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Safety assessment of the fruit extract of *Acacia nilotica* Linn. Willd ex Delile using rat models

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Key words: *Acacia nilotica* fruit, Aqueous extract, Oral acute and subchronic toxicities, Safety

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Abstract

The *Acacia nilotica* fruits are commonly used for their medicinal and food merits. The present work was carried out to evaluate the innocuousness of an *Acacia nilotica* aqueous fruit extract (ANFE) using acute and sub-chronic oral administration in rats. ANFE doses of 1, 2, 4, 8 and 16 g/kg were orally administered to rats, the mortality and toxic effects were observed for 14 days. For sub-chronic test, ANFE doses of 200, 400, 800 mg/kg and satellite group (800 mg/kg) were orally given to rats daily for 35 days, control was distilled water. Food behaviour, body weight, food efficiency and weight of faeces were followed during the treatment. At the end of administration, all the rats were kept fasted overnight and then sacrificed under anaesthesia. Blood was then collected from jugular vein for haematological and biochemical analyses. Results show that a single dose of ANFEs oral administration did not cause any mortality; LD_{50} was > 16 g/kg. Daily oral administration of ANFE increased significantly the food and water consumption, but decreased the faeces weight after 2 weeks of treatment. However, no significant modifications were observed in the haematological values, markers of renal and hepatic functions, body weight, absolute and relative organ weights, structures of kidney and liver. There were remarked hypoglycaemic, hypocholesterolaemic and hypolipidaemic effects of ANFE. These results suggest that oral administration of ANFE does not induce oral acute and sub-chronic toxicities. Therefore, these safety levels of *A. nilotica* fruit aqueous extract can be helpful for its optimal utilisation.

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Introduction

Acacia nilotica Linn Willd ex Delile (syn. *Acacia arabica* Lam. var. *nilotica* L. Benth) is a multipurposee tree, locally known in Cameroon as "Gavdé" or "Gavdi", belonging to the Mimosaceae or Fabaceae family. Many parts of *Acacia nilotica* are widely used in traditional medicine to treat various diseases (Malviya *et al*., 2011).

A. nilotica bark, gum, roots and leaves are very helpful in traditional medicine, but increasing attention has been directed toward *A. nilotica* (AN) fruits (pods and seeds) for their medicinal, food, technological and ecological benefits. Studies have shown the antihypertensive, antispasmodic, antioxidant, anti-quorum sensing, antidiarrheal and anti-inflammatory effects of pods (Gilani *et al*., 1999; Singh *et al*., 2009; Sanni *et al*., 2010; Sokeng *et al*., 2013), the antiplasmodial activity of husk and seed (El-Tahir *et al*., 1999), anthelmintic effects of the whole fruit (Bachaya *et al*., 2009).

AN fruits are traditionally used to treat several pathologies, such as gastrointestinal disorders, asthma, haemorrhoids, fever, sore throat, diarrhoea, hypertension, obesity, early ageing and toothaches (Malviya *et al*., 2011; Koubé *et al*., 2016). In addition, we reported that AN fruits are often combined with other plants, such as *Allium sativum*, *Euphorbia hirta*, *Bidens pilosa* and *Vernonia amygdalina* for the prevention and treatment of cancers, infertility, diabetes, cardiovascular diseases, viral, bacterial, parasitical and fungal diseases. Grilled seed is a spice for special sauces, while the whole fruit is a good source of feed for goats, sheep and camel during the dry season. The seeds are used for tanning and dyeing of leather (Arbonnier, 2000).

As medicinal and food uses of AN fruits are gaining interest, studies have been carried out to evaluate its toxicity. Al-Mustafa and Dafallah (2000) reported a low toxicity potential in rats fed with a diet containing 2 % and 8 % of AN pods for 4 weeks. In contrast, single administration of the ethanol AN fruit extract was toxic in rats via intraperitoneal route at doses of 75 - 500 mg/kg, with LD_{50} of 215.36 mg/kg corresponding to a mortality of 20-100% (El-Hadiyah *et al*., 2011). These authors have observed the renal and hepatic toxicities in rats at a dose of 60 mg/kg, via intraperitoneal route regarding repeated administration during 3 weeks.

Although the oral use of AN fruit is widespread by decoction or infusion, there is lack of systematic study to establish its levels of safety. Therefore, the aim of this study was to investigate the oral acute and sub-chronic toxicities of AN fruit aqueous extract in rats.

Materials and methods

Plant material

The dried fruits (pods with seeds) of *Acacia nilotica* Linn Willd ex Delile were collected in December in Maroua town, Far North Region of Cameroon. The plant was identified by Pr. P.M. Mapongmetsem of the Department of Biological Sciences (University of Ngaoundéré) and authenticated by Mr. J. Nana of the Institute of Agricultural Research for Development by comparing with the specimen stored in the National Herbarium of Yaoundé (Cameroon), number N.H.C.8582. A voucher specimen was deposited in the Department of Biological Sciences, University of Ngaoundéré, Cameroon.

Preparation of A. nilotica fruit extract

A. nilotica fruit extract (ANFE) was prepared according to traditional use. The fruits (pods and seeds) of *Acacia nilotica* (AN) was rinsed with tap water, kept at room temperature for 48 h, hand grind into fine powder in an electric cereal mill. The decoction was made by boiling the AN fruit powder (1kg) in 10 L of distilled water for an hour and cooled. After decantation, the supernatant was filtered, concentrated at 55°C by evaporating in a vacuum rotary evaporator and dried to a constant weight in an oven set at 40°C. The dried extract gave a yield of 14.9 % (w/w) and stored at 4°C until used.

Animals

Female and male Wistar Albino (WA) rats used in this study were obtained from the animal core facility of the Faculty of Science, University of Ngaoundere, Cameroon.

The animals were clinically, healthy and randomly assigned to control and treated groups. They were housed in cages of 5 per group and maintained in controlled conditions (23 ± 1°C, 60% relative humidity, and 12 h dark-light cycle). All rats were allowed free access to food (68.5% corn, 11.5 fish, 10% crab cotton, 9.5% palm oil and 0.5% salt) and tap water *ad libitum*. The acclimatization period lasted 7 days, and then the animals were fasted overnight with free access to water prior to dosing. Treatment was given by gavage of 10 mL/kg rat body weight (BW) using oro-gastric tube. All of the experimental protocols in animals were carried out according to standard guidelines for the care and use of laboratory animals described by the Institute for Laboratory and Animal Research (ILAR, 2011).

Acute oral toxicity study

The acute oral toxicity of *Acacia nilotica* was carried out in accordance with the World Health Organization (WHO) guideline (WHO, 2000). Briefly, sixty rats (12 weeks old, 126 - 237 g body weight) were randomly divided into 6 groups of 10 animals each (5 females and 5 males). Animals of each group were administered doses of the ANFE (1, 2, 4, 8 and 16 g/kg) while the control group received vehicle (distilled water).They were not fed for 5 h following the dosing. Body weight, signs of toxicity and mortality were observed after the administration at the first, second, third, fourth and fifth hour and once daily for the next 14 days, and LD50 was estimated. On the fifteenth day, all the rats were kept fasted overnight and then sacrificed under anaesthesia for the gross pathological observations of the tissues.

Sub-chronic oral toxicity study

According to the WHO guideline (WHO, 2000), Wistar Albino rats (6 weeks old, 60-120 g) were randomly divided into 5 groups of 10 animals each (5 males and 5 females) and housed in cages of 5 per sex. The animals were daily treated at the ANFE doses of 200, 400 and 800 mg/kg/day for 35 days every afternoon while the control group received water vehicle (10 mL/kg). In order to assess reversibility or permanence toxic effects at high-dose, the ANFE at the dose of 800 mg/kg was given once daily to the fifth group of rats for 35 days, and kept for another 14 days post treatment (satellite group). Food and water consumption, faeces and body weight were daily measured every afternoon before administering the extract, and then food efficiency was calculated. The general signs of toxicity included vomiting, diarrhoea, spiting, fatigue, changes in skin, fur, piloerection, mucous membranes, occurrence of secretions and excretions, autonomic activity, changes in gait, posture and response to handling as well as the presence of tonic movements, stereotype or bizarre behaviour and mortality were observed daily during the experiment period. At the end of the treatment period, animals were fasted overnight with free access to water. They were exposed to chloroform anaesthesia. Blood was then collected from jugular vein for haematological and biochemical analyses. After blood collection, the rats were slaughtered under anaesthesia and organs such as heart, lung, pancreas, liver, spleen, brain, ovaries, testicles and kidneys were rapidly removed, weighed and examined macroscopically. The ratio of each organ to terminal body weight (relative organ weight) was calculated.

Measurement of the haematological parameters

Blood samples (2 mL) were taken in tubes containing Na+ EDTA (1.5 mg) for haematological analyses, using automated haematology analyser (BC 3000 Plus, SHENZHEN MINDRAY, Hambourg, Germany). Haematological study included red blood cell count (RBC), haemoglobin concentration (Hb), haematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean platelet volume, platelets (Plt), and white blood cell count (WBC), lymphocytes (Lymph) and granulocytes (Gran).

Biochemical indices

Biochemical analyses were carried out on serum using automatic chemistry analyser (UV spectrophotometer 3000 Plus Evolution, Hambourg, Germany). Blood samples were collected in tubes without additive and centrifuged at 7500×g at 5°C for 5 min to obtain serum.

The serum was analysed for biochemical indices including alanine aminotransferase (ALT),alkaline phosphatase (ALP) and aspartate aminotransferase (AST) activities, serum urea nitrogen (Ur), creatinine (Cr),chloride ions (Cl^-), sodium ions (Na^+), potassium (K^+), glucose (Glu), total protein (TP), albumin (Alb), total bilirubin (TB), triglycerides (TG), total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C) using specific commercial diagnostic kits (Chronolab systems, Barcelona, Spain).

Statistical analysis

Statistical analysis was carried out using the Statgraphics Plus software version 5.0.

The data are shown as the mean ± standard deviation (SD) and were analysed using one-way analysis of variance (ANOVA). Significant differences between the control and experimental groups were determined using a low significant difference (LSD) multiple comparison Duncan test at 5% level of confidence.

Results

Acute oral toxicity of ANFE

No visible signs of acute toxicity or mortality were noticed at 1, 2, 4, 8 and 16 g/kg oral single dose administration of ANFE during the 14 days of observation (Table 1).

Table 1. Acute toxicity of ANFE in rats.

Dose (mg/kg)	Sex[a]	Mortality (%)[b]	Toxicity signs [c]	Toxicity latency (h)
Control	F	0	None	-
	M			
1000	F	0	None	-
	M			
2000	F	0	Permanent grooming	24
	M			12
4000	F	0	Permanent grooming, dark and hard faeces	48
	M			36
8000	F	0	Permanent grooming, piloerection, stupor, dark and hard faeces.	72
	M			48
16000	F	0	Permanent grooming, piloerection, stupor, dark and hard faeces, anorexia	72
	M			72

[a] F : female, M : male

[b] Dead/treated (N) x 100. N= 10 rats

[c] Animals were observed during 14 days after the single dose administration of ANFE, to note any adverse effect.

Thus indicating that the oral LD_{50} of ANFE was higher than 16 g/kg in female and male rats.

Subchronic oral toxicity of ANFE

Food and water consumption, body mass and food efficiency

Table 2 Presents food and water consumption, body mass and food efficiency of female and male rats. No significant differences were observed in body mass between treated rats and control excluding the group of female rats receiving 200 mg/kg/day. A significant increase ($p < 0.05$) was observed in food and water consumption of female and male rats compared to control, while food efficiency decreased significantly ($p < 0.05$) except female and male rats treated at middle dose (400 mg/kg/day). However, in female and male rats, the values of food efficiency of satellite group were found similar to control.

Table 2. Food and water consumption, body mass and food efficiency of female and male rats in the 35 days daily oral gavage of ANFE.

Parameters	Dose (mg/kg/day)				
	Control	200	400	800	800^{S}
Female n= 5					
TWI5^{a}	418.2 ± 34.0	476.9 ± 21.4*	489.6 ± 19.0*	548.1 ± 27.1**	519.1 ± 29.0**
TWI7^{aa}	-	-	-	-	658.3 ± 26.2
TFI5^{b}	393.7 ± 7.9	409.8 ± 5.3	423.9 ± 4.1*	437.5 ± 10.5*	434.7 ± 6.6*
TFI7^{bb}	-	-	-	-	531.2 ± 15.1
IBM^{c}	66.0 ± 4.1	69.7 ± 3.8	65.8 ± 5.3	68.2 ± 7.3	68.1 ± 9.4
FBM5^{cc}	165.7 ± 7.3	156.3 ± 1.2	157.6 ± 17.5	158.9 ± 13.0	161.1 ± 8.2
FBM7^{ccc}	-	-	-	-	199.4 ± 13.1
BMG5^{d}	99.7 ± 6.5	86.6 ± 4.3*	91.8 ± 7.2	90.6 ± 8.1	92.9 ± 6.2
BMG7^{dd}	-	-	-	-	131,1 ± 6.2
FE5^{e}	0.25 ± 0.0	0.21 ± 0.0*	0.22 ± 0.0	0.21 ± 0.0*	0.21 ± 0.0*
FE7^{ee}	-	-	-	-	0.25 ± 0.0
Male n= 5					
TWI5^{a}	479.2 ± 23.5	506.1 ± 19.1*	534.4 ± 28.0*	567.0 ± 12.4*	583.9 ± 45.0**
TWI7^{aa}	-	-	-	-	646.3 ± 54.1
TFI5^{b}	402.7 ± 9.7	455.8 ± 22.3*	476.5 ± 17.7*	479.6 ± 19.0*	477.6 ± 15.1*
TFI7^{bb}	-	-	-	-	543.4 ± 13.4
IBM^{c}	91.2 ± 7.7	94.0 ± 6.5	95.4 ± 9.0	86.5 ± 5.6	82.4 ± 7.7
FBM5^{cc}	194.1 ± 11.5	191.7 ± 14.2	199.6 ± 21.1	187.3 ± 15.6	182.2 ± 18.0
FBM7^{ccc}	-	-	-	-	212.1 ± 10.1
BMG5^{d}	102.8 ± 8.7	97.7 ± 9.3	104.2 ± 12.0	100.8 ± 8.1	99.8 ± 7.3
BMG7^{dd}	-	-	-	-	129.6 ± 12.7
FE5^{e}	0.25 ± 0.0	0.21 ± 0.0*	0.22 ± 0.0	0.21 ± 0.0*	0.21 ± 0.0*
FE7^{ee}	-	-	-	-	0.24 ± 0.0*

S: A satellite group was treated with ANFE at 800 mg/kg/day for 5 weeks followed by no treatment for 2 weeks.
^{a} Total water intake on week 5 (mL), ^{aa} Total water intake on week 7 (mL), ^{b} Total food intake on week 5 (g), ^{bb} Total food intake on week 7 (g), ^{c} Initial body mass (g), ^{cc} Final body mass on week 5 (g), ^{ccc} Final body mass on week 7 (g), ^{d} Body mass gain on week 5 (g), ^{dd} Body mass gain on week 7 (g), ^{e} Food efficiency on week 5 (ggain.g^{-1} intake), ^{ee} Food efficiency on week 7 (ggain.g^{-1} intake).
Values are mean ± S.D.
*Significantly different ($p < 0.05$) compared to control as determined by Duncan's multiple range test
**Significantly different ($p < 0.01$) compared to control as determined by Duncan's multiple range test.

Faeces weight

There was no significant difference in faeces weight between treated animals and control up to 2 weeks in both female (Fig. 1A) and male (Fig. 1B) rats, except male rats at middle dose, in which faeces weight decreased significantly from the second week of treatment. From the third and fourth week of treatment, treated rats had significantly lower faeces weight than control ($p < 0.05$).

However, two weeks after daily oral gavage of ANFE, faeces weight of satellite group became comparable to control.

Haematological parameters

Table 3 shows the values of haematological parameters. After five weeks of daily oral gavage of ANFE, there were no significant changes in blood analysis of RBC, LYM, GRA, WBC, HGB, HCT, PLT, MCV, MCH, MCHC and MPV between treated animals compared to control in both sexes.

Indices of renal function

Biochemical values of renal function in female and male rats after five weeks of daily oral gavage of ANFE are presented in Table 4.

No significant differences were observed in serum creatinine, urea, chloride, sodium and potassium of treated rats of both sexes compared to control.

Table 3. Haematological values of female and male rats in the 35 days daily oral gavage of ANFE.

Parameters	Doses (mg/kg/day)				
	Control	200	400 mmmg/kg	800	800[S]
Female n= 5					
RBC[a]	6.49 ± 0.39	6.42 ± 0.68	6.16 ± 0.15	6.72 ± 0.74	6.50 ± 0.81
LYM[b]	4.82 ± 0.42	5.02 ± 0.42	4.96 ± 0.48	4.74 ± 0.31	4.63 ± 0.41
GRA[c]	4.87 ± 0.56	4.22 ± 0.17	4.91 ± 0.31	4.18 ± 0.33	4.30 ± 0.34
WBC[d]	8.32 ± 0.44	7.98 ± 0.56	8.26 ± 0.38	8.18 ± 0.18	7.71 ± 0.38
HGB[e]	17.24 ± 0.47	17.41 ± 0.57	17.17 ± 0.36	16.94 ± 0.67	16.78 ± 0.85
HCT[f]	41.85 ± 1.77	40.85 ± 2.24	38.44 ± 1.85	40.60 ± 1.98	39.18 ± 1.79
PLT[g]	724 ± 15.74	730.4 ± 37.46	710 ± 24.09	755.4 ± 35.54	773 ± 17.89
MCV[h]	51.14 ± 1.68	52.39 ± 3.21	51.69 ± 1.63	52.43 ± 1.49	54.0 ± 1.63
MCH[i]	19.78 ± 1.23	19.66 ± 1.33	20.56 ± 0.79	19.68 ± 0.46	19.50 ± 0.80
MCHC[j]	39.52 ± 1.46	39.32 ± 1.26	39.94 ± 0.95	40.62 ± 1.12	41.64 ± 0.39
MPV[k]	7.26 ± 0.53	7.03 ± 0.70	7.14 ± 0.96	7.16 ± 0.25	7.36 ± 0.47
Male n= 5					
RBC[a]	7.12 ± 0.41	7.92 ± 0.38	7.81± 0.45	8.75 ± 0.57	8.60 ± 0.59
LYM[b]	4.15 ± 0.63	4.37 ± 0.75	4.26 ± 0.18	4.04 ± 0.52	4.37 ± 0.38
GRA[c]	5.48 ± 0.98	4.71 ± 0.45	4.66 ± 0.54	4.62 ± 0.76	4.85 ± 0.45
WBC[d]	8.17 ± 0.21	7.98 ± 0.16	7.84 ± 0.38	7.12 ± 0.31	7.41 ± 0.46
HGB[e]	16.06 ± 1.76	16.12 ± 0.96	16.43 ± 0.97	14.98 ± 1.01	16.11 ± 0.95
HCT[f]	39.98 ± 1.87	43.03 ± 2.82	41.78 ± 2.58	42.91 ± 1.91	41.55 ± 3.15
PLT[g]	689 ± 15.56	683 ± 23.13	696 ± 19.66	715 ± 13.90	710 ± 24.09
MCV[h]	50.19 ± 2.42	50.48 ± 1.65	50.22 ± 2.88	49.80 ± 2.82	50.74 ± 2.13
MCH[i]	19.80 ± 1.37	18.28 ± 0.97	18.30 ± 0.79	18.66 ± 1.24	18.58 ± 1.48
MCHC[j]	38.09 ± 1.27	38.80 ± 1.19	40.18 ± 1.51	37.92 ± 1.93	38.72 ± 1.43
MPV[k]	8.06 ± 0.59	7.14 ± 0.96	8.42 ± 0.34	7.94 ± 0.47	8.04 ± 0.39

S:a satellite group was treated with ANFE at 800 mg/kg/day for 5 weeks followed by no treatment for 2 weeks.

[a]Red blood cell (10^{12}/L), [b]Lymphocyte (10^9/L), [c]Granulocyte (10^9/L), [d]White blood cell (10^9/L), [e]Haemoglobin concentration (g/dL), [f]Haematocrit (%), [g]Platelets (10^9/L), [h]Mean corpuscular volume (fL), [i]Mean corpuscular haemoglobin (pg), [j]Mean corpuscular haemoglobin concentration (g/dL), [k]Mean platelet volume (fL).

Values are mean ± SD.

Indices of hepato-biliary and pancreatic functions

No significant differences were noted in the activities of enzymes (ALP, ALT, AST and GGT); as well as in serum albumin, protein, globulin and bilirubin concentrations (Table 5).

There were significant decreases ($p<0.05$) in the serum glucose, total cholesterol, LDL-cholesterol and triglyceride (from low dose) concentrations while a significant increase ($p<0.05$) in serum HDL-cholesterol concentration was observed in all the treated animals (particularly from middle dose) compared to control.

Internal organ study

There was no significant change in the absolute and relative organ weight of the treated animals compared to control (Table 6). In female rats however, relative weights of brain and lungs were significantly lower in satellite group compared to control. No abnormalities were detected during the examination of the internal organs.

Discussion

LD_{50}

Despite the common use of the *A. nilotica* fruits as food or remedies, few scientific studies have been undertaken to ascertain its safety via oral route. In the first part of this study, safety conditions of ANFE were evaluated among a wide range of doses in rats for short-time using oral single administration.

Table 4. Biochemical values of renal function in female and male rats during the 35 days daily oral gavage of ANFE.

Parameters	Doses (mg/kg/jour)				
	Control	200	400	800	800[S]
Female n = 5					
Cr[a]	0.51 ± 0.08	0.52 ± 0.04	0.49 ± 0.04	0.48 ± 0.05	0.50 ± 0.02
Ur[b]	24.0 ± 2.12	24.20 ± 1.09	23.0 ± 2.34	22.40 ± 2.30	22.45 ± 2.07
Cl[c]	96.12 ± 4.06	93.09 ± 3.05	91.75 ± 6.05	92.56 ± 5.04	94.80 ± 9.04
Na[d]	132.20 ± 6.45	126.20 ± 5.89	125.60 ± 6.34	124.80 ± 13.14	128.80 ± 7.05
K[e]	4.44 ± 0.51	4.62 ± 0.22	4.06 ± 0.35	3.98 ± 0.19	4.12 ± 0.46
Male n= 5					
Cr[a]	0.52 ± 0.02	0.48 ± 0.04	0.49 ± 0.03	0.47 ± 0.04	0.50 ± 0.02
Ur[b]	23.12 ± 1.40	21.82 ± 1.27	20.76 ± 2.24	20.92 ± 1.56	21.14 ± 1.97
Cl[c]	97.07 ± 6.11	95.13 ± 7.02	94.25 ± 8.06	93.76 ± 9.04	94.71 ± 7.02
Na[d]	131.20 ± 3.04	127.50 ± 15.84	127.60 ± 4.94	126.80 ± 6.49	128.40 ± 5.08
K[e]	4.22 ± 0.19	3.96 ± 0.23	3.96 ± 0.19	3.92 ± 0.19	3.94 ± 0.21

S:a satellite group was treated with ANFE at 800 mg/kg/day for 5 weeks followed by no treatment for 2 weeks.

[a] Creatinine (mg/dL), [b] Urea (mg/dL), [c] Chloride ions (mM), [d] Sodium ions (mM), [e] Potassium ions (mM)

Values are mean ± SD

* $P < 0.05$ compared to control as determined by Duncan's multiple range test.

The LD50 did not determine with exactitude due to the absence of deaths. Similar result was described by Guta *et al.* (2007), suggesting that LD50 is indeterminable via oral route in mice treated with doses up to 7000 mg/kg of ethyl acetate fruit extract of *A. nilotica*. This might indicate that ANFE is a mixture of substances devoid of acute oral toxicity.

Sub-chronic oral toxicity of ANFE

In the second part, the effect of sub-chronic oral administration of ANFE have been examined in rats during five weeks, and satellite group was normally treated with ANFE at a higher dose followed by no treatment for two weeks. Sub-chronic oral administration of different doses of ANFE during 5 weeks did not produce any sign of toxicity except agitation and spiting, which were seen in a dependent dose. No case of deaths in female and male rats were recorded at any dose over 5 weeks of treatment.

Effect of ANFE on eating behaviour and defecation

Concerning the food behaviour, the increase of food and water consumption was notable with dose. These results suggest that the fruits of *A. nilotica* can be used as appetite enhancer. However, ANFE did not provoke a change in body weight, which can be used as first safety indicator of this extract at the doses used; since its reduction could be considered as the toxic manifestations. In contrast, the decrease of food efficiency was not dose-dependent, thus it cannot be directly attributed to the ANFE.

Table 5. Biochemical values of hepato-biliary and pancreatic functions in female and male rats in the 35 days daily oral gavage of ANFE

Parameters	Doses (mg/kg/jour)				
	Control	200	400	800	800[S]
Female n= 5					
GLU[a]	106.40 ± 7.50	100.60 ± 3.78	96.80 ± 5.11*	90.10 ± 2.70*	91.20 ± 8.64*
ALB[b]	4.10 ± 0.16	3.90 ± 0.17	3.72 ± 0.19	3.79 ± 0.15	3.82 ± 0.16
TP[c]	7.08 ± 0.37	6.78 ± 0.15	6.62 ± 0.26	6.64 ± 0.16	6.56 ± 0.37
GLO[d]	2.98 ± 0.38	2.88 ± 0.30	2.90 ± 0.18	2.60 ± 0.09	2.74 ± 0.33
TB[e]	0.44 ± 0.02	0.47 ± 0.03	0.48 ± 0.05	0.51 ± 0.06	0.47 ± 0.02
ALP[f]	56.81 ± 2.94	53.60 ± 2.07	54.80 ± 5.49	52.40 ± 4.04	52.80 ± 1.30
ALT[g]	96.94 ± 8.38	95.23 ± 9.75	93.60 ± 6.15	92.22 ± 6.19	95.54 ± 7.50
AST[h]	39.80 ± 1.92	37.20 ± 4.49	36.84 ± 3.33	37.64 ± 4.09	37.51 ± 2.23
GGT[i]	18.28 ± 0.70	16.40 ± 2.07	16.40 ± 1.78	16.72 ± 1.97	17.46 ± 1.24
TC[j]	74.32 ± 3.52	70.72 ± 4.79	63.94 ± 6.83*	59.74 ± 3.45*	61.64 ± 0.85*
HDL[k]	50.54 ± 2.92	55.56 ± 2.21	63.40 ± 4.28*	69.42 ± 3.19*	70.61 ± 3.47*
LDL[l]	36.40 ± 1.14	28.51 ± 1.66	24.18 ± 6.46*	21.62 ± 3.05*	24.80 ±
TG[m]	117.6 ± 10.53	92.06 ± 10.83*	75.80 ± 4.66*	74.80 ± 7.16*	80.52 ±
Male n= 5					
GLU[a]	103.86 ± 5.41	100. 04 ± 4.51	93.32 ± 3.92*	87.88 ± 4.60*	90.02 ±
ALB[b]	3.98 ± 0.08	3.72 ± 0.19	3.66 ± 0.28	3.71 ± 0.15	3.76 ± 0.11
TP[c]	6.98 ± 0.31	6.74 ± 0.22	6.68 ± 0.44	6.32 ± 0.19	6.68 ± 0.15
GLO[d]	3.00 ± 0.37	3.02 ± 0.22	3.02 ± 0.65	2.62 ± 0.13	2.92 ± 0.11
TB[e]	0.44 ± 0.04	0.46 ± 0.03	0.47 ± 0.03	0.48 ± 0.03	0.45 ± 0.03
ALP[f]	77.05 ± 13.65	75.32 ± 8.30	75.40 ± 7.50	72.60 ± 2.51	73.48 ± 2.24
ALT[g]	97.12 ± 7.01	94.62 ± 2.04	92.40 ± 2.96	93.34 ± 10.37	92.46 ±
AST[h]	42.40 ± 6.22	41.72 ± 4.94	37.74 ± 6.28	38.28 ± 3.59	37.22 ± 5.04
GGT[i]	21.24 ± 3.08	17.38 ± 2.26	18.32 ± 1.84	16.22 ± 1.92	17.42 ± 2.88
TC[j]	72.44 ± 4.44	68.40 ± 2.79	62.54 ± 3.10*	64.12 ± 3.40*	64.50 ± 1.51
HDL[k]	47.40 ± 2.07	51.81 ± 1.92	58.88 ± 0.87*	64.20 ± 2.86*	57.42 ± 1.17*
LDL[l]	35.41 ± 4.83	26.8 ± 6.30*	24.20 ± 6.59*	20.80 ± 3.11*	24.60 ± 2.31*
TG[m]	109.02 ± 6.91	92.14 ± 10.44*	86.60 ± 5.08*	87.66 ± 6.45*	90.94 ±

S:a satellite group was treated with ANFE at 800 mg/kg/day for 5 weeks followed by no treatment for 2 weeks.

[a]Glucose (mg/dL), [b] Albumin (g/dL), [c] Total protein (g/dL), [d] Globulin (g/dL), [e] Total bilirubin (mg/dL), [f] Alkaline phosphatase (U/L), [g] Alanine aminotransferase (U/L), [h] Aspartate aminotransferase (U/L), [i] Gamma glutamyltransferase (U/L), [j] Total cholesterol (mg/dL), [k] High density lipoprotein (mg/dL), [l] Low density lipoprotein (mg/dL) and [m] Triglycerides (mg/dL).

Values are mean ± SD

*P < 0.05 compared to control as determined by Duncan's multiple range test.

The decrease in faeces weight has been observed with the duration of treatment. Male rats were more sensitive than female. This observation may suggest that constipation is the adverse effect when the treatment goes on after two weeks. Since in the satellite group, the increase of faeces weight appeared after the interruption of treatment.

Table 6. Absolute (A) and relative (R) organ weights of female and male rats in the 35 days daily oral gavage of ANFE.

Organs		Doses (mg/kg/day)				
		Control	200	400 mmmg/kg	800	800^{s}
Female n= 5						
Brain	A	1.50 ± 0.04	1.40 ± 0.03	1.45 ± 0.07	1.46 ± 0.05	1.53 ± 0.04
	R	0.90 ± 0.02	0.89 ± 0.02	0.91 ± 0.04	0.91 ± 0.03	0.77 ± 0.02*
Heart	A	0.45 ± 0.06	0.43 ± 0.08	0.41 ± 0.03	0.44 ± 0.05	0.45 ± 0.05
	R	0.26 ± 0.03	0.27 ± 0.04	0.26 ± 0.02	0.27 ± 0.02	0.22 ± 0.03
Liver	A	5.65 ± 0.11	5.12 ± 0.07	5.46 ± 0.19	5.24 ± 0.12	6.16 ± 0.22
	R	3.38 ± 0.06	3.27 ± 0.05	3.46 ± 0.12	3.29 ± 0.06	3.09 ± 0.13
Ovaries	A	0.1 ± 0.02	0.09 ± 0.01	0.11 ± 0.02	0.09 ± 0.01	0.1 ± 0.02
	R	0.05 ± 0.00	0.05 ± 0.00	0.06 ± 0.01	0.05 ± 0.00	0.05 ± 0.00
Pancreas	A	0.26 ± 0.02	0.24 ± 0.03	0.25 ± 0.02	0.24 ± 0.02	0.25 ± 0.01
	R	0.15 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	0.12 ± 0.02
Lungs	A	1.19 ± 0.05	1.14 ± 0.07	1.17 ± 0.05	1.15 ± 0.12	1.18 ± 0.06
	R	0.71 ± 0.03	0.72 ± 0.04	0.74 ± 0.03	0.72 ± 0.07	0.59 ± 0.03*
Spleen	A	0.32 ± 0.03	0.30 ± 0.03	0.29 ± 0.04	0.33 ± 0.05	0.34 ± 0.02
	R	0.19 ± 0.02	0.19 ± 0.01	0.18 ± 0.03	0.20 ± 0.03	0.17 ± 0.01
Kidney	A	1.17 ± 0.05	1.11 ± 0.07	1.12 ± 0.05	1.10 ± 0.07	1.40 ± 0.08
	R	0.70 ± 0.03	0.71 ± 0.04	0.71 ± 0.02	0.69 ± 0.03	0.70 ± 0.04
Male n= 5						
Brain	A	1.53± 0.05	1.50 ± 0.03	1.55 ± 0.03	1.45 ± 0.02	1.60 ± 0.02
	R	0.79 ± 0.03	0.78 ± 0.02	0.77 ± 0.03	0.77± 0.02	0.75 ± 0.02
Heart	A	0.56 ± 0.05	0.54 ± 0.04	0.53 ± 0.03	0.53 ± 0.02	0.57 ± 0.03
	R	0.29 ± 0.03	0.28 ± 0.04	0.26 ± 0.02	0.28 ± 0.01	0.27 ± 0.02
Liver	A	6.14 ± 0.12	5.95 ± 0.14	5.94 ± 0.21	5.90 ± 0.32	6.20 ± 0.34
	R	3.16 ± 0.08	3.10 ± 0.10	2.97 ± 0.14	3.14 ± 0.22	2.92 ± 0.23
Epididymis	A	0.22 ± 0.01	0.21 ± 0.00	0.19 ± 0.01	0.20 ± 0.01	0.21 ± 0.01
	R	0.11 ± 0.02	0.10 ± 0.00	0.09 ± 0.00	0.10 ± 0.02	0.09 ± 0.02
Pancreas	A	0.23 ± 0.03	0.21 ± 0.02	0.20 ± 0.02	0.22 ± 0.02	0.24 ± 0.02
	R	0.12 ± 0.02	0.11 ± 0.01	0.10 ± 0.02	0.12 ± 0.02	0.11 ± 0.02
Lungs	A	1.20 ± 0.04	1.20 ± 0.03	1.23 ± 0.07	1.13 ± 0.04	1.30 ± 0.06
	R	0.61 ± 0.02	0.62 ± 0.02	0.63 ± 0.05	0.60 ± 0.03	0.61 ± 0.04
Spleen	A	0.54 ± 0.02	0.53 ± 0.05	0.52 ± 0.05	0.54 ± 0.06	0.56 ± 0.02
	R	0.28 ± 0.04	0.27 ± 0.03	0.26 ± 0.02	0.28 ± 0.02	0.26 ± 0.01
Kidney	A	1.20 ± 0.02	1.21 ± 0.02	1.21 ± 0.03	1.14 ± 0.07	1.31 ± 0.02
	R	0.62 ± 0.01	0.63 ± 0.01	0.60 ± 0.02	0.61 ± 0.05	0.61 ± 0.02
Testicles	A	1.17 ± 0.05	1.12 ± 0.07	1.16 ± 0.05	1.09 ± 0.07	1.21 ± 0.08
	R	0.60 ± 0.03	0.59 ± 0.04	0.58 ± 0.02	0.58 ± 0.03	0.57 ± 0.04

S:a satellite group was treated with ANFE at 800 mg/kg/day for 5 weeks followed by no treatment for 2 weeks. Values are mean ± SD. A (g) and R (g per 100 g body weight).

Haematoprotective effect of ANFE

The repeated oral administration of ANFE did not induce any significant modification in haematological parameters. Furthermore, the haematological values were within the standards. This indicating that ANFE had no haematotoxic effect at the doses used.

Hypoglycaemic effect of ANFE

As concern the general parameters in serum especially glucose, the oral administration of ANFE decreased the concentration of glucose in serum after five weeks of treatment. This hypoglycaemic effect can be attributed to ANFE which contains constituents such as tannins, flavonoids, saponins (Malviya *et al.*, 2011). These substances could interact with alimentary glucose and thus would reduce its absorption. Moreover, the ANFE will induce the stimulation of pancreas Langerhans β cells receptors.

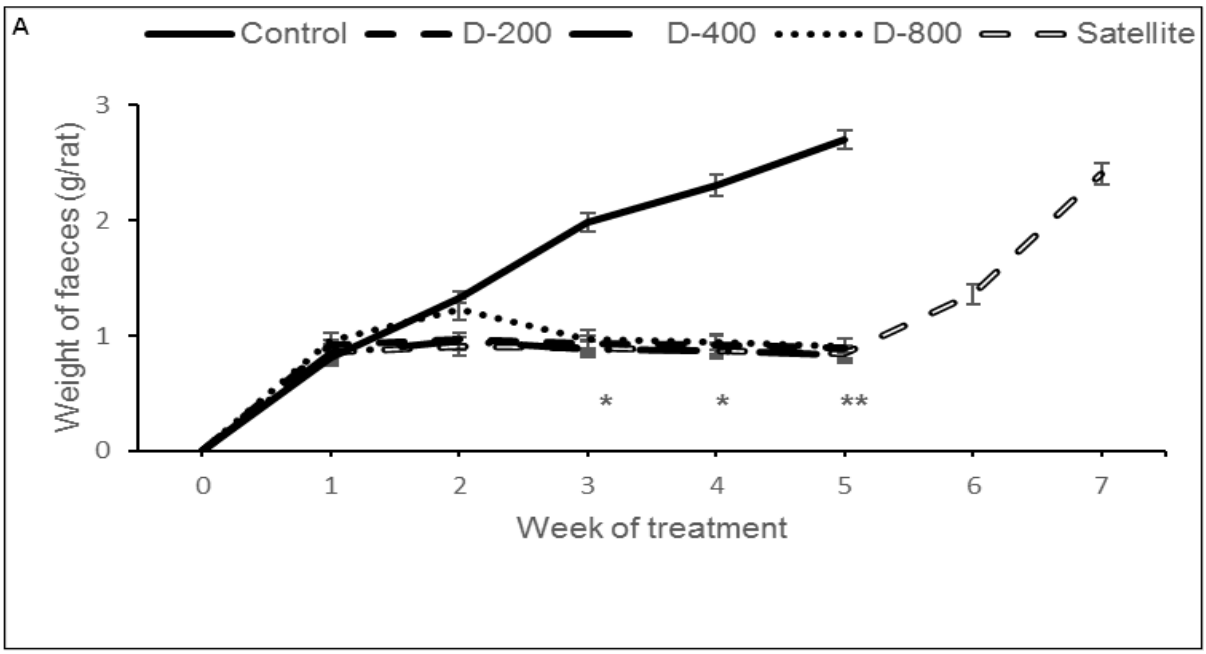

Fig. 1A. Faeces weight of female rats in the 35 days daily oral gavage of ANFE.

S: A satellite group was treated with ANFE at 800 mg/kg/day for 5 weeks followed by no treatment for 2 weeks

Values are mean ± S. D of 5 rats.

*Significantly different ($p < 0.05$) compared to control as determined by Duncan's multiple range test

**Significantly different ($p < 0.01$) compared to control as determined by Duncan's multiple range test.

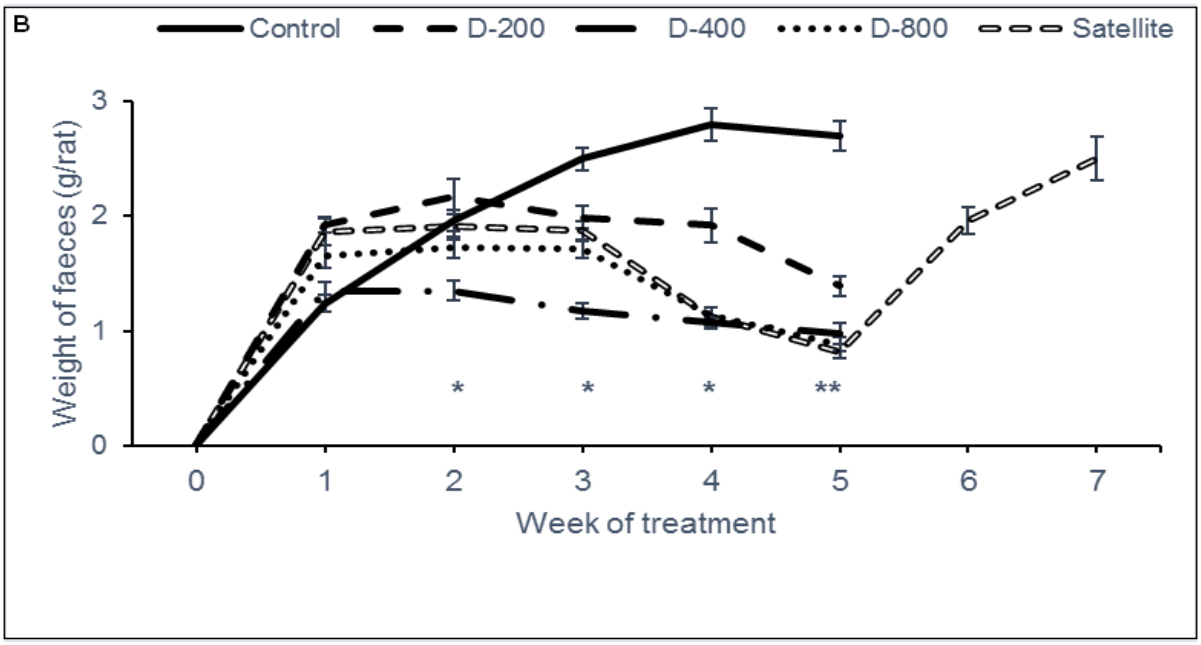

Fig. 1B. Faeces weight of male rats in the 35 days daily oral gavage of ANFE.

Satellite group was treated with ANFE at 800 mg/kg/day for 5 weeks followed by no treatment for 2 weeks

Values are mean ± S. D of 5 rats.

*Significantly different ($p < 0.05$) compared to control as determined by Duncan's multiple range test

**Significantly different ($p < 0.01$) compared to control as determined by Duncan's multiple range test

Effect of the repeated oral administration of ANFE on lipid profile

Lipid profile has been significantly modified by oral administration of ANFE, with hypocholesterolaemic and hypolipidemic tendencies. The decreases of total cholesterol and triglycerides can be attributed to the constituents such as tannins, flavonoids, saponins which would interact with them at the level of the intestine and limit the absorption of lipids. Furthermore, the substances will increase the transformation of hepatic cholesterol in biliary salts excreted by faeces. The reduction of LDL-cholesterol could be explained by the stimulation of LDL hepatic receptor, which would be induced by bioactive substances of ANFE.

Hepatic and renal protective effects of ANFE

Since the herbal medicine can affect the kidney or liver, biochemical analysis is directed to the markers of renal and hepatic functions. The renal impairment is linked to serum urea and creatinine elevations while hepatic damage is associated with GGT, ALP, AST and ALT elevations. Apart liver, ALP is also considered as marker for bone disease; it is the same thing for AST that is present in heart, skeletal muscle, kidney and brain. The study carried out by El-Hadiyah *et al.* (2011) showed that the alcoholic extract of *A. nilotica* fruits has renal and hepatic toxicities via intraperitoneal route at dose of 60 mg/kg. In the present work, the markers of renal and hepatic functions mentioned above did not change between control and treated rats, after 5 weeks of daily oral gavage of ANFE at doses ranging from 200 to 800 mg/kg. Similarly, the extract of *A. nilotica* flowers is found to be hepatoprotective (Wakte *et al*., 2012). These findings suggest that ANFE did not have the nephrotoxic and hepatotoxic effects at oral doses administered.

Conclusion

The present study provides precious data on the safety levels of *A. nilotica* fruit extract. A single oral administration of *A. nilotica* fruit extract showed that LD_{50} is higher than 16 g/kg, it is regarded as being devoid of acute oral toxicity. In the 35 days daily repeated oral administration at the doses ranging from 200 – 800 mg/kg, *A. nilotica* fruit extract was found to be appetite enhancer, hypoglycaemic, hypocholesterolaemic and hypolipidemic. The *A. nilotica* fruit extract did not modify the body weight, the markers of renal and hepatic functions as well as the values of haematological parameters. Data on the acute and sub-chronic studies might suggest that *A. nilotica* fruit aqueous extract is innocuous via oral route in rats at doses administered. Therefore, these safety levels of *A. nilotica* fruit aqueous extract can be helpful for its optimal utilisation.

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