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Research Article

PROTECTIVE EFFECT OF SENNA TORA, BOSCIASENEGALENSIS, HIBISCUS SABDARIFFA, DICHROSTACHYS GLOMERATA, ADANSONIA DIGITATA AND BALANITES AEGYPTIACA EXTRACTS AGAINST CCL_4 INDUCED OXIDATIVE STRESS IN RATS

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ABSTRACT

Objective: The aim of this study was to investigate the ability of extracts of *Senna tora*, *Bosciasenegalensis, Hibiscus sabdariffa, Dichrostachysglomerata*, *Adansoniadigitata* and *Balanitesaegyptiaca* to protect and improve the alterations causedbyCCL_4 in rats.
Material and methods: Aqueous and ethanolic extracts of these different plants were analyzed for total phenolic, total flavonoid content and *in vitro* antioxidant activity (DPPH and ABTSradical scavenging activity, total antioxidant capacity). For *in vivo* antioxidant activity, fourteen adult Wistar albino rats weighing 200-250 g were used divided into eight groups with each group constituted of 5 rats. For induction of oxidative stress, CCl4 was mixed with oliveoil by a 1% ratio, then was directly injected intraperitoneally (1 mL/kg/body weight). The Positive control group was fed with astandard diet during the experiment, negative control group was fed with standard diet for seven days and received a single dose of CCl4 solution intraperitoneally(1mL/kg), the rest of the groups were fed with a standard diet and were given the ethanolic extracts of each of the plants orallyata dose of250mg/kg for seven days and received CCl4(1 mL/kg) solution intraperitoneally on the eighth day. At the end ofthe experiment, lipid peroxidation, catalase and superoxide dismutase were evaluated.
Results: Rats which consumedethanolic extracts of the plants expressed the highest content of total phenols and flavonoids and lowest IC_{50} for DPPH and ABTS. Globally rats pretreated with ethanolic extracts of the plants presented asignificant reduction ($p<0.05$) of MDA levels compared to those of the negative control group. Pretreatment of rats with extracts of plants improvedenzymatic activities of catalase and superoxide dismutase. Fruits of *D. glomerata* exhibited the most significant positive effect.
Conclusion: The results of the present study showed that ethanolic extract of these different plants have antioxidant and scavenging activities as they ameliorated the effects produced by CCl4 in the experimental rats. Therefore, they could be used for the management of diseases associated with oxidative stress like cardiovascular diseases.

INTRODUCTION

Free radicals are molecules derived from various biological processes. Excessive production of Free radicals affects the equilibrium between pro-oxidants and antioxidants in biological systems, leading to modifications in genomes, proteins, carbohydrates, lipids and lipid peroxidation[1]. This damage generated by reactive species is combated by antioxidant defenses; enzymatic and non-enzymatic. Enzymatic antioxidants include superoxide dismutase (SOD) and catalase (CAT), while α-tocopherol (vitamin E), β-carotene (pro-vitamin A) and ascorbic acid (vitamin C) are some of the non-enzymatic antioxidants [2]. In normal situations, the human body produces antioxidants or procures them from the diet, either ways, they are capable of reducing the concentration of reactive species [3]. The imbalance between the production of reactive species and amount of antioxidants characterizes oxidative stress [4]. Damage caused by oxidative stress has been associated with the development of stroke, arteriosclerosis, cancer and cardiovascular diseases [5].Plant and plant products are rich sources of phytochemicals and have been found to possess a variety of biological activities including antioxidant potential [6].

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Fruits of *Dichrostachysglomerata* are commonly used as spices in a traditional soup in Cameroon [7].Kuate *et al*. [8]Studies showed that *D. glomerata* fruits exhibit *in vitro* and *in vivo* antioxidant activity. *Adansoniadigitata* is an African plant known as baobab tree, the African population use the baobab fruit to prepare decoctions, sauces and refreshing drink[9]. The pulp is a good source of ascorbic acid (2, 8-3g/kg MS) which is responsible for itsantioxidant activity[10].*Hibiscus sabdariffa* is an edible plant for which previous studies on alcoholic and aqueous extracts from its calyx reported anti-inflammatory, antioxidant, hypolipidemicactivities [11].The seed extracts of *Bosciasenegalensis* are rich in saponins and polyphenols, it has been shown that they have anti-inflammatory, anti-hyperglycemic, and antioxidant properties [12].The leaves of *Senna tora* are highly consumed in the north region of Cameroon. Methanolic extract of these leaves showed that they possess antioxidant activities [13]. *Balanitesaegyptiaca* also known as "desert date", is as pinytreeofupto l0 min height, distributedin Africaand South Asia. The fruits of this plant are known to containawidevariety of compounds, which showawiderange of biological and pharmacological properties such as antioxidant, anti-inflammatory, antimicrobial and cytotoxicactivities[14]. Many plants have many biological potentials but their protective effects are less studied.

The aim of this study was to investigate the ability of extracts of *Senna tora*, *Bosciasenegalensis, Hibiscus sabdariffa, Dichrostachysglomerata*, *Adansoniadigitata* and *Balanitesaegyptiaca* to protect and improve the alterations due toCCL_4in rats.

MATERIAL AND METHODS

Materials

Fruits of *Dichrostachysglomerata*, *Adansoniadigitata, Balanitesaegyptiaca, calyx of Hibiscus sabdariffa,* fresh leaves of *Senna tora and* seek of *Bosciasenegalensis* is were bought at small markets in Ngaoundere-Cameroon and transported to the Food Biophysics, Biochemistry and Nutrition Laboratory, of the National Advanced School of Agro-Industrial Sciences (ENSAI) of the University of Ngaoundere. Pulp of the fruits, calyx, and leaves were dried at 50°C, ground into fine powder using a hammer mill (culattiPolymix France) and sieved through a 500 µm sieve. The powder obtained was stored in sealed polyethylene sachets and stored at 4°C for further analyses.

Preparation of ethanolic and aqueous extracts

About 100 g of the powder was weighed into 1000mL of ethanol (98 %) for ethanolic extracts and 100 g of the powder into 1000mL and the mixture was stirred for 24 hours. The ethanol and aqueous soluble residue were filtered off and concentrated under a vacuum at room temperature using a rotary evaporator to yield extract according to conventional procedure. The obtained product after evaporation was frozen in a freezer and then lyophilized.

Determination of Phytochemical composition and In vitro antioxidant activity

Extraction of total phenolic compounds

Extraction of polyphenol powders was carried out using the method of Kim *et al*.[15] with some modifications. 2 g of each powder was macerated during 24 hours through stirring in 20 mL of methanol/Water 70/30 (v/v). The methanol/water extract was then centrifuged at 3500 rpm for 20 min. The supernatant layer was filtered, brought to 15 mL and stored at 4°C before analysis.

Total phenolic content

Total phenolic content was determined according to the method described by Wafa *et al*.[16]. 0.02 mL of samples were added to test tubes containing 2.98 mL of distilled water followed by an addition of 0.5 mL of folin-ciocalteu reagent solution (1/10) and 0.4 mL of sodium carbonate (Na_2CO_3, 20%). The sample was thoroughly mixed and vortexed. After 20 min of incubation at room temperature, absorbance was measured at 760 nm against a blank on a UV-visible spectrophotometer. Gallic acid (0 .2g/mL) were used for calibration and total phenolic content was expressed as gram of gallic acid equivalents per100 gram of dry weight (g GAE /100gDW).

Total flavonoid content

The determination of total flavonoid content was conducted as previously described by Dewanto *et al*.[17]. 0.1 mL of each extract were mixed with 2.4 mL of distilled water and 0.15 mL of 5% sodium nitrite (Na_2NO_2) solution. Subsesquently, the tubes were held at 25 °C for 6 min and 0.3 mL of 10 % aluminium chloride ($AlCl_3$,$6H_2O$) was added in each test tube and maintained at 25° C for 5 min. Lastly, 1 mL 1M of sodium hydroxide (NaOH) was added and vigorously mixed. Samples were immediately measured (510 nm) against a blank on a UV-visible spectrophotometer. Total flavonoid content was calculated as gram of rutin equivalent per 100 gram of dry weight (g RE/100g DW) against a standard curve of rutin (0.1g/L).

In vitro antioxidant activity

DPPH radical scavenging activity assay

The DPPH radical scavenging activity was evaluated according to the method described by (Zhang and Hamauzu [18].2mL of 0.025 g/L DPPH solution in methanol/water (70/30) was mixed with 0.5 mL plant extract. The reaction mixture was incubated in the dark for 1hour and its optical density was recorded at 517nm against the blank. For the control, 2 mL DPPH methanol/water solution was mixed with 0.5 mL of methanol/water (70/30). The DPPH radical scavenging activity was expressed in terms of IC_{50} values that refers to the smallest concentration of antioxidants required to scavenge 50% of the DPPH radical.

$$\text{Inhibition rate (\%)} = \frac{\text{Control absorbance} - \text{Extract absorbance}}{\text{Control absorbance}}$$

Total antioxidant capacity

Total antioxidant capacity was determined using a phosphomolybdenummethod.[20].0.3 mL of sample extract was mixed with 3 mL of reactive solution (0.6 M sulfuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate). Tubes were incubatedat 95°C for 90 Min. After cooling, absorbance was measured at 695 nm against the blank which contained 3 mL of reactive solution and 0.3 mL of methanol or water incubated in the same conditions as sample. Total antioxidant capacity was expressed in milligram equivalent ascorbic acid by gram of dry matter (mg Eq AA/ g DW).

Evaluation of in vivo antioxidant

Animals and Experimental design

Fourteenadult Wistar albino rats weighing about 200-250 g were used in the present study. The rats were obtained from the animal house of the National Advanced School of Agro-Industrial Sciences (ENSAI). They were kept at room temperature (25 ± 2°C). With a relative humidity of 44-56%, light and dark cycles of 12 and 12 h. The animals were acclimatized for one week before the beginning of the experiment and were fed with standard animal feed and water *ad* libitium. For induction of oxidative stress, CCl4 was mixed with olive oil by a 1% ratio, then directly injected intraperitoneally(1 ml/kg body weight). Animals were divided into eight groups of five rats each.

Groupe 1 (CP): positive control group was fed with standard diet during experiment

Groupe 2(CN): negative control group was fed with standard diet for seven days and received CCl4 solution intraperitoneally(1 mL/kg)

Groupe 3: was fed with standard diet and received ethanolic extract of *S.tora* orally at a dose of 250mg/kg for seven days and received CCl4(1 mL/kg)solution intraperitoneallyon the eighth day

Groupe 4: was fedwith standard diet and received ethanolic extract of *B.senegalensisorally*at a doseof250mg/kg for seven days and receivedasingle dose of CCl4 (1 mL/kg)solution intraperitoneallyon the eighth day

Groupe 5: was fedwith standard diet and received ethanolic extract of *H. sabdariffa*orallyat a dose of 250mg/kg for seven days and received a single dose of CCl4 (1 mL/kg)solution intraperitoneallyon the eighth day

Groupe 6: was fed with standard diet and received ethanolic extract of *D.glomerata*orallyat a dose of250mg/kg for seven days and received a single dose of CCl4 (1 mL/kg)solution intraperitoneallyon the eighth day

Groupe 7: was fedwith standard diet and received ethanolic extract of *A.digitataorally* at a dose of 250mg/kg for seven days and receivedasingle dose of CCl4 (1 mL/kg) solution intraperitoneallyon the eighth day

Groupe 8: was fed with standard diet and received ethanolic extract of *B.aegyptiacaorally* at a dose of 250mg/kg for seven days and receivedasingle dose of CCl4(1 mL/kg)solution intraperitoneally on the eighth day, after 48 hours all groups of rats were sacrificed.

Sample collection and biochemical assays

At the end of the experiment, rats were fasted for 14 h and the blood samples were collected, lung, heart, testis, liver and kidney were removed.

Tissue preparation and determination of invivo antioxidant activity

Livers and kidneys removed were rinsed with NaCl (0.9%) solution. Tissues were minced and homogenized (10% w/v) in ice-cold potassium phosphate buffer (0.1 M, pH 7.4). The homogenate was centrifuged at 3000g for 10 min at 4^0C; the resultant supernatant was used for the determination of antioxidant activity.

Measurement of lipid peroxidation

Lipid peroxidation was evaluated with Yagi method's [21].This method depends on the formation of malondialdehyde (MDA) as an end product of lipid peroxidation which reacts with thiobarbituric acid producing thiobarbituric acid reactive substance (TBARS). The amount of MDA was then measured by reaction with thiobarbituric acid at 532 nm using spectrophotometer. The values were calculated using the molar extinction coefficient of chromophore (1, 56×10^5 M^{-1} cm^{-1}).

Catalase activity

Catalase activity was assayed by the method of Sinha [22]which is based on the formation of chromic acetate from dichromate and glacial acetic acid in the presence of hydrogen peroxide. Chromic acetate produced was measured using a spectrophotometer at 620 nm, one enzyme unit was defined as the amount of enzyme which catalyzed the oxidation of 1 µmole H2O2 per minute under assay conditions. The activity was expressed in terms of units per milligram of protein.

Superoxide dismutase activity

The superoxide dismutase (SOD) activity was determined by the spectrophotometric method based on the inhibition of adrenaline oxidation to adrenochrome [23]. Briefly, 0.2 mL of sample was diluted in 3 mL of carbonate buffer, pH 10.2 and placed into quartz spectrophotometer cuvette. The reaction was started by adding 0.3mL of adrenalin 0,3 mM solution in 10 mM HCl. Adrenaline oxidation lead to the formation of the colored product, adrenochrome, which was detected by the spectrophotometer. In abasic pH the adrenalin was spontaneously oxidized, with the kinetics recorded by measuring the increase of absorbance at 480 nm over time. The kinetics of adrenalin oxidation in the presence of the sample was compared with the oxidation rate of adrenalin alone. A unit of SOD is defined as the amount of enzyme that inhibits the rate of adrenaline oxidation by 50%. The result was expressed in µUnit per milligram of protein. Protein was determined using the method of Lowry *et al*. [24].

Statistical Analysis

Each experiment and measurement was performed in triplicate. The results were presented as mean ± Standard deviation. One-way analysis of variance ANOVA was carried out (level of significance α=0.05) followed by multiple comparisons of DUNCAN using the Statgraphic package (Statgraphic Plus for windows, version 15.1.0.2 Manuggisticinc, USA).

RESULT AND DISCUSSION

Phenolic compounds

Total phenolic and flavonoid content of extracts of different samples are presented in Table 1. Results obtained showed that total polyphenol contentwasglobally higher in ethanolic extract compared to aqueous extract. Values are between 12.040 g GAE /g DW for ethanolic extract of *D.glomerata* and 0.589 g GAE /g DW for ethanolic extract of *B.aegyptiaca*. The contrary was observed with *A.digitata* whose total polyphenol aqueous extract (2.625 g GAE /g DW) was higher comparedto those in ethanolic extract (2.428 g GAE /g DW). Concerning flavonoids, same tendency was noted, ethanolic extract of *D.glomerata* presented the highest value 6.300 g RE / g DW and *B.senegalensis* the highest value of flavonoids (6.463 g RE / g DW) content in aqueous extract. With these first results

with respect to the concentration of polyphenols we conclude that polyphenols are concentrated in ethanolic extract.

Table 1 Phenolic compounds of ethanolic and aqueousextract of plants

Samples	Extract	Total Phenolics Contents (mgEGA/gDW)	Flavonoids contents (mgRE/gDW)
Adansoniadigitata	Ethanolic	2.43 ± 0.02[a]	1.02 ±0.04[a]
	Aqueous	2.63 ± 0.02[b]	1.44 ± 0.05[b]
Balanites aegyptiaca	Ethanolic	0.59 ± 0.02[b]	0.22 ± 0.05[a]
	Aqueous	0.45 ± 0.03[a]	0.31 ± 0.07[b]
Boscia senegalensis	Ethanolic	2.54 ± 0.01[b]	6.50 ± 0.08[b]
	Aqueous	2.07± 0.01[a]	6.04 ± 0.07[a]
Dichrostachysglomerata	Ethanolic	12.04 ± 0.01[b]	6.30 ± 0.38[b]
	Aqueous	10.69 ±0.08 [a]	5.79 ± 0.02[a]
Hibuscussabdariffa	Ethanolic	5.34 ± 0.01[b]	3.16 ±0.48[b]
Senna tora	Ethanolic	4.13 ± 0.12[b]	2.50 ± 0.20[b]
	Aqueous	2.07 ± 0.07[a]	1.57 ± 0.14[a]

EGA/gDW: gallic acid equivalent / gram of dry weight, RE/ DW: rutin equivalent / gram of dry weight. The data where mean ± standard deviation of triplicate (3). Means in the samecolumn for each plant followed by different letters were statisticaly different (p<0.05).

In vitro antioxidant activity

The antioxidant activities of extracts of different samples are presented evaluated by DPPH test and ABTS test are reported in **table 2**. The anti-radical activity with DPPH and ABTS were expressed in IC_{50}. It should be noted that the smaller the IC_{50} the greater is its antioxidant activity. The DPPH method was used to measure the ability of the antioxidants contained in the different extracts to stabilize the DPPH radical. Thus it appears from Table 2 that the ability to inhibit the DPPH radical varies significantly (p <0.05) from one extract to another with IC50 falling between 18.312 and 883.03µg/ mL respectively for ethanolic extract of *D.glomerata* and ethanolic extract of *B.senegalensis*. Several previous studies have also shown that the alcohol solvents were those which exhibited the highest anti-radical activity due to their content of phenolic compounds [25]. Indeed, the increase in the antioxidant activity is due to the fact that alcohol solvents better extract the antioxidant compounds. Figures 1, 2,3, 4, 5 and 6 show inhibition percentage at different concentration of extracts, we note a dose–response relationship in DPPH radical scavenging effect tests; thus the effect increased as the concentration increased for each individual extract. Stabilization of the inhibition percentage is attained for each extract of sample at a precise concentration. The concentration of stabilization of ethanolic extract is low compared to those of aqueous extract, this could be explained by their different contents in polyphenols as shown table 1. Concerning total antioxidant capacity presented by Table 2, the results follow logic observed with DPPH radical scavenging activity. Therefore, for the *in vivo* antioxidant activities we have usedethanolic extracts for experimentation.

Table 2 I*n vitro*antioxidant activities of extracts different plants

Samples	Extract	DPPH IC_{50} (µg/mL)	TAC (mg Eq AA/gDW)
Adansoniadigitata	Ethanolic	0.412 ± 0.02 [a]	42.96 ± 1.35[b]
	Aqueous	0.49 ± 0.01 [b]	26.70 ± 1.80[a]
Balanites aegyptiaca	Ethanolic	1.40 ± 0.02[a]	40.01 ± 0.92[b]
	Aqueous	1.47 ± 0.02 [b]	35.80 ± 0.52[a]
Boscia senegalensis	Ethanolic	0.39 ± 0.02[a]	41.80 ± 0.91[b]
	Aqueous	0.84 ± 0.01[b]	15.60 ± 1.35[a]
Dichrostachysglomerata	Ethanolic	0.02 ± 0.01[a]	98.17 ± 1.35[b]
	Aqueous	0.05 ± 0.03[b]	58.10 ± 2.25[a]
Hibuscussabdariffa	Ethanolic	0.06 ± 0.01[a]	25.97 ± 1.38[b]
	Aqueous	0.31 ± 0.02[b]	21.66 ± 0.49[a]
Senna tora	Ethanolic	0.48± 0.04[a]	17 .84 ± 1.31[b]
	Aqueous	0.64 ± 0.02[b]	15.91 ± 0.18[a]

TAC: Total antioxidant capacity, Eq AA/gDW **:** acid ascorbic equivalent / gram of dry weight. The data where mean ± standard deviation of triplicate (3). Means in the same column for each plant followed by different letters were statistically different (p<0.05).

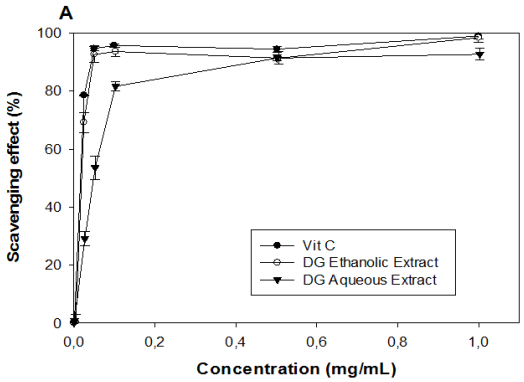


Figure 1 DPPH-free radical scavenging effect (percent) of ethanolic extract of *Dichrostachysglomerata*

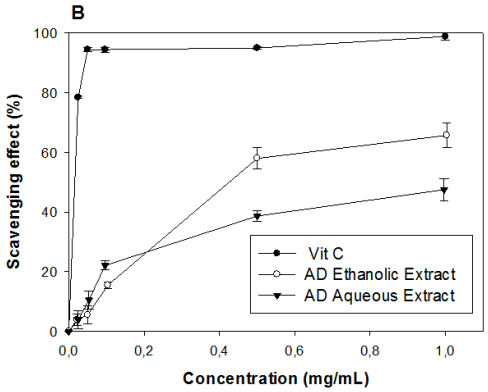


Figure 2 DPPH-free radical scavenging effect (percent) of ethanolic extract of*Adansoniadigitata*

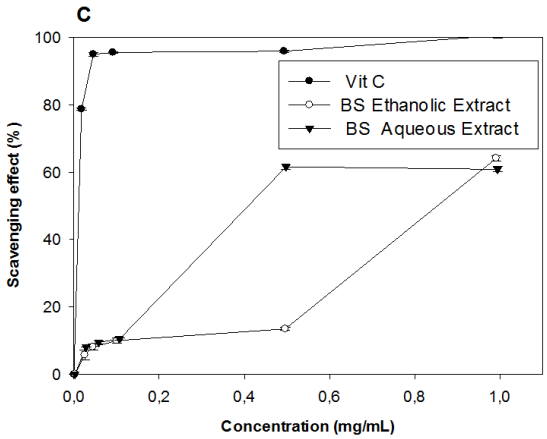


Figure 3 DPPH-free radical scavenging effect (percent) of ethanolic extract of *Boscia senegalensis*

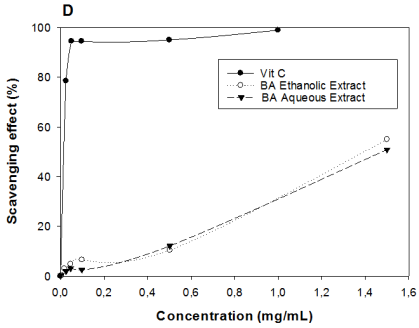


Figure 4 DPPH-free radical scavenging effect (percent) of ethanolic extract of *Balanitesaegyptiaca*

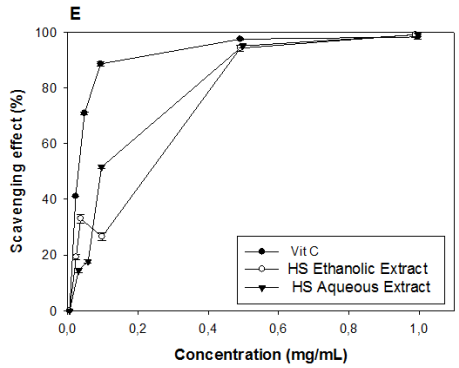


Figure 5 DPPH-free radical scavenging effect (percent) of ethanolic extract of *Hibiscus sabdariffa*

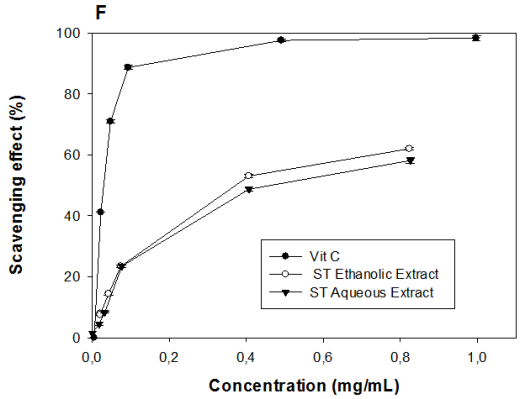


Figure 6 DPPH-free radical scavenging effect (percent) of ethanolic extract of *Senna tora*

In vivo antioxidant activity

Effect of ethanolic extract of different samples on organ-to-body weight ratios of rats

Effects of ethanolic extract of different samples on organ- to-body weight ratios of rats is shown in Table 3, all the organ-to-body weight ratios except testis of negative control group (group taken only CCl4) is significantly higher ($p < 0.05$) compared to those of the othergroups. This resultalready demonstrates thenegative effect of CCl4 on organs which will be confirmed by results of biochemical parameters.

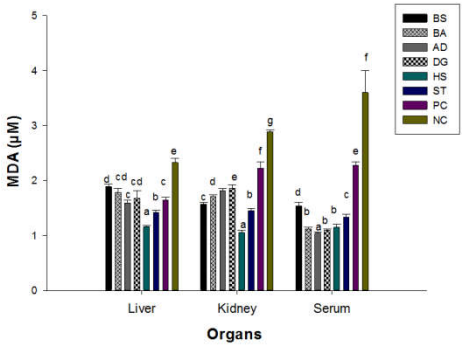
Table 3 Relative mass of rat organstreatedwith the variousethanolic plant extracts

Samples	Liver	Kidney	Heart	Lunch	Testis
Positif Control	3.25 ± 0.52^{b}	0.59 ± 0.11^{ab}	0.30 ± 0.07^{ab}	0.60 ± 0.04^{a}	1.05 ± 0.28^{a}
Negatif Control	4.29 ± 0.92^{c}	0.72 ± 0.13^{b}	0.35 ± 0.04^{b}	0.85 ± 0.24^{b}	0.92 ± 0.27^{a}
D. glomerata	3.24 ± 0.19^{b}	0.58 ± 0.07^{ab}	0.29 ± 0.40^{ab}	0.64 ± 0.17^{ab}	1.06 ± 0.14^{a}
A. digitata	3.24 ± 0.48^{b}	0.51 ± 0.07^{a}	0.28 ± 0.06^{ab}	0.53 ± 0.05^{a}	1.05 ± 0.10^{a}
B. aegyptiaca	3.20 ± 0.38^{b}	0.58 ± 0.05^{ab}	0.27 ± 0.03^{a}	0.51 ± 0.04^{a}	1.05 ± 0.10^{a}
B. senegalensis	3.26 ± 0.25b	0.58 ± 0.07^{ab}	0.28 ± 0.03^{ab}	0.56 ± 0.11^{a}	1.04 ± 0.07^{a}
H. sabdariffa	3.25 ± 0.37^{b}	0.54 ± 0.08^{a}	0.29 ± 0.05^{ab}	0.67 ± 0.23^{ab}	1.05 ± 0.18^{a}
S. tora	0.54 ± 0.08^{a}	0.53 ± 0.12^{a}	0.28 ± 0.03^{ab}	0.64 ± 0.07^{ab}	1.03 ± 0.21^{a}

The data were expressed as mean ± standard deviation of triplicate (n=3). Means in the same columm with different letters were statistically different ($p<0.05$).

Effect of ethanolic extract of different samples onlipid peroxidation

Malondialdehyde (MDA) is considered to be one of the end products of the oxidation of polyunsaturated fatty acids mediated by free radicals. The high level of MDA therefore marksthe presence of oxidative stress. Administration of 0.5 ml/kg body weight of CCl4 has been reported to elevate malondialdehyde, a product of lipid peroxidation in liver of rats treated with CCl4 only[26].They attributed the increase in MDA levels to enhanced lipid peroxidation, leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals. This in turn alters the ratio of polyunsaturatedfatty acidsto other fatty acids, thus, leading to a decrease in the membrane fluidity which may be sufficient to cause cell death[27].The level of Malondialdehyde in the serum, the kidneys and the liver of rats is presented infigure g, thehistogram of this figureshowsthat ethanolicextractsof different plants have negative effectson the production of MDAin the serum, the kidneys and the liver. Globally rats pretreated with ethanolic extracts present significant reduction ($p < 0.05$)of levels of MDA compared to those receiving CCl4 only. Thesesignificant reductions are higher in extracts *D.glomerata* of (1.67949 µM/mg, 1.08654 µM) and *A.digitata* (1.56838 µM/mg, 1.03686 µM/mg) to those of negative control group (2.32532µM, 3.59615 µM) respectively in the serum and the liver. In the kidneys, this reduction was significant with extracts of *B.senegalensis* (1.575 µM) and *B*.aegyptiaca (1.705 µM/mg) compared to negative control group (2.885 µM). The reduction of levels of MDA is in accord withresults of*in vitro* antioxidant activities ofethanolic extract.In fact, polyphenols work againstlipid peroxidationin two voices: by the protection of targeted lipids against the initiators of the oxidationor bystabilizationof the propagation phase.



NC: negative control group, PC: positive control group, BS: *Boscia senegalensis*, BA: *Balanitesaegyptiaca*, AD:*Adansoniadigitata*, DG: *Dichrostachysglomerata*, HS: *Hibiscus sabdariffa*, ST: *Senna tora*

Figure 7 Malondialdehyde levels of rats pretreated with ethanolic extract of plants

Percentages of protection of ethanolic extract of plants are presented in table 4. The results reveal that all ethanolic extract of different plants protect all organ against oxidation. Liver is a center of metabolism, ethanolic extract of *A.digitata,H. sabdariffa*and*S. tora* protect liver signicantly but*H. sabdariffa* exhibit the highest protection (50%).

Table 4 Protective percentage of ethanolicextracts of differentsamples

Samples	Organs	Protective Percentage (%)
	Liver	18.83
B. senegalensis	Kidney	45.55
	Serum	57.22
	Liver	23.38
B. aegyptiaca	Kidney	40.84
	Serum	68.89
	Liver	31.82
A. digitata	Kidney	37.17
	Serum	71.11
	Liver	27.92
D. glomerata	Kidney	35.60
	Serum	69.44
	Liver	50.14
H. sabdariffa	Kidney	63.61
	Serum	68.06
	Liver	38.96
S. tora	Kidney	49.74
	Serum	63.06

Effect of ethanolic extract of different sampleson catalase and superoxide dismutase activities

Carbon tetrachloride (CCl4) is assumed to initiate the biochemical processes leading to oxidative stress, which is the direct cause of many pathological changes intissues by producing free radicals [28]. The study of numerous compounds that could be useful antioxidants has generated increasing interest in the field of nutrition and medicine. Antioxidants have various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction and radical scavenging [29].

Table 5shows the activity of superoxide dismutase and catalase in the serum, kidneysand liver of rats having receivedethanolicextractsof samples. Superoxide dismutase and catalase are the important antioxidant enzymes in the body's defense system. Catalase catalyzes the reduction of hydrogen peroxide H_2O_2 into water molecules while superoxide dismutase catalyzes the disproportionation of the superoxide $O_2^{\bullet -}$ anion into H_2O_2 and oxygen. These enzymes therefore reduce the toxic effects of free radicals in the body[30]. In this study we note that, pretreatment of rats with extractsof plants improve enzymatic activities of catalase and superoxide dismutase.

Catalase activities in liver and serum (table 6) of rats pretreated with ethanolicextract of *D.glomerata* showed increases of 96.21% and 110.240%respectively compared to the negative control group. While in kidneys, group pretreated with ethanolic extract of *A. digitata*showed anincreaseof 115.54%. The lowest activities were observed in rats pretreated with extract of *B*.senegalensis in liver and serum with increasesof 0.82% et 14.59% respectively compared to the negative control group($p < 0.05$). The results for superoxide dismutase activities follow same logic asthose of catalase activities. In fact, group of rats treated with extract of *D.glomerata* present the highest increase incatalase activities in kidneys (60.714%), followed by extract of*A. digitata* which showed an increaseof 60% compared to the negative control group($p < 0.05$).The results obtained from this study clearly indicate that the antioxydant effect of ethanolicextacts of ourplants resulted in the protection of tissues against CCl4.

Table 5 SOD activity (µU/mg of protein) of ethanolicextracts of differentsamples

Samples	Liver	Kidney	Serum
B. senegalensis	73.93 ± 0.41^{b}	95.7857 ± 1.567^{a}	105.43 ± 0.63^{a}
B. aegyptiaca	101.43 ± 0.82^{c}	148.21 ± 4.54^{c}	160.27 ± 8.41^{b}
A. digitata	159.27 ± 7.42^{e}	150.00 ± 16.50^{cd}	161.21 ± 3.69^{b}
D. glomerata	167.21 ± 5.69^{e}	150.71 ± 5.77^{cd}	169.27 ± 5.39^{b}
H. sabdariffa	114.57 ± 3.63^{d}	161.96 ± 2.68^{e}	170.27 ± 6.39^{b}
S. tora	112.50 ± 6.19^{d}	120.29 ± 1.15^{b}	130.32 ± 2.18^{c}
Positif Control	178.43 ± 8.08^{f}	180.93 ± 11.96^{c}	189.96 ± 11.86^{d}
Negatif Control	56.36 ± 4.87^{a}	60.00 ± 3.30^{a}	70.00 ± 3.30^{e}

The data were expressed as mean ± standard deviation of triplicate (n=3). Means in the same columm with different letters were statistically different ($p<0.05$).

Table 6 Catalase activity (U/mg of protein) of ethanolicextracts of differentsamples

Samples	Kidney	Liver	Serum
B. senegalensis	238.04 ± 22.17^{a}	357.56 ± 27.49^{a}	291.13 ± 6.68^{a}
B. aegyptiaca	427.76 ± 53.72^{e}	457.20 ± 15.62^{bc}	310.75 ± 4.35^{c}
A. digitata	439.46 ± 0.58^{f}	440.09 ± 21.79^{b}	327.36 ± 3.19^{b}
D. glomerata	338.78 ± 5.78^{cd}	464.50 ± 20.56^{bc}	386.57 ± 6.10^{d}
H. sabdariffa	480.70 ± 11.68^{g}	477.83 ± 13.91^{c}	431.53 ± 16.96^{f}
S. tora	372.12 ± 12.44^{d}	460.72 ± 17.16^{bc}	414.74 ± 9.33^{e}
Positif Control	528.79 ± 3.47^{h}	545.76 ± 30.80^{d}	471.54 ± 9.84^{g}
Negatif Control	324.09 ± 2.90^{b}	368.38 ± 2.19^{a}	276.53 ± 2.32^{a}

The data were expressed as mean ± standard deviation of triplicate (n=3). Means in the same columm with different letters were statistically different ($p<0.05$).

CONCLUSION

In conclusion, the results of the present study showed that ethanolicextractsof fruits of *D. glomerata*, *A. digitata, B. aegyptiaca*fruits*, calyx of Hibiscus sabdariffa,* fresh leaves of*Senna tora, and* seek of *Bosciasenegalensis* have antioxidant and scavenging activities as theyameliorated the effects produced by CCl4 in the experimental rats. Therefore they couldbe usedfor the management of diseases associated with oxidative stress like cardiovascular diseases.Since the data reported in this study wasgenerated for short-term treatment with these plants, it is recommended that long-term animal studies be carried out to evaluate not only the effects of these extracts on biomarkers of oxidative stress but also the biochemical mechanismsinvolving xenobiotic enzymes.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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