggest that GAE was well accepted. Changes in body weight have been described as an important indicator of adverse effects of drugs and phytomedicines (OECD, 2001). A previous study reported high amino acid content in *G. africanum,* with only tryptophan not present (Ali *et al*., 2011). Amino acids are needed for growth and differentiation. Possibly the rats given GAE had access to these amino acids present in the extract, unlike those in the control group. This could explain such better growth and weight gain observed in GAE fed rats.

Relative liver weights of rats in the control group was significantly ($p < 0.05$) higher than that of treated groups (Table 2) and the weights obtained in the control group agrees with that recorded for control rats in a previous study (Ali *et al.*, 2012). The liver is the biggest of all the organs studied in this work, and the high relative weights in the control rats is an indication that the increase in weight seen in GAE treated rats was not due to increase in organ weight, rather, it was due to an increase in actual body mass. Liver is involved in metabolic degradation of both

natural and synthetic chemicals, during which process hepatotoxic metabolites may be generated leading to liver injury which could be seen as increase in liver size (Frenzel & Teschke, 2016). The extract did not cause increase in relative liver weight. This suggests that it is not harmful to the liver. Also, there were no significant ($p > 0.05$) differences between relative kidney weights of rats in the control and treated groups. Again this suggests that the plant is safe as the kidney is involved in excretion of harmful metabolites from the body was not adversely affected. Constant excretion of these metabolites could lead to kidney abnormalities which will be seen grossly as increases in relative kidney weights.

Hematological study was conducted on rats given GAE for 90 days as well as the control to ascertain the effect of this extract in the blood when taken for a long time. The increase in red blood cell count observed at days 60 and 90 agree with Nubila *et al*. (2013) and Ufelle *et al*., (2016) who reported increases in RBCs of rats fed *G. africanum* at 3 and 30 days, respectively (Nubila *et al*., 2013; Ufelle *et al*., 2016). This suggests that *G. africanum* could enhance erythropoiesis. As a result of the higher RBC count in the treated groups, MCV and MCH which are indices of the RBC count were significantly ($p < 0.05$) lower in these groups. However, there were no complementary increases in the packed cell volume (PCV) of treated rats. More importantly, rats given GAE in feed had no significant ($p < 0.05$) difference between their white blood cell counts and the control. Since increase in total WBC count is an indication of inflammation, it is safe to conclude that the extract did not cause any injury to the organs studied as also corroborated in the absence of increases in relative weights of these organs.

In conclusion, chronic oral administration of crude methanol leaf extract of *Gnetum africanum* in feed of rats increased body weight of rats without increasing relative organ weights, and also increased red blood cell count of treated rats while the white blood cell count was not increased. More studies are required to determine the actual effect of this plant on PCV. Therefore, chronic intake of this plant as remedy for disease conditions can be considered as a fairly safe practice.

References

Ali A, Khan HA, Abbas F & Inayet O (2012). Comparison of body and liver weights in albino rats after treatment with tamoxifen and taurine. *Journal of Medical Sciences*, **20**(4): 171-175.

Ali F, Assanta MA & Robert C (2011). *Gnetum africanum:* A wild food plant from the African forest with many nutritional and medicinal purposes. *Journal of Medicinal Foods,* **14(**11): 1289 – 1297.

Burkill HM (1994). *Useful Plants of Tropical Africa*. Vol. 2 families E-I. Royal Botanical Gardens, Kew. Pp 648.

Estridge BH, Reynolds AP & Walters NJ (2000). *Basic Medical Laboratory Techniques*, fourth ed. Delmar: Thomson Learning, Albany, New York. Pp 209-210.

Frenzel C & Teschke R (2016). Herbal Hepatotoxicity: clinical characteristics and listing Compilation. *International Journal* of *Molecular Sciences*, **17**(5): 588-519.

Iwu MM (2010). *Handbook of African Medicinal Plants*, second ed. Taylor and Francis Group, Florida. Pp 224-226.

Nubila T, Ukaejiofo EO, Nubila EI, Iyare EE, Shu EN, Chijioke CP & Ibok IF (2013). Subacute effects of methanolic leaf extract of *Gnetum africanum* on haematological profile in albino wistar rats. *International Journal of Medicinal and Aromatic Plants,* **3**(2): 220-225.

Okafor JC (1997). Conservation and use of traditional vegetables from woody forest species in Southeastern Nigeria. In: Guarino L (Ed). *Proceedings of the IPGRI international workshop on genetic resources of traditional vegetables in Africa: Conservation and use 1995 Conference.* Kenya: ICRAF. Pp 31-38.

OECD (1998). Guidelines for the Testing of Chemicals: Repeated dose 90-day Oral Toxicity Study in Rodents. 408. Adopted 21st September 1998. http://oecd-library.org/environment/test-no-408-repeated -dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en, retrieved 30-05-2016.

Organization of Economic Co-operation and Development (2001). OECD guidelines 423: Oral toxicity study in rodents. *OECD Guideline for the Testing of Chemicals*. Pp 1-8.

Schalm OW, Jain NC & Carroll EJ (1975). *Veterinary Haematology*, third ed. Lea and Fabiger, Philadelphia. Pp 15-81.

Ufelle SA, Achukwu PU, Ikegwuonu IC & Ghasi S (2016). Haematological effects of *Gnetum africanum* leaf extract on wistar rats. *International Journal of Ethnomedical Pharmacognosy,* **3**(1): 14-19.