

## Comparative Study of the Protective Effect of *Cola anomala* and *Coffea arabica* Against Induced Toxicity in Rats

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### Abstract

This work was aimed at evaluating the effects of *C. anomala* and *C. arabica* on Methotrexate (MTX) induced metabolic disorders. For this, the aqueous extract (AE) of *C. anomala* and *C. arabica* were prepared and their polyphenols, flavonoids and alkaloids contents determined as well as their antiradical and total antioxidant capacity. An animal experimentation using female rats was carried out for 14 days. Rats were divided into 6 groups; a negative control group receiving water; a positive control group receiving 12mg/Kg Bw of MTX; four tests groups receiving 12mg/Kg Bw of MTX and one of the extracts at a dose of 200mg/Kg Bw or 400mg/Kg Bw. At the end of the experiment, plasmas and hemolysates were prepared as well as liver and kidney homogenates for the evaluation of oxidative status (catalase, total protein and malondialdehyde (MDA)), liver toxicity (alanine amino transferase (ALT)) and renal toxicity (creatinine and urea) and lipid profile (triglycerides, total cholesterol and HDL-cholesterol). Weight gain in extract-treated rats was better with the *C. anomala*. Concerning oxidative status, MDA levels were generally lower in *C. anomala*-treated groups compared to *C. arabica* while catalase level was higher in *C. anomala*-treated rats. As for lipid profile, it is *C. arabica* that showed more or less better results. Both extracts led to an amelioration of toxicity markers compared to exclusive treatment with MTX. The results of this study suggest that *C. anomala* and *C. arabica* may reduce metabolic disorders associated with the intake of MTX during cancer treatment; *C. anomala* protecting better *C. arabica*.

**Keywords:** *Cola anomala*, *Coffea Arabica*, Methotrexate, metabolic disorders

### 1. Introduction

Caffeine is responsible for the stimulatory power of coffee and kola nut. The seeds of these two plants are highly consumed for this property. Traditionally, in western Africa, kola nut is consumed by mastication to fight physical and intellectual fatigue and to combat depression. It has also been recognised to ameliorate cognitive vigilance and performance (Bureau, 2013). The relationship between caffeine and cancer is very controversial. Some researchers have proven that the consumption of too much caffeine could affect the risk of developing cancer through various mechanisms. Still, other studies have shown the reduced risk of cancer with the consumption of coffee. The consumption of 3 to 4 cups of coffee per day is thought to reduce by 50% the risk of breast cancer in menopausal women and by 60% the risk of ovarian cancer. While for prostate cancer, this consumption could rather increase the risk of cancer development (Nehlig, 2012).

Recent research is geared at portraying the health benefits of these two plants that are attributable to the presence of bioactive secondary metabolites in their composition. It has been proven that the grains of *Coffea arabica* are an important source of biologically active metabolic molecules with many therapeutic properties. *C. arabica* contains phenolic compounds and their derivatives such as chlorogenic acid (Affonso *et al.*, 2016) that are responsible for cardio protective, hepatoprotective, neuroprotective, nephroprotective properties. The presence of trigonelline in this plant has attributable antioxidant and antimicrobial properties (Najib, Ahmad & Labadjo, 2015). Plants of the family of Sterculiaceae (in which we find kola nut species) contain many bioactive molecules amongst which we find alkaloids, flavonoids and polyphenols (Fabunmi & Arotupin, 2015). Other species of kola nut have been proven to contain tannin that condenses into phlobaphenes that are responsible for the red color of the nuts. Xanthines (caffeine (triméthylxanthine) being the most abundant (2-3.5 %)), nitrogen

containing molecules are the most represented groups of alkaloids. These xanthines have been proven to be responsible for their antidiarrhetic properties. Their polyphenols provide their strong antioxidant potential. In Asia, some decaffeinated extracts are thought to be responsible for their anti-inflammatory and chemopreventive properties through their action on androgenic receptors (Bureau, 2013). Studies carried out on *Cola verticillata* showed, antiproliferative and anti-toxic properties of this species (Mbong *et al.*, 2013, 2014).

Chemotherapy is one of the most used therapies in the treatment of cancers. However, side effects associated with this treatment are usually the worries of patients (Tecza, Pamula-Pilat, Lanuszewska & Grzybowska, 2015). Indeed, the toxicity of antineoplastic agents to rapidly replaceable cells such as bone marrow cells causes a large number of adverse effects in the body (Gorrini, Harris & Mak, 2013). Free radical generation and lipid peroxidation are expressed as some complications of these effects. Methotrexate (MTX) is used as a chemotherapeutic agent to treat certain cancers and auto-immune diseases. Many studies have demonstrated its toxic effects involving most body organs like the liver, kidney and blood parameters. Its side effects could be controversially related to the production of ROS (Boussios, Pentheroudakis, Katsanos & Pavlidi, 2012). Biologically, MTX causes lipid peroxidation indicated by high levels of Thiobarbituric acid reactive substances (TBARS). It also lowers levels of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione reductase indicating oxidative stress and thus decreasing the antioxidant defense systems responsible for its many side effects (Montasser *et al.*, 2017). Oxidative stress-induced lipid peroxidation leads to the deterioration of membrane permeability barrier and loss of functions. It has also been reported that oxidized lipids are markers of pathologic mechanisms in a variety of disease states (Volinsky & Kinnunen, 2013; Bochkov, Oskolkova & Birukov, 2010).

Treatment with MTX has been reported to induce in rats critical changes in biological, biochemical and hematological markers (Saka & Aouacheri, 2017).

Increasing attention is directed towards the role of plant bioactive molecules with antioxidants on the modulation of intracellular levels of reactive oxygen species resulting from cancer treatments (Saha *et al.*, 2017). Plant bioactive compounds such as polyphenols, flavonoids and alkaloids can reduce oxidative stress through various mechanisms including trapping of these reactive oxygen species (Meher and Mishra, 2017). *Cola anomala* and *Coffea arabica*, two plants of the family of *Malvaceae* and *Rubiaceae* respectively, are widely produced in Cameroon and are used in particular for their stimulating properties. These plants have many bioactive compounds including polyphenols, flavonoids, alkaloids that give them their antioxidant properties (Najib, 2015). *C. anomala* is abundantly present in Cameroon but its consumption is more or less limited given that it is generally consumed by the elders and mainly during “traditional gatherings” compared to *C. arabica* that is more present in modern diets and is undoubtedly the most consumed beverage worldwide. Many biological activities have been attributed to both of these species and it was thought important to equally evaluate their possible biological benefits during treatment with a chemotherapeutic agent.

## 2. Methods

### 2.1 *In vitro* Study

This part of the study was carried out to determine the amounts of targeted bioactive molecules as well as the antioxidant potential of aqueous extracts of both plants.

#### 2.1.1 Collection of Biological Material

The nuts of *C. anomala* were harvested in July 2017 in *Bandjoun*, Western region of Cameroon. The botanical identification was done in comparison to specimen N° 113 collected in *Nkongmenek* corresponding to the sample *C. anomala* K.Schum at the Cameroon National Herbarium under voucher number 48706/HNC. After this, the nuts were washed, shade-dried, and powdered using a standard propeller crusher. The powder of roasted seeds of *C. arabica* was bought from a local seller in the Yaounde neighborhood in Cameroon.

#### 2.2.2 Preparation of Aqueous Extracts

The extracts for this study were prepared depending on the plant sample. *C. anomala* was prepared by maceration. 300 g of powdered *C. anomala* nuts were macerated in 1.8L of distilled water for 12H at room temperature. After the maceration period, the filtrate was recuperated using Whatman paper no 3, Whatman International Limited, Kent, England. The filtrate was then oven-dried at 50 °C for 72H. *C. arabica* on the other hand was prepared by infusion. 300 g of *C. arabica* powder was infused in hot water (90 °C) for 10 min. Following this, the mixture was filtered using Whatman paper N° 3, Whatman International Limited, Kent, England. The obtained filtrate was equally oven-dried at 50 °C for 72H. Both extracts were stored in polystyrene bags for further use.

### 2.2.3 Estimation of Bioactive Constituents and Antioxidant Potential

#### Determination of total polyphenols

The Folin-Ciocalteu reagent method described by Singleton & Rossi in 1965, was used to measure total phenolic content of the extracts. For this, 0.1 mL of extracts (4 mg/mL) was mixed with 0.75 mL of Folin-Ciocalteu (0.2N) in opaque tubes. After 5min at room temperature, 0.75 mL of sodium carbonate (6%) was added. The tubes were mixed and incubated for another 90min before measuring the optical densities at 725nm. Gallic acid was used as standard (0-1000 µg/mL). The test was done in triplicate for each extract and total phenol content was expressed in mg equivalent Gallic acid per gram dry weight of extract (mgGAE/g).

#### Determination of total flavonoids

The Aluminum chloride colorimetric method of Aiyegoro and Okoh, 2010 with slight modifications was exploited to assay the flavonoids content of the extracts. Briefly, 0.5 mL of various extracts (4 mg/mL) were added to 1.5 mL of methanol followed by addition of 0.1 mL of aluminum chloride (AlCl<sub>3</sub>, 10%), 0.1 mL of potassium acetate (CH<sub>3</sub>COOK, 1 M) and 2.8 mL of distilled water. The mixture was well shaken and incubated for 30 min at room temperature and its absorbance was measured at 415 nm. Quercetin was used as standard (0-1000 µg/mL) and the total flavonoids content were expressed as mg equivalent quercetin per gram dry weight of extract (mgQuerE/g).

#### Determination of alkaloids

The amount of alkaloids in the extracts was determined using a modified method described by Singh *et al* (Singh, Vats, Suri, Shyram, Ranganathan & Sriatharan, 2004). Summarily, 10mL of 95% ethanol were added to 100mg of extract powder after which the mixture was centrifuged at 5000 rpm for 10 min. 1 mL of Iron-III Chloride [FeCl<sub>3</sub> (0.025 M) + HCl (0.5M)] and 1 mL of ethanolic solution of 1,10 phenanthroline (0.05 M) were added to 1 mL of the obtained supernatant. The mixture obtained was incubated for 30 min at 70 °C. The absorbance of the red complex was measured at 510 nm. Quinine was used as standard at different concentrations (0-100 µg/mL) and the total alkaloids content was expressed as mg equivalent quinine per gram dry weight of extract (mg QuiE/g).

#### Free radical scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH)

The free radical scavenging activity of the extracts of *C. anomala* and *C. arabica* was evaluated using the method described in 2004 by Katalini & collaborators (Katalini & Milos, Modun & Boban, 2004). Briefly, 50 µL of sample solution at different concentrations (0.5 to 7.5 mg/mL) was added to 1.950 mL of fresh methanolic solution of DPPH (3.2 mg/100 mL), mixed and kept protected from light at 37 °C for 30 min. The decrease of absorbance was recorded at 517 nm. The percentage of DPPH inhibition was calculated as follows: DPPH Scavenging Effect (%) =  $((A_0 - A_1) / A_0) \times 100$ , where A<sub>0</sub> was the absorbance of the DPPH control solution and A<sub>1</sub> was the absorbance of the assay (extract in presence of DPPH solution). The IC<sub>50</sub> value was calculated by the table curve method.

#### Total antioxidant capacity

This activity was assessed by the phosphomolybdenum assay method as described by Prieto *et al* (Prieto, Pineda & Aguilar, 1999). Briefly, an aliquot of 100 µL of crude extract at different concentrations (0.5 to 7.5 mg/ml) was combined with 1 mL of reagent [(0.6M sulphuric acid (H<sub>2</sub>SO<sub>4</sub>), 28mM sodium phosphate (Na<sub>3</sub>PO<sub>4</sub>) and 4mM ammonium molybdate ((NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>.4H<sub>2</sub>O)]. The mixture was incubated at 95 °C for 90 min after which it was cooled to room temperature. The variation in absorbance was measured at 765 nm. Ascorbic acid was used as standard and the total antioxidant capacity was expressed in mg equivalent ascorbic acid per gram dry weight of extract (mg AAE/g).

### 2.2 In vivo Study

#### 2.2.1 Experimental Design and Protocol

*Wistar* rats used for this study were reared in the animal house of the Laboratory of Nutrition and Nutritional Biochemistry (LNNB) of Department of Biochemistry, University of Yaounde 1, Cameroon. Female albino *wistar* rats of average weight 175 ± 10 g were housed in a standard animal facility under controlled environmental conditions at room temperature. The rats were exposed to a 12h light-dark cycle and received a standard chow diet and water *ad libitum*. For the experimentation, rats were kept in stainless steel standard cages and were acclimatized for 2 weeks in this environment before the conduction of experiments. Distilled water was used for the oral administration of the drug and plant extracts. The study protocol for laboratory animal use and care was legitimated by the Animal Ethics Committee of the Faculty of Sciences, University of Yaounde I,

Cameroon.

Before the conduction of this experiment, the rats were weighed, divided into six groups of five rats each and labelled. Each group received a different treatment by oral administration (through esophageal gavage). The groups were:

Negative Control (NC): receiving distilled water by gavage

Positive Control (PC): receiving 12 mg/kg.Bw of MTX + distilled water by gavage

Assay 1 (E1): receiving 12 mg/kg.Bw of MTX + 200 mg/kg.Bw *C. anomala* by gavage

Assay 2 (E2): receiving 12 mg/kg.Bw of MTX + 400 mg/kg.Bw *C. anomala* by gavage

Assay 3 (E3): receiving 12 mg/kg.Bw of MTX + 200 mg/kg.Bw *C. arabica* by gavage

Assay 4 (E4): receiving 12 mg/kg.Bw of MTX + 400 mg/kg.Bw *C. arabica* by gavage

Both extracts as well as the drug were dissolved in distilled water. All groups received a daily treatment in the morning. The cancer drug (MTX) was administered once per week. The rats were weighed on Days 1, 4, 8 and 12 during the experimentation period using an electronic weighing balance. After 14 days of experimentation, overnight fasted rats were sacrificed by cervical dislocation under slight anesthesia with diethyl-ether and blood was collected in EDTA tubes for preparation of plasma and hemolysates of erythrocytes. The liver and kidneys of each rat was collected, washed and rinsed with ice-cold saline solution (0.9% NaCl). The homogenates were prepared at a concentration of 10% (w/v) in normal saline (0.9% NaCl). All prepared biological samples were stored at -20 °C.

### 2.2.2 Biochemical Analysis

Measurement of lipid profile parameters

Total cholesterol (TC), triglycerides (TG) and HDL-cholesterol (HDL-c) concentrations were estimated using Chronolab kits according to the methods described by the manufacturer. The concentration of LDL-cholesterol was calculated using Friedewald's formula:  $LDL\text{-cholesterol (LDL-c)} = TC - (HDL\text{-c} + TG/5)$  (Friedewald, Levy & Fridrickson, 1972).

Evaluation of oxidative stress markers

Lipid peroxidation

In this method, thiobarbituric acid reactive substances (TBARS) were estimated by measuring the pink chromophore formed by the reaction of thiobarbituric acid with malondialdehyde (MDA) according to the method of Yagi (1976).

Catalase

The activity of catalase was assayed according to the method of Sinha (1972). The activity of this enzyme was expressed in mM hydrogen peroxide/min/mg of protein.

Total proteins

The level of total protein was assayed using the colorimetric method described by Gornall and collaborators (Gornall, Bardawill & David, 1949). The optical density was recorded at 540 nm. Bovine Serum Albumin (BSA) was used as standard at the concentration of 1 mg/mL.

Evaluation of hepatic and renal markers of toxicity

Hepatic marker

The activity of a transaminase (Alanine amino transferase (ALT) was determined using a CHRONOLAB kit according to the method described by the manufacturer. Pyruvate was used as standard and the activity was estimated using a standard curve. The results were expressed in IU/mL.

Renal markers

The levels of creatinine and urea in plasma were estimated using standard kits (Chronolab) according to the method described by the manufacturer. The obtained results were expressed in mg/dL.

### 2.2.3 Statistical Analysis

Results were expressed as means  $\pm$  standard deviation. Statistical analysis was carried out using SPSS 20.0 for Windows. One way ANOVA (analysis of variance) followed by LSD (*Least Significant Difference*) and post hoc one factor was used to compare the means of different groups. Results were significant for  $p \leq 0.05$ . The

Microsoft office excel software was used to treat the data and plot the graphs.

### 3. Results

#### 3.1 In vitro Study

This study revealed the presence of phenolic compounds, flavonoids and alkaloids in the aqueous extracts of *C. anomala* and *C. arabica* (Table 1). Alkaloid levels were found to be more important compared to the other two families of bioactive compounds. The amounts of phenolic compounds were similar in both extracts while flavonoid content was higher in *C. arabica* and alkaloids higher in *C. anomala*.

Table 1. Bioactive content of *C. anomala* and *C. Arabica*

Extracts	Polyphenols (mg GAE/g DM)	Flavonoids (mg Quer E/g DM)	Alkaloids ( $\mu$ g Qui E/g DM)
<i>C. anomala</i>	1910 $\pm$ 8.16	94.38 $\pm$ 0.17	614.0 $\pm$ 3.2
<i>C. Arabica</i>	1928.89 $\pm$ 5.67	159.58 $\pm$ 3.06	441.1 $\pm$ 5.6

The change in color from purple to yellow was found to be concentration dependent in both extracts for the scavenging of the DPPH free radical. Figure 1 is a representation of the inhibition percentages for both extracts. The extract of *C. anomala* exhibited best scavenging activity expressed by inhibition percentages varying from 7.34 to 64.14 % against inhibition percentages varying from 11.98 to 39.25% for *C. arabica*. The IC<sub>50</sub> value of *C. anomala* was found to be 4.01 mg/mL while the IC<sub>25</sub> value for *C. arabica* was found to be 2.33mg/mL.

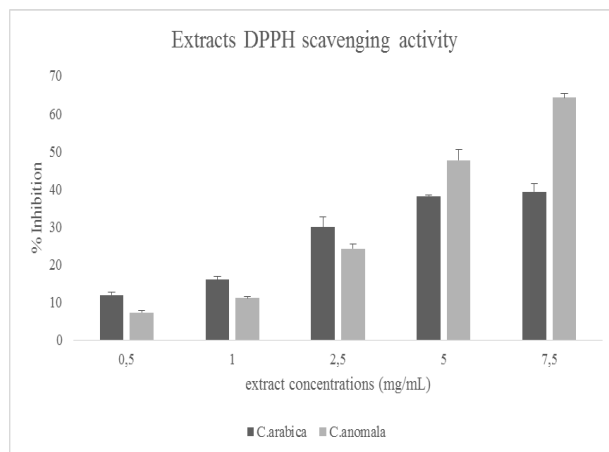


Figure 1. DPPH scavenging activity of extracts

As can be noticed in Figure 2, both extracts showed antioxidant activity and this was found to increase with increasing concentration of extract. For this study, the extract of *C. anomala* showed the highest reducing capacity at different concentrations compared to the extract of *C. arabica*. Depending on the extract concentration, this activity varied from 0.43 to 1.32 mg mgAAE/g DM for *C. anomala* extract against 0.16 to 1.17 mgAAE/g DM for *C. arabica* extract.

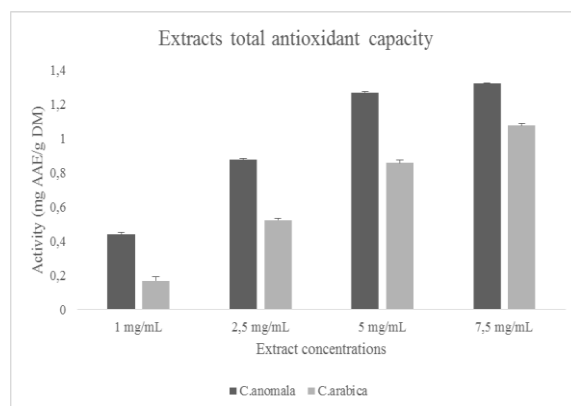


Figure 2. total antioxidant capacity of extracts plants

### 3.2 In vivo Study

The results obtained (Figure 3), revealed that feeding animals with chow diet in association with a treatment with MTX reduced weight gain in rats since weight gain in the positive control group was found to be lower than for the negative control (Group not receiving any treatment). Both extracts were found ameliorate weight gain in rat treated with MTX. The treated rats that also received *C. anomala* were found to gain weight better than rats those receiving MTX and the extract of *C. arabica*. The weight gain for both extracts was not dose-dependent.

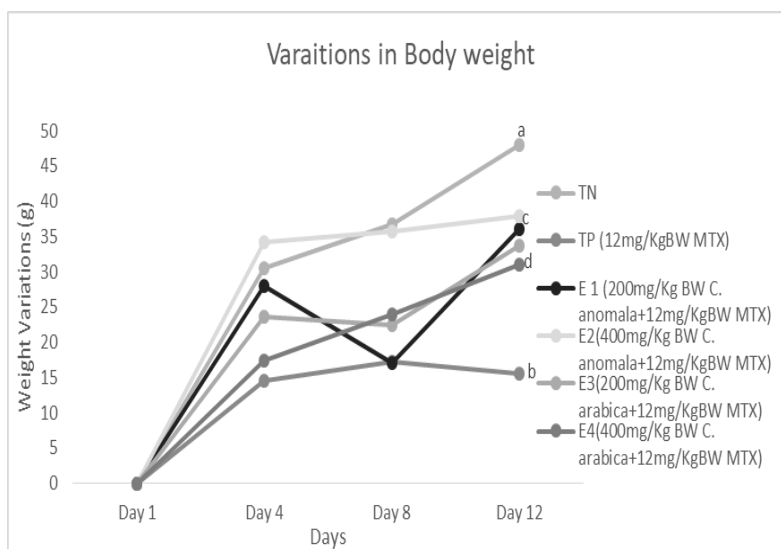


Figure 3. Effect of extracts on body weight of rats during Treatment

The results in Figure 4 show that there was no significant difference between the positive and negative control groups for the concentrations of TC, TG and HDL-c levels in plasma; only LDL-c was higher in the group receiving only MTX ( $p < 0,005$ ). In the treated groups, a significant decrease of TC, HDL-c and TG and increase of LDL-c was observed in rats that were administered either of the aqueous extracts. Generally, so far as the lipid profile is concerned, treatment with MTX did not affect parameters while the administration of both aqueous extracts helped ameliorate these parameters especially for rats that received the higher dose of *C. arabica*.

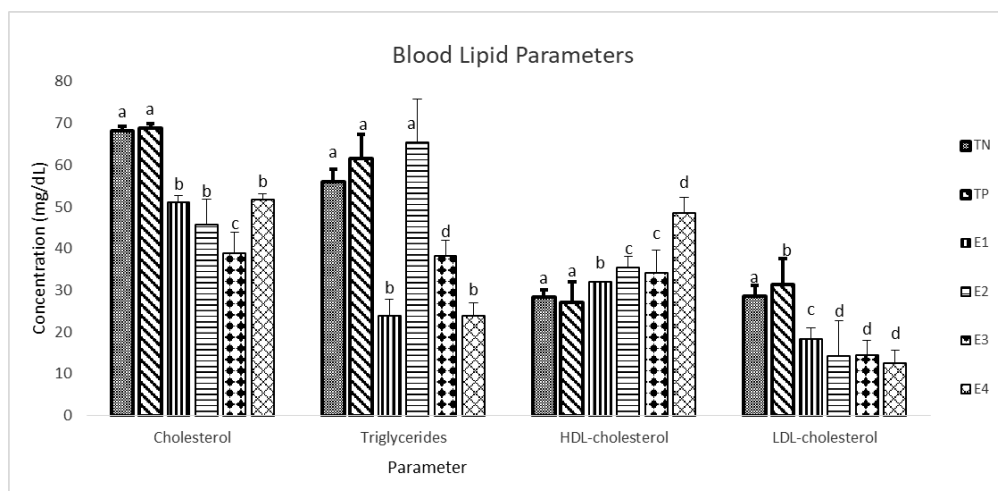


Figure 4. Effects of extracts of *C. arabica* and *C. anomala* on blood lipid parameters of experimental rats

The values are expressed as Mean  $\pm$  Standard deviation. TN : Negative Control (normal rats) ; TP: Positive Control (Cow diet + 12 mg/Kg MTX) ; E1 : Cow diet + 12 mg/Kg MTX + 200 mg/Kg *C. anomala* ; E2 : Cow diet + 12 mg/Kg MTX + 400 mg/Kg *C. anomala* ; E3 : Cow diet + 12 mg/Kg MTX + 200 mg/Kg *C. arabica* ; E4 : Cow diet +12 mg/Kg MTX + 200 mg/Kg *C. arabica*. The values affected with different letters (a, b, c, d) were significant.

It can be observed on Figure 5 an increase in MDA levels ( $p < 0,05$ ) in the positive control compared to the negative control group in kidney homogenates. In comparison with the positive control, the results obtained show ( $p < 0,05$ ) reduction of plasmatic, hepatic and kidney levels of MDA in treated groups that received the extract of *C. anomala*. In the kidney of rats treated with extracts, MDA levels were reduced to values comparable to that of the control group that received no treatment. These levels were also found to be lower in the plasma of rats treated with the aqueous extract of *C. anomala*. These results were similar in the liver but for the lower dose of the aqueous extract of *C. arabica*.

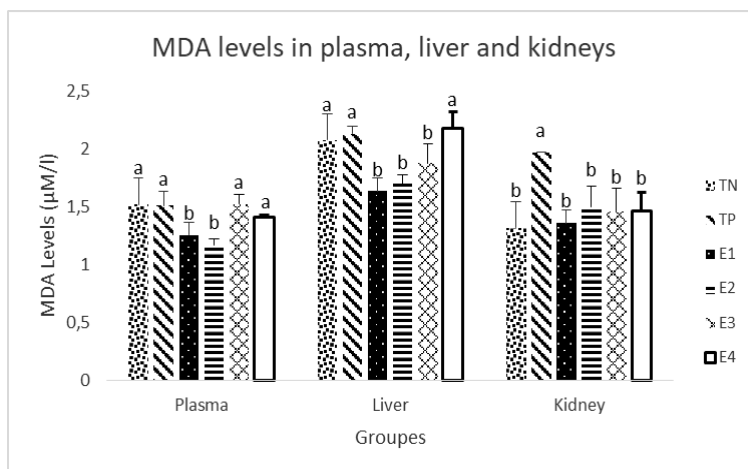


Figure 5. Effects of extracts on antioxidants markers

The values are expressed as Mean ± Standard deviation. TN : Negative Control (normal rats) ; TP : Positive Control (Cow diet + 12 mg/Kg MTX) ; E1 : Cow diet + 12 mg/Kg MTX + 200 mg/Kg *C. anomala* ; E2 : Cow diet + 12 mg/Kg MTX + 400 mg/Kg *C. anomala* ; E3 : Cow diet + 12 mg/Kg MTX + 200 mg/Kg *C. arabica* ; E4 : Cow diet +12 mg/Kg MTX + 200 mg/Kg *C. arabica*. The values affected with different letters (a, b, c, d) were significant.

As concerns the activity of catalase as can be observed in Figure 6, treatment with MTX reduced the activity of catalase when compared to non-treated rats. Nonetheless, rats that received MTX treatment and either of the extracts at both doses saw their catalase activity boosted. It was at least noted that the extract of *C. anomala* showed better results when compared to *C. arabica* ( $p < 0,05$ ).

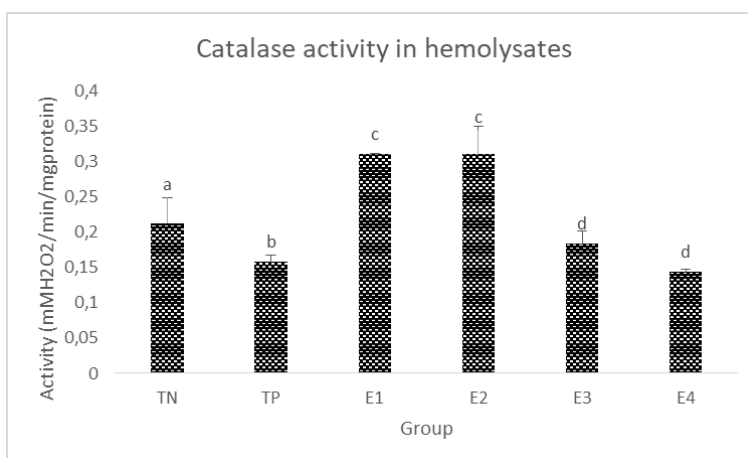


Figure 6. Effects of aqueous extracts of *C. anomala* and *C. arabica* on catalase activity in experimental rats

The values are expressed as Mean ± Standard deviation. TN : Negative Control (normal rats) ; TP : Positive Control (Cow diet + 12 mg/Kg MTX) ; E1 : Cow diet + 12 mg/Kg MTX + 200 mg/Kg *C. anomala* ; E2 : Cow diet + 12 mg/Kg MTX + 400 mg/Kg *C. anomala* ; E3 : Cow diet + 12 mg/Kg MTX + 200 mg/Kg *C. arabica* ; E4 : Cow diet +12 mg/Kg MTX + 200 mg/Kg *C. arabica*. The values affected with different letters (a, b, c, d) were significant.

Figure 7 is a representation of plasma ALT activity in experimental rats. The activity of this enzyme was found to be higher in the positive compared to the negative control group. The combination of MTX with extracts reduced the activity of this enzyme to levels comparable to non-treated rats. This was not true for the 200mg dose of *C. anomala* where activity was found to be comparable to the activity of the positive control group.

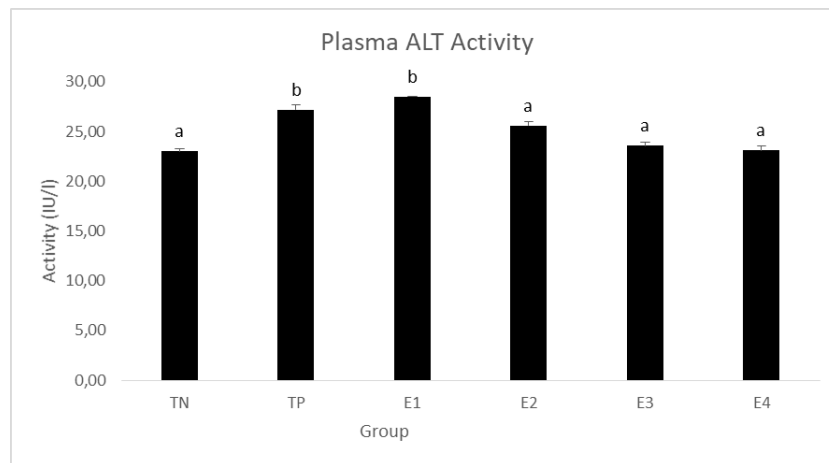


Figure 7. Effects of aqueous extracts of *C. arabica* and *C. anomala* on plasmatic Alanine amino-transferase (ALAT) levels in albino wistar rats

The values are expressed as Mean  $\pm$  Standard deviation. TN : Negative Control (normal rats) ; TP : Positive Control (Cow diet + 12 mg/Kg MTX) ; E1 : Cow diet + 12 mg/Kg MTX + 200 mg/Kg *C. anomala* ; E2 : Cow diet + 12 mg/Kg MTX + 400 mg/Kg *C. anomala* ; E3 : Cow diet + 12 mg/Kg MTX + 200 mg/Kg *C. arabica* ; E4 : Cow diet + 12 mg/Kg MTX + 200 mg/Kg *C. arabica*. The values affected with different letters (a, b, c, d) were significant.

In Figure 8, it can be seen that the level of plasmatic creatinine was more important in the positive control group compared to the negative control ( $p < 0,05$ ). Treated rats that equally received the aqueous extract of *C. anomala* had creatinine levels that were lower than for the negative control ( $p < 0,05$ ) while *C. arabica* group values were more or less similar to the negative control group of rats ( $p < 0,05$ ).

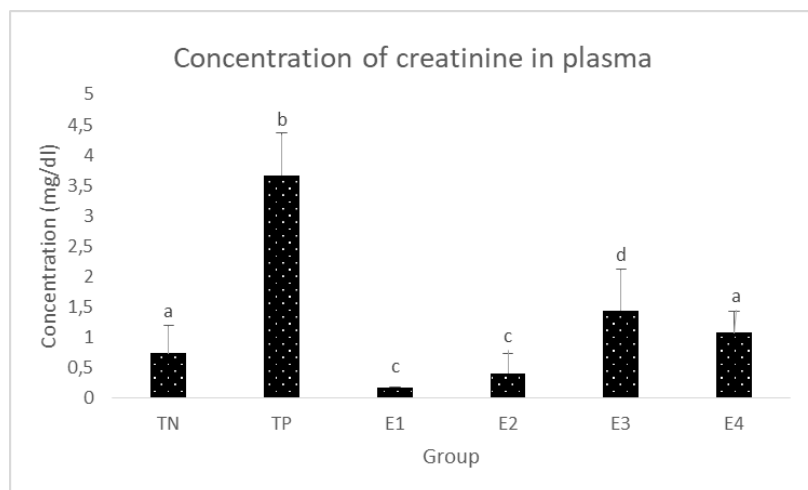


Figure 8. Effects of aqueous extracts of *C. anomala* and *C. arabica* on creatinine levels of plasma in experimental rats

The values are expressed as Mean  $\pm$  Standard deviation. TN: Negative Control (normal rats) ; TP : Positive Control (Cow diet + 12 mg/Kg MTX) ; E1 : Cow diet + 12 mg/Kg MTX + 200 mg/Kg *C. anomala* ; E2 : Cow diet + 12 mg/Kg MTX + 400 mg/Kg *C. anomala* ; E3 : Cow diet + 12 mg/Kg MTX + 200 mg/Kg *C. arabica* ; E4 : Cow diet + 12 mg/Kg MTX + 200 mg/Kg *C. arabica*. The values affected with different letters (a, b, c, d) were significant.



An increase in plasmatic urea levels was also observed after administration of MTX compared to the group that did not receive MTX (Figure 9). The extract-treated groups showed reduced levels of urea in the plasma of treated rats and the obtained values were comparable to those of the negative control. However, the dose 200 mg/kg.Bw of *C. arabica* showed a better reduction of urea level compared to all the other groups.

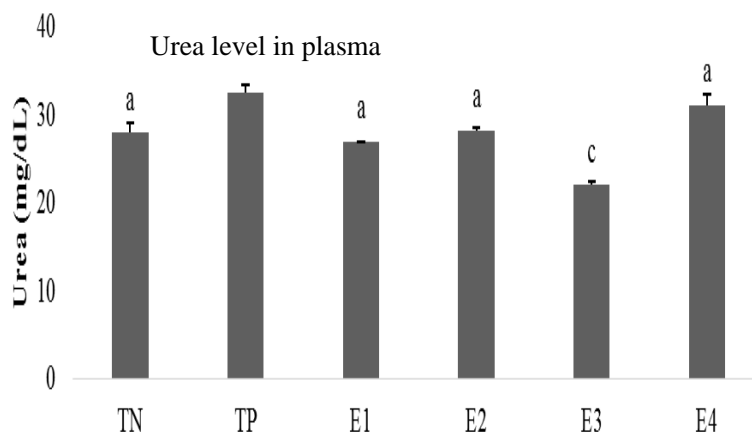


Figure 9. Effects of the aqueous extracts of *C. arabica* and *C. anomala* on the urea concentrations of the plasma of experimental rats

The values are expressed as Mean  $\pm$  Standard deviation. TN: Negative Control (normal rats) ; TP: Positive Control (Cow diet + 12 mg/Kg MTX) ; E1 : Cow diet + 12 mg/Kg MTX + 200 mg/Kg *C. anomala* ; E2 : Cow diet + 12 mg/Kg MTX + 400 mg/Kg *C. anomala* ; E3 : Cow diet + 12 mg/Kg MTX + 200 mg/Kg *C. arabica* ; E4 : Cow diet +12 mg/Kg MTX + 200 mg/Kg *C. arabica*. The values affected with different letters (a, b, c, d) were significant.

#### 4. Discussion

Weight loss is one of the most observed complications of chemotherapy due to an increase in treatment-related side effects (Bonomi, Batus, Fidler & Borgia, 2017). The results showed that even if weight gain in extract treated rats was not comparable to weight gain in non-treated rats, rats that received extracts in addition to MTX had better weight evolution compared to those that received MTX exclusively, suggesting a protective effect of the extracts against the limitations of weight gain induced by MTX. MTX is able to bind to neuropeptide Y (NPY) to decrease drug resistance by cancer cells (B dñme, Krieghoff & Beck-Sickinger, 2016). However NPY is a peptide neurotransmitter involved in the induction of food intake. Its association with MTX leads to a decrease in its activity resulting in a decrease in dietary intake, which is a direct consequence on weight gain in the positive control group compared to the negative control. On the other hand, the alkaloids present in our extracts with their stimulating action, could stimulate the neurotransmitter system and thus modulate the inhibitory action exerted by MTX on the NPY. Hussain *et al.* (2018) have shown that alkaloids thanks to their antioxidant and anti-amyloid as well as acetylcholine esterase inhibition, improve the functioning of the neurotransmitter system.

The antioxidant potential of each extract was evaluated using two methods: total antioxidant capacity (TAC) and scavenging of DPPH free radical. Both plant extracts were found to scavenge the DPPH free radical and reduce molybdate even if these activities varied according to the plant extracts. The activities observed could be linked to the presence of bioactive compounds that were found to be present in the prepared aqueous extracts. It has been demonstrated that polyphenols, flavonoids and alkaloids are important constituents that serve as powerful antioxidants due to the hydrogen-donating ability of their hydroxyl groups as well as their ability to donate electrons to stop the free radicals production involved in oxidative stress (Lin *et al.*, 2016; Gul çin *et al.*, 2010). For both tests, the extract of *C. anomala* was found to have a better antioxidant potential when compared to the extract of *C. arabica*. The amount of polyphenol was similar in both extracts while the amount of flavonoids was more important in the *C. arabica* extract and alkaloids more important in the extract of *C. anomala*. The activity of bioactive compounds depends more on the hydrogen and electron donating capacity than the amount of bioactive compound present in the sample. From this it could be thought that the antioxidant potential that was more important in *C. anomala* is due to the higher amount of alkaloids or due to bioactive compounds that are more active. This activity could also be due to the presence of other bioactive compounds that were not evaluated in this work. The above mentioned findings indicate that both *C. anomala* and *C. arabica* are sources

of phenolics, flavonoids and alkaloids and may therefore play a critical role as a singlet oxygen quencher and free radical scavenger to reduce molecular damage in the cell.

There is evidence that diseases such as cancer multiforms are highly related to oxidative stress. Also, oxidative stress may have a role in therapeutic mechanisms of anticancer agents like MTX. MTX decreases the effectiveness of the antioxidant defense system resulting in an increase in oxidative stress and MTX-induced oxidative stress has been observed in tumor tissues (Kolli, Nataraja, Isaac, Selvakumar & Abraham, 2014; El-sheikh, Morsy & Hamouda, 2016; Mahmoud, Hussein, Hozayen & El-Twab, 2017). Therefore, preventing oxidative damage and increasing antioxidants, could be an asset during cancer treatments. In this study, we investigated the influence of MTX treatment on the antioxidant defense system by evaluating the MDA levels and catalase activity in rats treated with MTX and MTX combined with either of our extracts. Associating both plants with MTX treatment reduced the MDA levels and increased catalase activity in experimental rats. It has been proven that the use of MTX induces lipid peroxidation with liberation of high levels of reactive oxygen and nitrogen species. The use of MTX has also been linked to the lowering of the activity of antioxidant enzymes such as catalase, superoxide dismutase and glutathione reductase (Montasser, Saleh, Ahmed-Farid, Saad & Mohamed-Assem, 2017). It can also cause an increase in the activity of these enzymes due to increased utilization for the scavenging of ROS generated due to oxidative stress (Saravanan, Ponmurugan, Sathiyavathi, Vadivukkarasi & Sengottuvelu, 2013). Catalase activity in this study was found to be lower in rats treated exclusively with MTX, meaning this treatment caused a reduction in its activity. The association of extracts to the MTX treatment protected the functioning of this enzyme as in this rats its activity was found to be higher. This mechanism can be further explained by the levels of MDA that was not found to be higher in rats that received MTX in combination with an extract compared to those that received only MTX. As a matter of fact, associating MTX with *C. anomala* provided lower levels of MDA levels for higher catalase activity (this was not the case with *C. arabica* where MDA levels were similar but catalase activity was higher than for the MTX exclusively treated group). From these results, the antioxidant defense system was found to be more protected by *C. anomala* than *C. arabica* in experimental rats. These results correspond to the *in vitro* results in this study where we found that the scavenging of DPPH free radical and the reduction of molybdate was more important with the aqueous extracts of *C. anomala* compared to the aqueous extract of *C. arabica*.

The role of bioactive molecules in the protection of the antioxidant defense system as is the case with *C. anomala* and *C. arabica* is of great importance in protecting biological systems from the deleterious effects of reactive oxygen and nitrogen species that are generated during cancer treatment with a chemotherapeutic drug. These reactive species have been found to increase cell damage by influencing the activity of the mitochondrial respiratory chain, causing inflammation, all leading to the damage of other biological molecules, like proteins and DNA (Lea, Tung & Zhou, 2015; Halliwell, 2002). All these are capable of leading to disorders in metabolic pathways. In this study was also evaluated the effect of our extracts on MTX-induced lipid metabolism disturbances by measuring some parameters of the lipid profile. MTX is a drug that is known to cause hepatic steatosis (Shetty *et al.*, 2017) that leads to modifications in blood lipid parameters. Treatment with MTX did not affect these parameters except for LDL-cholesterol that was found to be increased ( $p < 0.05$ ). It could be that the treatment was not long enough for disorders in these parameters to be observed. This interpretation can be supported by the fact that rats that received MTX in combination with extracts were found to have parameters that were better than those of rats that did not receive any treatment. The decrease in TG and LDL-cholesterol as can be observed in Figure 4 may be due to the activation of hepatic lipase which hydrolyzes TG-rich lipoproteins (LDL-c, VLDL-c) thus causing an increase in HDL-c (Abdulazeez, 2011). Also, decrease in total cholesterol may be due to a possible inhibition of the enzyme HMG-CoA reductase; the inhibition of this enzyme has been reported to prevent the production of hepatic cholesterol (Ahmadi, Ghorbanhagho & Argani, 2017). Comparing both extracts, showed that the aqueous extract of *C. arabica* ameliorated lipid parameters better than *C. anomala* especially with the 400mg dose ( $p < 0.05$ ).

The evaluation of protein, urea and creatinine levels and the activity of ALT were carried out to determine the level of damage that MTX treatment could have caused on the liver and kidneys of experimental rats.

The results showed that the total protein level was not different between groups. However, a significantly ( $p < 0.05$ ) elevated plasma ALT activity was observed in the group that received only MTX. Montasser and collaborators in 2017 also showed that treatment with MTX elevated plasma levels of ALT indicating liver toxicity since the release of this enzyme into the blood stream is an indicator of liver damage due to the destruction of liver cells through the loss of the functional integrity of hepatocytes leading to a leakage of its content into the blood stream (Montasser, Saleh, Ahmed-Farid, Saad & Mohamed-Assem, 2017). Both doses of *C. arabica* and the 400mg dose of *C. anomala* helped reduce ALT to levels that were comparable to that of

non-treated rats. It can thus be suggested that the aqueous extract of *C. arabica* protected the liver more than *C. anomala*. In this experimentation, MTX also increased the levels of creatinine and urea both indicators of renal toxicity. Harms *et al.* (2017) also carried out a study where plasma creatinine and urea levels were high after treatment with MTX (Harms, Khawaja, Taylor, Han & Mrug, 2017). As can be observed in Figures 8 and 9, the levels of these metabolic wastes were found to be lowered by associating MTX with either of the extracts. From these results, it can be suggested that both extracts improved glomerular filtration there by reducing the deleterious effects of MTX on the kidney. It is thought that this drop could be promoted by phenolic compounds contained in these extracts through their ability to protect the kidney from MTX-induced oxidative stress (Ahmed, Zaki & Nabil, 2015). Creatinine was lower in *C. anomala*-treated groups while urea was lower only for the 200mg dose of *C. arabica*.

## 5. Conclusion

The aim of this work was to compare the ability of *C. anomala* and *C. arabica* to protect rats against MTX-induced toxicity. Concerning the parameters evaluated in this study, both aqueous extracts of *C. anomala* and *C. arabica* protected against metabolic disorders that were generated by MTX. It appears that, these extracts contain important amounts of bioactive compounds with good scavenging and reducing capacity. These compounds helped increase antioxidant status of experimental rats; the consequence of this being the protection of rats exposed to MTX from renal and liver damage as well as improved lipid metabolism parameters. To a certain level, *C. anomala* protected the rats better against these deleterious effects compared to *C. arabica* at least for the two doses (200 and 400 mg/kg.Bw) and for the parameters investigated. All doses exhibited hepatoprotective and nephroprotective effects.

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