Print ISSN 2319-2003 | Online ISSN 2279-0780

IJBCP International Journal of Basic & Clinical Pharmacology

doi: 10.5455/2319-2003.ijbcp20141034

Research Article

Hepatoprotective and antioxidant activities of *Hibiscus sabdariffa* petal extracts in Wistar rats

Abba P. Obouayeba[1]*, Lydie Boyvin[2], Gervais M. M'Boh[2], Sekou Diabaté[4], Tanoh H. Kouakou[3], Allico J. Djaman[1, 2], Jean D. N'Guessan[1]

[1]Department of Biosciences, Laboratory of Biochemical Pharmacodynamic, Félix Houphouët Boigny University of Abidjan, 22 BP 582 Abidjan 22, Côte d'Ivoire, [2]Department of Clinical and Fundamental Biochemistry, Pasteur Institute of Côte d'Ivoire, 01 BP 490 Abidjan 01, Côte d'Ivoire, [3]Department of Natural Sciences, Laboratory of Biology and Crop Improvement, Nangui Abrogoua University of Abidjan, 02 BP 801 Abidjan 01, Côte d'Ivoire, [4]Unity of Physio/Phytopathology Central Laboratory of Biotechnology, National Agricultural Research Center, 01 BP 1740 Abidjan 01, Côte d'Ivoire

Received: 15 August 2014
Accepted: 23 August 2014

***Correspondence to:**
Abba P. Obouayeba,
Email: obouapac@yahoo.fr

ABSTRACT

Background: *Hibiscus sabdariffa* is a medicinal plant rich in phytochemical compounds, which is the source of its biological properties. This study on the aqueous extract of *H. sabdariffa* (AEHS) was conducted to assess its hepatoprotective and antioxidant properties.

Methods: It was carried out with 25 Wistar rats divided into five groups. Two groups were treated with a solution of NaCl 0.9%. One group was treated with silymarin at a dose of 25 mg/kg body weight (BW). Two other groups were treated with the AEHS at different doses (100 and 200 mg/kg BW). The treatments were carried out via oral route and at single dose for 7 days. After injection of 2,4-dinitrophenylhydrazine (DNPH), blood samples were collected for the carrying out of biochemical analyses of oxidative stress markers (thiobarbituric acid reactive substances, ferric reduction antioxidant parameter, and 2,2-diphenyl-1-picrylhydrazyl) and hepatotoxicity (albumin, total and direct bilirubin, alanine transaminase, aspartate aminotransferase, and lactate dehydrogenase).

Results: Three major results were obtained. The hepatotoxicity of DNPH expressed by the rats of Group 1 was significantly different ($p<0.05$) from those of the other groups (control, 2-4) for both hepatotoxicity and oxidative stress markers. The hepatoprotective and antioxidant properties of the AEHS and confirmation of those of silymarin through the rats of Groups 2-4 were statistically identical ($p<0.05$) to the control group for markers of hepatotoxicity and oxidative stress.

Conclusion: These results confirm and reinforce certain therapeutic virtues of *H. sabdariffa*.

Keywords: Antioxidant activity, Aqueous extract, Hepatoprotective activity, *Hibiscus sabdariffa*, Petals, Wistar rats

INTRODUCTION

The use of plants in therapy is certainly very old, but it is currently experiencing a renewed interest among the population despite advances in modern medicine.[1] According to the World Health Organization, more than 80% of the world population use traditional medicine to cope with health problems.[2] Among those medicinal plants, *Hibiscus sabdariffa* (Malvaceae) is an annual herbaceous plant originating from Central and West Africa as well as South East Asia.

The petals of *H. sabdariffa*, which extracts contain almost the same phytochemical compounds[3] as the flowers and sepals deserve that some pharmacological properties including antioxidant and hepatoprotective activities be assessed; insofar as if those properties appeared to be true, its extracts could actively and efficiently participate in the fight against liver diseases that are growing. These petals are used in the preparation of local nonalcoholic cold beverage and as a hot drink highly appreciated in Côte d'Ivoire. This nonalcoholic drink called bissap prepared from the red petals is popular and highly appreciated by population in most of the West African countries.

H. sabdariffa is also used in traditional medicine for its antihypertensive, diuretic, and laxative properties.[4] It is grown for food, economic interests, and its various pharmacological properties. These pharmacological properties are due to the presence of the phytochemical constituents of the plant. Indeed, *H. sabdariffa* contains several phytochemical compounds including organic acids, phenolic acids, anthocyanins, flavonoids, trace elements, and vitamins.[5,3] Our previous works[3] had revealed that the major compounds present in the aqueous extract obtained with the petals of *H. sabdariffa* were gossypetin, hibiscetin, quercetin, and sabdaretin (flavonoids) while delphinidin 3-O-sambubioside and cyanidin 3-O-sambubioside were the major anthocyanins. These secondary metabolites of interests present in the petals may confer potent functional properties like free radicals scavenging and liver protection.[6-8]

The liver is, indeed, a very important vital organ, due to the vital role it plays in various biochemical and physiological processes, notably the metabolic and detoxification functions.[9] The latter action of the liver is responsible for various diseases of this organ including hepatitis, liver cirrhosis, liver abscess, and liver cancer.[10] Liver diseases are numerous and mainly caused by infections (viral, bacterial, fungal, and parasitic), hepatotoxic substances, excessive intake of alcohol and toxins.[11] The use of plants, especially *H. sabdariffa*, in the treatment and prevention of liver diseases is confirmed and justified by very recent and important works[11,12] which showed that generally, medicinal plants play a crucial role in protecting the liver. In addition to that, the availability of this species and especially the use of its petals as food fall has become a habit for African populations. Since the juice obtained from the petals of this plant is used extensively in various ceremonies in West Africa in general and in Côte d'Ivoire in particular. Hence, the present study aims to assess the antioxidant and hepatoprotective activities of *H. sabdariffa* petal extracts in Wistar rats.

METHODS

Plant material

The petals of *H. sabdariffa* were used as plant material in the present study. The material was purchased from a local market in Adjamé (Abidjan, Côte d'Ivoire). The petals were cut, cleaned, washed thoroughly under running tap water, drained, and oven-dried at 55°C for 12 hrs. The samples were packed in polyethylene bags and stored at 4°C for laboratory analysis.

Animals

The animals used in this study were Wistar rats which average weight was 185±15 g. These animals which came from the animal house of the Pasteur Institute of Adiopodoumé (Abidjan, Côte d'Ivoire) were housed in cages in the animal house of the Biosciences Training and Research Unit, at room temperature. They had free access to food (pellets from Faci, Côte d'Ivoire) and water. 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 analyses met the quality criteria in accordance with international standards. It were 2, 4-dinitrophenylhydrazine, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), 2, 4, 6-tris (2-pyridyl)-S-triazine (TPTZ) and 1,1,3,3-tetramethoxypropane purchased from Sigma-Aldrich (Steinheim, Germany). The trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA), ferric chloride (FeCl3, 6H2O), ferrous sulfate (FeSO4, 7H2O) and acetonitrile were obtained from Merck (Darmstadt, Germany). The silymarin originated from Madaus GmbH (Cologne, Germany). The doxorubicin originated from SC Sindan-Pharma (Bucharest, Romania).

Extract preparation

100 g petals of *H. sabdariffa* were extracted from 200 mL of acidified methanol with trifluoroacetic acid 0.1% (v/v) for 24 hrs at 4°C. The macerate was filtered successively on cotton wool and Whatman paper. After low-pressure vacuum evaporation of the methanol in BÜCHI Rotavapor

R-114 at 38°C, we obtained a dry extract. 200 mL of distilled water was added to the dry extract, and the aqueous extract was submitted to a filtration on gel XAD7, in order to eliminate sugars and chlorophyll pigments. 100 mL of pure methanol was poured over the gel XAD7 and the methanolic filtrate obtained was resubmitted to low-pressure vacuum evaporation in BÜCHI Rotavapor R-114 at 38°C. The dry extract obtained was dissolved in 100 mL of distilled water. The aqueous extract was lyophilized with the freeze dryer CHRIST ALPHA 1-2. The dried extract obtained represented the aqueous extract of *H. sabdariffa* (AEHS) which was used to carry out the different studies.[3]

Assessment of hepatoprotective and antioxidant activities in vivo

Experimental protocol

The assessment of the hepatoprotective activity of the AEHS was carried out with 25 Wistar rats. The animals were divided into five groups of five rats according to the method described by Ologundudu et al., 2009[7] with some modifications as follows:

1. Control group: 0.5 mL of 0.9 % NaCl
2. Group 1: 0.5 mL of 0.9 % NaCl + 3 mg/kg body weight (BW) of 2,4-dinitrophenylhydrazine (DNPH)
3. Group 2: 25 mg/kg BW of silymarin + 3 mg/kg BW of DNPH
4. Group 3: 100 mg/kg of AEHS + 3 mg/kg BW of DNPH
5. Group 4: 200 mg/kg of AEHS + 3 mg/kg BW of DNPH.

The rats of the control group and Group 1 were treated with 0.5 mL of a solution of 0.9% NaCl for 1 week. The rats of Group 2 were treated with silymarin (25 mg/kg BW) dissolved in 0.9% NaCl for 1 week. The rats of Groups 3 and 4 were treated with the AEHS at different concentrations (respectively, 100 and 200 mg/kg BW) dissolved in NaCl 0.9% for 1 week. The different administrations were made via oral route at single dose. 1 hr after the last treatments, the rats of Groups 1-4 received the DNPH via intraperitoneal route (3 mg/kg BW) dissolved in 0.9% NaCl solution. 24 hrs after injection of DNPH, the animals were sacrificed after ether anesthesia. Blood samples were taken at the carotid artery of each animal separately in tubes without anticoagulant (dry tubes). The serum was then separated by centrifugation at 2500 rpm for 10 mins before being used for determination of the biochemical parameters of hepatotoxicity and oxidative stress. Similarly, liver samples of the sacrificed animals were collected, rinsed with distilled water, weighed and kept in 10% formaldehyde (binding agent) for the histopathological study. The relative weight of the rats was determined by the following formula:

$$RLW\ (\%) = (LW/BW\ D8) \times 100$$

RLW: Relative liver weight
LW: Liver weight
BW D8: BW at the 8th day

Biochemical parameters of hepatotoxicity

Serum biochemical parameters of hepatotoxicity used in this study are of two types: biochemical substrates (albumin [ALB], total bilirubin [T-BIL] and direct bilirubin [D-BIL]) and enzymatic parameters (alanine aminotransferase [ALT], aspartate aminotransferase, [AST] and lactate dehydrogenase [LDH]. These hepatotoxicity markers were measured out with an automatic analyzer (Roche/Integra) using experimental kits (Cobas Integra) following the methods described by the manufacturers.

Antioxidant activity in vivo

Estimation of lipid peroxidation

The estimation of lipid peroxidation was made in accordance with the method of Satoh, 1978.[13] Lipid peroxidation, a major indicator of oxidative stress, was estimated by TBA reactive substances (TBARS) assay.

Thus, 2.5 mL of TCA 20% (m/v) was added to 0.5 mL of serum to precipitate serum proteins. After centrifugation at 3000 rpm for 10 mins, 2.5 mL of sulfuric acid (0.05 mol/L) and 2 mL of TBA 0.2% were added to the sediment. This mixture was then stirred and incubated afterwards in a boiling water bath for 30 mins. After adding 4 mL of n-butanol, the reaction mixture was centrifuged again at the same speed, and then cooled to room temperature. The supernatant was then collected, and absorbance was read in a spectrophotometer (Spectronic Genesys 5, USA) at 532 nm. The calibration curve was obtained using different concentrations of 1,1,3,3-tetramethoxypropane (1.9-30.5 µmol/L) as a standard to determine the concentration of TBA-malondialdéhydes (MDA) adducts in the sample.

Total antioxidant capacity (TAC) assay

The TAC assay was made using the method described by Benzie and Strain, 1996.[14] The serum TAC was determined by measuring its ability to reduce ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) by the ferric reduction antioxidant parameter (FRAP) method. This method enables to read at 593 nm, the change in absorbance of a blue compound (Fe (II)-tripyridyltriazine) resulting from the reducing action of antioxidants. The FRAP reagent was a mixture consisting 300 mmol/L acetate buffer (pH=3.6), 10 mmol/L TPTZ in 40 mmol/L HCl, and 20 mmol/L of $FeCl_3$, $6H_2O$ according to the ratio 10/1/1.

On that respect, 20 µL of serum was added to 300 µL of freshly prepared FRAP reagent and preheated at 37°C. After incubation of the reaction medium at 37°C for 10 mins, the absorbance of the blue complex was read in a spectrophotometer (Spectronic Genesys 5, USA) at 593 nm against a blank (300 µL FRAP reagent + 10 mL

distilled water). Standard Fe^{2+} solutions were prepared at concentrations ranging from 1.56 to 100 mmol/L from ferrous sulfate ($FeSO_4$, $7H_2O$) in distilled water. The results were expressed in µmol ferric ions reduced to the form of ferrous ion per liter (FRAP value).

DPPH radical scavenging activity

The antiradical activity of the serum was carried out according to the method of Yokozawa et al., 1998[15] with some modifications. It is a method that enables to measure the ability of the serum to inhibit the free radicals produced by the DPPH.

A volume of 200 µL of acetonitrile (60% in distilled water) was added to 200 µL of serum in order to deproteinize the samples. The mixture was then incubated for 2 mins at room temperature and then centrifuged at 4000 rpm for 10 mins. 200 µL of supernatant was then added to 200 µL of a methanolic DPPH solution (100 mmol/L), and the reaction mixture was supplemented with 1 mL of methanol and stirred vigorously. After incubation at room temperature for 10 mins, the absorbance was read in a spectrophotometer (Spectronic Genesys 5, USA) at 517 nm. The serum-free acetonitrile solutions were used as control.

The ability of the serum to inhibit the free radicals produced by the DPPH was calculated using the following formula:

DPPH inhibition (%) = ([absorbance of blank - absorbance of sample]/absorbance of blank) × 100

Where absorbance of blank is the absorbance of the serum-free DPPH solution and absorbance of the sample, the absorbance of the reaction mixture containing DPPH and deproteinized serum.

Statistical analysis

Data were processed using statistical SPSS package version 7.5 (SPSS Inc. Chicago IL). Analysis of variance was performed, and means were separated by Newman–Keuls range test at $p<0.05$. Data are expressed as mean±standard deviation, n=5.

RESULTS

Assessment of hepatoprotective activity

Effects of the AEHS and silymarin on the BW, the weight and the relative liver weight of rats after injection of DNPH

The results of this study are shown in Table 1. The analysis of this Table 1 shows that the action of DNPH has significantly affected the liver (target organ) of rats. Indeed, these results showed that the weight and the relative weight of the liver of the rats of Group 1 were statistically superior ($p<0.05$) to those of animals of the other groups (control 2-4). However, the treatments with AEHS and silymarin inhibit the action of DNPH. Thus, the weight and the relative weight of the rats in the control group and Groups 2-4 were statistically identical ($p<0.05$). On the other hand, the DNPH had no effect on the BW of animals. The BW of rats was statistically the same before and after injection of DNPH.

Effects of the AEHS and silymarin on biochemical substrates after injection of DNPH in rats

The results of this study are presented in Table 2. These results show that after injection of the DNPH, the rats of Group 1 were significantly different ($p<0.05$) from those of the other groups (control, 2-4) which were identical for all the analyzed parameters (ALB, T-BIL and D-BIL). The value of the ALB of rats in Group 1 was statistically inferior ($p<0.05$) to those of rats in the other groups (control 2-4) which values were of the same order of magnitude. Meanwhile, the values of T-BIL and D-BIL of the animals in Group 1 were statistically superior to those of animals in the other groups (control, 2-4) which showed values of the same importance. Our results show that the values of the three parameters studied in rats of Groups 2-4 were statistically identical ($p<0.05$) to those of the control group in each case. The AEHS and silymarin inhibited the toxicity of DNPH.

Effects of the AEHS and silymarin on enzymatic parameters after injection of DNPH in rats

The results of this study are presented in Table 3. After injection of DNPH, we noticed that the rats of Group 1 differ significantly ($p<0.05$) from those of the other groups (control, 2-4) regardless of the enzymatic parameter analyzed (ALT, AST, and LDH). Indeed, the values of the parameters studied in Group 1 were statistically superior, regardless the parameter analyzed, to those of rats in the other groups (Control, 2-4) which values were statistically identical ($p<0.05$).

Antioxidant activity in vivo

The results of this study are shown in Table 4. After injection of DNPH, regardless of the oxidative stress parameter studied (TBARS, FRAP, and DPPH), these results enable to assert that the rats of Group 1 were statistically different ($p<0.05$) from those of the other groups (control, 2-4). In the case of FRAP and DPPH tests, the value of Group 1 for each parameter was significantly lower ($p<0.05$) than the control group. However, concerning the values of TBARS, we noticed that the value of Group 1 was significantly superior ($p<0.05$) to that of the control group. The values of the parameters tested in rats of the control group and Groups 2-4 were statistically identical ($p<0.05$).

DISCUSSION

The injection of the DNPH caused a significant increase ($p<0.05$) of weight and relative liver weight of rats of Group 1

Table 1: Effects of the AHES and silymarin on body weight, the weight, and relative liver weight of rats after injection of DNPH.

Groups	Treatments	BW D7 (g)	BW D8 (g)	LW (g)	RLW (%)
Control group	0.5 mL of NaCl 0.9%	215.2±07.6^{a}	212.6±08.4^{a}	4.40±0.32^{b}	2.10±0.27^{b}
Group 1	0.5 mL of NaCl 0.9% + 3 mg/kg BW of DNPH	228.8±10.2^{a}	218.6±12.5^{a}	5.70±0.27^{a}	2.61±0.42^{a}
Group 2	25 mg/kg BW of silymarin + 3 mg/kg BW of DNPH	211.0±10.5^{ab}	206.2±11.8^{a}	4.46±0.35^{b}	2.16±0.54^{b}
Group 3	100 mg/kg BW of AEHS + 3 mg/kg BW of DNPH	226.9±09.3^{a}	219.4±10.6^{a}	4.62±0.19^{b}	2.10±0.55^{b}
Group 4	200 mg/kg BW of AEHS + 3 mg/kg BW of DNPH	217.3±08.1^{a}	213.2±09.2^{a}	4.50±0.43^{b}	2.11±0.36^{b}

The values of the parameters studied are expressed as mean±SD, n=5. In the same column values, studied parameter followed by the same letter are not significantly different ($p<0.05$). BW D7: Body weight 7th day, BW D8: Body weight 8th day, LW: Liver weight, RLW: Relative liver weight. AEHS: Aqueous extract of *Hibiscus sabdariffa*, SD: Standard deviation, *H. sabdariffa: Hibiscus sabdariffa*, DNPH: 2,4-dinitrophenylhydrazine

Table 2: Effects of the AEHS and silymarin on biochemical substrates after injection of DNPH in rats.

Groups	Treatments	ALB (g/L)	T-BILI (mg/L)	D-BILI (mg/L)
Control group	0.5 mL of NaCl 0.9%	41.30±2.87^{a}	4.18±0.50^{b}	1.09±0.17^{b}
Group 1	0.5 mL of NaCl 0.9% + 3 mg/kg BW of DNPH	26.84±3.23^{b}	13.27±1.63^{a}	3.70±0.40^{a}
Group 2	25 mg/kg BW of silymarin + 3 mg/kg BW of DNPH	39.10±3.42^{a}	5.35±0.69^{b}	1.24±0.30^{b}
Group 3	100 mg/kg BW of AEHS + 3 mg/kg BW of DNPH	37.52±2.34^{a}	5.60±0.48^{b}	1.32±0.15^{b}
Group 4	200 mg/kg BW of AEHS + 3 mg/kg BW of DNPH	40.02±2.15^{a}	4.54±0.44^{b}	1.16±0.18^{b}

The values of the parameters studied are expressed as mean±SD, n=5. In the same column values, studied parameter followed by the same letter are not significantly different ($p<0.05$). ALB: Albumin, T-BILI: Total bilirubin, D-BILI: Direct bilirubin. AEHS: Aqueous extract of *Hibiscus sabdariffa*, SD: Standard deviation, *H. sabdariffa: Hibiscus sabdariffa*, DNPH: 2,4-dinitrophenylhydrazine

Table 3: Effects of the AEHS and silymarin on enzymatic parameters after injection of DNPH in rats.

Groups	Treatments	ALT (UI/L)	AST (UI/L)	LDH (UI/L)
Control group	0.5 mL of NaCl 0.9%	42.16±2.92^{b}	55.04±4.77^{b}	107.40±9.14^{b}
Group 1	0.5 mL of NaCl 0.9% + 3 mg/kg BW of DNPH	130.48±8.65^{a}	96.38±7.23^{a}	184.80±8.80^{a}
Group 2	25 mg/kg BW of silymarin + 3 mg/kg BW of DNPH	50.28±4.58^{b}	63.22±6.05^{b}	118.32±5.84^{b}
Group 3	100 mg/kg BW of AEHS + 3 mg/kg BW of DNPH	52.76±5.89^{b}	65.92±5.66^{b}	124.74±7.17^{b}
Group 4	200 mg/kg BW of AEHS + 3 mg/kg BW of DNPH	45.02±3.25^{b}	59.40±3.45^{b}	115.20±8.37^{b}

The values of the parameters studied are expressed as mean±SD, n=5. In the same column values, studied parameter followed by the same letter are not significantly different ($p<0.05$). AEHS: Aqueous extract of *Hibiscus sabdariffa*, SD: Standard deviation, *H. sabdariffa: Hibiscus sabdariffa*, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, LDH: Lactate dehydrogenase, DNPH: 2,4-dinitrophenylhydrazine

Table 4: Effects of the AEHS and silymarin on oxidative stress parameters after injection of DNPH in rats.

Groups	Treatments	TBARS (µmol/L)	FRAP (µmol/L)	DPPH (%)
Control group	0.5 mL of NaCl 0.9%	0.97±0.09^{c}	140.88±4.35^{a}	93.37±5.31^{a}
Group 1	0.5 mL of NaCl 0.9% + 3 mg/kg BW of DNPH	5.02±0.33^{a}	80.40±6.83^{b}	19.72±2.98^{b}
Group 2	25 mg/kg BW of silymarin + 3 mg/kg BW of DNPH	1.33±0.15^{bc}	134.96±9.25^{a}	88.34±6.49^{a}
Group 3	100 mg/kg BW of AEHS + 3 mg/kg BW of DNPH	1.40±0.10^{bc}	129.51±5.43^{a}	83.60±5.02^{a}
Group 4	200 mg/kg BW of AEHS + 3 mg/kg de BW of DNPH	1.12±0.09^{c}	138.22±7.95^{a}	90.00±7.04^{a}

The values of the parameters studied are expressed as mean±SD, n=5. In the same column values, studied parameter followed by the same letter are not significantly different ($p<0.05$). AEHS: Aqueous extract of *Hibiscus sabdariffa*, SD: Standard deviation, *H. sabdariffa: Hibiscus sabdariffa*, TBARS: Thiobarbituric acid reactive substances, FRAP: Ferric reduction antioxidant parameter, DHHP: 2,2-Diphenyl-1-picrylhydrazyl, DNPH: 2,4-dinitrophenylhydrazine

relative to those of the rats of the other groups (control, 2-4). It also induces an increase in values of the T-BIL and D-BIL and decreases that of ALB. The enzymatic parameters (ALT, AST, and LDH) are also increased in each case in the rats of Group 1 compared to those of rats in the other groups (control, 2-4). These effects of DNPH are an indication that the action of DNPH has significantly affected the liver (target organ) sparing the rest of the body of rats during the time of observation.

The results of this study corroborate the works of several authors[16-18] who have shown that following the injection of a substance inducing hepatotoxicity like DNPH, an increase of liver weight of rats, as well as the relative liver weight of rats were observed. The increase in value of T-BIL and D-BIL[9,19] and decrease of ALB[12,20] are also associated to DNPH induced liver toxicity.[21] Hepatic cytolysis, loss of biochemical functions generate the inhibition of ALB production, expressed by a decrease in ALB. ALB, the major serum protein, plays two main roles, maintenance of the oncotic pressure and transport of various substances such as iron, fatty acids, calcium, hormones, and bilirubin. The latter cannot, therefore, be transported to the hepatocytes to undergo different transformations explaining the accumulation the T-BIL and D-BIL are high. The hepatic cytolysis is accompanied by the alteration of the cell membrane with the loss of the functions thereof. These results are in line with those obtained by several authors.[9,18,20] These results enable to assert once again the hepatotoxicity of DNPH[7,22] and highlight the important role of the liver in detoxification of toxic substances.[23,19]

Our results had shown that the treatments with the AEHS (Groups 3 and 4) and silymarin (Group 2) inhibited the toxicity of DNPH. Indeed the different doses of *H. sabdariffa* had attenuated the side effect of DNPH like reductions of relative liver weight, ALB, and inhibition of serum liver biomarkers (ALT, AST, and LDH). *H. sabdariffa* had also normalized the concentration of the T-BIL and D-BIL. This action of *H. sabdariffa* is used to assert the hepatoprotective property of AEHS as already shown some authors.[6-8] It also confirms that of silymarin (reference molecule) according to the works of several authors.[9,12,19,20]

The hepatoprotective properties of AEHS are probably due to the presence of major phytochemicals put in evidence by our previous work on the same extract.[3] These compounds are gossypetin, hibiscetin, quercetin, sabdaretin, delphinidin 3-O-sambubioside, and cyanidin 3-O-sambubioside shown in our previous work on the same samples.[3] Indeed, some authors have been reported the hepatoprotective property of *H. sabdariffa* extracts rich in anthocyanins.[6,7] Similarly, other authors have shown the hepatoprotective property of flavonoids including quercetin.[24-26]

The mechanism of DNPH-mediated tissue damage suggests an underlying process of oxidation. Therefore, the hypothesis on which this investigation was based, is that if the anthocyanin extract of dried flowers of *H. sabdariffa* possesses antioxi-dant properties, therefore, it would prevent lipid peroxidation and other metabolic side effects of DNPH caused by its oxidant action. Present results demonstrated reasonably well that treatment of rats with the AEHS prior to DNPH intoxication significantly inhibited its cytotoxic and other metabolic side effects in the liver. The injection of the DNPH caused a significant increase ($p<0.05$) of the TBARS value of rats in Group 1 relative to those of rats in other groups (control, 2-4). It induces a decrease of the value of FRAP and the percentage inhibition of DPPH of rats in Group 1 compared to those of rats in other groups (control, 2-4).

The results of lipid peroxidation test are in concordance with those obtained by some authors.[6-8] The high value of TABRS in Group 1, significantly different ($p<0.05$) from that of the control group, indicates lipid peroxidation of polyunsaturated membrane leading to cell necrosis with accumulation of MDA in the serum of rats.[6-8] The production of MDA in biological tissues is mainly due to free radicals attacks during oxidative stress. There is an alteration of the cell membrane which is the basis of the loss of biochemical and physiological functions of the cell that occurs in cell necrosis.[6,7,23] The results that show the TAC (FRAP test) of the AEHS corroborate those of Ajuwon et al., 2012.[27] They show a FRAP value in rats treated by DNPH significantly inferior ($p<0.05$) to that in rats from the control group. These results would mean that the injection of DNPH causes an oxidative stress with an excessive production of free radicals at the origin of the disequilibrium of the balance antioxidants/pro-oxidants in favor of the latter. They could also be explained by the fact that the injection of DNPH would lead to a failure of the antioxidant defense system through the inactivation of enzymes, biochemical substrates, and trace elements. The results of the measurement of inhibition of DPPH radicals show that the rats from Group 1 are significantly different ($p<0.05$) from the ones in the control group. These results probably reflect the fact that the injection of DNPH has brought about an oxidative stress responsible for the failure of the natural antioxidant defense system due to inactivation of enzymes, biochemical substrates, and trace elements. The results of these tests (TBARS, FRAP, and DPPH) clearly show that the DNPH-induced oxidative stress in the liver is well-correlated with the observed hepatotoxicity through the increase or decrease of the values of the various parameters studied.

Nevertheless, treatments of the aqueous extract to *H. sabdariffa* (Groups 3 and 4) and silymarin (Group 2), reference molecule have identical values statistically ($p<0.05$) than the control group regardless either test (TBARS or FRAP or DPPH), indicate the inhibitory effect of these on the oxidative stress induced by the DNPH. These results are in line with those of several authors.[6,7,28,29] They reflect the antioxidant properties of the extract of *H. sabdariffa*[6,7,8,22] and confirm that of silymarin in line with the conclusions of the works of some authors.[28,29] These in vivo antioxidant activities of AEHS are in accordance with our previous study[3] which had demonstrated the in vitro antioxidant activity of the aqueous extract of the petals of *H. sabdariffa* based on the presence of flavonoids and anthocyanins.

CONCLUSION

H. sabdariffa is a medicinal plant rich in phytochemical compounds responsible for its pharmacological properties. The juice of flowers of *H. sabdariffa* L., commonly known as Bissap is used in the preparation of local nonalcoholic cold beverage and as a hot drink. In Côte d'Ivoire, the production of a nonalcoholic drink called Bissap that is prepared from the red petals is popular. The in vivo hepatoprotective and antioxidant properties of this plant can consequently offer a liver protection to the population who consume it and prevent liver injuries.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Ethical Committee of Health Sciences, Félix Houphouët Boigny University of Abidjan

REFERENCES

1. Aboughe AS, Mathouet H, Souza A, Bivigoua F, Eyelé MMC, Lamidi M. Quelques plantes utilisées en médecine traditionnelle pour le traitement de la stérilité chez des femmes au Gabon. Ethnopharmacology. 2009;43:52-58.
2. Farnsworth NR. Biological and phytochemical screening of plants. J Pharm Sci. 1966;55(3):225-76.
3. Obouayeba AP, Djyh NB, Diabaté S, Djaman AJ, N'guessan JD, Koné M, et al. Phytochemical and antioxidant activity of Roselle (*Hibiscus sabdariffa* L.) petal extracts. Res J Pharm Biol Chem Sci. 2014;5:1453-65.
4. Okonkwo TJN. *Hibiscus sabdariffa* anthocyanins: a potential two-colour end-point indicator in acid-base and complexometric titrations. Int J Pharm Sci Rev Res. 2010;4:123-8.
5. Carvajal-Zarrabal O, Barradas-Dermitz DM, Orta-Flores Z, Hayward-Jones PM, Nolasco-Hipolito C, Aguilar-Uscanga MG, et al. *Hibiscus sabdariffa* L., roselle calyx, from ethnobotany to pharmacology. J Exp Pharmacol. 2012;4:25-39.
6. Wang CJ, Wang JM, Lin WL, Chu CY, Chou FP, Tseng TH. Protective effect of *Hibiscus* anthocyanins against tert-butyl hydroperoxide-induced hepatic toxicity in rats. Food Chem Toxicol. 2000;38(5):411-6.
7. Ologundudu A, Ologundudu AO, Ololade IA, Obi FO. The effect of *Hibiscus* anthocyanins on 2, 4-dinitrophenylhydrazine-induced hepatotoxicity in rabbits. Int J Phys Sci. 2009;4:233-7.
8. Ahmad-Raus R, Jamal P, Mohd-Isa ES. *Hibiscus sabdariffa* aqueous extracts prevents progression of acute liver injury induced by acetaminophen. Pertanika J Trop Agric Sci. 2012;35:511-20.
9. Bagban IM, Roy SP, Chaudhary A, Das SK, Gohil KJ, Bhandari KK. Hepatoprotective activity of the methanolic extract of *Fagonia indica* burm in carbon tetra chloride induced hepatotoxicity in albino rats. Asian Pacific J Trop Biomed. 2012;S1457-60.
10. Kumar SV, Sanjeev T, Ajay S, Kumar SP, Anil S. A review on hepatoprotective activity of medicinal plants. Int J Adv Res Pharm Bio Sci. 2012;2:31-8.
11. Pandey G. Medicinal plants against liver diseases. Int J Res Pharm. 2011;5:115-21.
12. Ahmed B, Alam T, Varshney M, Khan SA. Hepatoprotective activity of two plants belonging to the Apiaceae and the Euphorbiaceae family. J Ethnopharmacol. 2002;79(3):313-6.
13. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta. 1978;90(1):37-43.
14. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239(1):70-6.
15. Yokozawa T, Chen CP, Dong E, Tanaka T, Nonaka GI, Nishioka I. Study on the inhibitory effect of tannins and flavonoids against the 1,1-diphenyl-2 picrylhydrazyl radical. Biochem Pharmacol. 1998;56(2):213-22.
16. Uzma N, Kumar BS, Anees S. Red wine ameliorates CCl4-induced acute liver injury in rats. Aust J Biomed Sci. 2011;1:1-7.
17. Ezejindu DN, Okafor IA, Anibeze CIP, Ihentuge CJ. Histological effects of carotenoid on carbon tetrachloride induced hepatotoxicity in adult Wistar rats. Int J Eng Sci Invent. 2013;2:58-63.
18. Douhri B, Idaomar M, Senhaji NS, Ennabili A, Abrini J. Hepatoprotective effect of *Origanum* elongatum against carbon tetrachloride (CCl4) induced toxicity in rats. Eur J Med Plants. 2014;4:14-28.
19. Neharkar VS, Gaikwad KG. Hepatoprotective activity of *Cassia alata* (Linn.) leaves against paracetamol-induced hepatic injury in rats. Res J Pharm Biol Chem Sci. 2011;2:783-8.
20. Palanisamy D, Syamala, Kannan E, Bhojraj S. Protective and therapeutic effects of the Indian medicinal plant *Pterocarpus santalinus* on D-galactosamine-induced liver damage. Asian J Tradit Med. 2007;2:51-7.
21. Singh A, Bhat TK, Sharma OP. Clinical biochemistry of hepatotoxicity. J Clin Toxicol. 2011;S4:001.
22. Olusola AO, Olusola AO, Bada SO, Obi FO. Comparative study on the effect of *Hibiscus sabdariffa* calyx anthocyanins and ascorbate on 2,4-dinitrophenylhydrazine-induced damage in rabbits. Am J Biochem. 2012;2:1-6.
23. Samaresh PR, Devendra S, Tushar P, Shastry CS, Gheewala N, Goutam S, et al. Antioxidant and hepatoprotective activity of *Madhuca longifolia* (koenig) bark against CCl4-induced hepatic injury in rats: in vitro and in vivo studies. Res J Pharm Biol Chem Sci. 2010;1:1-10.
24. Gulati RK, Agarwal S, Agrawal SS. Hepatoprotective studies on *Phyllanthus emblica* Linn. and quercetin. Indian J Exp Biol. 1995;33(4):261-8.
25. Oh H, Kim DH, Cho JH, Kim YC. Hepatoprotective and free radical scavenging activities of phenolic petrosins and flavonoids isolated from *Equisetum arvense*. J Ethnopharmacol. 2004;95(2-3):421-4.
26. Abo-Salem OM, Abd-Ellah MF, Ghonaim MM. Hepatoprotective activity of quercetin against acrylonitrile-induced hepatotoxicity in rats. J Biochem Mol Toxicol. 2011;25(6):386-92.
27. Ajuwon OR, Katengua-Thamahane E, Van Rooyen J, Oguntibeju OO, Marnewick JL. Protective effects of Rooibos (*Aspalathus linearis*) and/or red palm oil (*Elaeis guineensis*) supplementation on tert-butyl hydroperoxide-induced oxidative hepatotoxicity in wistar rats. Evid Based Complement Alternat Med, 2013;2013:984273.
28. Subash KR, Ramesh KS, Charian BV, Britto F, Rao NJ, Vijaykumar S. Study of hepatoprotective activity of *Solanum nigrum* and *Cichorium intybus*. Int J Pharmacol. 2011;7:504-9.
29. Hurkadale PJ, Shelar PA, Palled SG, Mandavkar YD, Khedkar AS. Hepatoprotective activity of *Amorphophallus* paeoniifolius tubers against paracetamol-induced liver damage in rats. Asian Pac J Trop Biomed. 2012;2(Suppl 1):S238-42.

doi.: 10.5455/2319-2003.ijbcp20141034
Cite this article as: Obouayeba AP, Boyvin L, M'Boh GM, Diabaté S, Kouakou TH, Djaman AJ, N' Guessan JD. Hepatoprotective and antioxidant activities of *Hibiscus sabdariffa* petal extracts in Wistar rats. Int J Basic Clin Pharmacol 2014;3:774-80.