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## Cardioprotective and anti-inflammatory activities of a polyphenols enriched extract of *Hibiscus sabdariffa* petal extracts in wistar rats

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**Abstract**

*Hibiscus sabdariffa* is a medicinal and food plant rich in phytochemical compounds which are the source of its biological properties. This study was conducted to assess its cardioprotective and anti-inflammatory properties. The study of the cardioprotective activity was made with 20 Wistar rats divided into four groups. The treatments were carried out via oral route and at single dose for one week. After injection of doxorubicin, blood samples were collected for the dosage of biochemical markers of cardiotoxicity (alanine aminotransferase, aspartate aminotransferase, creatine kinase MB and lactate dehydrogenase). The anti-inflammatory activity was also conducted with 20 Wistar rats divided into four groups. After administration of treatments via oral route, the volume of the legs of the animals was measured. The results had shown that the cardiotoxicity of doxorubicin observed in rats of group 2 was significantly different ( $P < 0.05$ ) from the other groups (control, 3 and 4) treated with the plant extract as shown by the biochemical markers of cardiotoxicity. The markers were statistically identical ( $P < 0.05$ ) for groups 3, 4 and control. Induction of inflammation by carrageenan and anti-inflammatory property of *Hibiscus sabdariffa* with the paws of the rats in control group statistically larger than ( $P < 0.05$ ) those of the animals of other groups (2, 3 and 4) regardless of the observation time. These results confirm and reinforce the position of *Hibiscus sabdariffa* as a medicinal and food plant with several therapeutic potential.

**Keywords:** Hibiscus sabdariffa, aqueous extract, cardioprotective activity, anti-inflammatory activity, 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 [13]. 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 [10]. 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 [2, 8]. Our previous works [25] had revealed that the major compounds presents 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.

The petals of *Hibiscus sabdariffa* rich in flavonoids ( $03.50 \pm 0.85$  mg/g) and anthocyanins ( $16.53 \pm 1.10$  mg/g) and containing several bioactive phenolics (gossypetin, hibiscetin, quercetin, sabdaretin, delphinidin 3-O-sambubioside, and cyanidin 3-O-sambubioside) [25] deserve that some pharmacological properties including cardioprotective and anti-inflammatory activities be assessed. Moreover, our previous work had pointed out the hepatoprotective and *in vivo* antioxidant properties of the aqueous extract obtained with the petals of *Hibiscus sabdariffa* [26]. 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.

A number of works had shown that medicinal plants play a crucial role in the prevention of cardiovascular and inflammatory diseases [28, 29, 27]. 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 [10]. 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 cardioprotective and anti-inflammatory activities of *Hibiscus sabdariffa* petal extracts enriched in polyphenols in Wistar rats.

## 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. The trifluoroacetic acid (TFA), methanol (MeOH), carrageenan and indomethacin were obtained from Merck (Darmstadt, Germany). The doxorubicin originated from SC Sindan-Pharma (Bucharest, Romania).

### 2.2 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.

### 2.3 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.

### 2.4 Extract preparation

The extract was prepared according to the method of Kouakou *et al.* (2009) [22]. 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 aqueous extract of *H. sabdariffa* (AEHS) which polyphenols content and compounds were previously determined by (Obouayeba *et al.*, 2014a) [25].

### 2.5 Acute toxicity

The acute toxicity study of aqueous extract of *Hibiscus sabdariffa* (EAHS) was made in accordance with the method described by Bidié *et al.* (2011) [7]. Thirty (30) rats with 15 females and 15 males were divided into five groups of six rats in equal numbers by sex. Four groups of rats were treated with the extract of *Hibiscus sabdariffa* at different doses (250, 500, 1000 and 2000 mg/kg body weight). The rats of group 1 were treated with 0.9% NaCl solution that was used to prepare the concentrations of the aqueous extract of *Hibiscus sabdariffa* with corresponding doses. The Different administrations were made via oral route at single dose. The animals were observed for 24 hrs and then six days to note any pathological disorders and deaths.

### 2.6 Assessment of cardioprotective activity

#### 2.6.1 Experimental protocol

The assessment of cardioprotective activity of the aqueous extract of *Hibiscus sabdariffa* (AEHS) was carried out with 20 rats using the method described by Zanwar *et al.* (2011) [35]. 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 body weight (BW) of Doxorubicin

Group 3: 100 mg/kg body weight of AEHS + 15 mg/kg body weight of Doxorubicin

Group 4: 200 mg/kg body weight of AEHS + 15 mg/kg 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 aqueous extract of *Hibiscus sabdariffa* 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 8<sup>th</sup> day.

#### 2.6.2 Biochemical parameters of cardiotoxicity

Serum Biochemical parameters of cardiotoxicity 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 anti-inflammatory activity

### 2.7.1 Experimental protocol

The assessment of anti-inflammatory activity of the aqueous extract of *Hibiscus sabdariffa* (AEHS) induction of paw edema by carrageenin rat was performed according to the method described by Winter *et al.* (1962) [34] 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  $\mu$ L of carrageenan 0.1%

Group 2: 10 mg/kg body weight of indomethacin + 50  $\mu$ L of carrageenan 0.1%

Group 3: 100 mg/kg body weight of AEHS + 50  $\mu$ L of carrageenan 0.1%

Group 4: 200 mg/kg body weight of AEHS + 50  $\mu$ L 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 BW dissolved in NaCl 0.9%. The rats of groups 2 and 3 were treated with the aqueous extract of *Hibiscus sabdariffa* (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  $\mu$ L 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:

$$A = \text{PPG} - \text{PPD}$$

Where A: edema diameter

### 2.7.2 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.*, (2004) [16].

$$\text{Inhibition (\%)} = [(A_0 - A_1)/A_0] \times 100$$

## 2.8 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  $\pm$  standard deviation (SD),  $n = 5$ .

## 3. Results

### 3.1 Acute toxicity

The administration of doses ranging from 250 to 2000 mg/kg BW showed no clinical signs of intoxication and did not cause any death in rats for 24 hours of observation and even after a week after treatment. Table 1 shows the percentage mortality of the rats depending on the dose of the aqueous extract of *Hibiscus sabdariffa*. The results were used to estimate the maximum tolerated dose (MTD) of this extract at 2000 mg/kg BW.

**Table 1:** Mortality of male rats (M) and female rats (F) as a function on the dose of the aqueous and *Hibiscus sabdariffa* extract time.

| Treatments            | Group 1    |     | Group 2      |     | Group 3      |     | Group 4       |     | Group 5       |     |
|-----------------------|------------|-----|--------------|-----|--------------|-----|---------------|-----|---------------|-----|
|                       | 0 mg/kg BW |     | 250 mg/kg BW |     | 500 mg/kg BW |     | 1000 mg/kg BW |     | 2000 mg/kg BW |     |
| Number of animals     | M 3        | F 3 | M 3          | F 3 | M 3          | F 3 | M 3           | F 3 | M 3           | F 3 |
| Time after treatments |            |     |              |     |              |     |               |     |               |     |
| 15 min                | 0          | 0   | 0            | 0   | 0            | 0   | 0             | 0   | 0             | 0   |
| 30 min                | 0          | 0   | 0            | 0   | 0            | 0   | 0             | 0   | 0             | 0   |
| 1 h                   | 0          | 0   | 0            | 0   | 0            | 0   | 0             | 0   | 0             | 0   |
| 4 h                   | 0          | 0   | 0            | 0   | 0            | 0   | 0             | 0   | 0             | 0   |
| 8 h                   | 0          | 0   | 0            | 0   | 0            | 0   | 0             | 0   | 0             | 0   |
| 12 h                  | 0          | 0   | 0            | 0   | 0            | 0   | 0             | 0   | 0             | 0   |
| 16 h                  | 0          | 0   | 0            | 0   | 0            | 0   | 0             | 0   | 0             | 0   |
| 20 h                  | 0          | 0   | 0            | 0   | 0            | 0   | 0             | 0   | 0             | 0   |
| 24 h                  | 0          | 0   | 0            | 0   | 0            | 0   | 0             | 0   | 0             | 0   |
| 6 days                | 0          | 0   | 0            | 0   | 0            | 0   | 0             | 0   | 0             | 0   |
| Mortality (%)         | 0          |     | 0            |     | 0            |     | 0             |     | 0             |     |

## 3.2 Cardioprotective activity

### 3.2.1 Effect of aqueous 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 2. 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 aqueous extract of *Hibiscus sabdariffa* 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 2:** Effect of the aqueous extract of *Hibiscus sabdariffa* on body weight and heart weight in rats after injection of doxorubicin.

| Groups        | Treatments  | BW D7 (g)                 | BW D8 (g)                 | HW (mg)               | RHW                      |
|---------------|---|---------------------------|---------------------------|-----------------------|--------------------------|
| Control group | 0,5 mL of NaCl 0,9 %                              | 200.6 ± 8.5 <sup>ab</sup> | 197.5 ± 09.2 <sup>a</sup> | 756 ± 17 <sup>b</sup> | 3.82 ± 0.27 <sup>b</sup> |
| Group 2       | 0,5 mL of NaCl 0,9 % + 15 mg/kg BW of Doxorubicin | 220.6 ± 9.2 <sup>a</sup>  | 208.0 ± 12.5 <sup>a</sup> | 910 ± 16 <sup>a</sup> | 4.37 ± 0.42 <sup>a</sup> |
| Group 3       | 100 mg/kg BW of AEHS + 15 mg/kg BW of Doxorubicin | 206.7 ± 9.8 <sup>a</sup>  | 199.8 ± 11.2 <sup>a</sup> | 778 ± 14 <sup>b</sup> | 3.89 ± 0.54 <sup>b</sup> |
| Group 4       | 200 mg/kg BW of AEHS + 15 mg/kg BW of Doxorubicin | 210.4 ± 7.2 <sup>a</sup>  | 206.1 ± 08.6 <sup>a</sup> | 764 ± 15 <sup>b</sup> | 3.71 ± 0.35 <sup>b</sup> |

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 7<sup>th</sup> day; BW D8: body weight 8<sup>th</sup> day; HW: Heart weight; RHW: relative heart weight.

EAHS: Aqueous extract of *Hibiscus sabdariffa*; SD: standard deviation.

### 3.2.2 Effect aqueous extract of *Hibiscus sabdariffa* on enzymatic parameters after injection of doxorubicin in rats

The results of effect of the aqueous extract of *Hibiscus sabdariffa* on enzymatic parameters after injection of doxorubicin in rats are presented in table 3. 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 aqueous extract of *Hibiscus sabdariffa* (groups 3 and 4) on the toxicity induced by doxorubicin through the concentrations of the parameters studied (CK-MB, LDH, ALT and AST) of animals in groups 3 and 4 which were statistically identical (P<0.05) to control group and.

**Table 3:** Effect of the aqueous extract of *Hibiscus sabdariffa* on enzymatic parameters after injection of doxorubicin in rats.

| Groups        | Treatments   | CK-MB                 | LDH                  | ALAT                | ASAT                |
|---------------|--|-----------------------|----------------------|---------------------|---------------------|
|               |  | (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 <sup>c</sup>  | ± 12.41 <sup>c</sup> | ± 2.77 <sup>b</sup> | ± 4.32 <sup>b</sup> |
| Group 2       | 0,5 mL of NaCl 0,9 % + 15 mg/kg BW of Doxorubicin  | 484.4                 | 241                  | 72.22               | 190.04              |
|               |  | ± 12.73 <sup>a</sup>  | ± 15.54 <sup>a</sup> | ± 5.49 <sup>a</sup> | ± 9.56 <sup>a</sup> |
| Group 3       | 100 mg/kg BW of AEHS + 15 mg/kg BW of Doxorubicine | 258.28                | 137.6                | 45.5                | 68.9                |
|               |  | ± 11.04 <sup>bc</sup> | ± 14 <sup>bc</sup>   | ± 3.81 <sup>b</sup> | ± 3.31 <sup>b</sup> |
| Group 4       | 200 mg/kg BW of AEHS + 15 mg/kg BW of Doxorubicin  | 162.3                 | 114.5                | 42.94               | 63.48               |
|               |  | ± 12.98 <sup>c</sup>  | ± 9.30 <sup>c</sup>  | ± 5.39 <sup>b</sup> | ± 4.06 <sup>b</sup> |

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; ALAT: alanine aminotransferase; ASAT: Aspartate aminotransferase.

EAHS: Aqueous extract of *Hibiscus sabdariffa*; BW: Body weight; SD: standard deviation.

### 3.3 Anti-inflammatory activity

The results of this study are shown in table 4. 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 (2, 3 and 4) regardless of the observation time. Regarding the appearance of the legs of rats in groups 2, 3 and 4, 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 AEHS 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.

**Table 4:** Effects of the aqueous extract of *Hibiscus sabdariffa* and indomethacin after injection of carrageenan in rats.

| Groups        | Treatments                                | 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 <sup>a</sup>    | 1.07 ± 0.06 <sup>a</sup> | 1.60 ± 0.05 <sup>a</sup>  | 1.80 ± 0.07 <sup>a</sup> |
| Group 2       | 10 mg/kg BW of Indomethacin + Carrageenan | 0.52 ± 0,02 <sup>b</sup>    | 0.59 ± 0.03 <sup>b</sup> | 0.70 ± 0.03 <sup>bc</sup> | 0.40 ± 0.04 <sup>c</sup> |
|               |   | (36.58)                     | (44.86)                  | (56.25)                   | (77.77)                  |
| Group 3       | 100 mg/kg BW of AEHS + Carrageenan        | 0.60 ± 0.05 <sup>b</sup>    | 0.68 ± 0.04 <sup>b</sup> | 0.82 ± 0.05 <sup>b</sup>  | 0.55 ± 0.03 <sup>b</sup> |
|               |   | (26.82)                     | (36.45)                  | (48.75)                   | (69.44)                  |
| Group 4       | 200 mg/kg BW of AEHS + Carrageenan        | 0.50 ± 0.03 <sup>b</sup>    | 0.57 ± 0.02 <sup>b</sup> | 0.65 ± 0.03 <sup>c</sup>  | 0.38 ± 0.04 <sup>c</sup> |
|               |   | (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.

AEHS: Aqueous extract of *Hibiscus sabdariffa*; BW: Body weight; SD: standard deviation

## 4. Discussion

The results of acute toxicity of aqueous extract of *Hibiscus*

*sabdariffa* show any death at the dose of 2000 mg/kg BW in Wistar rats indicating no toxicity at this dose for the extract.

These results corroborate with those Sireeratawong *et al.* (2013) [32] who have identified a maximal tolerated dose (MTD) of 5000 mg/kg BW for the aqueous extract of *Hibiscus sabdariffa* Wistar rats via oral route. Furthermore, the works of Jeroh *et al.* (2012) [18] revealed the non-toxicity of the aqueous extract of *Hibiscus sabdariffa* in the kidneys of Wistar rats. Our results confirm that *Hibiscus sabdariffa* is a medicinal plant having no toxicity. The MTD of extract of *Hibiscus sabdariffa* (2000 mg/kg BW) compared to extracts of other plants of the Ivorian pharmacopoeia obtained via oral route in Swiss mice as the total aqueous extract of *Mansonia altissima* (150 mg/kg BW) (Djyh *et al.*, 2010) [12], the methanol extract of *Mitragyna ciliata* (500 mg/kg BW) (Bidié *et al.*, 2010) [6], the methanol extract of *Chrysophyllum perpulchrum* (500 mg/kg BW) (Bidié *et al.*, 2011) [7] indicates the very low toxicity of this plant. Indeed, the MTD of *H. sabdariffa* extract is 13 times less than the total aqueous extract of *Mansonia altissima* and four times less than the methanol extracts of *Mitragyna ciliata* and *Chrysophyllum perpulchrum*. Our results are a guarantee of security for the high use of this plant by the African people not only in traditional medicine, but also for the production of non-alcoholic drink « Bissap » and the production of pulp and sauce as a dish [10].

The results of the cardioprotective activity of aqueous extract of *H. sabdariffa* 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.* (2009) [21] and Fathiazad *et al.* (2001) [14]. 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 (Zanwar *et al.*, 2011; Andreadou *et al.*, 2007; Radhika *et al.*, 2013; Meenakshi *et al.*, 2014) [35, 4, 30, 23] who attributed the increase in the concentration of enzymes (CK-MB, LDH, ALT and AST) 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 (ALT, AST, CK-MB and LDH) 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 Andreadou *et al.* (2007) [4], Naiyra *et al.* (2010) [24] and Firoz *et al.* (2011) [15]. Furthermore, our results showed that treatment with the aqueous extract of *H. sabdariffa* 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 (CK-MB, LDH, ALT and AST) in rats treated groups and control group. This inhibitory action confirms the cardioprotective property of the aqueous extract *Hibiscus sabdariffa* in accordance with the works of Jonadet *et al.* (1990) [19], Olatunji *et al.* (2005) [28] and Ojeda *et al.* (2010) [27]. The cardioprotective property of the aqueous extract of *H. sabdariffa* 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 [20, 36, 33]. The results of the anti-inflammatory activity of the aqueous extract of *H. sabdariffa* 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.* (2008) [3] and Paschapur *et al.* (2009) [29] 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 aqueous extract of *Hibiscus sabdariffa* maintained unchanged the paw diameter of rats, compared to the control group rats. The aqueous extract of *Hibiscus sabdariffa* inhibits the inflammatory activity of carrageenan in accordance with the works of Dafallah and Al-Mustafa (1996) [11], Christian *et al.* (2006) [9] and Arunachalam *et al.* (2009) [5]. This anti-inflammatory property of aqueous extract of *Hibiscus sabdariffa* 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 [31]. This action of flavonoids prevents normal development of the inflammatory process that should lead to apoptosis if the body becomes unable to defend itself [17]. The aqueous extract of *Hibiscus sabdariffa* 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 a medicinal and food plant rich in polyphenolics compounds of interest responsible for its pharmacological properties. This study which aimed to evaluate cardioprotective and anti-inflammatory activities of the aqueous extract of *Hibiscus sabdariffa* in Wistar rats revealed three important elements. The demonstration of the cardiotoxicity of doxorubicin and the inflammatory action of carrageenan. The cardioprotective and anti-inflammatory properties of the aqueous extract of *Hibiscus sabdariffa* supported by its polyphenolics compounds. The confirmation of indomethacin as the anti-inflammatory 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 aqueous extract of *H. sabdariffa* red petals help to protect the heart, and the reduction of inflammation in population who consume it.

## 6. References

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