Journal of Food Research; Vol. 3, No. 3; 2014
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Protective Effect of Hydroethanolic Extracts of *Solanum scabrum* and *Cola verticillata* Against Cyclophosphamide Induced Toxicity in Female Rats

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Received: September 3, 2013 Accepted: February 9, 2014 Online Published: March 31, 2014

doi:10.5539/jfr.v3n3p18 URL: http://dx.doi.org/10.5539/jfr.v3n3p18

Abstract

The aim of this study was to evaluate the protective effects of hydroethanolic extracts of *S. scabrum* and *C. verticillata* against cyclophosphamide induced toxicity. In this light, female albino wistar rats were treated by intraperitoneal administration of 100 mg/kg BW of cyclophosphamide or distilled water every other day for 7 days associated with oral gavage using hydroethanolic extract of *C. verticillata*/*S. scabrum* at a dose of 200 or 400 mg/kg BW or not every day for the same 7 days. On the 8th day, blood and organs (liver, heart and kidney) were collected for analyses of toxicity-related and oxidative stress markers. Cyclophosphamide treatment induced significant toxicity as shown by liver enzymes, urea and creatinine levels. The administration of extracts helped reduce the levels of these markers. The antioxidant effect of these extracts also helped or not to ameliorate oxidative stress markers (MDA, NO, hydroperoxides, catalase, thiols, GPx) depending on the extract and on the dose administered. These results suggest that administration of hydroethanolic extracts of *S. scabrum* and *C. verticillata* can help prevent or reduce toxicity that is brought about by treatment with cyclophosphamide due to their ability to upregulate antioxidant mechanisms.

Keywords: *S. scabrum*, *C. verticillata*, oxidative damage, toxicity

1. Introduction

Chemotherapy has been used for many years and is still today, one of the most common treatments for cancer. In general, chemotherapeutic drugs act by interfering with the growth of cancer cells (cytoxicity). However, their toxicity is not always limited to these cells but also extended to normal cells leading to various types of disorders in the organism that receives the treatment. An example to such chemotherapeutic agents is cyclophosphamide. Due to its toxic metabolites notably acrolein and phosphoramide, it has been proven to have serious side effects that include liver and renal damage as well as genotoxicity (Gustafsson et al., 1996; Kopecna, 2001; Senthikumar, Ebenezer, Sathish, Yogeeta, & Devaki, 2006). The mechanisms through which cyclophosphamide causes toxicity are poorly understood; however, numerous studies have shown that cyclophosphamide exposure enhances production of intracellular reactive oxygen species (ROS), suggesting that biochemical and physiological disturbances may result from oxidative stress (Ghosh, Das, Ghosh, Mallick, & Debnath, 2002; Manda & Bhatia, 2003). As a matter of fact, its cytotoxicity is mediated through a concomitant decrease in the activities of enzymes and the levels of non-enzymatic antioxidants (C. Revnic, F. Revnic, & Botea, 2005; Selvakumar, Prahalathan, & Varalakshmi, 2006).

Natural products and herbal medicines have been used traditionally for various ailments to avoid side effects due to treatments (Abdel-Hamid, Nazmy, Mahmoud, Fawzy, & Youssof, 2011). Plants produce significant amounts of antioxidants such as polyphenols, phenols and flavonoids (S. C. Sati, N. Sati, Rawat, & O. P. Sati, 2010). Due to their hydrogen-donating and metal-chelating capacities, these compounds are potential chemopreventive

agents (Grzegorczyk, Matkowski, & Wysokinska, 2007). Moreover, some studies have shown that plant base natural products could provide protection against toxicities caused by cyclophosphamide administration (Sati et al., 2010; Nithya, Chandrakumar, & Senthilkumar, 2012).

Cola verticillata whose seeds are consumed in the name of kola nut and *Solanum scabrum* locally called "Njama-njama" whose leaves are consumed as vegetable are two plants that are very present in Cameroonian diets. *C. verticillata* belongs to the family of Sterculiaceae; Most members of this family have been found to have high antioxidant potential (Endrini, MarsiatiHimm, Suherman, Fauziah, & Asmah, 2009; Momo, Ngwa, Fomekong, & Oben, 2009) and also to be cytotoxic on some cancer cell lines (Endrini et al., 2009). *S. scabrum* is highly consumed in the North West Region of Cameroon as a vegetable. It is locally used as analgesic, febrifuge, narcotic and purgative by local population. *S. nigrum* a specie closely related to *S. Scabrum*, has been proven to have hepatoprotective (Sarwat, Shahid, & Mohammad, 1995), neuroprotective (R. M. Perez, , J. A. Perez, Garcia, & Sossa, 1998), and antiproliferative properties (Son et al., 2003). Recently, we have shown that both *C. Verticillata* and *S. Scabrum* exhibit antiproliferative properties on an ovarian cancer cell line (Mbong et al., 2013). In order to further evaluate the potential role of plant foods in the management of cancer, we decided in this study to evaluate the ability of *S. scabrum* and *C. verticillata* extracts to attenuate cyclophosphamide treatment related toxicity.

2. Methodology

2.1 Reagents

Cyclophosphamide, butylated hydroxytoluene (BHT), Xylenol orange, ammonium sulphate, methanol, sulfuric acid, trichloroacetic acid (TCA), thiobarbituric acid (TBA), hydrochloric acid (HCl), N-1-naphtylethylenediamine dichloride (NED), sulfanilamide, orthophosphoric acid, sodium nitrite, Tris, 5,5-dithiobis (2-nitrobenzoic acid) (DNTB), dibasic sodium phosphate, monobasic sodium phosphate, sodium Azid, oxidized glutathione, β-mercaptoethanol, hydrogen peroxide, metaphosphoric acid and sodium citrate.

2.2 Preparation of Extracts

Leaves of *S. scabrum* were harvested in Babangui, in the North West Region of Cameroon in March 2010 while fruits of *C. verticillata* were harvested in Bamena, in the West Region of Cameroon in April 2010. They were washed then shade-dried until constant weight was attained. After which they were grinned and the obtained powders were used to prepare hydroethanolic extracts. For this, 500 g of each material was macerated for 48H in 2000 ml of 50% ethanol; the filtrates were recuperated and concentrated by air drying at 40 °C to obtain crude hydroethanolic extracts of *C. verticillata* and *S. scabrum*.

2.3 Animals and Treatment

30 adult female albino wistar rats weighing between 160 and 210 g, provided by the Department of Biochemistry of the University of Yaounde I, Cameroon, were used in this study. The animals had free access to standard diet and water. They were equally maintained at room temperature conditions throughout the experimental period. After an acclimation period of 3 days, they were divided randomly into six groups of five animals each. Extracts were administered by oral gavage every day; the re-suspension solvent being 1% ethanol and cyclophosphamide was administered by intraperitoneal injection every other day; the suspension solvent being distilled water. The six groups were treated as follows;

Group I (Non-treated Control; NC): 1% ethanol by gavage plus distilled water intraperitoneally.

Group II (treated control; PC): 1% ethanol by gavage plus 100mg/kgBW cyclophosphamide intraperitoneally.

Group III (200Ss): 200 mg/kgBW *S. scabrum* by gavage plus 100 mg/kgBW cyclophosphamide intraperitoneally.

Group IV (400Ss): 400 mg/kgBW *S. scabrum* by gavage plus 100 mg/kgBW cyclophosphamide intraperitoneally.

Group V (200Cv): 200 mg/kgBW *C. verticillata* by gavage plus 100 mg/kgBW cyclophosphamide intraperitoneally**.**

Group VI (400Cv): 400 mg/kgBW *C. verticillata* by gavage plus 100 mg/kgBW cyclophosphamide intraperitoneally.

After an experimentation period that lasted 7 days, they were all dispatched and their blood collected in EDTA tubes. Collected blood was centrifuged at 3400 rpm for 15 minutes; the supernatant was collected then stored at -20 °C for further studies.

Homogenates of liver, kidney and heart were prepared after the animals were dissected and the organs recuperated and put on ice. They were grinned then centrifuged at 3400 rpm for 10min. The homogenates were prepared at a concentration of 10% w/v in normal saline (0.9% NaCl) and then stored at -20 °C.

For preparation of hemolysates of erythrocytes, after centrifuging total blood, 100 µl of pellets were pipetted into another tube then washed twice with 2 ml 0.9% NaCl and centrifuged at 3400 rpm for 10 min at room temperature. Hemolysates were got by adding 2ml of distilled water, then the obtained supernatant was stored at -20 °C.

2.4 Biochemical Analyses

2.4.1 Protein Concentration

Protein concentration was evaluated in all collected samples (hemolysate of erythrocytes, plasma and homogenates of heart, kidney and liver) using the "total protein colorimetric test Biuret" kit commercialized by Cypress Diagnostics according to the protocol provided by the manufacturer.

2.4.2 Plasma Aspartate amino transferase (AST) and Alanine Amino Transferase (ALT) Activity

AST and ALT activities were evaluated in plasma of treated rats using GOT (AST) and GPT (ALT) "Liq U.V kinetic test according to IFCC. Liquid." kit of Cypress Diagnostics and the kit was used according to the protocol provided by the manufacturer.

2.4.3 Plasma Creatinine

Plasma creatinine levels were measured using the "Creatinine kinetic test Jaffe without deproteinization." kit manufactured by Cypress Diagnostics and according to the protocol provided by the manufacturer.

2.4.4 Plasma Urea Levels

The amount of urea in plasma was determined using the "Urea Liq U.V. kinetic test Urease-GLDH Liquid" kit commercialized by Cypress Diagnostics and according to the protocol provided by the manufacturer.

2.4.5 Hydroxyl (OH) Radical Level

It was measured in blood plasma and homogenates of organs according to the protocol described by Jiang and collaborators in 1992 without any modification. This method is based on the principle that; in acid medium, the peroxide ion oxidizes Fe^{2+} into Fe^{3+} which reacts with xylenol orange to form a complex that absorbs at 560 nm.

2.4.6 Malondialdehyde (MDA) Levels

MDA levels were determined in plasma and homogenates of organs following the protocol described by Yagi in 1976 and it was exploited without any modification. The principle of the method is as follows; carbonylated molecules like malondialdehyde generated from the decomposition of hydroperoxydes, react with TBA to form a pink chromophore with absorbance at 532 nm

2.4.7 Nitric Oxide (NO) Radical Levels

It was measured in heart homogenates and plasma following the diazotation reaction described by Griess in 1879 according to the method described by Manish and coworkers in 2006.

2.4.8 Thiols Protein Levels

Thiols protein levels were measured in all prepared biological samples according to the method described by Ellman in 1959 which is based on the fact that thiols carried by proteins are measured by following the evolution of the concentration of TNB which is a yellow complex formed by the reduction of DTNB (Ellman's reagent) (412-415 nm).

2.4.9 Catalase (CAT) Activity

The activity of CAT was evaluated according to the method described by Sinha in 1972 based on the fact that H_2O_2 remaining after the action of CAT combines with potassium dichromate to form an unstable blue-green complex which is later decolorized into a green complex on heating which absorbs light at a wavelength of 570 nm.

2.4.10 Glutathione Peroxidase (GPX) Activity

The activity of GPx was evaluated according to the method described by Rotruck, Pope, and Ganther in 1973 by the principle that; glutathione peroxidase catalyzes the reduction of H_2O_2 into water. The hydrogen atoms necessary for this reduction is provided by the oxidation of glutathione which then has its thiols quantified using the method of Ellman (Ellman, 1959).

2.4.11 Lipid Profile

For lipid profile, total, HDL and LDL cholesterols were determined as well as triglycerides level in plasma of experimental rats: CHRONOLAB® kits were used. Plasma total cholesterol and triglycerides were evaluated using the cholesterol and triglycerides kits respectively. For evaluation of HDL cholesterol, the HDL cholesterol precipitating reagent IVD kit was used for precipitation of VLDL and LDL cholesterol after which the cholesterol kit was used to determine HDL cholesterol levels. LDL cholesterol was estimated using Friedewald's formula (Friedewald, Levy, & Fredrickson, 1972). All used kits were exploited according to the protocols provided by the manufacturer.

2.5 Statistical Analysis

Data are expressed as mean ± SE. SPSS version 16.0 for Windows was used to analyze results. One way ANOVA followed by the post hoc test Least Significance Difference (LSD) was exploited to determine difference between groups and results were considered significantly different when $p < 0.05$.

3. Results

Toxicity markers evaluated during this experimentation included AST, ALT Urea and creatinine. As concerns hepatic toxicity markers, the highest levels of AST and ALT were found in rats treated only with 100 mg/kg BW of cyclophosphamide ($p < 0.05$). For AST, those supplemented with 200 mg/kg BW *S. scabrum* had the same levels as non-treated rats (Table 1). Amongst rats treated with extracts, the highest amounts were found with 200 mg/kg BW *C. verticillata* treated rats and it was higher than for rats which did not receive any treatment. For ALT, the lowest amounts were still obtained with *S. scabrum* but this time with the higher dose while the highest amount was with 200 mg/kg BW of *C. verticillata*. Cyclophosphamide and cyclosphosphamide+400 mg/kg BW *S. scabrum* were the only two groups to have AST/ALT ratios higher than one; for all the other groups, this ratio was lower than one.

Table1. Liver toxicity marker levels measured in plasmas of experimental rats

	AST (U/l)	ALT (U/l)	AST/ALT
NC	7.16 ± 1.29^{a}	8.75 ± 0.00^{a}	0.81 ± 0.14^{a}
PC	23.70 ± 5.48^{c}	18.35 ± 5.75^{c}	1.32 ± 0.11^{d}
200 Ss	1.96 ± 0.23^{b}	6.75 ± 2.60^{a}	0.33 ± 0.13^{b}
400 Ss	5.71 ± 0.50^{a}	4.44 ± 0.89^{b}	1.31 ± 0.14^{d}
200 Cv	12.18 ± 3.75^{e}	12.21 ± 2.84^{d}	0.98 ± 0.07^{ac}
400 Cv	3.96 ± 1.86^{b}	5.39 ± 1.78^{b}	0.72 ± 0.51^{ac}

NC=negative control or non-treated control; PC=positive control; Ss= S. scabrum; Cv= C. verticillata.

For each column, values with different superscripts are significantly different ($P < 0.05$) according to the LSD post hoc test.

As can be seen in Table 2, urea level was highest in cyclophosphamide-treated rats even if this amount was not significantly higher than for cyclophosphamide+400 mg/kg BW *S. scabrum* ($p > 0.05$). Non-treated rats and those supplemented with 200 mg/kg *S. scabrum* showed similar levels; this also being true for creatinine. 200 mg/kg BW *C. verticillata* treated rats showed the highest amount of creatinine ($p < 0.05$).

Table 2. Renal toxicity markers measured in plasmas of experimental rats

	Urea (mg/l)	Creatinine (mg/l)
NC	2.50±1.64[a]	0.52±0.02[ad]
PC	5.50±2.74[b]	0.58±0.03[acb]
200 Ss	2.33±2.06[a]	0.47±0.07[d]
400 Ss	5.00±0.29[ab]	0.61±0.02[b]
200 Cv	3.67±0.52[ab]	0.87±0.1[e]
400 Cv	4.67±2.00[ab]	0.63±0.10[b]

NC=negative control or non-treated control; PC=positive control; Ss= S. scabrum; Cv= C. verticillata

For each column, values with different superscripts are significantly different ($P < 0.05$) according to the LSD post hoc test.

Some markers of the antioxidant defense system were equally evaluated during this study notably, CAT, GPx and thiols proteins.

Looking at liver thiols levels, non-treated rats had the same levels with all groups ($p > 0.05$); this amounts varied only between treated groups (Table 3).

Table 3. Thiols protein levels in homogenates of organs

Thiols (µM/g)	Liver	Heart	Kidney
NC	0.41±0.22[abcd]	0.36±0.01[a]	0.32±0.01[a]
PC	0.23±0.01[ac]	0.82±0.54[b]	0.63±0.20[b]
200 Ss	0.11±0.00[bc]	0.52±0.04[ab]	0.31±0.10[a]
400 Ss	0.58±0.31[d]	0.59±0.19[ab]	0.62±0.18[b]
200 Cv	0.49±0.29[de]	0.50±0.11[ab]	0.75±0.18[bd]
400 Cv	0.32±0.01[abce]	0.38±0.17[a]	0.59±0.05[bc]

NC=negative control or non-treated control; PC=positive control; Ss= S. scabrum; Cv= C. verticillata

For each column, values with different superscripts are significantly different ($P < 0.05$) according to the LSD post hoc test.

Plasma thiol levels were the same for all groups (Table 4) except for those supplemented with *C. verticillata*. In erythrocytes, this was also true except for supplementation with 200 mg/kg BW of *S. scabrum* and 400 mg/kg BW of *C. verticillata* ($p < 0.05$).

Table 4. Thiols protein levels in blood compartments

Thiols (µM/g)	Plasma	Erythrocytes
NC	4.46±0.04[b]	1.60±0.30[a]
PC	3.83±0.32[b]	1.81±0.39[a]
200 Ss	3.96±0.96[b]	0.56±0.09[b]
400 Ss	2.55±1.09[b]	0.99±0.31[a]
200 Cv	7.03±3.05[a]	0.86±0.29[a]
400 Cv	8.62±4.06[a]	0.78±0.01[ab]

NC=negative control or non-treated control; PC=positive control; Ss= S. scabrum; Cv= C. verticillata

For each column, values with different superscripts are significantly different ($P < 0.05$) according to the LSD post hoc test.

The amount of CAT was measured in erythrocytes as it is in this blood compartment that its activity is highest. CAT level was seen to be most important in the group of rats treated with cyclophosphamide+400 mg/kg BW of *S. scabrum* but this amount was found to be significantly different only from the group of rats treated with cyclophosphamide+200 mg/kg BW of *S. scabrum* ($p < 0.05$) (Figure 1).

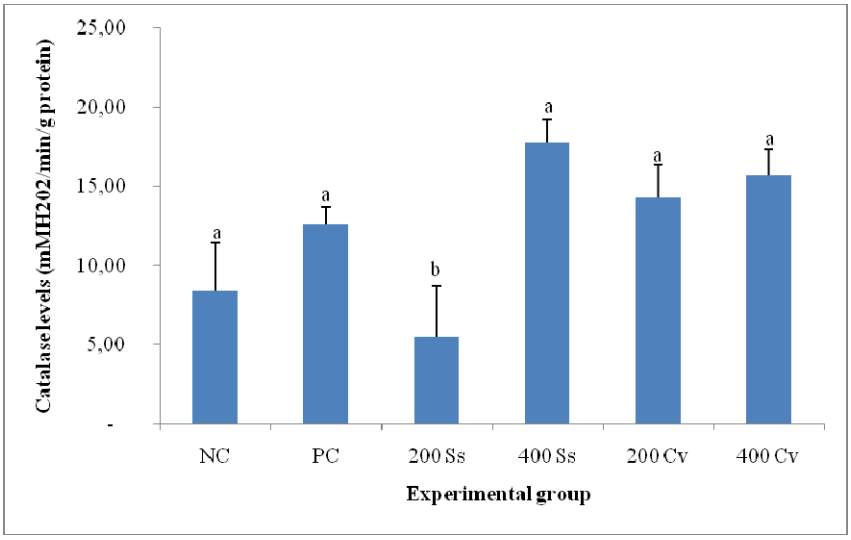

Figure 1. Blood catalase levels as obtained in hemolysates of erythrocytes

NC=negative control or non-treated control; PC=positive control; Ss= S. scabrum; Cv= C. verticillata

Histograms with different superscripts are significantly different ($P < 0.05$) according to the LSD post hoc test.

Figure 2 is a representation of GPx levels as measured in homogenates of liver, heart and kidney as well as in erythrocytes. It was found to be significantly highest in non-treated rats when compared to treated rats ($p < 0.05$). The lowest amount of GPx in erythrocytes was found in the cyclophosphamide+400 mg/kg *C. verticillata* group ($p < 0.05$).

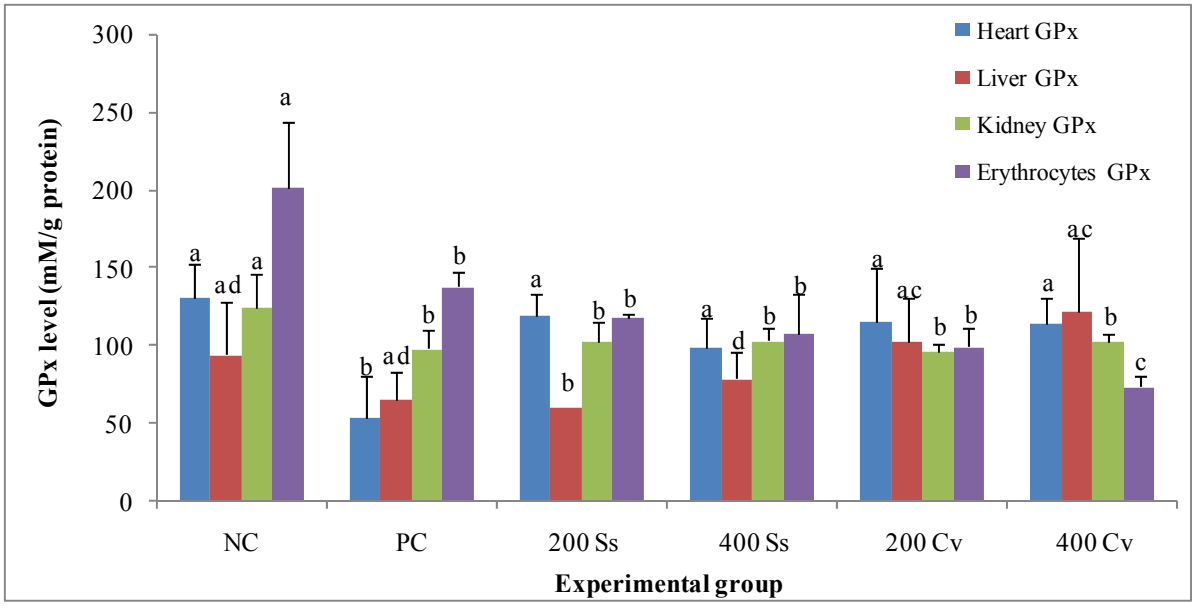

Figure 2. Glutathione peroxidase levels as obtained in hemolysates and homogenates of organs (heart, liver and kidney)

NC=negative control or non-treated control; PC=positive control; Ss= S. scabrum; Cv= C. verticillata.

Histograms with different superscripts are significantly different ($P < 0.05$) according to the LSD post hoc test.

In the liver, non-treated rats and those treated with *C. verticillata* and/or cyclophosphamide showed similar results ($p > 0.05$). The lowest ($p < 0.05$) GPx liver level was obtained with the cyclophosphamide+200mg/kgBW *S. scabrum* groups. In the kidney, the highest levels of GPx were found in non-treated rats ($p < 0.05$) compared to all other treated groups which showed similar results (Figure 2). In heart homogenates these levels were similar in all groups except in those treated exclusively with cyclophosphamide ($p < 0.05$).

MDA, NO and hydroxyl radical levels were evaluated as oxidative stress markers. Plasma MDA level was lowest in non-treated and cyclophosphamide+400 mg/kg BW *C. verticillata* groups ($p < 0.05$); the highest value being with cyclophosphamide exclusively treated rats ($p < 0.05$).

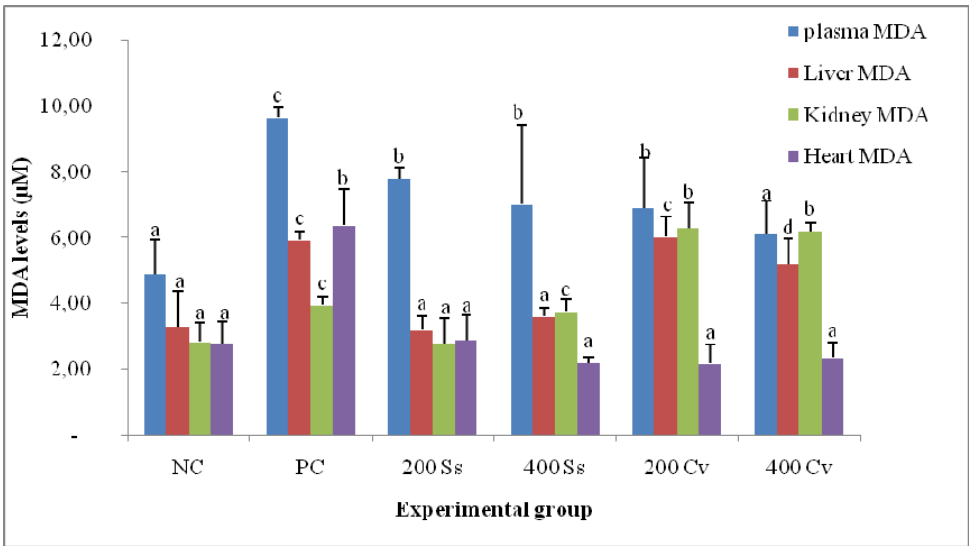

Figure 3. Malodialdehyde levels (MDA) plasma and homogenates of liver, heart and kidney

NC=negative control or non-treated control; PC=positive control; Ss= S. scabrum; Cv= C. verticillata.

Histograms with different superscripts are significantly different ($P < 0.05$) according to the LSD post hoc test.

Looking at MDA levels in liver and kidney homogenates, similar amounts ($p > 0.05$) were obtained from non-treated and cyclophosphamide+200 mg/kg BW *S. scabrum* supplemented rats. Cyclophosphamide treated rats and cyclophosphamide+200 mg/kg BW *C. verticillata*-treated rats showed similar MDA levels that were significantly highest compared to all the other groups (Figure 3). In the kidney, they were found to vary according to the treatment received by rats. Moreover, in this organ, cyclophosphamide and cyclophosphamide+400 mg/kg BW *S. scabrum* were similar. *C. verticillata* treated rats had kidney MDA levels that were higher compared to all other groups ($p < 0.05$). In the heart, only rats treated exclusively with cyclophosphamide showed significantly high amounts ($p < 0.01$).

NO was a parameter analyzed in plasma and heart homogenates of experimental rats. It was seen to be similar in non-treated rats, cyclophosphamide+200 mg/kg BW *S. scabrum* and cyclophosphamide+400 mg/kg BW *C. verticillata*. Concerning plasma NO levels, lower values were obtained with non-treated rats, cyclophosphamide+200 mg/kg BW *S. scabrum*, and both doses of *C. verticillata*. The highest amount was found with cyclophosphamide-treated rats ($p < 0.05$). Rats treated with cyclophosphamide+400 mg/kg *S. scabrum* provided us with the lowest plasma NO levels ($p < 0.05$).

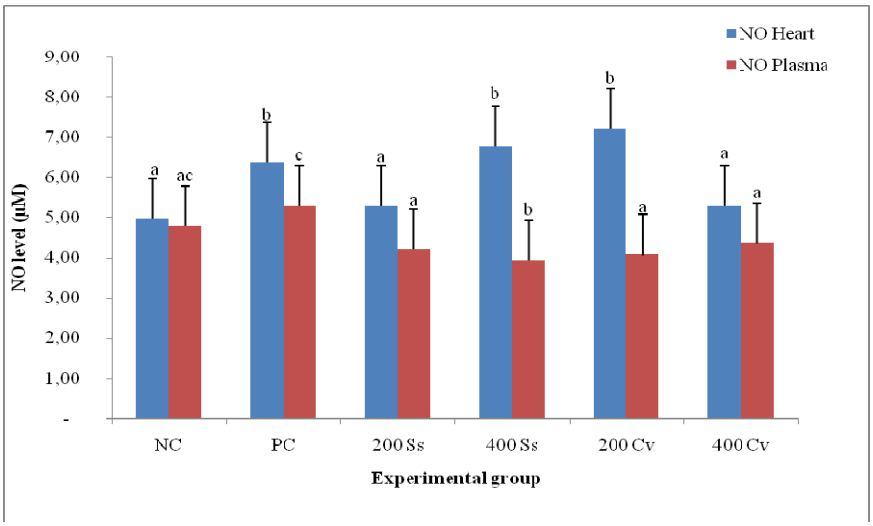

Figure 4. Nitric oxide (NO) radical levels in plasma and heart homogenates

NC=negative control or non-treated control; PC=positive control; Ss= S. scabrum; Cv= C. verticillata

Histograms with different superscripts are significantly different ($P < 0.05$) according to the LSD post hoc test.

As concerns plasma OH levels (Figure 5), they were the same in treated and non-treated rats ($p > 0.05$) and these amounts were found to be very low when compared to organ levels. They were highest in kidney. In this organ, the highest amount was obtained with cyclophosphamide+200 mg/kg BW *C. verticillata* ($p < 0.05$) followed by cyclophosphamide+400 mg/kg BW *S. scabrum* ($p < 0.05$). The lowest ($p < 0.05$) level was obtained in rats treated with cyclophosphamide+200 mg/kg BW *S. scabrum*. In the liver the lowest amount was got with non-treated rats and cyclophosphamide+200 mg/kg BW *S. scabrum* treated rats. The highest amount was with cyclophosphamide-treated rats and groups treated with *C. verticillata* ($p < 0.05$). In the heart, certain levels were comparable to liver OH levels. The highest amounts were with cyclophosphamide-treated and cyclophosphamide +400 mg/kg BW *S. scabrum* -treated rats ($p < 0.05$).

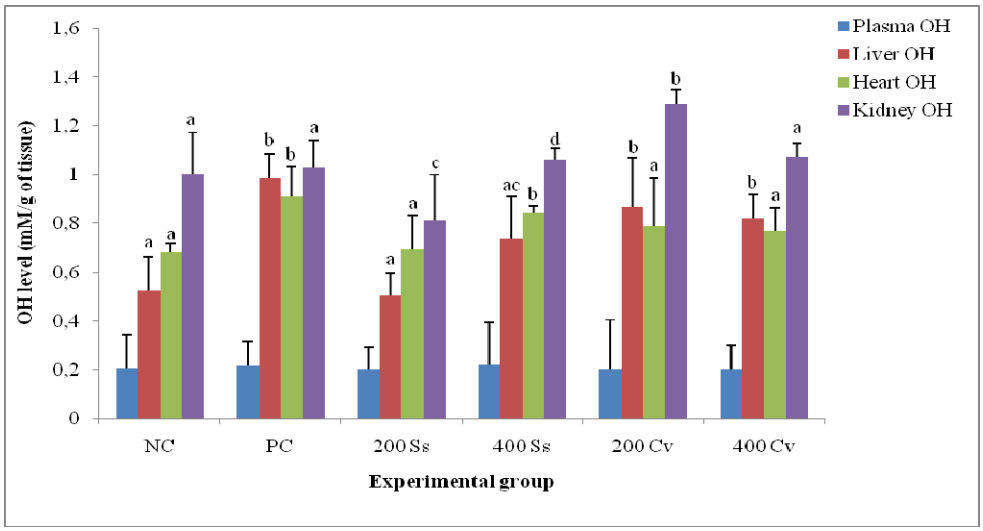

Figure 5. Hydroxyl (OH) radical levels obtained in plasma and homogenates of selected organs

NC=negative control or non-treated control; PC=positive control; Ss= S. scabrum; Cv= C. verticillata

Histograms with different superscripts are significantly different ($P < 0.05$) according to the LSD post hoc test.

When doing toxicity studies using plant extracts, the evaluation of lipid levels are often considered as markers of

peroxidative damage. For total cholesterol, the highest amount was obtained with non-treated rats and this value was found to be significantly different from the amounts in all other groups ($p < 0.05$). Apart from cyclophosphamide+200 mg/kgBW *S. scabrum* which had a significantly higher level ($p < 0.05$), all the other groups provided us with similar amounts ($p > 0.05$) (Figure 6). As for LDL cholesterol, non-treated rats had the highest LDL cholesterol levels ($p < 0.05$) followed by the cyclophosphamide+200 mg/kgBW *S. scabrum* ($p < 0.05$). The lowest levels were found with both doses of *C. verticillata*. These LDL cholesterol levels at least corresponded to total cholesterol levels.

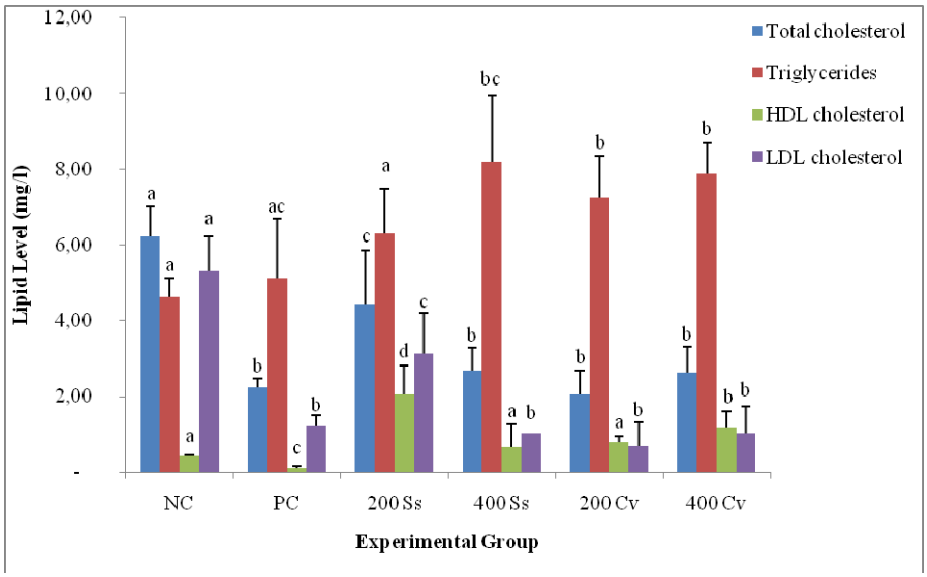

Figure 6. Plasma lipid profile parameters of experimental rats

NC=negative control or non-treated control; PC=positive control; Ss= S. scabrum; Cv= C. verticillata

Histograms with different superscripts are significantly different ($P < 0.05$) according to the LSD post hoc test.

For HDL cholesterol, the highest level was found in cyclophosphamide+200 mg/kg BW *S. scabrum* -treated rats ($p < 0.05$) followed by cyclophosphamide+400 mg/kg BW *C. verticillata*-treated rats ($p < 0.05$). Non-treated rats and those treated with the higher dose of *S. scabrum* and the lower dose of *C. verticillata* had similar HDL cholesterol levels ($p > 0.05$). The lowest level was in rats treated exclusively with cyclophosphamide ($p < 0.05$). As concerns triglycerides (Figure 6), the highest level was found in cyclophosphamide+400 mg/kg BW *S. scabrum* even if similar to cyclophosphamide+200 mg/kg BW *S. scabrum* and non-treated rats.

4. Discussion

Blood levels of AST and ALT were evaluated as toxic markers and they were found to be significantly higher in the plasma of rats treated with cyclophosphamide when compared to non-treated rats. It could be thought that treatment of rats with this drug led to the leakage of these enzymes into the blood stream (Senthikumar, Ebenezer, Sathish, Yogeeta, & Devak, 2006). ALT and AST are thus indicative of the status of liver cells. Also, the ratio of AST/ALT > 1 was obtained only with cyclophosphamide exclusively treated rats and supplementation with the higher dose of *S. scabrum*. AST/ALT ratio is more indicative of liver function than AST taken separately. Studies have shown the protective effects of plant extracts on liver cells after intoxication with an agent (Bose, Mazumder, Kumar, Sivakumar, & Kumar, 2007; Nithya, Chandrakuma, & Senthikumar, 2012).

Elevations in blood urea and creatinine can serve as clinical indicators of poor kidney function (Hayes, 2008). Acrolein produced during cyclophosphamide metabolism is known to be nephrotoxic (Luczaj & Skrzydlewska, 2003). In this study, only supplementation with 200 mg/kgBW *S. scabrum* maintained the level of urea similar to that of non-treated rats showing that it protected this organ from the toxic effects of this drug. The other combinations with cyclophosphamide were either similar or higher comparatively to the positive control group. Creatinine levels on the other hand were not modified by treatment ($p > 0.05$) except for the combination of cyclophosphamide with the 200 mg dose of *C. verticillata* which was found to be highest.

In proteins, acrolein preferentially attacks free -SH groups of cysteine residues,-amino groups of lysine residues

and histidine residues. It is well-documented that the conjugation of the β-carbon of acrolein with sulfhydryl groups is rapid and essentially irreversible (Cao, Hardej, Trombetta, Trush, & Li., 2003), and leads to a decrease in glutathione (GSH). Apart from depleting GSH levels, acrolein also leads to oxidation of proteins (Uchida et al., 1998). In this study plasma thiol levels were significantly higher in groups supplemented with *C. verticillata* implying protection against GSH depletion. High GSH levels have been associated to protection against peroxidative damage.

To overcome the assaults of oxidative stress caused by ROS, cells have developed a number of defense strategies including various protein disulfide reductase enzymes as well as multifunctional DNA repair and thiol-reducing proteins. There are also antioxidant mechanisms such as GSH, radical-scavenging vitamins E and C and ROS-metabolizing enzymatic systems such as superoxide dismutase, CAT GPx (Yousefipour, Ranganna, Newaz, & Milton, 2005).

In our study, CAT and GPx levels were evaluated. CAT is considered a primary antioxidant as it is involved in the direct elimination of ROS. CAT levels were similar in all experimental groups except for those treated with cyclophosphamide+200 mg/kgBW *S. scabrum* which was higher than all others. CAT scavenges H_2O_2 generated by SOD. As concerns GPx, in the heart, non-treated rats had higher levels compared to cyclophosphamide-treated rats but this level was brought to levels similar to non-treated rats by association with extracts. In erythrocytes and kidney, treated rats had lower levels while in the liver treating rats did not improve or lower glutathione peroxidase levels. The difference observed in various compartments (liver, kidney, erythrocytes, heart) could be attributed to the different oxidative exposures to which are subjected the different organs. This enzyme has been reported to be an adaptative response to peroxidative stress conditions (Rathore, Kale, John, & Bhatnagar, 2000).

Many chemotherapeutic drugs are known to act by increasing ROS levels (Schumacker, 2006). Acrolein synthesized during treatment with cyclophosphamide is known to increase the level of oxygen free radicals through glutathionyl propionaldehyde. It is also oxidized by xanthine oxidase and aldehyde dehydrogenase to produce acrolein radicals and O_2• (Adams & Klaidman, 1993). Free radicals thus play an important role in cyclophosphamide induced oxidative stress in organs like the brain, liver and kidney and the etiology of toxicity of this drug is associated to it (Akbar, Banji, & Deshmukh, 2010). The hydroxyl radical and malondialdehyde were evaluated in this study as markers of cellular lipid peroxidation. From both results, the drug exposed cells to lipid peroxidation and its association to extracts especially the 200 mg/kgBW *S. scabrum* limited this process probably due to its antiradical activity. Lipid profile parameters were also evaluated as markers of lipid peroxidation. HDL was found lowered by administration of cyclophosphamide but supplementation with both extracts at both doses helped improve this level. HDL-cholesterol has protective effect against lipid peroxidation (Ray, Panditb, Dasb, & Chakrabortya, 2011).

In plasma, treatment with cyclophosphamide did not increase NO levels may be due to the treatment period that lasted only 7 days while its association with extracts reduced NO levels. Contrarily, NO levels measured in the heart showed higher levels for treated rats except for those associations that included 200 mg *S. scabrum* and 400mg *C. verticillata* which had levels similar to non-treated rats. Cyclophosphamide is used in cancer treatment and its ability to increase NO levels is thought to be advantageous as its association to O_2- is known to form peroxynitrite which has tumoricidal functions. Controversially, high levels of NO could cause oxidative injury to cells still through peroxynitrite (Perwez, Lorne, & Curtis, 2003) leading to vascular toxicity. It has been shown that the pro-oxidant versus antioxidant outcome of NO depends critically on the relative concentrations of the individual reactive species in the presence of high NO levels. The pro-oxidant reactions of NO occur with superoxide, whereas its antioxidant effects are a consequence to direct reactions with alkoyl and peroxyl radical intermediates during lipid peroxidation (Gisone, Dubner, Del Pérez, Michelin, & Puntarulo, 2004).

From these results we notice that exogenously administered antioxidants (present in extracts) can be useful to overcome drug-induced oxidative stress, thus reducing its deleterious effects on organs like the liver and kidney. Other research works corroborate with this one (Lakshmi, Rudrama, & Madhavi, 2009; Saber, Hoda, & Hawazen, 2011; Okwuosa, Achukwu, Azubike, & Abah, 2012). It was also noticed that depending on the extract (hydroethanolic extracts of *S. scabrum* and *C. verticillata*) and on the dose (200 or 400 mg/kgBW) associated to cyclophosphamide, the level could be increased or decreased depending on the biochemical parameter evaluated.

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