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Full Length Research Paper

Effects of ethanolic extract of *Waltheria indica* aerial parts on some liver and kidney function indices in albino rats

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This study evaluated the toxicological effects of chronic treatment with ethanolic extract of *Waltheria indica* aerial portion on body weight, hematological and biochemical parameters in albino rats using standard methods. Rats treated with 400 mg/kg body weight (bw)/day of the extract showed no behavioural changes. However, there was general reduction of activity in rats given 800 and 1,600 mg/kg bw/day of the extract. Also, the LD_{50} treated rats exhibited hypoactivity, grooming, prostration and irritation during treatment in the third and fourth weeks of treatment period. The data on body weight changes indicated that there were no significant differences in body weight between the control group and groups that received different doses of the extract ($p<0.50$). Haematological results for the red blood cell, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration in extract treated rats showed no significant changes at all doses of treatments when compared with controls in female rats. However, data on mean corpuscular haemoglobin in the male rats treated with 1,600 mg/kg showed a significantly decreased value when compared with controls. On the other hands, white blood cell counts decreased significantly after treatment with 400 and 800 mg/kg bw/day extract in the male rats. Lymphocytes count was also decreased significantly in males treated with 800 mg/kg bw/day of extract. Alanine transaminase (ALT), total bilirubin and creatinine increased significantly, respectively at 800 and 1,600 mg/kg bw/day doses of extract when compared with the control rats ($p<0.05$). In conclusion, the overall data of this study suggest that the oral administration of *W. indica* extract did not induce any toxic effects, especially when administered at low doses; however, further investigation is needed to evaluate its chronic toxicity.

Key words: *Waltheria indica,* Wistar rats, evaluation, toxicological, biochemical, haematological, parameters.

INTRODUCTION

Waltheria indica (Synonymn *Waltheria americana*) is a plant widespread throughout the tropics (Burkill et al., 2000). It belongs to the family Sterculiaceae and it is commonly called sleeping morning. Locally, the plant is

called “hankufa” in Northern Nigeria and “Konkodi” in the south. The plant has been used in traditional medicine for treatment of several pathologies (Olajuyugbe et al., 2011). In Nigeria, *W. indica* roots and aerial parts have been used mainly against pain, inflammation, conditions of inflammation, diarrhea, dysentery, wounds, anaemia, epilepsy, convulsion, and asthma (Heinrich et al., 1992; Hamidu et al., 2008). Whole plant is used to treat peptic ulcer (Oluranti et al., 2012), while decoction of aerial parts may be taken to treat anaemia (Gbadamosi et al., 2012). The use of medicinal plants has received great attention in the world as an alternative to conventional drugs partly due to perceived therapeutic efficacy and low side effect profile of natural products from plants (Aluko, 2016) and the demand for these remedies has recently increased (Mhuji et al., 2016). The World Health Organization (WHO) estimated that about 80% of the population of most developing countries relies on herbal medicines for their primary health care (Dharm and Pramod, 2017). Some of these traditional medicines involve the use of crude plant extracts in the form of infusion, decoction or tincture which may contain an extensive diversity of molecules often with indefinite biological effects (Olowa and Nuneza, 2013; Brenda et al., 2016). Novel clinically active drugs are been isolated from higher plants; however, there are limited scientific evidence as to the efficacy and safety to support the continued therapeutic application of these medications (Amrit et al., 2014; Taiwo and Joel, 2015).

Studies providing an evidence for local and traditional uses of *W. indica* have been documented. Yerra et al. (2005) reported that flavonoids isolated from *W. indica* effectively inhibited the production of nitric oxide (NO), turmor necrosis factor alpha (TNF-α) and interleukin 12 (IL 12); this supports the use of the plant for the treatment of inflammatory diseases in traditional medicine. Hamidu et al. (2013) also reported that the ethyl acetate fraction of the plants significantly reduced oedema size, suggesting anti-inflammatory activities. Also reported are anti-microbial activities of the plant (Zailani et al., 2010) and analgesic effects (Yaro et al., 2007). Although, there are diverse potentially clinical utility and scientific studies published on *W. indica*, toxicity reports on this plant are sparse in literature; and it has been suggested that the safety of plants-based medicine needs to be evaluated essentially before recommending for human consumption (Poonam et al., 2014; Oloro et al., 2016). Toxicological studies in form of acute, sub-acute and sub-chronic are requirements for many products used as medicines (Oloro et al., 2016). The liver is the site of detoxification and deamination; the determination of the activity of certain enzymes was employed in knowing the toxic effects or level of plants extracts used as medicines (Asadu et al., 2015). This work reports the effects of ethanolic extract of the plant on haematologial and biochemical parameters in rats.

MATERIALS AND METHODS

Plant

Fresh samples of aerial parts (flowers, leaves and stems) of *W. indica* were collected from the vicinity of the university dam, Ahmadu Bello University, Zaria in the month of July 2016. The plant material was identified by taxanomic means through comparison with the herbarium specimen and authenticated by Dr. Mujtaba Abubakar of Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria. A voucher specimen (NPR 2006) was prepared and deposited in the herbarium of the same department.

Preparation for extraction

Fresh plant material was washed, dirt removed, air-dried and then oven-dried for 2 h in the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria. It was pounded into powder and sieved.

Extraction

Powdered material (500 g) was exhaustively extracted with aqueous ethanol (60% v/v) using Soxhlet apparatus. The aqueous ethanolic extract upon concentration yielded a yellowish green residue hitherto called the extract (15.3% w/w). The extract was suspended in water and defatted with petroleum ether. The solvents were then removed at 52°C under reduced pressure in a rotavapour. The solid sample was stored in a refrigerator until needed for experimentation.

Animals

The animals used in this study were young adult albino mice weighing 21.5 to 27.0 g and Wistar albino rats (191 to 215 g) of both sexes obtained from the animal house of the Department of Pharmacy, Ahmadu Bello University, Zaria, Nigeria. The animals were maintained under standard nutritional and environmental conditions, having access to water and food *ad libitum*. Feeding was withdrawn 12 h before experimentation.

Acute toxicity test

The acute toxicity (oral LD_{50}) of the extract of *W. indica* aerial portion was earlier established in previous communications in 25 albino mice using standard method of Lorke (1983).

Sub-acute toxicity study

For the purpose of this study, adult Wistar rats of both sexes were

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allotted randomly to 4 groups, each consists of 6 rats. The extract was administered by gavage to three groups (II, III and IV, respectively) at 400, 800, and 1,600 mg/kg doses on alternate days for 28 days (4 weeks) between 08:00 and 09:00 h each day. Animals in group I (control) were given normal saline (2 ml/kg) orally. Animals were observed for clinical signs and symptoms, behaviour alterations, food and water intake and body weight changes. All experimental animals were observed twice daily for mortality during the 28 days period of study. Weight of each rat was recorded at day zero and at weekly intervals throughout the duration of the study. The group mean weights were calculated and recorded.

At the end of the 28 days period, the animals were fasted overnight; then the following morning each animal was heparinized and blood samples collected from orbital sinusis. Samples were collected after 24 h of the last doses of the extract.

Haematology and blood chemistry examination: Hematological analysis

The hematological parameters (red blood cell [RBC], haemoglobin [HB], packed cell volume [PVC], white blood cell [WBC], differential leucocyte count [DLC], mean corpuscular volume [MCV], mean corpuscular haemoglobin [MCH], and mean corpuscular haemoglobin concentration [MCHC]) were measured using standard methods. Analysis was performed on all samples immediately after collection (TO). RBC counts were done using Neubauer haemocytometer (Shah and Altindag, 2005). 20 µl of each whole blood was diluted with 0.98 ml of Dacie's fluid (1 ml of 40% formaldehyde that is full strength, 3.13 g trisodium citrate, 0.1 g brilliant cresyl blue, dissolved in 100 ml of distilled water). The solution was gently mixed to dispense the cells; this provided a 1:5 dilution of the blood. The mixed solution was drawn into a disposable plastic pipette. The first few drops were discarded and one drop touched the edge of a Neubauer haemocytometer between the cover slip and counting chamber. Capillary action draws the sample under the cover slip (Handy and Depledge, 1999).

RBCs were counted on microscope in 5 of the secondary squares (model DM750, Leica Microsystems GmbH-wetzlar, Germany) at ×640. RBC was expressed as 10^6 mm^{-3}. WBC was counted by using a neubaver laemocytometer (Shah, 2010). Blood was diluted 1:20 with Turk's diluting fluid (1% glacial acetate solution and Gentian violet 0.3% w/v dissolved in distilled water). Total number of WBC expressed as 10^3 mm^{-3} (Wintrobe and Lee, 1967). PCV was determined by microhaematocrit centrifugation. The length of columns containing packed red cells and the packed red cells plus supernatant were measured and PVC was expressed as percentage. Haemoglobin concentration (Hb) was measured with haemoglobin test kit (Roach GmbH mannhein, Germany) using the cyanmethemoglobin method (Larsen and Snieszko, 1961). MCV, MCH and MCHC were indirectly calculated from the obtained values of Hb, PCV and RBC as described by Francesco et al. (2012).

Differential leucocyte count

A blood smear was prepared dried and stained with Leishman's stain slide placed on microscope and scanned at low power to find a distribution of cells. A drop of oil is placed on the slide and cells were examined with the oil immersion objective. Percentage of each type of white blood cell was determined and recorded.

Biochemical analysis

Serum alanine (ALT) and aspartate (AST) were colorimetrically assayed using the methods of Reitman and Frankel (1957). Total bilirubin, urea and creatinine were assayed using the sulphanilic reaction, diacetylmonoxine reaction and Jaff's reaction, respectively as described by Kaplan et al. (1988). The biuret test (Henry et al., 1974) was used for total protein estimate, while chloride and bicarbonate were estimated by titrimetric method (Harold, 1988). Potassium and sodium levels were estimated by flame photometric method.

Statistical analysis

Data were expressed as mean±standard error of mean (SEM). The data were analyzed using the student's t-test and the differences were considered significant when $P<0.05$.

RESULTS

The oral LD_{50} of *W. indica* extract (875 mg/kg) was as earlier reported by previous studies (Hamidu et al., 2013).

Table 1 shows the body weight changes in the rats during the 28 days study periods. The data indicated that there were no significant differences in body weight between the control group and the groups that received different doses of the plant extract ($p>0.05$).

Sub-acute toxicity studies

The effects of *W. indica* aerial parts extract on haematological values of male and female rats in the sub-acute test are shown in Table 2. The table showed no significant difference in female rats treated with various doses as compared to the controls. Values in the table however showed that the MCV, significantly decreased ($p<0.05$) in the male rats treated with 1,600 mg/kg. DLC values are shown in Table 3. The table indicated that in female rats treated with 1,600 mg/kg, there was a significant increase ($p<0.05$) in neutrophil count. In the male rats, a significant increase in white blood cell was observed in the groups that received 800 and 1,600 mg/kg; and a significant decrease in lymphocyte in male rats treated with 1,600 mg/kg when compared with controls ($p<0.05$).

The effects of administration of *W. indica* extracts on indices of liver and kidney function are shown in Tables 4 and 5, respectively. Indices of liver functions (AST, ALT and total bilirubin) did not show increases by the extract following 4 weeks of administration. The groups administered 800 and 1,600 mg/kg however produced higher values as compared to the controls, though not statistically significant.

DISCUSSION

The oral LD_{50} of the ethanolic extract of *W. indica* aerial portion (stem, leave, flowers) was reported by our earlier

Table 1. Weekly body weight changes in sub-acute oral administration test.

Group	Week 1	Week 2	Week 3	Week 4
Female				
Control	196.02±6.50	216.10±4.81	221.03±6.21	231.05±5.17
400 mg/kg	197.05±7.00	210.70±5.31	217.12±5.30	228.11±6.00
800 mg/kg	195.10±5.71	203.50±3.90	216.30±7.00	230.33±5.40
1,600 mg/kg	190.80±5.60	197.01±5.90	211.50±5.30	227.59±5.01
Male				
Control	209.17±8.01	227.50±8.61	234.71±5.91	249.21±5.14
400 mg/kg	202.21±7.05	224.31±7.51	231.26±7.31	237.10±6.71
800 mg/kg	189.54±6.90	220.51±7.01	220.31±6.80	230.80±5.71
1,600 mg/kg	190.3±7.60	210.59±7.51	211.5±7.01	230.70±6.17

Values are mean±SEM, n=6 for both sexes.

Table 2. Effects of *Waltheria indica* aerial parts extract on haematological values of male and female rats in sub-acute test.

Haematological value	***W. indica* extract doses**			
	Control (2 ml/kg normal saline)	**400 mg/kg**	**800 mg/kg**	**1,600 mg/kg**
Female				
Red blood cell ($\times 10^6$ μl^{-1})	6.98±0.03	6.88±0.10	7.07±0.08	7.01±0.17
Haemoglobin (g/dl)	14.93±0.09	14.75±0.9	14.90±0.10	15.01±0.80
Packed cell volume (%)	45.02±0.25	43.20±0.15	43.50±0.51	46.05±0.62
MCV (fl)	60.76±0.30	60.58±0.20	59.00±0.25	60.80±0.90
MCH (pg)	20.01±0.50	19.95±0.51	20.15±0.30	19.08±0.51
MCHC (g/dl)	33.52±0.20	33.80±0.25	34.81±0.53	32.95±0.62
Male				
Red blood cell ($\times 10^6$ μl^{-1})	9.05±0.15	8.90±0.01	8.71±0.54	8.41±0.51
Haemoglobin (g/dl)	15.59±0.21	16.98±0.18	15.98±0.58	15.56±0.18
Packed cell volume (%)	50.01±0.60	52.20±0.09	50.95±0.37	50.52±0.29
MCV (fl)	60.25±0.45	60.91±0.14	59.84±0.63	59.96±0.60
MCH (pg)	19.01±0.61	18.90±0.25	17.90±0.25*	18.01±0.52
MCHC (g/dl)	33.01±0.20	32.14±0.10	33.21±0.18	33.50±0.49

Values are expressed as Mean±SEM, n=6. *Significantly different from control, P<0.05.

Table 3. Effects of *W. indica* aerial part extract on differential leucocytes count values in rats in sub-acute studies.

Haematological value	***W. indica* extract doses**			
	Control (2 ml/kg normal saline)	**400 mg/kg**	**800 mg/kg**	**1,600 mg/kg**
Female				
White blood cell ($\times 10^3$ μl^{-1})	3.01±0.40	2.69±0.22	3.40±0.30	3.41±0.22
Neutrophil (%)	15.00±0.35	17.00±0.54	18.95±1.41	13.95±0.38
Lymphocyte (%)	70.95±0.50	70.50±0.61	67.30±2.31	70.01±0.58
Monocyte (%)	9.10±0.90	8.94±0.90	10.54±3.10	9.95±0.61
Eosinophil (%)	3.95±0.80	4.90±0.85	4.06±1.33	5.90±0.29
Basophil (%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Male				
White blood cell ($\times 10^3$ μl^{-1})	4.90±0.50	7.01±0.08*	6.68±0.81*	4.56±0.73

Table 3. Contd.

Neutrophil (%)	26.02±0.38	29.05±3.01	31.94±2.55	23.08±2.20
Lymphocyte (%)	66.00±1.26	58.67±3.21	61.05±3.00*	69.50±1.80
Monocyte (%)	9.50±0.21	10.25±1.40	9.58±0.90	8.80±0.84
Eosinophil (%)	2.80±0.32	3.10±0.55	3.78±0.57	2.90±0.62
Basophil (%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

All values are expressed as mean±SEM, n=6.

Table 4. Effects of *W. indica* aerial part extract on biochemical indices of liver function in rats.

Parameter	I (Controls)	II (200 mg/kg)	III (400 mg/kg)	IV (800 mg/kg)	V (1,600 mg/kg)
ALT (iU/L)	62.50±2.50[a]	69.00±3.20[a]	68.95±3.15[a]	98.50±2.02[b]	110.5±3.61[b]
AST (iU/L)	57.46±4.00	58.52±3.80	60.02±2.75	59.05±2.62	59.50±3.00
Total Protein (g/L)	69.55±2.80	72.05±1.60	73.50±2.25	72.85±3.60	75.05±2.11
Total Bilirubin (Umol/L)	8.55±1.50[a]	9.10±2.50[a]	9.50±2.00[a]	10.55±2.55[a]	16.51±2.6[b]

[ab]Values with different superscripts on the same horizontal row are significantly different (P<0.05), values in parentheses are daily doses of extract.

Table 5. Effects of *W. indica* aerial part extract on some biochemical indices of kidney function in rats.

Parameter	I (Controls)	II (200 mg/kg)	III (400 mg/kg)	IV (800 mg/kg)	V (1,600 mg/kg)
Na^+ (nmol/L)	143.90±1.85	143.00±2.64	143.55±1.16	143.50±1.50	143.05±3.00
K^+ (nmol/L)	5.90±3.50	6.51±2.25	5.60±3.15	5.90±3.11	8.55±2.60
HCO_3^- (nmol/L)	61.33±2.35	60.10±3.00	61.00±2.85	61.05±3.00	62.00±3.0
Cl^- (nmol/L)	142.00±2.00	142.55±1.80	146.00±1.50	148.00±1.70	149.00±2.20
Urea (nmol/L)	7.55±1.85	7.60±2.00	7.60±2.70	8.00±1.50	8.11±2.80
Creatinine (nmol/L)	56.10±4.00[a]	62.25±3.50[a]	91.05±3.00[b]	92.00±2.00[b]	103.00±3.80[b]

[ab]Values with different superscripts on the same horizontal row are significantly different (P<0.05), values in parentheses are daily doses of extract.

studies (Hamidu et al., 2013). The study found that LD_{50} of the extract was 875 mg/kg; suggesting that the extract is relatively safe. In addition, no physical symptom of toxicity based on the oral LD_{50} value recommended by organization for economic co-operation and development (1998): very toxic ≤5 mg/kg, toxic >5≤50 mg/kg, harmful >50≤500 mg/kg and no label >500≤2000 mg/kg.

Body weight changes after administration of various doses of the extract showed no significant difference from values obtained from control animals (p>0.05).

Analysis of blood parameters is a relevant risk evaluation; since change in haematological and biochemical system has a higher predictive value for human toxicity in humans when data are translated from animal study (Raza et al., 2002). It has been demonstrated that *W. indica* contain various bioactive principles with pharmacological potential, which can cause beneficial and/or harmful effects on human health; furthermore, it was documented that the general concern of users for lack of scientific evidence has favored to conduct studies regarding the toxicity and harmful effects of plants used by people as natural drugs (Carlos et al., 2015). From the result of the haematological analysis, all the parameters analyzed: RBC, Hb, PCV, MCV, MCH, and MCHC showed no significant difference in female rats given various doses of the extract when compared with controls (p>0.05). However, MCH values significantly decreased (p>0.05) in male rats treated with 1,600 mg/kg for 28 days (Table 2). MCH, MCHC and MCV are related to individual RBC, while parameters like Hb and PVC are associated with total population of RBCs (Mishra and Tandon, 2012). Therefore, significant decrease in these parameters as seen in this study after treatment with high doses (800 and 1,600 mg/kg) of the extract for 28 days may mean that either the incorporation of Hb into RBC or the morphology and osmotic fragility of RBCs were altered. DLC also showed significant increase (p<0.05) of neutrophils in female rats administered 1,600 mg/kg

of the extract (Table 3).

Results of all the parameters analyzed in the kidney function: sodium, potassium, bicarbonate, chloride, urea and creatinine, indicated no significant changes ($p<0.05$) in serum level of rats given various doses as compared to controls. Normally, urea and creatinine determine the general function of the kidney, whereas the electrolytes: sodium (Na^+), potassium (K^+), bicarbonate (HCo_3^-) and chloride (Cl^-) are determinants of tubular function. The values of urea and creatinine showed no significant changes ($p>0.05$) in rats treated with various doses from the control groups; suggesting extract is not nephrotoxic at least in rats. Histomorphological studies of kidney tissues are being studied to confirm the biochemical results reported in this study.

In conclusion, the overall data of this study suggest that the oral administration of *W. indica* extract did not induce any toxic effects, especially when administered at low doses; however, further investigation is needed to evaluate its chronic toxicity.

CONFICT OF INTERESTS

The authors have not declared any conflict of interests.

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ABBREVIATIONS

ALT, Alanine transaminase; **AST,** aspartate transaminase; **DLC,** differential leucocyte count; **Hb,** haemoglobin; **MCV,** mean corpuscular volume; **MCH,** mean corpuscular haemoglobin; **MCHC,** mean corpuscular haemoglobin concentration; **PVC,** packed cell volume; **RBC,** red blood cell; **WBC,** white blood cell.

REFERENCES

Aluko BT (2016). Evaluation of the safety profile of the leaves of *Gutenbergia nigritana* Benth. E. J. Med. Plants 15(3):1-7.

Amrit KS, Dharam PA, Prakash D, Suchita D, Tanveer N, Balgangashar R (2014). Acute and sub - cute toxicity studies of pharmacologically active *seabuckthorn* leaf extract. Int. J. Pharm. Pharm. Sci. 6(6):414-419.

Asadu CL, Abong O, Anosike CA, Robert U (2015). *In vivo* toxicological studies on methanolic leaf extract of *Lantana camera*. Am. Eurasian J. Toxicol. Sci. 7(2):115-122.

Brenda AB, Nadiah Z, Waisadin B, Sergio AG, Dounia K, Hateem F, Abdelhamid E (2016). Plants extracts: from encapsulation to application. Expert Opin. Drug Deliv. 13(8):1165-1175.

Burkill HM, Corey SC, Moore AK (2000). The useful plants of West Tropical Africa. 2nd Edn., Royal Botanical Garden, Kew. pp. 293-294.

Carlos GE, Raphael PP, Alfonso AA, Luicita KR, Nancy AH, Eleazor CM (2015). Effects of aqueous and ethanolic extract of dried leaves of *pseudocalymma allianceum* (Bignonaceae) on hematological and biochemical parameters of wistar rats. Asian Pac. J. Reprod. 4(2):129-134.

Dharm N, Pramod KS (2017). Ethnobotanical importance and herbal medicine in Vidhya region of Eastern Utter Pradesh, India. J. Med. Plant Res. 11(25):403-413.

Francesco F, Satheeshkumar P, Kumar DS, Caterina F, Giuseppe P (2012). A Comparative study of haemoglobin and blood Chemistry of Indian and Italian Grey Mullet (*Mugil Cephalus Linneaus* 1758). HOAJ Biol. 1:1-5.

Gbadamosi IT, Mood JO, Yekini AO (2012). Nutritional composition of ten ethnobotanicals used for treatment of anaemia in South West Nigeria. Eur. J. Med. Plants 2(2):140-150.

Hamidu JL, Ayo JO, Adelaiye AB, Abubakar MS (2008). Sedative and anticonvulsant effects of ethyl acetate fraction of *Waltheria indica* in mice. J. Pharmacol. Toxicol. 3:261-266.

Hamidu JL, Ayo JO, Adelaiye AB Abubakar MS (2013). Evaluation of *Waltheria indica* aerial parts extract for analgesic and anti-inflammatory activities. Eur. J. Sci. Res. 110(2):295-302.

Handy RD, Depledge MH (1999). Physiological responses: Their measurements and uses as environmental biomarkers in ecotoxicology. Ecotoxicology 8:329-349.

Harold S (1988). Practical Clinical Biochemistry. CBS Publ. New Delhi, India. P 132.

Heinrich M, Kuht M, Write CW, Rimper H, Phillipson JD, Schandelmaer A, Warhist DC (1992). Parasitology and Microbiological evaluation of Mixed Indian Medicinal Plants. J. Ethnopharmacol. 36:81-85.

Henry RJ, Cannon DC, Winkelman JW (1974). Clinical Chemistry; Principles and techniques, Harper and Row 2nd Edn. pp. 943-949.

Kaplan LA, Szabo LL, Opherin EK (1988). Clinical Chemistry: Interpretation and techniques, 3rd edn. Leo and Febinger, Philadelphia. pp. 112-231.

Larsen HN, Snieszko SF (1961). Comparison of various methods of determination of haemoglobin in trout blood. Prog. Fish-Culturist 23(1):8-17.

Lorke D (1983). A new approach to Practical acute toxicity testing. Arch. Toxicol. 54:275-287.

Mhuji K, Patrick A, Chacha M (2016). Acute oral toxicity study of *Mystroxylon aethiopicum* root bark aqueous extract in albino mice. J. Med. Plants Res. 10(37):656-661.

Mishra N, Tandon VL (2012). Hematological effects of aqueous extract of ornamental plants in male swiss albino mice. Vet. World 5(I):19-23.

Olajuyugbe OO, Babalola AE, Afolabi AJ (2011). Antibacterial and Phytochemical screening of crude ethanolic extract of *Waltheria indica* Linn. Afr. J. Microbiol. Res. 5:3760-3764.

Oloro J, kuguli JM, Nabirunbi R, Tanayen JK, Laurence I, Francis B, Amon AG (2016). Acute and sub - acute toxicity evaluation in rats of PPOJ5 and ADOJS herbal remedies used traditionally in the management of HIV infection. E. J. Med. Plants 16(4):1-6.

Olowa LF, Nunez OM (2013). Brime Shrimp Lethality assay of the ethanolic extracts of selected species of medicinal plants from Iligan City, Philippines. Int. Res. J. Biol. Sci. 2(II):74-77.

Oluranti OO, Olawuyi OJ, Jonathan SG (2012). Therapeutic properties of some Nigerian higher fungi. Nat. Sci. 10(10):135-143.

Poonam S, Priyanka B, Tasleem A, Imran K, Ramir S (2014). Pharmacology, phytochemistry and safety of aphrodisiac medicinal plants: A review. Res. Rev. J. Pharmacol. Toxicol. Stud. 2:1-8.

Raza M, Al-Shabanah OA, El-Hadiya TM, Al-majeed AA (2002). Effect of prolonged vigabatrin treatment on hematological and biochemical parameters in plasma, livers and kidneys of swiss albino mice. J. Sci. Pharm. 70(2):135-145.

Reitman S, Frankel SA (1957). A calorimetric method for determination of Serum Glutamic Oxaloacetic and Glutamic Pyruvic transaminase. Am. J. Clin. Pathol. 28:56-60.

Shah SL (2010). Haematological changes in *Tinca tinca* after exposure to lethal and sub -lethal doses of mercury, cadmium and lead. Iran. J. Fish Sci. 9:434-443.

Shah SL, Altindag A (2005). Alternations of immunological parameters of tench (*Tinca tinca*) after acute and chronic exposure to lethal and sub lethal treatment with mercury, cadmium and lead. Turks J. Vet. Anim. Sci. 29:1163-1168.

Taiwo OE, Joel OO (2015). Acute toxicity and histopathological assessment of methanolic extract of *Cleome viscosa* (Linn.) whole plant. J. Med. Plant Res. 9 (11):360-369.

Wintrobe MM, Lee GR (1967). Clinical haematology, 6th edn. Philadelpia: Library of congress, Lea and Febinger. P 28.

Yaro AH, Mohammed Z, Shok, M, Ilyas N, Musa K Y (2007). Analgesic activity of *Waltheria indica*. Eur. J. Sci. Res. 16(1):1-6.

Yerra KR, Shih-Hua F, Yew-Min T (2005). Anti-inflammatory activities of flavonoids isolated from *Caesalpina Pulcherrima*. J. Ethnopharmacol. 100(3):249-253.

Zailani AH, Jada SM, Wurochekke UA (2010). Antimicrobial activity of *Waltheria indica*. J. Am. Sci. 6(12):1590-1599.

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Presence of *Bacillus thuringiensis* (Bt) gene in cereals and cereal-based products in local markets and supermarkets of Yaoundé, Cameroon

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The use and importation of genetically modified (GM) crops and products derived from these crops are regulated by national and international policies, which unfortunately are often not properly implemented in some countries. Given the ongoing globalization of trade and increasing availability of GM plant products, countries like Cameroon with a weak system to regulate the importation of these products face the threat of these products entering local markets. This study investigated the presence of GM cereals and cereal-based products circulating in the local markets and supermarkets in Yaoundé, Cameroon. An inventory of cereal based products from these markets was conducted and one of the products was labeled as being derived from GM cereals crops. DNA was extracted from 26 products with a protocol using SDS guanidine thiocyanate to assess the presence of the *Bacillus thuringiensis* (Bt) gene. Polymerase chain reaction (PCR) was used to amplify the DNA fragment associated with Bt gene. Majority of the products were maize-soya based and wheat-soya based. The Bt gene was present in four of the 14 maize based products tested. The presence of the Bt gene in these cereal based products suggest the need for these products to be labeled according to international regulations.

Key words: Bt gene, invertase gene, polymerase chain reaction, guanidine thiocyanate cereal, cereal based products.

INTRODUCTION

The exponential growth in human population during the last century has left various challenges, especially in the domain of feeding and health in many countries. Given this rapid growth and some environmental and agricultural challenges in many countries (Kishore et al., 1999), much effort was needed to address threats that impeded crop production. Traditional and modern biotechnology has been used to improve agronomic traits in plants. As reported, one advantage of growing genetically modified (GM) crop is the reduction in the use

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Table 1. Sequences of primers used in this study.

Primers	Length (bp)	Sequence 5'to 3'	Target gene	Annealing temperature (°C)
Ivr 1A	25	CCGCTGTATCACAAGGGCTGGTACC	Maize invertase	50
Ivr 1B	25	GGAGCCCGTGTAGAGCATGACGATC		50
Cry 1As	25	TGGGGAACAGGCTCACGATGTCCAG	Bt-maize specific	74 and 69
Cry1Ab	25	ACCATCAACAGCCGCTACAACGACC		74 and 69

Ivr 1A = Primer for the forward strand and Ivr 1B = primer for the reverse strand of the invertase gene; Cry 1As = primer for the forward strand and Cry 1Ab = primer for the reverse strand of the Bt gene.

of chemicals to control pests, leading to increased productivity and profitability (Halford et al., 2000; Engel et al., 2002; Gomez-Barbero et al., 2006).

However, many concerns have been raised on the use of GM crops. The negative consequences of the widespread use of GM plants have been divided into two categories involving: (1) adverse effects on the environment and (2) harmful effect on human and animal health (Halford et al., 2000; FAO, 2003; Qaim, 2009). The most commonly cited risks have been transfer to non-target species (Halford et al., 2000; Cartagena Protocol on Biosafety, 2000) and the possible long term undesirable consequence of biodiversity erosion through the loss of traditional crops (Firbank et al., 2006).

The negative socioeconomic effects caused by the corporate control of the food chain have also been reported. Considering the dominance of the biotech industry by large multinational corporations (MNCs), the shift in seed control from a local to a corporate level reduces farmer's involvement in seed enhancement as well as control over "food sovereignty" (FA0, 2003; Azadi et al., 2015). Considering the controversy, policies have been proposed to insure a free and informed adoption and control of genetically modified products (Hogan et al., 2001; Nair et al., 2002; MINEP, 2003; Vacher et al., 2009). Before the introduction of these products in Africa, ethical, cultural environmental, health, economic and ecological concerns were raised (MINEF/UNEP/GEF, 2003). Today, African countries have adopted GM products for commercialization (James, 2008).

Cameroon has ratified the Cartagena Protocol on Biosafety, but has not yet adopted GM crops for commercialization. Given the globalization of trade, the wide-spread adoption of GM crops, and the weak system to regulate GM food products in Cameroon, it was necessary to identify such foods in the market. This study investigated the presence of GM cereals and cereal-based products circulating in the local markets and supermarkets in Yaoundé, Cameroon.

MATERIALS AND METHODS

Sample collection and preparation

An inventory of maize, wheat and soya based products or combination from local markets and supermarkets was carried out using a structured questionnaire. Data recorded included the manufacturer, expiry date, manufacturing date, ingredients of the product, composition of the product, physical form of the product, place of manufacturing and packaging form. Processed products and seeds were collected for maize and soya-based products. The maize grain collected from the field was used to standardize the protocol for DNA extraction. Non-powdered products were separately milled and 30 g of each sample was placed in a plastic bag, sealed and stored at 25°C in a dry place.

DNA extraction

Total genomic DNA was extracted as indicated by Edwards et al. (1991) for the cell lysis steps and Chomczynski and Mackey (1995) for protein and nucleic acids precipitation steps. Note that, the cell lysis solution was added to the mixture followed by proteinase K (20 mg/ml). After an overnight incubation of this mixture, RNAse H 250 UI was added followed by incubation at 37°C, as the objective was the extraction of total DNA. The obtained DNA pellet washed using 70% ethanol with the pellet air-dried, was re-suspended in 200 µl of 1xTris-EDTA buffer. DNA samples were stored at -20°C until further analysis.

The polymerase chain reaction and optimization of reaction conditions

Amplification profiles for the invertase and Bt genes were established through two different PCR procedures; the touchdown PCR and the multiplex PCR. These methods were applied to determine the best annealing temperatures. The multiplex PCR was used to check if the two genes could be amplified under the same conditions. The method of Brinegar et al. (2004) was used with modifications. The PCR mixture consisted of 4 µl of DNA extract, 0.25 µl of 5 U/ml Taq polymerase, 0.25 µl of each primer (Table 1), 0.5 µl of 10 mM dNTPs, 2.5 µl of 10x thermopol buffer in a final volume of 16 µl. The amplifications were performed using a T3 thermal cycler (Biometra, UK) with the following conditions: 3 min at 94°C (pre-denaturation), 45 cycles of 45 s at 94°C (denaturation), 45 s at 69°C for Ivr gene and 50°C for Bt gene (annealing), and 30 s at 72°C (elongation), with a terminal step of 5 min at 72°C. The mixture was held at 4°C.

Electrophoresis of PCR products

PCR amplification products were separated by gel electrophoresis using 2.5% agarose gels (Seakem) with 1×Tris-Borate-EDTA (TBE) running buffer and stained with ethidium bromide. DNA fragments were visualized on an ultraviolet trans-illuminator and photographed with a digital camera.

Table 2. Sample distribution per manufacturer/country.

Country	Cameroon	France	Ivory Coast	Republic of South Africa	Turkey	Unknown
Percentage of products	39.29	39.29	7.14	3.57	3.57	7.14

Table 3. Sample distribution per type of cereals.

Sites of collection	Maize-based Product	Wheat-based Product	Soya-based Product	Maize-and soya based Product	Wheat- and soya-based Product	Multicereal Product	Total
Local market	3	0	1	0	0	0	4
Super-market	6	10	1	3	1	1	22

Table 4. Distribution of Ivr and Bt-genes among the study samples.

Gene Id	Number of products tested	Number of products with the gene	Number of products without the gene
Ivr gene	26	14	12
Bt gene	14	4	9

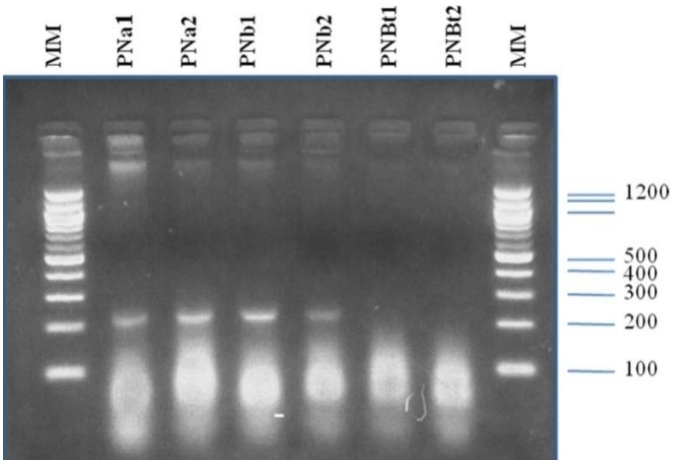

Figure 1. Bands for Ivr gene using the two different annealing temperatures (PNa=74°C – PNb=69°C). -, No bands for Bt gene with the annealing temperature of 69°C; MM = 100 base pair molecular weight marker (MM); PNa1 = PN sample code; a = annealing temperature of 69; 1 = the first tube.

RESULTS

Source and characteristics of study samples

Samples collected were from various places of manufacture (Table 2). Four products were collected from local markets and 22 from supermarkets (Tables 3 and 4).

DNA extraction and PCR assay

DNA was successfully extracted from 14 products using SDS and guanidine thiocyanate. Amplification of the Ivr gene in a sample was used as an indicator for a successful DNA extraction procedure. DNA was not successfully extracted from wheat-based products. Invertase gene was used as an internal positive control for DNA extraction and PCR conditions. Only Ivr-positive samples were tested for Bt-gene. Four of the 14 products tested were Bt-positive (Figure 1). The product used to standardize the protocol for DNA extraction was also tested for Bt gene and was found positive. Band sizes for amplified Ivr gene and Bt genes were 240 bp for Ivr gene and 200 bp respectively (Figure 2). Ivr-positive products were maize and soya based products. No amplification of Ivr gene was obtained with wheat-based products and multi-cereal products. Bt gene was mostly found in maize-based products.

DISCUSSION

Cameroon, although it has ratified the Cartagena protocol on biosafety, has not yet adopted GM crops and derived foods for commercialization. Actually, the quality control scheme of GMOs and derived products is being built. In this process, a comprehensive organization is needed. Laws have been enacted but yet few are implemented. For instance, it is accepted globally and recommended

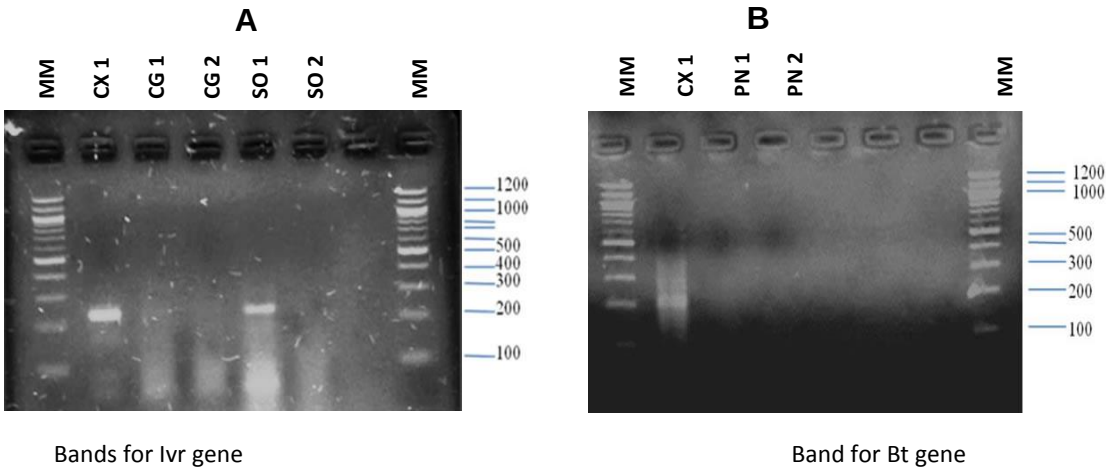

Figure 2. PCR fragments for Ivr (A) and Bt genes (B).

that GMOs and derived products should be labeled to insure a free and informed consumer choice. The presence of just one sample label that conforms to this law reveals the limited practical implementation of the regulation in use in Cameroon (Decree No. 2007/0737/PM of the 31st May 2007). Even though each country or region need to establish threshold value for GM-based product labeling application, Cameroon and even the Central region of Africa has not yet defined the percentages at which a product can be label as containing genetically modified ingredients. It might mean that no matter the percentages of ingredients present in a food product, according to the law, it should be labeled.

In the process of food control, laboratories analyses play major role as quality results are needed for good and right decisions to be taken in the regulatory processes (see "Elements for a National Biotechnology Policy Framework for Cameroon"). Using PCR techniques, the purification of nucleic acids from the sample is often the deciding factor in the production of meaningful results. The combined protocol used in this study can be used with success for DNA extraction from various food types. However, the method was best suited for isolation of DNA from less processed foodstuffs such as corn flour and corn seeds. Improvements are needed to isolate DNA from highly processed foodstuffs that might also contain PCR-inhibitory substances. This was the case of wheat-based products which consistently failed (Tengel et al., 2001). It will also be of use to evaluate the power of the method in terms of quantities and quality of DNA obtained. This will allow the establishment of standards for each product type.

Successful amplification of Bt-gene in cereals and cereal-based products made in Cameroon suggest the use of Bt maize seeds by farmers and in the industry; the source and origin are still to be identified. However, the identification of Bt-positive products was based on the qualitative-PCR analysis using species-specific primers. The obtained molecular sizes were not very different from those reported in the literature (Tengel et al., 2001; Brinegar et al., 2004). Coupled with other techniques as the quantitative-PCR analysis, Western blot, the method used in this study would allow a broad application (Roger et al., 2014). Amplification of the Ivr and Bt genes fragments demonstrated that DNA of integrity sufficient for PCR analysis can be purified using the described techniques.

The finding of this study shows the need to strengthen the national bodies in charge of control at different levels, with capacities and equipments for an efficient and effective action. Given the role of laboratory analysis in the established regulation processes for GMOs, laboratories should be equipped with appropriate trainings and equipments. A broad range assessment of various food types in their various forms would allow a global view at national scale and the standardization of laboratory techniques for GMO detection in Cameroon.

As Cameroon is considered a leading country in the Central region of Africa, the presence of National Laboratories for GMOs testing would be a valuable asset in the northern region and in the southern part of the country, as these laboratories would serve for the Central Africa zone. Quality control for GMOs testing should be added to the quality control system of the country at the various customs posts, especially at the national ports.

Conclusion

The finding of this study indicates that many of the maize-, wheat- and soya-based products present in local markets and supermarkets in Yaoundé are not labeled according to the regulation in use for genetically modified products in Cameroon. Many of which are GM-derived

food products; it will be necessary for the Cameroon government to revise this regulation to meet this challenge.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Azadi H, Samiee A, Mahmoudi H, Jouzi Z, Rafiaani Khachak P, De Maeyer P, Witlox F (2015). Genetically modified crops and small-scale farmers: main opportunities and challenges. Crit. Rev. Biotechnol. 7:1-13.

Brinegar C, Levee D (2004). A Simple Method for Detecting Genetically Modified Maize in Common Food Products. BAMBED 32:35-38.

Cartagena Protocol on Biosafety to the Convention on Biological Diversity, text and annexes, Montréal 2000.

Chomczynski P, Mackey K (1995b). Short technical reports. Modification of the TRI reagent procedure for isolation of RNA from polysaccharide- and proteoglycan-rich sources. Biotechnology 19:942-947.

Edwards K, Johnstone C, Thompson C (1991). A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res. 19:1349.

Engel KH, Frenzel Th, Miller A (2002). Current and future benefits from the use of GM technology in food production. Toxicol. Lett. 127:329-336.

FAO (2003). Weighing the GMO arguments: against, cited 2013-02-08, available at: http://www.fao.org/english/newsroom/focus/2003/gmo8.htm

Firbank LG, Rothery P, May MJ, Clark SJ, Scott RJ, Stuart RC, Boffey CWH, Brooks DR, Champion GT, Haughton AJ, Hawes C, Heard MS, Dewar AM, Perry JN, Squire GR (2006). Effects of genetically modified herbicide-tolerant cropping systems on weed seed banks in two years of following crops. Biol. Lett. 2:140-143.

Gomez-Barbero M, Rodriguez-cerezo E (2006). Economic Impact of Dominant GM Crops Worlwide : A review. European commission DG JRC-IPTS.

Halford NG, Shewry PR (2000). Genetically modified crops: methodology, benefits and public concerns. Br. Med. Bull. 56:62-73.

Hogan RM, Walters DS (2001). Quality control in the development of transgenic crop seed products. Crop Sci. 41:1381-1389.

James C (2008). Etat des PGM cultivées: 2008. ISAAA. 2008.

Kishore GM, Shewmaker C (1999). Biotechnology: Enhancing human nutrition in developing and developed worlds. Proc. Natl. Acad. Sci. USA 96:5968-5972.

MINEP/UNEP/GEF (2003). Proceedings of socio-economic impact of GMOs, Limbe Botanical and zoological Garden, 9-10 December 2003

Ministère de l'Environnement et de la Protection de la Nature (MINEP), Loi n° 2003/006 du 21 Avril 2003 portant Régime de sécurité en matière de Biotechnologie moderne au Cameroun.

Nair RS, Fuchs RL, Shuette SA (2002).Current methods for assessing Safety of Genetically Modified Crops as examplified by Data on Roundup Ready Soybeans. Toxicol. Pathol. 30:117-125.

Qaim M (2009). The Economics of Genetically Modified Crops*",* Ann Rev Resour Econ. 1:665-693.

Roger DD, Gone S (2014) Evidence of the Presence of Genetically Modified Foods in the Sudano-Sahelian Zones of Cameroon. Food Nutr. Sci. 5:922-928.

Tengel C, Schüßler P, Setzke1 E, Balles J, Sprenger-Haußels M (2001). PCR-Based Detection of Genetically Modified Soybean and Maize in Raw and Highly Processed Foodstuffs . BioTechniques 31:426-429.

Vacher C, Bourguet D, Marion D, Lemarié S, Ambec S, Hochberg ME (2006). Fees or refuges: which is better for the sustainable management of insect resistant to transgenic Bt corn? Biol. Lett. 2:192-202.

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