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Evaluation of antiradical activity and acute toxicity of healing clays from Côte d'Ivoire

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ABSTRACT

Clays have been used since prehistoric times to treat various ailments around the world because of their many virtues including oxidative stress diseases. Several studies have shown the efficiency of natural antioxidants compounds in the treatment and prevention of oxidative stress diseases such as arterial hypertension, cancer, diabetesand rheumatism. This study aimed at checking if clays AK1 and AK2 used to treat various diseases in Ivory Coast possess an antiradical activity and are non-toxic. The antiradical activity was achieved by the DPPH free radical inhibition method. The two clays AK1 (IC_{50} =0.025 ± 0.0002 µg/mL) and AK2 (IC_{50} = 0.0423 ± 0.0001 µg/mL) showed antioxidant power; but the best value was that of AK1 whose antiradical activity was greater than that of the reference molecule Vitamin C (IC_{50} = 0.035 ± 0.0004 µg/mL). The study of their acute toxicity was carried out according to the OECD 423 method at limit dose of 5000 mg/kg of body weight. After fourteen days of observation, the results show that both clays are not toxic to rats, even in large doses (5000 mg/kg of body weight). Therefore, they could be used for effective therapeutic purposes.

Keywords: Clays; antioxidant; DPPH; OECD 423.

INTRODUCTION

Excessive production of reactive oxygen species (ROS) in cells, due to exposure to tobacco, alcohol, radiation or environmental toxins, disrupts the balance between oxidation and antioxidation, which leads to certain chronic and degenerative diseases (Li et al., 2015; Wang et al., 2016;Zhou et al., 2016; Xu et al., 2017) such as Alzheimer's disease, rheumatism, diabetes, cardiovascular diseases (Konan et al., 2019), skin diseases (Bene et al., 2017), etc. Increasing the intake of

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antioxidants, mainly from edible plants (fruits, vegetables,...) and medicinal plants would mitigate the damage caused by oxidative stress by delaying or inhibiting the oxidative process by blocking the initiation or propagation of the oxidative chain reaction caused by oxidative stress (Fereidon and Ambigaipalan, 2015; Konan et al., 2019). In view of their important effects on health, several researches have been initiated to discover new non-synthetic antioxidants to limit this aggression on cellular constituents and associated pathologies. Clay minerals have been used for therapeutic purposes since prehistoric times (Carretero and Pozo, 2009, Carretero and Pozo, 2010). They are used to treat digestive system (internal) and skin diseases (external) such as Buruli ulcer, heartburn, diarrhoea, intestinal infection (Williams et al., 2008; Coulibaly et al., 2013, Sylla Gueye et al., 2016). Research showed antibacterial and bactericidal properties of clays (Haydel et al., 2008; Magaña et al., 2008; Ma'or et al., 2006; Tong et al., 2005; Williams et al., 2004). However, no study concerning their antiradical properties is mentioned in the literature. In Ivory Coast, clays are used to treat several diseases by some naturotherapists. This is the case of clays (referenced AK1 and AK2) used in the treatment of skin diseases and gastrointestinal system by Aboutou Poterie (Kouakou et al., 2012). Previous studies have shown that these clays have antibacterial properties by inhibiting the growth of certain bacteria through their chemical composition (Kouakou et al., 2014). Thus, considering their use in the health field, it would be important to evaluate their safety. Thus, this study aimed at contributing to the search for new antioxidants of natural origin by studying the antiradical properties using DPPH method and acute toxicity of clays AK1 and AK2 using OECD 423 method.

MATERIALS AND METHODS

Clay raw materials

The two clays referenced AK1 and AK2 (Figure 1)were collected in Aboutou's Pottery office, a center of naturotherapy recognized officially by the state of Ivory Coast since August 06th, 2009 under the number MSHP/PNPMT/ N° 2355. At Aboutou's office, AK1 and AK2 are used to treat affections of the digestive system, skin diseases, wounds, etc. as well by internal ways as externals. They are from the Bocanda region in the center part of Ivory Coast at 7° 03′ 49″ North latitude and 4° 29′ 57″ West longitude.AK1 and AK2 have been characterized physicochemical and mineralogical by Kouakou et al. (2012).The results of the chemical composition and of the semi-quantitative composition calculation according to Yvon et al. (1982) methodology are given in Table 1.

Antiradical activity

Determination of DPPH radical scavenging capacity

Principle

The 2,2′-diphenyl-1-picrylhydrazyl (DPPH) is characterized by its ability to produce stable free radicals. This stability is due to the delocalization of free electrons within the molecule. The presence of these DPPH• radicals gives rise to a dark purple coloration of the solution. The reduction of DPPH• radicals with an antioxidant causes a discoloration of the solution (Fereidon and Ambigaipalan, 2015) into a yellow colored compound (the diphenyl-picrylhydrazine) (Bene et al., 2017). The color change can be followed by spectrophotometry at 517 nm and in this way the antioxidant potential of a substance or a plant extract can be determined (Molyneux, 2004; Popovici et al., 2010).

Protocol

The *in vitro* antiradical activity of clays from Ivory Coast (AK1 and AK2) was measured by the 2,2'- diphenyl-1-picrylhydrazyl (DPPH) test, according to the method of Parejo et al. (2002) with some modifications. Slowly, 2 mL of an ethanol solution (70/30) containing DPPH (100 µM) was mixed with 2 mL of different dilutions of the extracts (0, 1.5625, 3.125, 6.25, 12.5, 25, 50, 100 and 200 µg/mL). The same concentrations were used for vitamin C (used as reference). The resulting mixture is then kept away from light at room temperature for 30 minutes. The absorbance is then measured at 517 nm against a control consisting of 2 mL of the DPPH solution and 2 mL of the ethanol solution. Samples and references are prepared under the same operating conditions. The

decrease in absorbance is measured spectrophotometrically and the Percent of inhibition (% PI) is calculated according to the formula below (Kanfon et al., 2018; Konan et al., 2019) :

$$\mathbf{PI=}\frac{\mathbf{(A0-A1)x100}}{\boldsymbol{A0}}$$

PI (%): inhibition power in %

A0: absorbance of the DPPH solution in the absence of the extract (white)

A1: absorbance of the DPPH solution in the presence of the extract (test)

The concentration of extracts or vitamin C, that is responsible of the inhibition of 50% (IC_{50}) of DPPH radicals, was determined by projection from 50% on the graph representing the percentage inhibition of DPPH, depending on the concentrations of the clays from Ivory Coast (AK1 and AK2) and vitamin C [% Inhibition DPPH = f (extract concentrations)].

Acute toxicity assessment method

The acute toxicity of clays from Ivory Coast (AK1 and AK2) was determined in rats using OECD (2001) Method 423. Rats fasted for 16 hours were randomly assigned to groups of three. Graduated doses of the extract (5, 50, 300 and 2000 mg/kg bw) were administered separately to the rats in each group using a curved steel needle (Figure 2). All rats were then allowed free access to food and water. They were observed for 24 hours during 14 days to look for signs of acute toxicity. The number of deaths during this period was recorded.

• 0 mg/kg (0 mg/mL) < category 1 ≤ 5mg/kg (0.25 mg/mL)

• 5 mg/kg (0.25 mg/mL) < category 2 ≤ 50mg/kg (2.5 mg/mL)

• 50 mg/kg (2.5 mg/mL) < category 3 ≤ 300mg/kg (15 mg/mL)

• 300 mg/kg (15 mg/mL) < category 4 ≤ 2000 mg/kg (100 mg/mL)

• 2000 mg/kg (100 mg/mL) < category 5 ≤ 5000 mg/kg (250 mg/mL)

Animal material and method of administration

Eighteen rats (Wistar) weighing between 160 and 240 g were used in this study. They were purchased and maintained at Félix HOUPHOUËT-BOIGNY University pet store for experimental purposes. The animals were kept under controlled conditions of the temperature (23 ± 2)°C, humidity (50 ± 5)% and 12-hours light-dark cycles. All animals were acclimatized for seven days prior to the study. Animals were randomized into groups (experimental and control) and individually housed in sanitized polypropylene cages containing a sterile paddy wrap as bedding. They had free access to standard pellets as a staple diet and water at will. The animals were accustomed to the laboratory conditions 48 hours prior to the experimental protocol in order to minimize any non-specific stress.

AK1 **AK2**

Figure 1: Clay samples AK1 and AK2.

Table 1: Chemical and mineralogical composition of AK1 and AK2.

AK1						AK2					
Oxides	**%**	**Trace elements**	**ppm**	**Phase**	**%**	**Oxides**	**%**	**Trace elements**	**ppm**	**Phase**	**%**
SiO_2	61.99	As	20.56	Kaolinite	22.6	SiO_2	52.24	As	15.42	Kaolinite	43
Al_2O_3	16.78	Cd	<L.D	Quartz	41.1	Al_2O_3	22.13	Cd	0.20	Quartz	26.2
Fe_2O_3	8.47	Co	56.24	Illite	18.1	Fe_2O_3	10.85	Co	30.83	Illite	13.4
MnO	0.02	Cr	120.5	Montmorillonite	6.5	MnO	0.03	Cr	122.5	Goethite	12.1
MgO	1.23	Cu	54.39			MgO	0.23	Cu	39.11		
CaO	0.15	Ga	19.37			CaO	0.28	Ga	26.94		
Na_2O	0.13	Mo	0.68			Na_2O	0.06	Mo	2.53		
K_2O	2.14	Ni	47.82			K_2O	1.59	Ni	26.45		
TiO_2	0.77	Pb	10.26			TiO_2	0.93	Pb	14.34		
P_2O_5	0.06	Zn	64.32			P_2O_5	0.09	Zn	32.97		
		V	122.9					V	135.3		
		Sn	1.23					Sn	2.29		

< L.D : limite detection

Source: Kouakou et al., 2012.

Figure 2: Gavage of rats with a curved steel needle.

RESULTS

Antiradical activity

In this experiment, the scavenging ability of clays on DPPH free radical was examined in the concentration range of 50-500 µg/mL using the DPPH colorimetric assay. The results are given in Table 2. The scavenging ability of clays compared to those of vitamin C is shown in Figure 3. The results show that all substances reduce the DPPH radical in a dose-depedent manner, i.e. the percentage of DPPH inhibition increases with the concentration of the extracts up to a threshold where the percentage of inhibition stabilises with the increase in the concentration of the extracts. The two clays used have antiradical activities. In fact, they discolour the DPPH solution (from purple to yellow). Among these clays, AK1 (IC_{50} = 0.025 ± 0.0002 µg/mL) was more active than Vitamin C (IC_{50} = 0.035 ± 0.0004 µg/mL), which is more active than AK2 (IC_{50} = 0.0423 ± 0.0001 µg/mL). Indeed, a lower IC_{50} corresponds to a better activity of the extract.

Acute toxicity

The oral administration to rats of graduated doses (5, 50, 300 and 2000 mg / kg bw) of clays AK1 and AK2 did not show any significant change in behavior, respiration, skin effects, the sensory or gastrointestinal nervous system responses during the observation period (Lebri et al., 2015). No mortality or toxic reaction was recorded in the different groups, 24 hours after administration during 14 days. Clays from Ivory Coast (AK1 and AK2) are safe up to a dose of 2000 mg/kg of body weight.

Weight development

After administration of clays to rats of the different categories, their weights were measured every 7 days for a period of 14 days. All treated animals showed a positive weight change. The evolution of the weight of the animals during the 14 days of the experiment is recorded in Tables 3,4 and 5.

Observations for 24 hours and 14 days

Table 6 gives the observations for 24 hours and 14 days.

The different clays are non-toxic whatever the dose used.

Table 2: DPPH radical scavenging activities of AK1, AK2 and Vitamin C.

	Vitamin C	AK1	AK2
IC_{50} (µg/mL)	0.035 ± 0.004	0.025 ± 0.0002	0.0423 ± 0.0001

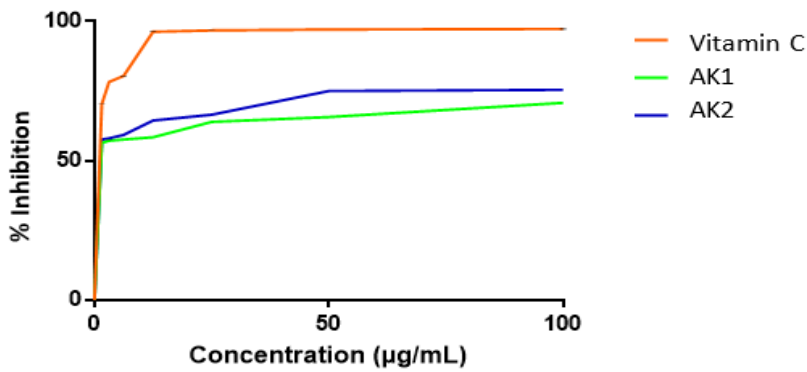

Figure 3: The scavenging effects of AK1, AK2 and Vitamin C.

Table 3: Control rats D0-D7-D14.

Animals	Weight (g)		
	(D0)	(D7)	(D14)
Rat 1	195±1	200±2	210±1
Rat 2	200±2	206±1	210±1
Rat 3	190±0	195±0	203±2

Table 4: Change in average animal weight Doses administered (mg / Kg) average body weight of animals (g) D0-D7-D14 (Clay AK1).

5 mg/kg body weight			
Animals	Weight (g)		
	(D0)	(D7)	(D14)
Rat 1	200±2	207±1	215±1
Rat 2	160±0	165±2	170±0
Rat 3	210±2	220±1	227±2

50 mg/kg body weight			
Animals	Weight (g)		
	(D0)	(D7)	(D14)
Rat 1	170±2	175±1	180±1
Rat 2	240±1	247±1	252±0
Rat 3	199±0	203±0	210±0

300 mg/kg body weight			
Animals	Weight (g)		
	(D0)	(D7)	(D14)
Rat 1	207±2	215±2	221±1
Rat 2	218±1	220±0	226±3
Rat 3	199±1	204±2	210±0

2000 mg/kg body weight			
Animals	Weight (g)		
	(D0)	(D7)	(D14)
Rat 1	210±4	210±0	216±0
Rat 2	250±2	250±1	257±1
Rat 3	207±1	207±1	215±2

Table 5: Change in average animal weight Doses administered (mg/Kg) average body weight of animals (g) D0-D7-D14 (Clay AK2).

5 mg/kg body weight			
Animals	Weight (g)		
	(D0)	(D7)	(D14)
Rat 1	200±2	209±1	216±1
Rat 2	207±0	215±2	223±0
Rat 3	186±2	192±1	200±2

50 mg/kg body weight			
Animals	Weight (g)		
	(D0)	(D7)	(D14)
Rat 1	178±3	186±1	195±1
Rat 2	175±2	180±2	189±2
Rat 3	200±0	207±1	215±3

300 mg/kg body weight			
Animals	Weight (g)		
	(D0)	(D7)	(D14)
Rat 1	206±1	213±1	220±3
Rat 2	202±0	209±2	217±2
Rat 3	201±1	204±1	210±0

2000 mg/kg body weight			
Animals	Weight (g)		
	(D0)	(D7)	(D14)
Rat 1	202±2	210±0	216±1
Rat 2	203±0	207±2	212±2
Rat 3	201±1	205±1	211±0

D0: Day 0, D7: Seventh day, D14: fourteenth day.

Table 6: Different observations made in 24 hours during 14 days.

Observations	Control lot		Experimental lot	
	24 hours	**14 days**	**24 hours**	**14 days**
Skin and Fur	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal
Mucous membranes	Normal	Normal	Normal	Normal
Diarrhea	Absence	Absence	Absence	Absence
Salivation	Absence	Absence	Absence	Absence
Lethargy	Absence	Absence	Absence	Absence
Heartbeats	Normal	Normal	Normal	Normal
Aggressiveness	Absence	Absence	Absence	Absence
Sleepiness	No	No	No	No
Power supply	Yes	Yes	Yes	Yes
Mobility	Yes	Yes	Yes	Yes
Mortality	No	No	No	No

DISCUSSION

The evaluation of acute toxicity consists in measuring and recording the various adverse effects that appeared after the administration of clays. Indeed, in this work, the animals which received 2000 mg / Kg did not show changes in behavior and neither more or less serious signs of intoxication (apathy, drowsiness, difficult movement of the animals during the experiment). No cases of mortality were recorded. At the end of the acute toxicity evaluation test, the LD50 is higher than 2000 mg/Kg orally. According to the Globally Harmonized System of Classification (GHS), the clays can be classified as non-toxic (Olson et al., 2000). The maximum tolerated dose is 2000 mg/kg bw/vol. This confirms the idea that the MTD is higher than the doses necessary to have pharmacological effects. Thus, thanks to its DMT of 5000 mg/kg bw/vol, the decoction offers an appreciable safety margin (Betti et al., 2012; El Hilaly et al., 2004). The scavenging ability of clays AK1 and AK2 compared to those of vitamin C indicated that AK1 and AK2 exhibited very significantly radical scavenging. The best antiradical activity was obtained with AK1 (IC_{50} = 0.025 ± 0.0002 µg/mL); whose activity was greater than that of vitamin C (IC_{50} = 0.035 ± 0.0004 µg/mL) taken as the reference molecule. The observed antiradical activity could be explained by the presence of Cu, Zn, Fe in AK1 and AK2 (Leung, 1998). Indeed, these minerals have been revealed in these different clays by Kouakou et al. (2012) (Table 1). The high proportions of copper (54.39 ppm) and zinc (64.32 ppm) in AK1 compared to AK2 [Copper (39.11 ppm) and Zinc (32.97 ppm)] could also explain the fact that the antiradical activity of AK1 is better than that of AK2. Concerning iron, it is free in AK1, unlike AK2 where it is involved in the composition of goethite (Table 1). Moreover, these elements (Fe, Cu and Zn) are not toxic at the tested doses (OMS, 1973).These results could justify the use of these different clays in traditional medicine.

Conclusion

This work aimed at encouraging the use of clays in traditional medicine in Ivory Coast. The results of the acute toxicity studies showed that a dose of 5000 mg / kg of body weight of clays administered by the oral route appeared to be non-toxic. The antiradical activity of

Ivory Coast clays was evaluated using DPPH methods; vitamin C was used as reference molecule. The best antioxidant activity was obtained with AK1. This clay was most active than vitamin C. This study confirms that it is safe for people who use it in the center of Ivory Coast as a medicine. To our knowledge, this is the first time that this activity has been carried out on the clays of Ivory Coast. As perspective, it is intended to continue this study, by carrying out the evaluation of antioxidant activities with other methods such as ABTS and FRAP.

COMPETING INTERESTS

The authors declare that they have no competing interest about the work reported in this paper.

AUTHORS' CONTRIBUTIONS

Conception and design of the study: LPM-SK, LNA, BIHGD, AFK, ALCK, NBYF, KLK, KBA, YJA-Y.

Data collection : LPM-SK, AFK, ALCK, NBYF.

Draft of the article : LPM-SK, LNA, BIHGD, AFK, ALCK.

Critical revision of the article for important intellectual content : LPM-SK, LNA, BIHGD, AFK, ALCK, NBYF, KLK, KBA, YJA-Y.

All authors read and approved the final version of the manuscript.

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