International Journal of Phytomedicine 7 (2015) 123-130
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Antioxidant activity and acute toxicity of a recipe used in traditional medicine for the treatment of high blood pressure

Amani Komenan Nazaire[1], Kouassi Konan[1], Bouagnon Rita[1], Bidie Alain Dit Philippe[1], Djaman Allico Joseph[1,2] N'guessan Jean David[1]

***Corresponding author:**

Amani Komenan Nazaire

[1]Laboratory of Biochemical Pharmacodynamy, Félix Houphouët-Boigny University of Abidjan, Côte d'Ivoire, (225)22 BP 582 Abidjan 22
[2]Pasteur Institute of Côte d'Ivoire, (225)01 BP 490 Abidjan 01

Abstract

The use of recipes using herbs in the treatment of high blood pressure is quite common in Cote d'Ivoire. Among these recipes, a decoction of a mixture of plants called "*Tanopati*" is one of the most commonly used. The objective of this study is to determine the chemical constituents, evaluate the *in vitro* antioxidant activity and acute toxicity in mice of lyophilized extract of "*Tanopati*" recipe.

The phytochemical constituents of '*Tanopati*" was determined by the use of common tests related to flavonoids, tannins, leuco-anthocyanins, anthracenosids, terpenoids, steroid and saponins. We have also quantified the total polyphenolics compounds by Folin-Ciocalteu reagent, flavonoids by aluminum trichloride reagent and condensed tannins by a method using iron trichloride. Antioxidant activity was assessed by DPPH test and an acute toxicity test was performed in mice for the determination of Letal dose 50 (LD 50) of "*Tanopati*".

The phytochemical screening revealed the presence of flavonoids, tannins, leuco-anthocyanin, anthracenosids, terpenoids, steroid and saponins. The quantitative determination of total polyphenols (232 µg EGA/mg), total flavonoids (58.48 µg EQ/mg) and condensed tannins (80.5 µg EC/mg) showed that "*Tanopati*" contained a significant amount of phenolic compounds. The reduction of DPPH radicals (IC_{50}= 28 µg/mL) and the capacity to reduce ferric ions to ferrous ions showed a considerable antioxidant activity of "*Tanopati*". Acute toxicity study carried out on "*Tanopati*" performed in mice (p.o.) showed that the recipe is not toxic (LD50 2000mg/kg b.w).

These results support the safely use of "*Tanopati*" as a traditional medicine for the care of hypertension. The antioxidant properties are undoubtedly an additional benefit for the usage of "*Tanopati*" as an antihypertensive drug.

Keywords: *Tanopati*, phytochemical screening, antioxidant activity, acute toxicity.

Introduction

The use of medicinal plants in disease treatment is an aged tradition and is currently gaining popularity among the population. According to the World Health Organization (WHO), more than 80% of the world population relies on traditional medicine in the treatment of their health problems [1]. This massive falling back on traditional medicine could be explained by the fact that many diseases are treated and cured by plants also at low cost [2].The use of traditional medicine is also favored by the non-availability of conventional medications due to their high cost, the fear of taking synthetic products, the desire to consume organic (natural) products etc. [3,4].

In view of these socio-economic facts, the search for the scientific bases of their efficacy and toxicity for their rational use becomes necessary. Indeed, the absence of a precise dosage and ignorance of the toxic effects of these plants is an obstacle to their systematic use. Thus, our research team focuses its work for several years to the promotion of medicinal plants through the study of their bio-tolerance, the discovery of new accessible molecules and formulation of drugs used in the treatment of major chronic diseases for example cardiovascular disease.

Several studies have shown the involvement of oxidative stress in the physiopathology of cardiovascular disease [5, 6,7]. These diseases, the most recurring being hypertension, are a major public health problem worldwide. The prevalence regarding hypertension was estimated in 2000 to 26.4% of the adult world population about 972 million people, of which 639 million live in developing countries. The projection on the number of hypertensive patients indicates 1.56 billion by 2025 [8]. In Côte d'Ivoire, the prevalence of hypertension was around 14% of the population [9]. The treatment management is usually a lifelong treatment and the cost of treatment is not always affordable to the majority of the population.

In the traditional Ivorian medicine, many plants are used by traditional healers as therapeutic recipe to treat cardiovascular diseases [10]. Among these recipes, one of the most commonly used is "*Tanopati*",a water brewage containing *Ageratum conyzoides, Eucalyptus camaldulensis, NewbouldiaLaevis,*

Phyllanthusmuellerianus, Aloe vera and *Cassia occidentalis*. The traditional use consists in the drinking of 250 mL of the brewage, twice a day corresponding to a dose of (17 mg/kg b.w. for one day).

In order to bring our contribution to the rationale of traditional medicine in Côte d'Ivoire, our present study focused to evaluate the *in vitro* antioxidant activity and acute toxicity of the "*Tanopati*" recipein mice.

Material and Methods

Animals

Thirty (30) white female mice of 8 weeks old were used for the acute toxicity test. These animals were brought from the animal house of the Faculty of Pharmaceutical and Biological Sciences at Félix Houphouët-Boigny University of Abidjan and weighed between 15 and 20 g. These mice were nultiparous and non-pregnant, derived from a non-inbred strain selected from a mouse line with characteristics of vigor and productivity called CF1 (Carworth Farms Strain 1) known as OF1 (Oncins France strain).

Plant Material

The plant material used is a recipe obtained from the decoction of roots, leaves and bark of plants of the Ivorian traditional medicine. They are: *Ageratum conyzoides*, *Eucalyptus camaldulensis, NewbouldiaLaevis*, *Phyllanthusmuellerianus*, *Aloe vera* and *Cassia occidentalis*. This recipe, called "*Tanopati*", was provided by Mr.ADOU TANO ALBERT, an Ivorian traditional practitioner. All these animals were housed in plastic cages where they had free access to water and food, and kept at a temperature of 22 ± 3 C with a relative humidity of 50.15%. The cycle of light and darkness was 12 h/12 h. All the experimental procedures were approved by the Ethical Committee of Health Sciences, Félix Houphouët-Boigny University of Abidjan. These guidelines were in accordance with the European Council Legislation 87/607/EEC for the protection of experimental animals.

Drugs and Chemicals

All reagents, solvents and chemical compounds used for analysis met the quality criteria in accordance with international standards. These aregallic acid,quercetin, catechin, vitamin C, trichloroacetic acid, ammonium chloride, potassium acetate, sodium carbonate, iron trichloride, vanillin, hydrochloric acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and potassium ferricyanide, obtained from Merck (Darmstadt, Germany).

Preparation of lyophilized extract of "*Tanopati*"

The recipe was given to us in its form of use (brewage) by the traditional health practitioner (THP). The decoction was then lyophilized and stored in the freezer for the study.

Phytochemical screening

The phytochemical analyses were performedby the methods described by Dohou*et al.*[11].Alkaloids were identified by Mayer, Wagner and Dragendorffreactions; flavonoids were highlighted by the reaction of Wilstater; leuco-anthocyanins by the Bate-Smith reaction; tannins by the ferric chloride reaction; coumarins by fluorescence reaction; anthracenosidsby Bornträgerreaction; steroids and triterpenes by the Liebermann-Burchard reaction, and saponins by the foam index.

Assessment of the phenolic content

Determination of total phenols

The total phenols content was determined in the extract of "*Tanopati*" by the Folin-Ciocalteu method [12]. To 0.5 mL of the extract of "*Tanopati*" (0.1 g/mL), were respectively added 5 ml of Folin-Ciocalteu reagent diluted 1/10 with distilled water and 4 mL of sodium carbonate (1M). After 15 min of incubation at room temperature, the optical density was measured on a spectrophotometer(HACH DR 2400) at 765 nm. Gallic acid prepared in a solvent mixture of methanol/water (50: 50, v/v) was used as a standard at concentrations ranging from 0 to 50 µg/mL. The total phenolic content of the "*Tanopati*" extract was expressed in terms of microgram of gallic acid equivalents per gram of extract (µg GAE/mg of extract).

Determination of total flavonoids

The colorimetric method of aluminum chloride was used to determine the flavonoids content of "*Tanopati*" extract according to the method described by Chang *et al.*[13].To 0.5 mL of the extract of "*Tanopati*" (0.1 g/mL) were successively added 1.5 mL of methanol; 0.1 mL of 10% ammonium chloride; 0.1 mL of potassium acetate (1M) and 2.8 mL of distilled water. After 30 min of incubation at room temperature, the optical density was measured in a spectrophotometer(Hach DR 2400) at 415 nm. Methanolic solution of quercetin was used as a standard at concentrations ranging from 0 to 80 µg/mL. The flavonoids content of "*Tanopati*" extract was expressed as microgram quercetin equivalent per gram of extract (µg QE/mg of extract).

Determination of condensed tannins (CT)

The dosage of the condensed tannins was performed according to the method of iron trichloride ($FeCl_3$) [14]. In an acid medium, condensed tannins react with vanillin and cause a green coloration of the solution. Thus, 3 mL of a methanol solution of 4% vanillin and 1.5 mL of concentrated hydrochloric acid were added to 400 µL of "*Tanopati*" extract. After 15 min of reaction, the absorbance was read at 550 nm. The concentration of condensed tannins was determined from a calibration curve established with catechin (0-100 mg/mL). The results were expressed in microgram catechin equivalents per milligram of extract (µg CE/mg of extract).

Evaluation of *in vitro*antioxidant activity

Measurement of anti-radical activity

The measurement of the anti-radical activity of "*Tanopati*" extract was performed through the inhibition assay of 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the method of Parejo *et al.*[15]. This method evaluates the ability of the extract to fix free radicals by measuring the decrease of the violet coloration due to the reduction of DPPH radicals.

From a stock solution of "*Tanopati*" extract (0.1 mg/mL), a range of concentrations was prepared by successive double dilution. A volume of 2 mL of methanol solution of DPPH (0.1 mM) was added to 2 mL of extract at various concentrations. After 30 min incubation at room temperature, the absorbance was read in a spectrophotometer at 517 nm against the blank sample. Ascorbic acid, prepared at concentrations ranging from 0to 0.1 mg/mL, was used as reference.The inhibition of DPPH radicals was calculated using the following formula:

$$\text{DPPH inhibition (\%)} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

where $Abs_{control}$ is the absorbance of the DPPH solution without plant extract and Abs_{sample}, the absorbance of the reaction medium containing DPPH and the plant extract or ascorbic acid.

The antioxidant capacity of the plant extract was determined from the IC_{50} (inhibitory concentration 50) which is the sample concentration required to reduce 50% of DPPH radicals. The IC_{50} was determined graphically on a curve showing inhibition of DPPH radicals depending on the concentrations of the extract studied.

Measurement of iron reducing power: FRAP (Ferric Reducing Antioxidant Power)

The reducing power of "*Tanopati*" extract was determined according to the method described by Yildirim *et al.*[16]. This method measures the ability of the extract to reduce ferric ions (Fe^{3+}) present in the complex $[K_3Fe(CN)_6]$ into ferrous ion (Fe^{2+}). An increase in absorbance corresponds to an increase in reducing power.

Thus, a volume of 0.5 mL of the extract at different concentrations (0.1-0.006 mg/mL) was mixed with 2.5 ml of phosphate buffer (0.2M; pH 6.6) and 2.5 mL of potassium ferricyanide $[K_3Fe(CN)_6]$ 1 %. The solution was incubated at 50 C for 30 min after addition of 2.5 mL of trichloroacetic acid (10%). The reaction mixture was centrifuged at 3000 rounds/min for 10 min. A volume of 2.5 mL of the supernatant was homogenized with 2.5 mL of distilled water and 0.5 ml of $FeCl_3$ (0.1%). After 10 min incubation at room temperature, the absorbance was measured at 700 nm against the blank sample. Ascorbic acid (0-100 mg/mL) was used as a positive control in this study.

Assessment of acute toxicity

Acute Toxic Class method described in the OECD Guideline 423 for chemical produce test was used. This method uses pre-determined doses and the results allow the classification of substances in the harmonized global classification system (GHS) causing acute toxicity.

Thirty (30) mice divided into 5 groups of 6 animals each were acclimatized for a week and fasted without water for 4 h (OECD 243, 2001). "*Tanopati*" extract, taken up with distilled water and prepared to doses of 5, 50, 300 and 2000 mg/kg body weight (b.w) was administered in a single dose by gavage to the mice of the experimental groups. Group 1 (control group) received by gavage 0.2 mL of distilled water. The treatment of groups 2, 3, 4 and 5 animals at the respective doses of 5, 50, 300 and 2000 mg/kg b.w,was done in 2 trials per dose. The first test called initial test involved 3 mice and the second called confirmatory test, also involved three mice. All 6 animals of the same experimental group were treated when the first test does not cause death in 2 days of observation. The administration of the plant extract at a higher dose was performed when the previous dose does not cause death, and so on until the last predetermined dose.

Statistical Analysis

The results were expressed as means ± standard error of means (SEM) of three replicates. Where applicable, the data were subjected to one way analysis of variance (ANOVA) and differences between samples were determined by Tukey's Multiple Comparison test using Graph Pad Prism 5.0 program. P values less than 0.05 were considered to be statistically significant.

Results

Phytochemical Screening

The results of the phytochemical screening are shown in Table 1. These results show that the extract of "*Tanopati*" contains tannins, flavonoids, anthocynosids, leuco-anthocyans, anthocyanins, steroids and triterpenes. However, alkaloids, coumarins are absent in this extract.

Table 1: Chemical composition of the lyophilized extract of "*Tanopati*"

Groups	Characteristic	Results
Alkaloids	No precipitate	-
Flavonoids	red-purple	+
Tannins	Blue-black	+
catechin tannins	Red precipitate	+
Gallic Tannins	Blue-black	+
Leuco-anthocyans	Cherry red	+
Coumarines	No fluorescence	-
Steroids and triterpens	Greenish	+
Saponosids	presence of foam	+
Anthracenosids	red	+

Phenolic compounds contents

The total phenolic content of *"Tanopati"* extractwas determined from the calibration curve of gallic acid with the equation y = 0.0093x with R = 0.9732. Those of total flavonoids and condensed tannins were determined respectively from the calibration curves of quercetin (y = 0.0002x; R = 0.9482) and catechin(y =0.0033x; R = 0.99). The results, shown in Table 2 indicate that the extract of "*Tanopati*" contains a significant amount of phenolic compounds.

Table 2: Phenolic compounds content of the extract of "*Tanopati*"

	Total phenol	Total Flavonoids	Condensed Tanins
Content	232 µg GAE/mg	58.48µg QE/mg	80.5 µg CE/mg

In vitro antioxidant activity

Free radical scavenging activity

The results of the free radical scavenging activity of the extract of "*Tanopati*" and ascorbic acid are presented in Figure 1. The values of concentration required to reduce 50 % of DPPH radicals (IC_{50}) are 0.028 mg/mL for "*Tanopati*" extract and 0.002mg/mL for ascorbic acid.

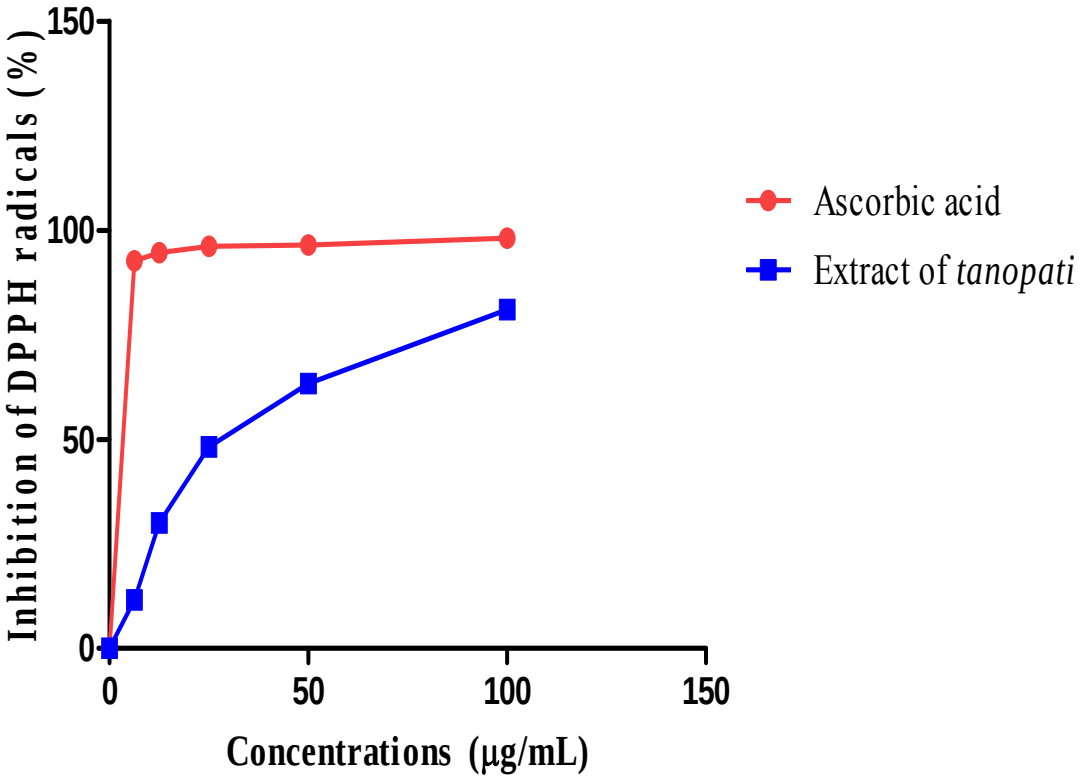

Figure 1: Inhibition of free radicals DPPH by extract of "*Tanopati*" and ascorbic acid.

Ferric reducing antioxidant power (FRAP)

The results of the reduction of the Fe^{3+} ion into Fe^{2+} ion by "*Tanopati*" extract and ascorbic acid are shown in Figure 2. This reduction of iron increases with increasing concentrations of the extract. The curve corresponding to the reducing activity of the extract of "*Tanopati*" has a slope weaker than that of ascorbic acid up to a concentration of 0.00315mg/mL where the reducing activity of these two samples looks nearly identical.

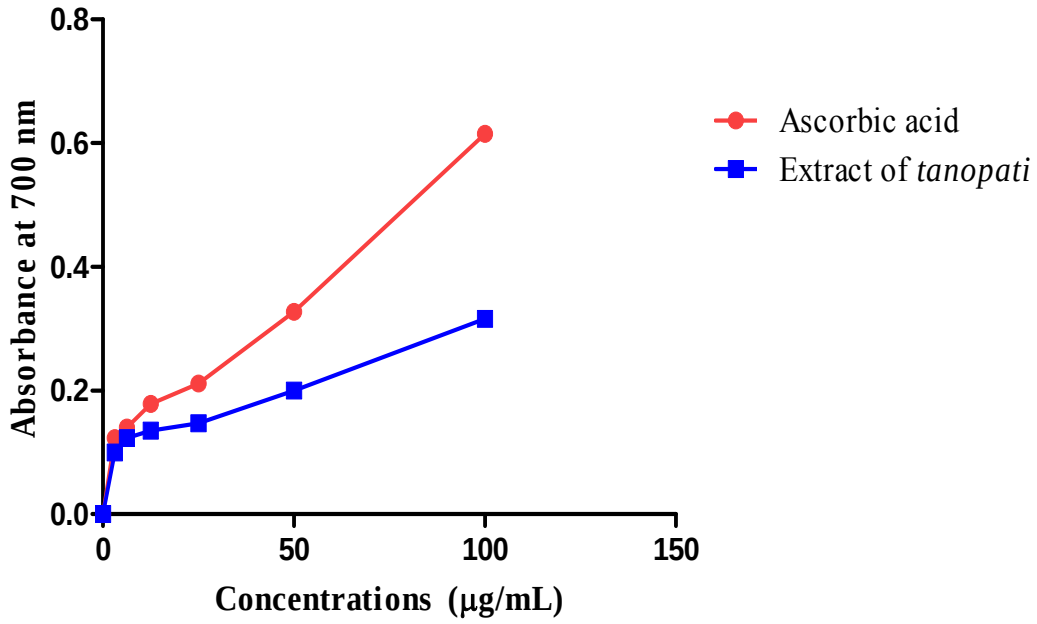

Figure 2: Reducing power of the extract of "T*anopati"* and ascorbic acid

Acute toxicity of extract of "*Tanopati*"

The sequential administration of the extract of "*Tanopati*" at doses of 5, 50, 300 and 2000 mg/kg of body weight (b.w.) revealed no major clinical signs of intoxication and caused no death in mice during the 3 weeks of observation (Table 3). However, behavioral problems such as drowsiness, apathy and travel difficulties were observed in animals at doses of 300 and 2000 mg/kg of body weight.These problems that appeared during the first hour after administration have faded after 2 hours.

Table 3:Mortality of mice based on the dose of the extract of "*Tanopati*"

Groups	Control	Group 2	Group 3	Group 4	Group 5
Doses (mg/kg b.w.)	0	5	50	300	2000
Number of mouse per group	6	6	6	6	6
Mortality (%)	0	0	0	0	0

The results of the effect of "*Tanopati*" extract on body weight of mice based on time are contained in Table 4.The average weight of the control group increases significantly after the second week of observation. Whereas in groups 2, 3 and 4 receiving respectively doses of 5, 50, 300 mg/kg b.w.of "*Tanopati*", there was no significant difference in mean weight.At a dose of 1000 mg/kg b.w., a significant decrease in the average weight of the mice was observed.

Table 4: Average weight of mice according to time of treatment

Groups	Groups1	Groups2	Groups3	Groups4	Groups5
Treatment	Distilled water (0,2 mL)	5 mg/kg b.w.of *Tanopati*	50 mg/kg b.w.of *Tanopati*	300 mg/kg b.w.of *Tanopati*	1000 mg/kg b.w.of *Tanopati*
Body weight(day0)	17,28±0,27^{a}	17,8 ±0,27^{ab}	18,46 ±0,34^{b}	19,71± 0,18^{cd}	20,27± 0,09^{d}
Body weight(day7)	18,36 ±0,3^{a}	18,10± 0,25^{a}	18,58 ±0,33^{a}	20,01± 0,19^{bc}	20,48± 0,08^{c}
Body weight(day14)	19,53 ±0,28^{a}	18,37 ±0,25^{a}	18,92 ±0,32^{ab}	20,26± 0,34^{cd}	20,36 ±0,08^{d}

Values are expressed as mean ± SEM (n=6). In the same column the values followed by the same letter are not significantly different (p□ 0.05).

Discussion

In our study, the antioxidant activity and acute toxicity of the extract of the recipe "*Tanopati*" were evaluated. The phytochemical screening allowed us to highlight in the extract of this recipe, tannins, flavonoids, leucoanthocyans, anthocyanins, terpenoids, steroids, anthocyanosids and saponosids. Our results are in accordance with those of Anuka*et al.*[17] on *Phyllantusmuellerianus*, Nyunaï*et al.*[18] on *Ageratum conyzoides*, Morin [19] on *Aloevera*,Purwar*et al.*[20],on *Cassia occidentalis*and those ofOlounladé[21] on *Newbouldialaevis*. These authors have shown that these plants mixed in "*Tanopati*" contain active compounds such as tannins, flavonoids, alkaloids, anthocyanins, quinonederivatives, saponins, steroids and triterpenoids. Previous studies had also shown that these plants are rich in phenolic compounds. "*Tonopati*" is a mixture containing these plants, so it can be said thatits phytochemical contents are derived from these plants.

The evaluation of the phenolic compounds content showed that "*Tanopati*" extract is rich in total phenols, total flavonoids and condensed tannins. In fact, phenolic compounds are indeed widely distributed in plant tissues where many antioxidant molecules are found [22].

As regard to the evaluation of the *in vitro*antioxidant activity, results showed that the extract of "*Tanopati*" can reduce DPPH free radicals and iron. This antioxidant activity or reducing power effect is due to the substantial content of phenolic compounds in the extract. It is well documented that polyphenols are compounds having antioxidant activity due to their redox properties [23]that allow them to neutralize free radicals by donating electrons or protons [24], to block the chain reaction of free radical by transfer of hydrogen atoms [25] and chelate metal ions [26]. This antioxidant activity of polyphenols is widely used in order to prevent and treat diseases related to oxidative stress. According to Han*et al.* [27], phenolic compounds have veinotonic and vascular-protective properties that could be beneficial in the prevention of vascular damage that may occur in hypertensive patients.

Previous studies have shown the importance of natural substances in the development of new pharmaceutical products or herbal medicines [28, 29]. However, other studies have shown the potential toxic effects of these products [30,31].The present study conducted on the extract of "*Tanopati*" indicates that this recipe has antioxidant properties which justify its development as a new compound of herbal medicine. Therefore, it is necessary to characterize its effect on biological systems, including its toxicological effects.

In the second part of our study, we investigated the toxicological effect of aqueous extract of "*Tanopati*". Acute toxicity study of the aqueous extract showed that with doses up to 2000 mg/kg body weight, no cases of deaths were observed. According to the globally harmonized system of classification (GHS) (OECD 243, 2001), our extract is classified into Category 5 (unclassified) corresponding to LD50 greater than or equal to 5000 mg/kg. According to Delongeas*et al.* [32], any product whose LD50 is greater than 5 g/kg is considered non-toxic. Thus, the safety margin of the aqueous extract of "*Tanopati*" is very wide. The daily dose used in traditional medicine (17 mg/kg, unpublished data) is well below this margin. Moreover during the study, we noticed a significant increase of the weight of mice in the control group while in the groups of handled mice, we did not observe significant difference of the variation of the middle weight. Our extract does not have significant effects on the weight gain. According to West *et al.* [33], the weight gainis associated withfat accumulationin adipose tissue. The polyphenols whichare the main constituentsof"*Tanopati*" are powerful antioxidantsthat can reduce the sizeof adipocytes[34], which would result in adecrease infat accumulation. Moreover, theactiveoxygen species(AOS) of oxidativestressmay interferewithmitochondrial functionanddisruptmitochondrial enzymesthus inhibitingoxidativemechanism[35].

Conclusion

At the end of our study, it appears that the extract of "*Tanopati*" used in traditional medicine as treatment recipe for high blood pressure has a remarkable free radical scavenging and antioxidant activity. The extract of this recipe is also non-toxic according to the globally harmonized system of classification (GHS).Its non-toxic, antioxidant and antiradical properties justify its use in the treatment of hypertension. Future *in vivo* studies for biochemical and histopathological exploration will be undertaken for more scientific informations about "*Tanopati*".

Abbreviations

b.w: body weight; DPPH: 2,2-diphenyl-1-picrylhydrazyl; EAG: gallic acid equivalents; EQ: quercetin equivalent ; GHS: harmonized global classification system; LD: lethal dose;OECD:Organization for Economic Co-operation and Development; SEM: standard error of means THP: traditional health practitioner.

Authors' contributions

AKN carried out toxicity analysis and drafted the manuscript. KK carried out the phytochemical analysis and performed the statistical analysis. BR carried out the phytochemical analysis and helped for the statistical analysis.BADP participated in the design of the study and performed the toxicity results interpretation. DAJ participated in the design of the study and helped to draft the manuscript. NJD conceived the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Disclosure of interests

The authors declare that they have no potential conflict of interest relevant to this article.

Acknowledgment

The authors are grateful to Mr. Albert Tano ADOU an Ivorian traditional practitioner for providing the recipe "*Tanopati*".

References

[1]. Farnsworth NR.Biological and phytochemical screening of plants.J PharmSci.1996; 55: 225-276.

[2]. Kamanyi A, Dkikeng A, Bopelet M. Contribution à la Phytothérapie -Les graines sèches de *Momordicafoetida*Schum.(Cucurbitacées) constituent une source potentielle des principes à effets anti-nidatoire et utérotonique. Revue Méd Pharm Afr.1997; 12: 17-26.

[3]. Duke JA.Medicinal plants and the pharmaceutical industry.In New Crops.Edited by JanickJ and SimonJE.John Wiley and Sons, Inc., New York; 1993.p. 664-669.

[4]. Cox PA,Balick MJ.The ethnobotanical approach to drug discovery.Sci Am. 1994;270(6): 82-87.

[5]. Dhalla NS, Temsah RM, Netticadan T. Role of oxidative stress in cardiovasculardiseases.*J Hypertens*, 2000; 18:655-673.

[6]. Fearon IM, Faux S P. Oxidative stress and cardiovascular disease: Novel tools give (free)radical insight.JMol Cell Cardiol.2009; 47:372-381.

[7]. Touyz RM and Briones AM.Reactive oxygen species and vascular biology: implications inhuman hypertension Hypertens Res. 2011; 34:5-14.

[8]. Beaufils M. HTA-INFO, Paris, Edition ImothepMedecine Sciences.2005; 19:16.

[9]. Comoe KC,Sess D, Guelain J. Etude de l'hypertension artérielle en milieu urbain de Côte d'Ivoire. Med Trop. 1993; 53 (2): 173-179.

[10]. Adjanohoun EJ, Ahyi MRA, Aké-Assi L, Akpagana K, Chibon P, Hadji-El A, Eyme J,Garba M, Gassita JN, Gbeassor M, Goudote E, Guinko S, Hodouto KK, Houngnon P, Keita A, Keoula Y, Kluga-Ocloo WP, Lo I, SiameviKM,Taffame KK.Médecine traditionnelle et pharmacopée. Contribution aux études ethnobotaniques et floristiques au Togo. Rapport ACCT, Paris, France. 1987.p. 225.

[11]. Dohou N, Yamni K, Gmira N, Idrissi Hassani LM. Etude de polyphénols des feuilles d'uneendémique ibéro Marocaine, *Thymelaealychroides*. Acta Bot Malacit.2003;29:233-239.

[12]. Mc Donald TF, Pelzer S, Trautwein W, Pelzer DJ. Regulation and modulation of calcium channels in cardiac, skeletal, and smooth muscle cells.Physiol Rev. 1994; 74:365-507.

[13]. Chang Q, Zuo Z, Harrisson F, Chow MS. Hawthorn.J ClinPharmacol.1998; 42:605-612.

[14]. Heimler D, Vignolini P, Din MG, Vinueri FF, Ronani A. Antiradical activity and polyphenol composition of local Brassiccaceae edible varieties. Food Chem. 2006; 99:464-469.

[15]. Parejo I, Codina C, Petraski C, Kefalas P. Evaluation of scavening activity assessed by co (II)/EDTA-induced luminal chemilunescence and DDPH (2,2-diphenyl-1-pycrilhydrazyl) free radical assay. J.Pharmacol. Toxicol. Method.2000; 44: 507-512.

[16]. Yildirim A, Mavi A, Kara AA. Determination of antioxidant and antimicrobial activities of *Rumexcrispus L.* extracts.J Agric Food Chem. 2001; 49: 411-420.

[17]. Anuka JA, Yaro AH, Wannang NN, Ezenwanne EB, Yakasai IA. Some *in vivo* and *in vitro* studies of the aqueous leaf extract of Phyllanthusmuellerianus (Euphorbiaceae) in laboratory animals. JPB.2005; 2(2): 93-99.

[18]. NyunaïN, AbdennebiEH, BickiiJ, Manguelle-DicoumAM, NjifutiéN. Acute and SubchronicOral Toxicity of Aqueous Extract of *Ageratum Conyzoides* Linn. W Afr J Pharmacol Drug Res. 2011;27: 32-40.

[19]. Morin E. *Aloevera (L.) Burm. f.:* Aspects pharmacologiques et cliniques. Thèse de Doctorat de Pharmacie. Université de Nantes, France, Faculté de Pharmacie ; 2008. p.274.

[20]. Purwar C, Rai R, Srivastava N, Singh J. New flavonoid glycosides from Cassia occidentalis. Section B -organic chemistry including medicinal chemistry. Indian J Chem.2003; 42:434-6.

[21]. Olounladé AP. Effets anthelminthiques des feuilles de *Newbouldialaevis*testées *in vivo* sur lesnématodes gastro-intestinaux (*Haemonchuscontortus*et *Trichostrongyluscolubriformis*) chez lesmoutons Djallonké. DEA, Université de Lomé, Togo ;2005. p.65.

[22]. Hsu CY, Chan YP, Chang J. Antioxidant activity of extract from *Polygomumcuspidatum*.BiolRes. 2007;40: 13-21.

[23]. Zeng W, Wang SY.Antioxidant activity and phenolic compounds in selected herbs.J Agric Food Chem. 2001;49: 5165-5170.

[24]. Chen CW, Ho CT. Antioxidant properties of polyphenols extracted from green tea and black tea. J Lipids.1995;2:35-46.

[25]. Meir S, Kanner J, Akiri B, Hadar SP.Determination and involment of aqueous reducing compounds in oxidative systems of various senescing leaves.J Agricfoodchem.1995; 43: 1813-1817.

[26]. Brown JE, Khodr H, Hider RC, Rice-Evans C. Structural dependence of flavonoïd interactions with Cu^{2+}ions: implication for their antioxidant properties.Biochem J. 1998;330: 1173-1178.

[27]. Han X, Shen T, Lou H.Dietary Polyphenols and Their Biological Significance. Int J Mol Sci.2007; 8(9): 950-988.

[28]. Gilani AH, Rahman AU. Trends in ethnopharmacology.J Ethnopharmacol.2005;100: 43-49.

[29]. Patwardhan B. Ethnopharmacology and drug discovery.J Ethnopharmacol.2005;100: 50-52.

[30]. Pak E, Esrason KT, Wu VH. Hepatotoxicity of herbal remedies: an emerging dilemma. Prog Transplant.2004; 14: 91-96.

[31]. Marcus DM, Snodgrass WR. Do no harm: avoidance of herbal medicines during pregnancy.Obs&Gynae. 2005;105: 1119-1122.

[32]. Delongeas JL, Burnel D, Netter P, Grignon M, Mur JM, Roger RJ,Grlgnon G. Toxicité et pharmacocinétique de l'oxychlorure de Zirconium chez la souris et le chat.JPharmacol.1983; 14(4):437-447.

[33]. West DB, York B.Dietary fat, genetic predisposition, and obesity: lessons from animal models.AmJ ClinNutr.1998; 67: 505-512.

[34]. Myoung NW, Song HB,Myung SC.Hypolipidimic and body fat-flowing effects of Fatcleanin rats fed a high-fat diet. Food ChemToxicol.2009; 47: 2076-2082.

[35]. Powers SK, Lennon SL. Analysis of cellular responses to free radicals: focus on exercise andskeletal muscle. ProcNutr Soc. 1999; 58:1025-1033.