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Pharmacological properties of *Hibiscus sabdariffa* petal extract rich in polyphenols

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Abstract
This study was conducted to assess its pharmacological proprieties. The studies were performed using Wistar rats divided into groups. Treatments were administered via oral route and at single dose for seven days, followed by injection of a hepatotoxic substance (DNPH) or cardiotoxic substance (doxorubicin). Blood samples were collected for the carrying out of biochemical analyses of markers of heart, markers of liver and makers oxidative stress. Regarding anti-inflammatory activity, after administration of treatments via oral route, the volume of the legs of the animals was measured. The results helped to highlight the hepatoprotective, cardioprotective and antioxidant properties of *Hibiscus sabdariffa*. The assessment of anti-inflammatory activity has led to two main results. Induction of inflammation by carrageenan and anti-inflammatory property of *Hibiscus sabdariffa*. These results confirm and reinforce the position of *Hibiscus sabdariffa* as a medicinal plant with several potential therapeutic and protective for the heart and liver.

Keywords: *Hibiscus sabdariffa*, petals extract, pharmacological proprieties, wistar rat

1. 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 (WHO), more than 80 % of the world population uses traditional medicine to cope with health problems [11]. Among those medicinal plants is *Hibiscus sabdariffa* (Malvaceae). It is an annual herbaceous plant growing in Central and West Africa as well as South East Asia. *Hibiscus sabdariffa* is also used in traditional medicine for its antihypertensive, diuretic and laxative properties [8, 22]. 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 this plant. Indeed, *Hibiscus sabdariffa* contains several phytochemical compounds including organic acids, phenolic acids, anthocyanins, flavonoids, trace elements and vitamins [22, 6]. Our previous works [20] had revealed that the major compounds presents in the petals 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 petals are used in preparation of local non-alcoholic cold beverage and as a hot drink highly appreciated in Côte d'Ivoire. This non-alcoholic drink called « Bissap » prepared from the red petals is popular and highly appreciated by populations in most of the West African countries [20].

A number of works had shown that medicinal plants play a crucial role in the prevention of cardiovascular, liver, oxidative stress and inflammatory diseases [23, 28, 29]. In addition, the availability of *Hibiscus sabdariffa* is not a limiting factor and especially the food use of its petals is rooted in the habits of African populations [8]. In regard of the presence of different polyphenolics compounds of interest and the extensive consumption of the juice of the petals of this plant in various ceremonies in West Africa in general and in the Côte d'Ivoire in particular, the present study aims to assess the hepatoprotective, cardioprotective, antioxidant and anti-inflammatory activities of *Hibiscus sabdariffa* petal extracts enriched in polyphenols in Wistar rats [22].

2. Materials and methods

2.1. Drugs and chemicals

All reagents, solvents and chemical compounds used for analysis met the quality criteria in accordance with international standards. It were 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 trifluoroacetic acid (TFA), thiobarbituric acid (TBA), ferric chloride ($FeCl_3$, $6H_2O$), ferrous sulfate ($FeSO_4$, $7H_2O$), acetonitrile methanol (MeOH), 2, 4-dinitrophenylhydrazine (DNPH), carrageenan and indomethacin were obtained from Merck (Darmstadt, Germany). The silymarin originated from Madaus GmbH (Cologne, Germany). The doxorubicin originated from SC Sindan-Pharma (Bucharest, Romania).

2.3. Plant material

The petals of *Hibiscus 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.

2.4. 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 Ivograins, 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.

2.5. Extract preparation

The extract was prepared according to the method of Kouakou *et al*. [16]. One hundred grams (100 g) petals of *Hibiscus 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. Two hundred milliliters (200 mL) of distilled water were 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. One hundred milliliters (100 ml) of pure methanol were 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 petals extract of *H. sabdariffa* (PEHS) which polyphenols content and compounds were previously determined by Obouayeba *et al*. [6]. This extract which was used to carry out the different studies.

2.6. Assessment of cardioprotective activity

2.6.1. Experimental protocol

The assessment of cardioprotective activity of the petals extract of *Hibiscus sabdariffa* (PEHS) was carried out with 20 rats using the method described by Zanwar *et al*. [39]. The animals were divided into four groups of five rats as follows:

Control group: 0.5 mL of 0.9 % NaCl
Group 2: 0.5 mL of 0.9 % NaCl + 15 mg/kg of body weight (BW) of Doxorubicin
Group 3: 100 mg/kg of body weight of PEHS + 15 mg/kg of body weight of Doxorubicin
Group 4: 200 mg/kg of body weight of PEHS + 15 mg/kg of body weight of Doxorubicin

The rats of the control group and group 2 were treated with 0.5 mL of a solution of 0.9 % NaCl for 7 days via oral route. The rats of groups 3 and 4 were treated with the PEHS respectively at doses of 100 and 200 mg/kg BW for 7 days via oral route. The different treatments were made at single dose. The rats of the groups (1, 2 and 3) received 0.5 mL of doxorubicin (15 mg/kg BW) via intraperitoneal route, one hour (1 hr) after the last treatment. Twenty-four hours (24 hrs) after injection of doxorubicin, blood samples were taken at the carotid artery of each animal separately in tubes without anticoagulant (dry tubes). The serum was then centrifuged at 2500 rpm/min for 10 min before being used for determination of the biochemical parameters of cardiotoxicity. The animals were sacrificed after anesthesia with ether. The heart of the sacrificed animals was similarly collected, rinsed with distilled water, weighed and kept in 10 % formaldehyde (binding agent) for histopathology study.

The relative weight of heart of rats was determined by the following formula:

RHW = HW/BW D8

Where RHW: Relative heart weight; HW: Heart weight; BW D8: Body weight at the 8th day.

2.6.2. Biochemical parameters of heart

Serum Biochemical parameters of heart used in this study are essentially enzyme namely alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase MB (CK-MB) and lactate dehydrogenase (LDH). These parameters were measured using an automatic analyzer (Roche/INTEGRA) using experimental kits (COBAS INTEGRA) following the methods described by the manufacturers.

2.7. Assessment of hepatoprotective and antioxidant activities *in vivo*

2.7.1. Experimental protocol

The assessment of the hepatoprotective activity of the petals extract of *Hibiscus sabdariffa* (PEHS) 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*. [25] with some modifications as follows:

Control group: 0.5 mL of 0.9 % NaCl
Group 1: 0.5 mL of 0.9 % NaCl + 3 mg/kg body weight of DNPH
Group 2: 25 mg/kg of body weight of silymarin + 3 mg/kg of body weight of DNPH
Group 3: 100 mg/kg of body weight of PEHS + 3 mg/kg of body weight of DNPH
Group 4: 200 mg/kg of body weight of PEHS + 3 mg/kg of body weight 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 one week. The rats of group 2 were treated with silymarin (25 mg/kg body weight) dissolved in 0.9% NaCl for one week. The rats of groups 3 and 4 were treated with the PEHS at different concentrations (respectively 100 and 200 mg/kg body weight) dissolved in NaCl 0.9 % for one week. The different administrations were made via oral route at single dose. One hour (1 hr) after the last treatments, the rats of groups 1, 2, 3 and 4 received the DNPH via intraperitoneal route (3 mg/kg body weight) dissolved in 0.9 % NaCl solution. Twenty-four hours (24 hrs) after injection of DNPH, the animals were sacrificed after ether anaesthesia. 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 min 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) x 100
RLW: Relative liver weight
LW: Liver weight
BW D8: Body weight at the 8th day

2.7.2. Biochemical parameters of liver
Serum biochemical parameters of liver used in this study are of two types: biochemical substrates (albumin, total and direct bilirubin) and enzymatic parameters (alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH). These hepatotoxicity markers were measured out with an automatic analyser (Roche/INTEGRA) using experimental kits (COBAS INTEGRA) following the methods described by the manufacturers.

2.8. Antioxidant activity *in vivo*
The assessment of the antioxidant activity was coupled with that of the hepatoprotective activity.

2.8.1. Estimation of lipid peroxidation
The estimation of lipid peroxidation was made in accordance with the method of [33]. Lipid peroxidation, a major indicator of oxidative stress, was estimated by thiobarbituric acid Reactive Substances (TBARS) assay. Thus, 2.5 mL of trichloroacetic acid (TCA) 20 % (m/v) was added to 0.5 mL of serum to precipitate serum proteins. After centrifugation at 3000 rpm for 10 min, 2.5 mL of sulfuric acid (0.05 mol/L) and 2 mL of thiobarbituric acid (TBA) 0.2 % were added to the sediment. This mixture was then stirred and incubated afterwards in a boiling water bath for 30 min. 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 by using different concentrations of 1, 1, 3, 3-tetramethoxypropane (1.9-30.5 µmol/L) as a standard to determine the concentration of TBA-MDA adducts in the sample.

2.8.2. Total antioxidant capacity assay
The total antioxidant capacity (TAC) assay was made using the method described by [5]. The serum total antioxidant capacity was determined by measuring its ability to reduce ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) by the FRAP method (ferric reduction antioxidant parameter). 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 of 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 were 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 min, 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).

2.8.3. DPPH radical scavenging activity
The antiradical activity of the serum was carried out according to the method of Yokozawa *et al*. [38] 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 min at room temperature and then centrifuged at 4000 rpm for 10 min. 200 µL of supernatant were 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 min, 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] x 100
Where absorbance of blank is the absorbance of the serum-free DPPH solution and absorbance of sample, the absorbance of the reaction mixture containing DPPH and deproteinized serum.

2.9. Assessment of anti-inflammatory activity
2.9.1. Experimental protocol
The assessment of anti-inflammatory activity of petals extract of *Hibiscus sabdariffa* (PEHS) induction of paw edema by carrageenin rat was performed according to the method described by Winter *et al*. [37] with 20 rats. The animals were divided into four groups of five rats as follows:
Control group: 0.5 mL of 0.9 % NaCl + 50 µL of carrageenan 0.1 %
Group 2: 10 mg/kg of body weight of indomethacin + 50 µL of carrageenan 0.1 %
Group 3: 100 mg/kg of body weight of PEHS + 50 µL of carrageenan 0.1 %
Group 4: 200 mg/kg of body weight of PEHS + 50 µL of carrageenan 0.1 %
The rats of the control group were treated with 0.5 mL of a solution of 0.9 % NaCl. The rats of group 2 were treated with indomethacin 10 mg/kg of BW dissolved in NaCl 0.9 %. The rats of groups 2 and 3 were treated with the PEHS (respectively at doses of 100 and 200 mg/kg BW dissolved in NaCl 0.9 %). The different treatments were carried out via oral route at single dose. One hour (1 hr) after treatment administration, inflammation was induced by injecting under the plantar fascia of the left hind paw of rats 50 µL of a solution of carrageenan 0.1 % in 0.9 % NaCl. One, two, three, and six hours after injection of carrageenan the animals paw diameter was measured using calipers. The increase of the inflamed paw (left hind paw: PPG), which received carrageenan relative to the diameter of healthy paw (right hind paw: PPD), was determined using the formula:
A = PPG – PPD

Where A: edema diameter

2.9.2. Determination of the percentage inhibition of inflammation

The percentage inhibition of inflammation was determined by comparing the mean increase of the animals of groups 2, 3 and 4 (A_1) with to those of animals of the control group (A_0) according to with the formula proposed by Garcia *et al*. [14] in accordance with subsequent work.

Inhibition (%) = $[(A_0 – A_1)/A_0] \times 100$

2.10. Statistical analysis

Data were processed using Statistica software package version 7.1 (StatSoft Inc., Tulsa, USA). Analysis of variance (One-way ANOVA) was performed and means were separated by Newman-Keuls range test at $p<0.05$. Data are expressed as mean ± standard deviation (SD), n = 5.

3. Results

3.1. Cardioprotective activity

3.1.1. Effect of petals extract of Hibiscus sabdariffa on body weight, weight and relative heart weight in rats after injection of doxorubicin

The results of this study are presented in table 1. The analysis of this table shows that the action of doxorubicin significantly affected the heart (target organ) rats. Indeed, these results showed that the weight and the relative heart weights of rats of group 2 are statistically superior ($p<0.05$) to those of animals of the other groups (control, 3 and 4). However, treatment with the PEHS inhibits the action of doxorubicin. Thus, the weight and the relative weight of the rats in the control group and groups 3 and 4 were statistically identical ($p<0.05$). Doxorubicin had no effect on body weight of animals. The body weight of rats was statistically the same before and after the injection of doxorubicin.

Table 1: Effect of petals extract of *Hibiscus sabdariffa* on body weight and heart weight in rats after injection of doxorubicin.

Groups	Treatements	BW D7 (g)	BW D8 (g)	HW (mg)	RHW
Control group	0.5 mL of NaCl 0.9 %	200.6 ± 8.5 ^{ab}	197.5 ± 09.2 ^{a}	756 ± 17 ^{b}	3.82 ± 0.27 ^{b}
Group 2	0.5 mL of NaCl 0.9 % + 15 mg/kg BW of Doxorubicin	220.6 ± 9.2 ^{a}	208.0 ± 12.5 ^{a}	910 ± 16 ^{a}	4.37 ± 0.42 ^{a}
Group 3	100 mg/kg of BW of PEHS + 15 mg/kg of BW of Doxorubicin	206.7 ± 9.8 ^{a}	199.8 ± 11.2 ^{a}	778 ± 14 ^{b}	3.89 ± 0.54 ^{b}
Group 4	200 mg/kg of BW of PEHS + 15 mg/kg of BW of Doxorubicin	210.4 ± 7.2 ^{a}	206.1 ± 08.6 ^{a}	764 ± 15 ^{b}	3.71 ± 0.35 ^{b}

The values of the parameters studied are expressed as mean ± SD, n = 5. In the same column the means followed by the same letter are not significantly different ($p<0.05$).

BW: Body weight; BW D7: Body weight 7th day; BW D8: body weight 8th day; HW: Heart weight; RHW: relative heart weight. PEHS: Petals extract of *Hibiscus sabdariffa*;

SD: standard deviation.

3.1.2. Effect petals extract of Hibiscus sabdariffa on enzymatic parameters after injection of doxorubicin in rats

The results of effect of the PEHS on enzymatic parameters after injection of doxorubicin in rats are presented in table 2. After injection of doxorubicin, it is observed that the rats of group 2 differ significantly ($p<0.05$) from those of the other groups (control, 3 and 4) regardless either the enzyme parameter analyzed (CK-MB, LDH, ALT and AST). Indeed, the concentrations of these various parameters of rats in group 2 were statistically superior ($p<0.05$) to those of rats in the other groups (control, 3 and 4). These results also show the inhibitory action of the PEHS (groups 3 and 4) on the toxicity induced by doxorubicin through the concentrations of the parameters studied of animals in groups 3 and 4 which were statistically identical ($p<0.05$) to control group and.

Table 2: Effect of petals extract of *Hibiscus sabdariffa* on enzymatic parameters after injection of doxorubicin in rats.

Groups	Treatements	CK-MB	LDH	ALT	AST
		(UI/L)	(UI/L)	(UI/L)	(UI/L)
Control group	0.5 mL of NaCl 0.9 %	150.22	103.4	40	60.22
		± 10.50 ^{c}	± 12.41 ^{c}	± 2.77 ^{b}	± 4.32 ^{b}
Group 2	0.5 mL of NaCl 0.9 % + 15 mg/kg of BW of Doxorubicin	484.4	241	72.22	190.04
		± 12.73 ^{a}	± 15.54 ^{a}	± 5.49 ^{a}	± 9.56 ^{a}
Group 3	100 mg/kg of BW of PEHS + 15 mg/kg of BW of Doxorubicin	258.28	137.6	45.5	68.9
		± 11.04 ^{bc}	± 14 ^{bc}	± 3.81 ^{b}	± 3.31 ^{b}
Group 4	200 mg/kg of BW of PEHS + 15 mg/kg of BW of Doxorubicin	162.3	114.5	42.94	63.48
		± 12.98 ^{c}	± 9.30 ^{c}	± 5.39 ^{b}	± 4.06 ^{b}

The values of the parameters studied are expressed as mean ± SD, n = 5. In the same column, the means followed by the same letter are not significantly different ($p<0.05$). CK-MB: Creatine Kinase MB; LDH: Lactate dehydrogenase; ALT: alanine aminotransferase; AST: Aspartate aminotransferase. PEHS: Petals extract of *Hibiscus sabdariffa*; BW: Body weight; SD: standard deviation.

3.2. Assessment of hepatoprotective activity

3.2.1. Effects of petals extract of Hibiscus sabdariffa and silymarin on the body weight, the weight and the relative liver weight of rats after injection of DNPH

The results of this study are shown in table 3. The analysis of this table 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 2 were statistically superior ($p<0.05$) to those of animals of the other groups (control, 3, 4 and 5). However, the treatments with PEHS and silymarin inhibit the action of DNPH. Thus, the weight and the relative weight of the rats in the control group and groups 3, 4 and 5 were statistically identical ($p<0.05$). On the other hand, the DNPH had no effect on the body weight of animals. The body weight of rats was statistically the same before and after injection of DNPH.

Table 3: Effects of petals extract of *Hibiscus sabdariffa* and silymarin on body weight, the weight and relative liver weight of rats after injection of DNPH.

Groups	Treatements	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 2	0.5 mL of NaCl 0.9 % + 3 mg/kg of BW of DNPH	228.8 ± 10.2^{a}	218.6 ± 12.5^{a}	5.70 ± 0.27^{a}	2.61 ± 0.42^{a}
Group 3	25 mg/kg of BW of silymarin + 3 mg/kg of BW of DNPH	211.0 ± 10,5^{ab}	206.2 ± 11.8^{a}	4.46 ± 0.35^{b}	2.16 ± 0.54^{b}
Group 4	100 mg/kg of BW of PEHS + 3 mg/kg of BW of DNPH	226.9 ± 09,3^{a}	219.4 ± 10.6^{a}	4.62 ± 0.19^{b}	2.10 ± 0.55^{b}
Group 5	200 mg/kg of BW of PEHS + 3 mg/kg of 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 seventh day, BW D8: Body weight eighth day, LW: Liver weight, RLW: Relative liver weight. PEHS: Petals extract of *Hibiscus sabdariffa*, DNPH: 2, 4-dinitrophenylhydrazine. BW: Body weight; SD: Standard deviation; Silymarin: reference molecule of hepatoprotective.

3.2.2. Effects of petals extract of Hibiscus sabdariffa and silymarin on biochemical substrates after injection of DNPH in rats

The results of this study are presented in table 4. These results show that after injection of the DNPH, the rats of group 2 were significantly different ($p<0.05$) from those of the other groups (control, 3, 4 and 5) which were identical for all the analyzed parameters (ALB, T. BIL and D. BIL). The value of the albumin of rats in group 2 was statistically inferior ($p<0.05$) to those of rats in the other groups (control, 3, 4 and 5) which values were of the same order of magnitude. Meanwhile, the values of total and direct bilirubin of the animals in group 2 were statistically superior to those of animals in the other groups (control, 3, 4 and 5) which showed values of same importance. Our results show that the values of the three parameters studied in rats of groups 3, 4 and 5 were statistically identical ($p<0.05$) to those of the control group in each case. The PEHS and silymarin inhibited the toxicity of DNPH.

Table 4: Effects of petals extract of *Hibiscus sabdariffa* and silymarin on biochemical substrates after injection of DNPH in rats.

Groups	Treatements	ALB	T BILI	D BILI
		(g/L)	(mg/L)	(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 2	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 3	25 mg/kg of BW of silymarin + 3 mg/kg of BW of DNPH	39.10 ± 3.42^{a}	5.35 ± 0.69^{b}	1.24 ± 0.30^{b}
Group 4	100 mg/kg of BW of PEHS + 3 mg/kg of BW of DNPH	37.52 ± 2.34^{a}	5.60 ± 0.48^{b}	1.32 ± 0.15^{b}
Group 5	200 mg/kg of BW of PEHS + 3 mg/kg of 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; PEHS: Petals extract of *Hibiscus sabdariffa*; DNPH: 2, 4-dinitrophenylhydrazine; BW: Body weight; SD: standard deviation; Silymarin: reference molecule of hepatoprotective.

Table 5: Effects of petals extract of *Hibiscus sabdariffa* and silymarin on enzymatic parameters after injection of DNPH in rats.

Groups	Treatements	ALT	AST	LDH
		(UI/L)	(UI/L)	(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 2	0.5 mL of NaCl 0.9 % + 3 mg/kg of BW of DNPH	130.48 ± 8.65^{a}	96.38 ± 7.23^{a}	184.80 ± 8.80^{a}
Group 3	25 mg/kg of BW of silymarin + 3 mg/kg of BW of DNPH	50.28 ± 4.58^{b}	63.22 ± 6.05^{b}	118.32 ± 5.84^{b}
Group 4	100 mg/kg of BW of PEHS + 3 mg/kg of BW of DNPH	52.76 ± 5.89^{b}	65.92 ± 5.66^{b}	124.74 ± 7.17^{b}
Group 5	200 mg/kg of BW of PEHS + 3 mg/kg of 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 ($p<0.05$). LDH: Lactate dehydrogenase; ALT: alanine aminotransferase; AST: Aspartate aminotransferase. PEHS: Petals extract of *Hibiscus sabdariffa*; DNPH: 2, 4-dinitrophenylhydrazine; BW: Body weight; SD: standard deviation; Silymarin: reference molecule of hepatoprotective.

Table 6: Effects of petals extract of *Hibiscus sabdariffa* and silymarin on oxidative stress parameters after injection of DNPH in rats.

Groups	Treatements	TBARS	FRAP	DPPH
		(µmol/L)	(µmol/L)	(%)
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 2	0.5 mL of NaCl 0.9 % + 3 mg/kg of BW of DNPH	5.02 ± 0.33^{a}	80.40 ± 6.83^{b}	19.72 ± 2.98^{b}
Group 3	25 mg/kg of BW of silymarin + 3 mg/kg of BW of DNPH	1.33 ± 0.15^{bc}	134.96 ± 9.25^{a}	88.34 ± 6.49^{a}
Group 4	100 mg/kg of BW of PEHS + 3 mg/kg of BW of DNPH	1.40 ± 0.10^{bc}	129.51 ± 5.43^{a}	83.60 ± 5.02^{a}
Group 5	200 mg/kg of BW of PEHS + 3 mg/kg of 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 ($p<0.05$). PEHS: Petals extract of *Hibiscus sabdariffa*; TBARS: Thiobarbituric acid reactive substances; FRAP: Ferric reduction antioxidant parameter; DHHP: 2, 2-Diphenyl-1-picrylhydrazyl; DNPH: 2, 4-dinitrophenylhydrazine; BW: Body weight; SD: standard deviation; Silymarin: reference molecule of hepatoprotective.

Table 7: Effects of petals extract of *Hibiscus sabdariffa* and indomethacin after injection of carrageenan in rats.

Groups	Treatements	Mean diameters of paws (mm)			
		1 h	2 h	3 h	6 h
Control group	0.5 mL of NaCl 0.9 %	0.82 ± 0.04 [a]	1.07 ± 0.06 [a]	1.60 ± 0.05 [a]	1.80 ± 0.07 [a]
Group 2	10 mg/kg BW of Indomethacin + Carrageenan	0.52 ± 0,02 [b]	0.59 ± 0.03 [b]	0.70 ± 0.03 [bc]	0.40 ± 0.04 [c]
		(36.58)	(44.86)	(56.25)	(77.77)
Group 3	100 mg/kg BW of PEHS + Carrageenan	0.60 ± 0.05 [b]	0.68 ± 0.04 [b]	0.82 ± 0.05 [b]	0.55 ± 0.03 [b]
		(26.82)	(36.45)	(48.75)	(69.44)
Group 4	200 mg/kg BW of PEHS + Carrageenan	0.50 ± 0.03 [b]	0.57 ± 0.02 [b]	0.65 ± 0.03 [c]	0.38 ± 0.04 [c]
		(39)	(46.73)	(59.37)	(78.89)

Values are expressed as mean volumes ± SD, n = 5. In the same column, the mean volumes followed the same letter are not significantly different ($p<0.05$). Values in parenthesis are the percentage of inhibition of rat paw edema. PEHS: Petals extract of *Hibiscus sabdariffa*; BW: Body weight; SD: standard deviation. Indomethacin: reference substance of anti-inflammatory.

3.2.3. Effects of petals extract of Hibiscus sabdariffa and silymarin on enzymatic parameters after injection of DNPH in rats

The results of this study are presented in table 5. After injection of DNPH, we noticed that the rats of group 2 differ significantly ($p<0.05$) from those of the other groups (control, 3, 4 and 5) regardless of the enzymatic parameter analyzed (ALT, AST and LDH). Indeed, the values of the parameters studied in group 2 were statistically superior, regardless the parameter analyzed, to those of rats in the other groups (control, 3, 4 and 5) which values were statistically identical ($p<0.05$).

3.3. Antioxidant activity *in vivo*

The results of this study are shown in table 6. 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 2 were statistically different ($p<0.05$) from those of the other groups (control, 3, 4 and 5). In the case of FRAP and DPPH tests, the value of group 2 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 2 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 3, 4 and 5 were statistically identical ($p<0.05$).

3.4. Anti-inflammatory activity

The results of this study are shown in table 7. After injection of carrageenan in the plantar aponeurosis of the left hind paw of the animals, it is found that the different groups of rats were significantly different ($p<0.05$) relative to the appearance of their paws. Edemas of variable diameter appear indeed to their paws on processing. The paws of the rats in the control group were statistically larger than ($p<0.05$) those of the other groups (3, 4 and 5) regardless of the observation time. Regarding the appearance of the legs of rats in groups 3, 4 and 5, there are three scenarios related to the observation time in line with the percentages of inhibition of rat paw edema. Until two hours (2 hrs) after the injection of carrageenan, all percentages of edema inhibition are less than 50 irrespective of the treatment. Three hours (3 hrs) after injection of carrageenan, the percentage inhibition of edema for each treatment with the exception of PEHS 100 mg/kg BW is higher than 50. Six hours (6 hrs) after injection of the carrageenan, all percentages of inhibition were above 50 whatever the treatment.

4. Discussion

The results of the cardioprotective activity of PEHS show that injection of doxorubicin induced a significant increase ($p<0.05$) of the weight and the relative weight of the heart of rats in group 2 compared to those of the rats in the other groups (control, 2, 3 and 4). Doxorubicin also induces an increase in the concentration of enzymes (CK-MB, LDH, ALT and AST) in each case in the rats of group 2 compared to those of rats in the other groups (control, 3 and 4). These effects are an indication that doxorubicin significantly affected the heart cells (target organ). These results are in concordance with those obtained by the works of Koti *et al*. [15]. These authors have shown that doxorubicin increases heart weight and relative heart weight of rats. Our results are also identical to those of various authors [18, 30, 39] who attributed the increase in the concentration of enzymes to cardiac toxicity. They had pointed out that the myocardial necrosis which usually accompanied the alteration of the heart cell membrane associated with the loss of function of the latter. Enzymatic proteins present in heart cells are found in the serum, where their high concentration statistically different ($p<0.05$) from that of the control group for each parameter studied. Our results therefore confirm the cardiotoxicity of doxorubicin as already reported by the works of Naiyra *et al*. [18] and Firoz *et al*. [12]. Furthermore, our results showed that treatment with the PEHS inhibits the toxicity of doxorubicin. Indeed, there is no significant difference ($p<0.05$) for the weight and the relative weight of the heart, the concentration of enzymes in rats treated groups and control group. This inhibitory action confirms the cardioprotective property of PEHS in accordance with the works of Olatunji *et al*. [24] and Ojeda *et al*. [23]. The cardioprotective property of PEHS is probably related to the presence of its major phytochemical compounds including flavonoids (gossypetin, hibiscetin, quercetin and sabdaretin) and anthocyanins (delphinidin 3-O-sambubioside and cyanidin 3-O-sambubioside). Thus, the works of different authors have demonstrated the cardioprotective property of phenolic compounds in particular anthocyanins and flavonoids [35, 40].

These findings corroborate the works of several authors [9, 10, 36]. In their studies, these authors showed an increase in liver weight in rats, as well as the relative weight of the liver of rats after injection of hepatotoxicity inducing substances. The results of this study highlight the hepatotoxicity of DNPH in accordance with the findings of several other authors [9, 25]. Our results provide supplementary proof for the *in vivo* hepatoprotective potencies of *Hibiscus sabdariffa*. Indeed, Wang *et al*. [36] had respectively pointed out the protective action of *H. sabdariffa* during the toxic effect induced by tert-butyl hydroxyperoxyde in liver. The same goes for silymarin in accordance with the works of

Palanisamy *et al*. [27]. These results highlight the important role of the liver in detoxification of toxic substances as revealed by the works of several authors [19, 31]. The results of this study corroborate those of several authors who attributed the increase in value of the total and direct bilirubin [19] and the decrease of albumin [27] to liver toxicity. Generally, during hepatic cytolysis, there is a loss of biochemical functions and consequently the reduction of albumin production, expressed by the decrease in albumin. Major serum protein, albumin plays two main roles, the maintenance of oncotic pressure and transport of various substances such as iron, fatty acids, calcium, hormones and bilirubin. The latter cannot therefore be transported towards the hepatocytes so as to undergo the different transformations. It accumulates in the serum which is why the values of the total and direct bilirubin are high. These elements enable to confirm the hepatotoxicity of DNPH according to the works of Ologundudu *et al*. [25] and Olusola *et al*. [26]. The results of this study also show the hepatoprotective property of PEHS demonstrated in our work and previously by Wang *et al*. [36] and Olusola *et al*. [26]. These results also confirm the hepatoprotective effects of silymarin (reference molecule) [19, 27]. These results are in line with those obtained by several authors [9, 27]. As these authors have shown that high values of enzymatic parameters (ALT, AST and LDH) expressed hepatotoxicity, our results illustrate a phenomenon of hepatotoxicity. The hepatic cytolysis is accompanied by the alteration of the cell membrane with the loss of functions. Enzymatic proteins are found in the serum, from which the high value in group 2, statistically different ($p<0.05$) from that of the control group for each enzymatic parameter. These elements enable to assert once again the hepatotoxicity of DNPH in accordance with the works of Ologundudu *et al*. [25] and Olusola *et al*. [26]. However, the results of this study also show the hepatoprotective property of PEHS [25] and confirm the one of silymarin [19, 27] through the values of enzymatic parameters of the control group and groups 3, 4 and 5 which are statistically identical ($p<0.05$). The hepatoprotective property of PEHS due to the presence of phytochemical compounds including phenolic acids, flavonoids, anthocyanins and vitamins as highlighted by several authors [6]. The same goes to silymarin, reference hepatoprotective substance [17].

The results of the lipid peroxidation test follow those obtained by the authors [25, 36]. The high value of TABRS in group 2, significantly different ($p<0.05$) from that of the control group, indicate a peroxidation of polyunsaturated membrane lipids leading to cell necrosis with accumulation of malondialdehydes (MDA) in the serum of rats [25, 36]. The formation of malondialdehydes 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 ends in cell necrosis [36]. These elements highlight the hepatotoxicity of DNPH as already shown by the works of Ologundudu *et al*. [25]. The same goes with hepatotoxic substances such as carbon tetrachloride [34], tert-butyl hydroperoxide [36]. However, the treatments with PEHS in animals (groups 4 and 5) and silymarin in animals (group 3), reference molecule have values statistically identical ($p<0.05$) to the control group. These results are in line with those of several authors [25, 35]. They reflect the antioxidant property of PEHS [25, 36] and confirmation of that of silymarin [34]. The results of the measurement of inhibition of DPPH radicals show that the rats from group 2 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 defence system due to inactivation of enzymes, biochemical substrates and trace elements. They could also be explained by the fact that the injection of DNPH leads to oxidative stress with an excessive production of free radicals which is the basis of disequilibrium of the balance antioxidants/pro oxidants in favour of the latter [21]. These results as well as the previous ones express the hepatotoxic action of DNPH in rats of group 2 as already mentioned by Ologundudu *et al*. [25]. The treatments of rats with the PEHS (groups 4 and 5) and silymarin (group 3) provide values statistically identical DPPH ($p <0.05$) to the ones in rats of the control group. These results are in accordance with conclusions of various authors [25, 36] enable to indicate that the PEHS has antioxidant properties and confirm those of silymarin [34]. The results that show the total antioxidant capacity (FRAP test) of the PEHS corroborate those of Ajuwon *et al*. [3]. They show a FRAP value in rats of group 2, 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 favour of the latter. They could also be explained by the fact that the injection of DNPH would lead to a failure of the antioxidant defence system through the inactivation of enzymes, biochemical substrates and trace elements. These results once again express a hepatotoxicity reaction in rats due to DNPH thus confirming the works of Ologundudu *et al*. [25] and Olusola *et al*. [26]. Nevertheless, the treatments with the PEHS in rats (groups 4 and 5) and silymarin in rats (group 3), show values statistically identical ($p<0.05$) to those in rats of the control group. These results highlight the antioxidant and hepatoprotective property of PEHS [34] and confirm that of silymarin in line with the conclusions of the works of Subash *et al*. [34]. These *in vivo* antioxidant activities are in accordance with our previous study [20] which had demonstrated the *in vitro* antioxidant activity of PEHS. Moreover, the same work had revealed the presence of potent antioxidant flavonoids (gossypetin, hibiscetin, quercetin and sabdaretin) and anthocyanins (delphinidin 3-O-sambubioside and cyanidin 3-O-sambubioside).

The results of the anti-inflammatory activity of PEHS show that the injection of the carrageenan induced a significant increase ($p<0.05$) of the paw diameter of rats in control group compared to those of rats in groups treated irrespective of observation time. These results are in line with Adedapo *et al*. [2] and Paschapur *et al*. [7] who showed the edema formation after injection of carrageenan. The occurrence of edema results in the inflammation induced by carrageenan, indicating that this substance is an inflammatory substance. Our results show that the PEHS maintained unchanged the paw diameter of rats, compared to the control group rats. The PEHS inhibits the inflammatory activity of carrageenan in accordance with the works of Christian *et al*. [7] and Arunachalam *et al*. [4]. This anti-inflammatory property of PEHS is certainly due to phenolic compounds especially flavonoids (gossypetin, hibiscetin, quercetin and sabdaretin) whose presence in this

plant has been demonstrated in our previous works one the same extract. Indeed, flavonoids inhibit key enzymes of the inflammatory reaction (cyclo-oxygenase, lipo-oxygenase and nitric oxide synthase), which gives them their anti-inflammatory properties [32]. This action of flavonoids prevents normal development of the inflammatory process that should lead to apoptosis if the body becomes unable to defend itself [13]. The PEHS has a similar effect to that of indomethacin (reference substance of anti-inflammatory) on carrageenan inflammatory effects regardless of the reaction time observation.

5. Conclusion

Hibiscus sabdariffa is medicinal and food plant rich in polyphenolic compounds of interest responsible for its pharmacological properties. This study which aimed to evaluate cardioprotective, hepatoprotective, antioxidant and anti-inflammatory activities of petals extract of *Hibiscus sabdariffa* in Wistar rats revealed three important elements. The cardioprotective, hepatoprotective, antioxidant and anti-inflammatory properties of petals extract of *Hibiscus sabdariffa* supported by its polyphenolic compounds. The confirmation of indomethacin as the anti-inflammatory reference molecule and the silymarin as the hepatoprotective reference molecule. This study allows us to say that in Côte d'Ivoire the local non-alcoholic drink commonly known as « Bissap », obtained from the red petals help to protect the heart, the liver, and the reduction of inflammation and oxidative stress in population who consume it.

6. References

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