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IN VITRO ANTIOXIDANT AND FREE SCAVENGING ACTIVITIES OF EXTRACTS AND SOLUBLE FRACTIONS, ACUTE AND SUBACUTE TOXICITY OF AQUEOUS EXTRACT FROM CYMBOPOGON CITRATUS (DC.) STAPF (POACEAE) AND LIPPIA MULTIFOLIA MOLDENKE (VERBENACEAE) LEAVES

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ABSTRACT

The present study was initiated in the evaluation of antioxidant activity of extracts and soluble fractions from *Cymbopogon citraus and Lippia multifolia* leaves *in vitro* against fivr selected reactive oxygen species, (ROS) and acute and subacute toxicity of aqueous extract from both selected medicinal plants *in vivo*. Results revealed that aqueous and methanol 80% extracts from *C. citratus and L. multifolia* leaves exhibited pronounced antioxidant activity against all selected ROS with IC50 values < 10 µg/ml. Their respective chloroform, ethylacetate, *n*-butanol and residual soluble fractions also exhibited pronounced antioxidant activity by producing IC_{50} values < 10 µg/ml against all selected ROS. In acute and subacute toxicity, both aqueous extracts had no significant influence on the levels of hematological and biochemical parameters of treated rats since all reported values were found to be in acceptable physiological limits. In addition, they did not cause mortality of treated rats since their LD_{50} were estimated to be greater than 5000 mg/kg bw and did not significantly modify the vital organ weights and electrolytes levels of treated Wistar rats. Both aqueous extracts form *C. citratus and L. multifolia* were considered practically non-toxic *per os* supporting their use for a lo time without the occurring of side effects.

KEYWORDS: *Cymbopogon citratus*, Poaceae, *Lippia multifolia*, Vernenaceae, Extracts, Soluble fractions, antioxidant activity, Acute and Subacute toxicity.

1. INTRODUCTION

The utilization of medicinal plants in different form as macerates, decoctions, infusions, etc by people from practioners for the treating of various deseases is very common and currently practiced in rural and urban areas often in many developing countries worldwide. Their use is due to their various pharmacological properties proven in scientific works or empirical known by practioners in their daily practices. These different pharmacological effects are due to the presence of different chemical groups such as phenolics (flavonoids, tannins, phenolic acids, proanthocyanidins, anthraquinones, anthocyanins, etc.) alkaloids, xanthones, steroids, terpenoids and polysaccharides, etc. which may scavenge free radicals and reduce oxidative stress (Huyut et al., 2017; Ziarno et al., 2021).

Medicinal plants have been considered as primary and principal source of natural secondary metabolites endowed with different biological activities among which antioxidant property. Nowadays many of them are scientifically investigated to pertain their antioxidant effects especially *in vitro* test (Ekin et al, 2017; Cimanga et al., 2018; Chavan et al., 2018; Cardoso, 2019; Al-Laith et al, 2019) an their acute and sub-acute toxicity in animal model (Ali et al., 2015; Madingou et al, 2016; Cimanga et al., 2015; Unuofin et al., 2018; Ugwah-Oguejohor et al., 2019).

Nowadays, herbal medicines have received greater attention as alternative to clinical therapy an their demand has currently increased (Cimanga et al., 2018). People are increasingly interesting in choice of products of natural origin or those containing natural substitutes for synthetic additives to maintain their health in good state. They draw many attentions to information of medicinal plant extracts and their derived commercially available plant material, which are often used in traditional medicine for the treatment of various illnesses

and popular medicinal plants can be recommended for their use by people (Koslowska et al, 2022).

Reactive oxygen are free radicals formed by exogenous chemicals and endogenous metabolic process in human body. They are able or capable of oxidizing macrobio-molecules viz nucleic acids, proteins, lipids and can initiate different degenerative diseases like cancer, emphysema, diabetes, asthma, rheumatism, cirrhosis, etc. They played an important role in the development of some illnesses such as arthritis asthama, cardiovascular diseases, dementia, inflammation, Parckinson's disease, etc. They reacted with various biological molecules such as protein, nucleic acids, and lipids resulting in the creation of imbalance between oxidants and antioxidants (Narayanaswamy and Balakrishna, 2011; Nigam and Sodhi, 2014).

On the other hand, antioxidant compounds are responsible for the defense mechanism against various pathologies associated to the attack of free radicals, and thus, the intake of plant-derived antioxidants is involved in the prevention of various degenerative diseases caused by oxidative stress (Kaksmibai and Amirham, 2018). Antioxidant compounds can either be natural or synthetic products. Natural antioxidants can be obtained through diet in the form of fruits, spices, vegetables, etc. Synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole diminish oxidation phenomenon, but they have been proved to be dangerous to humankind.

The present study was conducted to evaluatate of antioxidant activity of extracts and soluble fractions from *Cymbopogon citratus* and *Lippia multifolia* leaves *in vitro* and acute and subacute toxicity *in vivo* of both aqueous extracts from the two selected medicinal plants.

2. MATERIALS AND METHODS

2.1 Plant material

Leaves of *Cymbopogno citratus* (lemongrass) and *Lippia moltifolia* were collected in Talngai village, Dumi quarter in Kinshasa-DRCongo. The plats were identified at Institut National d'Eudes et Recherches en Agronomie (INERA) at Department of Biology, Faculty of Sciences and Technology, University of Kinshasa where voucher specimens of the plants were deposited in the herbarium of this institute. The plan materials were dried at room temperature for one week and the dried materials were reduced to powders kept in brown bottles to avoid contamination before use.

(A)

(B)

Fiure 2: *Cymbopon citratus* (A) and *Lippia multifolia* (B).

2.2 Preparation of Extracts and Fractionation

50 g of each powder leaves of *C. citratus* and *L. multifolia* were separately mixed with 200 distilled water and boiled on hot plate maintained at 100°C for 15 minutes. After cooling and filtration on sterile cotton and Whatman paper filter N°1, each filtrate was evaporated on rotative evaporator giving respective dried extracts denoted as Cc-1 (32.64 g) for *C. citratus* and LM-1 (35.35 g) for *L. mulifolia* leaves. An amount of 10 g of each dried extract were dissolved in 100 ml distilled water and filtered as described above and each resulting filtrate was separately extracted successively and exhaustively with solvents with different polarities such as chloroform, ethylacetate and *n*-butanol. All fractions and respective residual aqueous phase were evaporated as described above yielding corresponding dried extracts denoted as Cc-1.1 (2.05 g), Cc-1.2 (2.20 g), Cc-1.3 (2.23 g) and Cc-1.4 (3.21 g) for *C. citartus* and Lm-1.1 (2.15 g), Lm-1.2 (2.25 g), Lm-1.3 (2.45 g) and Lm-1.4 (3.01 g) for *L. multifolia* corresponding to respective chloroform, ethylacetate, *n*-butanol and residual aqueous soluble fractions (Nsaka et al., 2012; Cimanga et al., 2015).

2.3 Evaluation of activities of reactive oxygen species

2.3.1 2,2'-Diphenyl-1-picrazylhydrazyl (DPPH)

The effects of extracts and soluble fractions from *Raphia sese* against DPPH was evaluated using methods previously described by Manthal al., 2019. For this 2 mg of each sample (methanol solution) were mixed with 2 ml MeOH to have sock solution of 1 mg/ml. These were next diluted in two foldilutions to have a series of test concentrations from 1 to 20 µg/ml). In 2 ml of test sample dilution, 1ml of DPPH 0.04% DPPH methanolic solution was added, mixed gently and all test tubes were

incubated in obscurity for 30 minutes. After, the absorbance of each test sample in experimental conditions, was measure at 517 nm using UV-VIS spectrophotometer (Shimaduzu UV PC-1600, USA) against a blank (0.04% DPPH methanolic solution 0.04%, and IC_{50} of each extract was derived from linear curves. The percentage inhibitions of DPPH activity was calculated using the following formula:

% Inhibition of activity of DDPH = Anc – Ats/Anc x 100

Where Anc was the absorbance the negative control and Ats the absorbance of the test sample.

2.3.2 2,2-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid or 2,2-Azinobis-(3-ethylbenzothiazoline sulfonate): ABTS

Against ABTS, the effects of extracts and soluble fractions from *R. sese* ripe dried fruits was assessed using methods previously described by de Vargas et al, 2016 and Al-Laith et al, 2019. In fact, 3.75 mg of potassium persulfate was dissolved in 1 ml distilled water. A total volume of 44 µl of this solution were added to 9.7 mg of ABTS dissolved in 2.5 ml methanol so as to prepare ABTS solution. ABTS solution was allowed to stand in the dark for about 15h at the room temperature. The working solution was prepared by mixing 1 ml ABTS solution with 88 ml of 50% methanol, 2 mg of each test sample was dissolved with 2 ml methanol to have stock solution of 1 mg/ml and after diluted in two foldilutions to have a series of test concentrations from 1 to 20 µg/ml. In 2 ml of each test dilution sample, 250 µl of ABTS solution were added mixed gently and allowed to stand for 4 min. Tha absorbance was read at 734 nm od UV-VIS spectrometer described as above. The percentage inhibition of the activity of ABTS radical was calculated using the same formula described above.

2.3.3 Superoxide anions: $O^{2.}$

The superoxide anion scavenging activity was measured based on procedures previously described by Esmaelie et al., 2015 and Chava et al., 2018. Superoxide anions were generated in a PMS-NADH sysyem (phenazine methosulfate-nicotinamide adenine dinucleotide phosphate) by oxidation of NADH and assayed through reduction of NBT (nitro blue tetrazolium). In this experiment, the superoxide anions radicals were were generated in 3 ml sodium phosphate buffer (100 Mm, pH 7.4, containing 1 ml NBT (150 µM) solution, 1 ml NADH (nicotinamide adenine dinucleotide phosphate) (468 µM solution) and different concentrations of test samples I methanol. The reaction started by adding 1 ml of PMS (phenazine methosulphate) solution (60 µM) to the mixture. The reaction mixture was incubated at 25°C for 5 minutes, and the absorbances were measured against blank solution. He decrease in the extent of NBT reduction measured by the absorbance of the reaction mixture correlated with the superoxide anions radicals scavenging activity the test samples. Le percentage inhibition of the activity of the radical was calculated using the same formula described above.

2.3.4 Hydroxyl radical: $OH^{\cdot}$

Deoxyribose assay was used to determine the hydroxyl radical scavenging activity in an aqueous medium by methods previously described by Lalhminglui et al., 2018. The reaction mixture containing $FeCl_3$ (100 mM), EDTA (1 mM), H_2O_2 (1mM) and 2-desoxy-D-ribose (2.8 mM) mixed with or without test samples of various test concentrations in 1 ml final reaction volume made with potassium phosphate buffer (20 mM, pH 7.4) and incubated for 1h at 37°C. The mixture was heated in water bath for 15 minutes followed by the addition of 1 mml of TCA (tricloro acetic acid) (2.8%) and TBA (thiobarbituric acid) (0.5%) in 0.025 mM containing BHA (butylated hydroxyanisole acid). Finally, the reaction mixture was cooled on ice and centrifuged at 5000 rpm for 15 minutes. Absorbances of supernatant were measured at 532 nm on the same apparatus describe above. All readings were corrected for any interference from color of the tested samples or antioxidant by including appropriate controls. The negative control without any antioxidant was considered 100% deoxyribose oxidation, The percentage inhibitions of hydroxyl radical scavenging activity of test samples were determined accordingly in comparison with negative control using the same formula described above.

2.3.5 Assay against hydrogen peroxide scavenging activity

This activity was evaluated using methods previously proposed by Leyton et al., 2015 and Bayramoglu et al., 2017. Solution of hydroxide peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations (1-20 µg/ml) of ascorbic acid as reference antioxidant product as positive control and test samples were separately added to hydrogen peroxide solution (0.6 ml, 40 mM). The absorbances of all test samples were measured at 230 nm after determination 10 minutes against a blank solution containing phosphate buffer with hydrogen peroxide. The inhibitions percentages of hydrogen peroxide activity was calculated using the formula already described above. In all tests, gallic acid and ascorbic acid were used as reference antioxidant products.

2.4 Total phenolic compounds determination

The total content of phenolic compounds was determined by methods previously described by Al-Rifai et al., 2017 using Folin-Ciocalteus's (FC) or Denis's (D) reagent (phosphotungtate/phosphomolybdate). 1 ml of aqueous and methanol 80% was mixed with 2 ml FC reagent diluted in water in proportion 1:10 (v/v) and 2 ml sodium carbonate (75 g/L) were added were added after 3 minutes. The tubes were vortexed for 15 seconds and allowed to stand for 20 minutes at 30°C for color development. Absorbances of test samples were measured at 760 nm on UV-VIS spectrophometer (Shimaduzu, USA). Total phenolic compounds content were expressed in terms of gallic acid equivalent (GAE) mg (mg GAE/100 g of extract) using gallic acid as reference standard (1-20 µg/ml).

2.5 Total flavonoids determination

Total flavonoids determination was estimated using aluminium chloride reagent (ACl_3 5% in methanol) by colorimetric assay previously % extracts described by Rahman et al., 2015 and Al-Rifai et al., 2017. To 1 ml of aqueous and methanol, and catechin (1-20 µg/ml) used as reference, 150 µl of 5% sodium nitrate and 2.5 ml of distilled water were added and mixed gently. After 5 minutes, 0.3 ml of $AlCl_3$ 5% was added and gently mixed again. At 6 min after, 1 ml NaOH 0.001M and 0.55 distilled water were added to the mixture, gently mixed and left at room temperature for 15 min. Absorbances of test samples and reference product were measured at 510 nm. Total flavonoids contents were expressed in terms of catechin equivalent mg (CAE)/100 g of dry extract.

2.6 Acute and Subacute toxicity

Two groups were used as followed:

-Group I was administered orally 5 ml distilled water as negative control,

- Group II (10 rats weighing 150-160 g body weight (bw) was given separately by the same way highest oral dose of 5000 mg/k bw of aqueous extracts Cc-1 and LM-1 from *Cymbopogon citratus* and *Lippia multifolia* leaves in acute toxicity In subacute toxicity, the groups of rats and the administration way were the same as in acute toxicity. Aqueous Cc-1 and Lm-1 extracts were separately administered daily at oral doses of 500, 1000 and 5000 mg/kg bw. In both toxicity tests, all rats were observed daily for the occurring sides effects and the mortality was recorded if possible (Unuofin et al, 2018; Ugwah-Oguejohor et al, 2018; Cimanga et al., 2021).

2.7 Estimation of Hematological and Biochemical

Bloods from rats having received highest oral dose of 5000 mg/kg bw in subacute toxicity od aqueous Cc-1 and Lm-1extracts from *C. citratus and L. multifolia* leaves was collected from tail vein on Day-28 at the end of experimental toxicity for analysis. For biochemical parameters, blood was centrifuged at 4000 g for 5 min to obtain plasma, which was stored at -20°C: glucose, creatinine, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), uric acid, total cholesterol, low-density lipoproteins (LGL), high density of lipoproteins (HDL) and other were quantified using Architect (Abottâ) automation with Boehringen Ingelhemâ biochemical kits. Total proteins were estimated using Biuret's method (Saha et al., 2011). Hematological parameters were analyzed using an automatic hematological analyzer (coulter STK, Beckam) with appropriated kits. The differential leucocytes count was performed with optical microscopy after staining and in each case, 100 cells were counted (Akpamabiatu et al., 2013).

2.8 Determination of serum electrolytes

All rats in subacute toxicity having received 5000 mg/kb bw of aqueous Cc-1 and Lm-1 extracts from *C. citratus and L. multifolia* leaves were sacrificed. The blood of each rats was collected by cardiac puncture with sterile syringes and emptied into prelabeled centrifuge tubes for serum separation. They were left in stand at room temperature for 30 min before centrifuging at 4000 rpm for 5 min in 80-1 electric centrifuge (Bram Scientific an Instrument Co, England).

Electrolytes potassium, calcium and chloride estimation was carried out by colorimetric method using Bioxer reagent kits (UK-BXCO141A for K, BXCO291A for Ca and BXCO281A for Cl^-, carefully following the instructions and specifications of the manufacturer. The estimation of bicarbonate (HCO^{3-}) and sulphate SO^{4-}) level was carried out by titration and that of sodium (Na) by photometer (IL 943). The remaining other electrolytes were determinated using and electrolyte auto-analyzer Landwid LW E60B used also by Imo et al., 2019 and by flame photometer (AOAC, 1984; Cimanga et al., 2021).

2.9 Evaluation of vital organs weights

Vital organs like kidneys, liver, pancreas, lungs, spleen, ovaries, testicles or testis and heart of treated rats having had 5000 mg/kg bw of aqueous Cc-1 and Lm-1 extracts, were removed on Day-29 and plentifully washed with distilled water, placed on a saline soaked gauze and dried at 50°C in a hot and weighted until constant weight (Abubakar et al., 2019; Cimanga et al., 2021).

3. RESULTS AND DISCUSSION

3.1 Antioxidative active of Extracts and Soluble fractions

This antioxidant activity of extracts and soluble fractions from *R. sese* was evaluated and following criteria were taken account for good understanding of reported results; IC_{50} < 10 µg/ml; pronounced activity, 10 < IC_{50} < 20 µg/ml, good activity, 20 < IC_{50} < 30: moderate activity, 30 < IC_{50}< 50 µg/ml, low or weak activity, IC_{50} > 50 µg/ml; inactive.

For *C. citartus* samples, it was firstly observed that aqueous Cc-1 and methanol 80% CC-2 extracts exhibited pronounced antioxidant activity against all selected reactive oxygen species (ROS) by producing IC_{50} values of 4.55±0.08, 4.25±0.04, 5.02 ± 0.04, 7.16 ± 0.04 and 7.22 ± 0.03 µg/ml while methanol 80% extract Cc-2 exerted the same activity with IC_{50} values 3.20 ± 0.4, 4.23 ± 0.00, 5.53 ± 0.088, 7.06 ± 0.05, 7.06 ± 0.05 and 6.54 ± 0.01 µg/ml respectively. The IC50 values developed by methanol extract Cc-2 were in general low as sign of its high activity compared to aqueous extract Cc-1 suggesting the influence of the solvent used (water versus methanol 80%). In the same order, soluble fractions chloroform Cc-1.1 rich in steroids and terpenoids, ethylacetate Cc-1.2 rich in flavonoids, *n*-butanol Cc-1.3 rich in saponins and residual aqueous Cc-1.4 rich in other phenolic compounds than flavonoids, acted also in same manner by displayed pronounced antioxidant activity against all selected ROS by producing varying IC_{50} values from 3.20±0.04 to

11.2±0.04 µg/ml, excepted well chloroform Cc-11 soluble fraction showing moderate activity against hydrogen peroxide H_2O_2. Among these fractions, ethylacetate soluble fraction rich in flavonoids showed high activity compared to other soluble fractions since its IC_{50} values were low compared to those produced by other fractions (Table 1).

By examination IC_{50} values of some soluble fractions, their values were gradually an suggested that they exhibited similar activity such as soluble fraction Cc-1.2 compared to Cc-.1.4. showing that Cc-1.2 acted against DPPH with IC_{50} value of 5.00±0.05 and Cc-1.4 with

Table 1: Effects of Extracts and Soluble fractions from *C. citratus* leaves on reactive oxygen species (IC_{50}, µg/ml).

Sample codes	DPPH	ABTS	$O^{2.}$	$OH^{\cdot}$	H_2O_2
Cc-1	4.55±0.08	4.25±0.04	5.02±0.04	7.16±0.04	7.22±0.03
Cc-1.1	6.65±0.4	7.15±0.06	8.06±0.05	9.25±0.05	11.2±0.04
Cc-1.2	5.00±0.05	4.04±00.7	4.22±0.04	6.45±0.07	7.31±0.03
Cc-1.3	7.25±0.08	8.84±0.03	9.05±0.04	9.32±004	9.86±0.06
Cc-1.4	5.02±0.05	4.12±003	4.35±0.05	6.66±0.05	7.74±0.06
C-2	3.20±0.04	4.23±0.00	5.53±0.08	7.06±0.05	6.54±0.01
TPC	2.75±0.03	2.05±0.04	4.11±0.05	5.32±0.03	5.15±0.02
TF	3.05±06.04	2.85±0.01	3.75±0.03	4.56±0.05	4.73±0.04
Gallic acid	2.06±0.02	3.12±0.02	4.65±0.00	8.47±0.00	7.82±0.03
Ascorbic acid	3.53±0.00	2.67±0.01	7.15±0.02	7.55±0.02	8.02±0.01

Cc-1: aqueous extract, Cc-1.1 to 1.4: chloroform, ethylacetate, *n*-butanol and residual aqueous soluble fractions respectively from the partition of Cc-1 extract, TPC: total phenolic compound, TF: total flavonoid.

IC_{50} of 5.02±0.03 µg/ml, against ABTS, Cc-1.2 with IC_{50} value of 4.04±0.08 and Cc-1.4 with 4.12±0.03 µg/ml, against hydrogen, Cc-1.2 with 6.45±0.07 and Cc-1.4 with 6.66±0.05 µg/ml,. against superoxide anion IC1.2 with 4.22±0.04 and Cc-1.4 with 4.35 ±0.05 µg/ml and against hydrogen peroxide Cc-1.2 with 7.31 and Cc-1.4 with 7.74 µg/ml with no significant difference Soluble fraction Cc-1.3 rich in saponins showed high IC_{50} values against all tested ROS as a sign of its low activity compared to other soluble fractions, although it showed high activity against hydrogen peroxide compared to Cc-1 soluble fraction rich in steroids and terpenoids.

In addition, all soluble fractions showed low activity compared to the parent aqueous extract Cc-1 suggesting that these soluble fractions could act in synergistic manner to restore the high activity of the parent extract as also previously reported by Cimanga et al., 2020; 2021.

By comparison the activity of tested samples from *C. citratus* to reference products, it was observed that Cc-1 (5.02±0.04 µg/ml), Cc-1.2 (4.22±0.04 µg/ml), Cc-1.4 (4.33±0.05 µg/ml), C-2 (5.53±0.08 µg/ml, TPC (5.32±0.03 µg/ml) and TF (4.56±0.05 µg/ml) exhibited high activity against superoxide anion compared to ascorbic acid (7.15±0.02 µg/ml), Cc-1 (7.16±0.04 µg/ml), Cc-1.2 (6.45±0.07 µg/ml), Cc-1.4 (6.66±0.05 µg/ml), C-2 (7.06±0.05 µg/ml, TPC (5.32±0.03 µg/ml- and TF (4.56±0.06 µg/ml) showed high against hydroxyl radical activity compared to ascorbic acid (7.55±0.02 µg/ml) and gallic acid (8.47±0.00 µg/ml), and Cc-1 (7.22±0.03 µg/ml), Cc-1.2 (7.31±0.03 µg/ml), Cc-1.4 (7.74±0.06 µg/ml), C-2 (6.54±0.01 µg/ml), TPC (5.15±0.02 µg/ml) and TF (4.73±0.04 µg/ml) displayed high activity against hydrogen peroxide compared to ascorbic acid (8.02±0.02 µg/ml) and gallic acid (7.82±0.03 µg/ml. But the activity of gallic acid and ascorbic acid against DDPH and ABTS radical was high compared to all samples from *C. citratus* leaves (Table 1).

Moreover, C-1 (7.16±0.04 µg/ml, Cc-1.2 (6.45±0.07 µg/ml), Cc-1.4 (6.66±0.05 µg/ml), C-2 (7.06±0.05 µg/ml, TPC (5.32±0.03 µg/ml and TF (4.36±0.05 µg/ml) showed high activity compared to gallic acid (8.47±0.00 µg/ml) against hydroxyl radical and C-1 (7.22±0.03 µg/ml), Cc-12 (7.31±0.03 µg/ml) Cc-1.4 (7.74±0.06 µg/ml, C-2 (6.54±0.01 µg/ml), TPC (5.15±0.02 µg/ml and TF (4.73±0.04 µg/ml) exhibited high activity against gallic acid (7.82±0.03 µg/ml) against hydrogen peroxide. Cc-1.2 (4.22±0.04 µg/ml), Cc-1.4 (4.35±0.06 µg/ml), TPC (4.11±0.05 µg/ml) and TF (3.75±0.03 µg/ml) showed high activity compared to gallic acid (4.65±0.00 µg/ml) against superoxide anion radical. Only TPC extract (2.05±0.04 µg/ml and 2.67±0.01 µg/ml exhibited high activity compared to gallic acid (3.12±0.02 µg/ml and ascorbic acid (2.67±0.01 µg/ml) respectively.

Results indicated that aqueous and methanol 80% extracts Lm-1 and Lm-2 displayed pronounced antioxidant activity with IC_{50} values ranging from 4.05±0.08 to 7.02±0.05 µg/ml for aqueous extract Lm-1 and from 3.80±0.04 to 6.24±0.01 for methanol 80% extract Lm-2. Lm-2 extract showed high activity compared to Lm-1 extract for the same reason evoked above.

Moreover, its soluble fractions chloroform Lm-1.1 rich in steroids and terpenoids, ethylacetate Lm-1.2 rich in flavonoids, *n*-butanol Lm-1.3 rich in saponins and

residual aqueous Lm-1.4 rich in other phenolic compounds than flavonoids exerted also pronounced activity when tested against all selected ROS with varied IC_{50} values ranging from 4.82±0.05 to 9.55±0.02 µg/ml. Ethyacetate Lm-1.2 soluble fraction showed high activity since its IC_{50} values produced against all tested ROS were low compared to the remaining soluble fractions (Table 2). It was also observed that Lm-1.2 (4.54±0.06 µg/ml) and Lm-1.4 (4.62±0.07 µg/ml) and Lm-1.2 (6.11±0.07 µg/ml and Lm-1.4 (6.45±0.05 µg/ml) presented similar antioxidant activity when tested against ABTS and H_2O_2 radicals respectively since no significant difference was deduced ($p < 0.05$). As observed for *C. citratus* soluble fractions, those from *L. multifolia* exerted low activity compared to the parent aqueous extract Lm-1 due to the same reasons evoked above.

Table 2: Effects of Extracts and Soluble fractions from *L. multifolia* leaves on reactive oxygen species (IC_{50}, µg/ml).

Sample codes	DPPH	APTS	$O^{2\cdot}$	$OH^{\cdot}$	H_2O_2
Lm-1	4.05±0.08	3.25±0.07	5.12±0.07	6.06±0.02	7.02±0.05
Lm-1.1	6.55±0.05	7.02±0.02	8.00±0.06	9.15±0.08	10.7±0.00
Lm-1.2	4.82±0.05	4.54±00.6	4.11 ± 0.04	5.36. ±0.02	6.11±0.07
Lm-1.3	7.05±0.08	8.64±0.03	9.15±0.05	9.12±007	9.55±0.02
Lm-1.4	5.12±0.05	4.02±007	4.45±0.05	6.15 ± 0.08	6.45±0.05
Lm-2	3.80±0.04	3.03±0.00	5.03±0.06	5.76±0.05	6.24±0.01
TPC	2.75±0.03	2.05±0.04	4.11±0.05	5.32±0.03	5.15±0.02
TF	3.05±06.04	2.85±0.01	3.75±0.03	4.56±0.05	4.73±0.04
Gallic acid	2.06±0.02	3.12±0.02	4.65±0.00	8.47±0.00	7.82±0.03
Ascorbic acid	3.53±0.00	2.67±0.01	7.15±0.02	7.55±0.02	8.02±0.01

Lm-1: aqueous, Lm-1.1 to 1.4: chloroform, ethylacetate, n-butanol and residual aqueous soluble fractions from the partition of Lm-1 extract, C-2: methanol 80% extract, TPC: total phenolic compounds, TF: total flavonoids.

Total phenolic compounds (TPC) exerted antioxidant activity against DPPH, ABTS, $O^{2\cdot}$, $OH^{\cdot}$ and H_2O_2 radicals with IC_{50} values of 2.75±0.03, 2.05±0.04, 4.11±0.05, 5.32±0.03 and 5.15±0.02 µg/ml respectively while total flavonoids (TF) acted the same manner against the same radicals by producing IC_{50} values of 3.05±0.05, 2.85±0.01, 3.75±0.03, 4.56±0.05 and 4.73±0.04 µg/ml respectively. TPC showed high activity compared to TF because the first extract contained more different phenolic compounds compared to TF extract containing only one category of phenolic compound being flavonoids.

By the comparison of the antioxidant activity developed by samples from *L. multifolia* leaves to those shown by reference products, it appeared that Lm-1 (5.12±0.07 µg/ml), Lm-1.2 (4.11±0.04 µg/ml), lm-1.4 (4.45±0.05 µg/ml) and Lm-2 (5.03±0.00 µg/ml) exerted high activity against superoxide anion compared to ascorbic acid (7.5±0.02 µg/ml). Lm-1 (6.06±0.02 µg/ml), Lm-1.2 (5.35±0.02 µg/ml), Lm-1.4 (6.15±0.06 µg/ml) and Lm-2 (5.76±0.05 µg/ml) exhibited high activity against hydroxyl radical compared to ascorbic acid (7.55±0.02 µg/ml) and gallic acid (7.82±0.03 µg/ml). Lm-1 (7.02±0.05 µg/ml), Lm-12. (6.11±0.07 µg/ml), Lm-1.4 (6.45±0.05 µg/ml) and Lm-2 (6.24±0.01 µg/ml) showed high activity against hydrogen peroxide radical compared to ascorbic acid (8.02±0.01 µg/ml) and gallic acid (8.47±0.00 µg/ml). Howerver, some samples from *C. citratus* and *L. multifolia* showed similar antioxidant activity compared to ascorbic and gallic gallic because their respective IC_{50} were almost similar (Table 1 and 2). These reference products ascorbic acid and gallic acid presented high activity compared to the remaning samples not mentioned in this step.

3.2 Estimation of total phenolic compounds and flavonoids content

Total phenolic compounds and flavonoids were determined by colorometric methods as described above. Results indicated that that aqueous Cc-1 of *C.citratus* extract contained 20.36 mg of total phenolic compounds and 6.22 mg of total flavonoids while methanol 80% Cc-2 extract contained 30.13 mg of total phenolic compounds and 10.51 mg of total flavonoids expressed in gallic acid and catechin respectively. Aqueous extract Lm-1 of *L. multifolia* contained 28.03 mg of phenolic compounds and 12.02 mg of total flavonoids while methanol extract Lm-2 contained 32.32 mg of total phenolic compounds and 10.02 mg of total flavonoids. Methanol 80% Cc-2 and Lm-2 extracts contained high amount of both constituents compared to aqueous Cc-1 and Lm-1 extract due probably to the influence of the used solvent extractive (water versus methanol 80%).

3.3 Acute and Subacute toxicity

Results revealed no any toxic symptom signs of effects at the oral administration of highest dose of 5000 mg/kg bw of both aqueous extracts Cc-1 and Lm-1 form *C. citratus* and *L. multifolia* leaves, after 28 days of observation. Wistar rats having received this high dose gained body weight compared to negative control as illustrated in figure 2a an b.

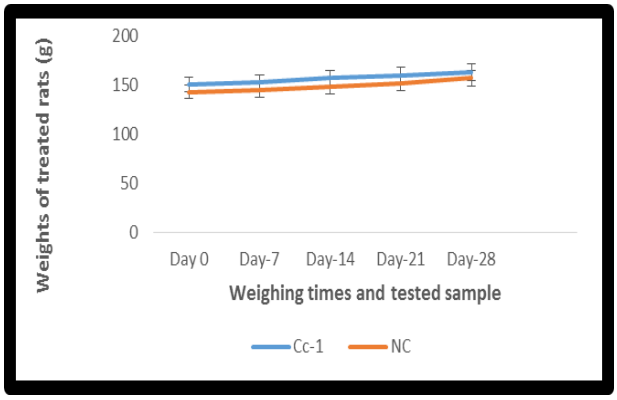

Figure 2a: Variation of treated wistar rats weights to administered aqueous extract Cc-1 of *Cymbopogon citratus* leaves.

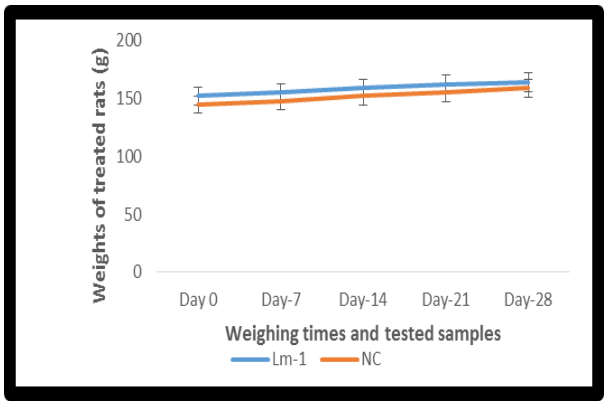

Figure 2b: Variation of treated Wistar rats weights to administered aqueous extract Cc-1 of *Lippia multifolia* leaves.

No mortality of treated rats was recorded and the lethal dose 50 (LD_{50}) of both aqueous extracts was estimated to be greater than 5000 mg/kg bw. According with Kennedy at al., 1986, the studied aqueous extracts Cc-1 and Lm-1 from *C. citrtatus a*nd *L. multifolia* leaves were considered as practically non-toxic and safe by oral route.

3.4 Effects of aqueous Cc-1 from *C. citratus* on hematological parameters of treated Wistar rats at highest oral dose of 5000 mg/kg bw

Results indicated that no basophil was found in negative control and treated rat groups with aqueous Cc-1 and Lm-1 extracts from *C. citratus* ad *L. multioflia* leaves at highest oral dose of 5000 mg/kg bw and was found to be in intervals of reference values. Eosinophils knew slight increase without significant difference compared to negative control ($p < 0.05$) while the levels of hematocrit and hemoglobin in treated rats were significantly increased compared to negative control. The increase of hemoglobin level may be due to the absorption of iron and copper in high amount by treated rats from the administered aqueous extract and to the

Table 3: Effects of aqueous Cc-1 extract from *C. citratus* on hematological parameters of treated Wistar rats at highest oral dose of 5000 mg/kg bw.

Parameters	Negative control	Cc--1: 5000 mg/kg bw	Reference values
Basophils (%)	0.00±0.00	0.00±0.00	0.00±0.00
Eosinophils (%)	0.13±0.06	0.015±0.05	0.01-0.16
Hematocrit (%)	44.67±0.05	48.03±0.04	40.4-50
Hemoglobin (g/dl)	16.08±0.06	17.46±0.04	15.0-18.28
Lymphocytes (%)	88.52±0.05	90.03±0.05	58.8-94
Monocytes (%)	0.28 ±0.03	0.32±0.04	0.0-0.40
Platelets (x 103 μl^{-1})	1202.36±0.08	1196.45±0.06	995-1713
Segmented leucocytes (%°	16.32±0.06	18.11±0.04	-
RBC (x 106 μL^{-1})	8.71±0.08	8.85±0.06	7.6-10.29
WBC (x 103 μL^{-1})	15.85±0.03	17.75±0.06	6.6-20.5

Immunopotentianting effects of both auqeous extracts as also previously reported by Tamwar et al, 2019; Cimanga et al., 2020; 2021).

This observation was also valid for the levels lymphocytes and monocytes compared to negative control. A significant decrease was recorded for platelets in treated rats compared to negative control suggested the administered aqueous extract from *R. sese* ripe dried fruits could precipitate thrombocytopenia that was one of the common hematological disorders, characterized by an abnormally high number of number of platelets from multiple causes (Izak and Brussel, 2014).

Significant increase of segmented Leucocytes and white blood cells red blood cells level were observed in treated rats while red blood level showed slight increase compared to negative control.

Table 4: Effects of aqueous Cc-1 extract from *Lippia multifolia* on hematological parameters of treated Wistar rats at highest oral dose of 5000 mg/kg bw.

Parameters	Negative control	Lm-1: 5000 mg/kg bw	Reference values
Basophils (%)	0.00±0.00	0.00±0.00	0.00±0.00
Eosinophils (%)	0.14±0.06	0.015±0.05	0.01-0.16
Hematocrit (%)	45.67±0.07	47.08±0.05	40.4-50
Hemoglobin (g/dl)	15.08±0.04	17.86±0.04	15.0-18.28
Lymphocytes (%)	86.52±0.03	92.03±0.05	58.8-94
Monocytes (%)	0.26 ±0.03	0.30±0.05	0.0-0.40
Platelets (x 103 μl^{-1})	1212.36±0.08	1208.40±0.06	995-1713
Segmented leucocytes (%°	16.22±0.06	18.31±0.04	-
RBC (x 106 μL^{-1})	8.51±0.05	8.82±0.06	7.6-10.29
WBC (x 103 μL^{-1})	15.55±0.03	17.85±0.06	6.6-20.5

3.5 Effects of aqueous Cc-1 extract from *C. citratus* leaves on biomedical parameters of treated Wistar rats at highest oral dose of 5000 mg/kg bw

Results of the effects of administered aqueous Cc-1 and Lm-1 extracts were presented in Table 4 and 5. They revealed a significant increase in the levels of albumin, alkaline phosphate (ALP), alanine transferase (ALT); aspartate transferase (AST) and creatinine in treated rats compared to untreated rats with significant difference ($p < 0.05$).

Albumin was proteins produced in the liver and had high concentration in plasma. Its decrease in serum may arise from liver diseases mainly in cirrhosis (Carvaldho and Machado, 2018; Bai et al., 2019). ALT and AST were well known as good indicator liver enzymes for this organ and the elevation of their level in blood can indicate the damage of parenchymal liver cells and perturbation of the function of this organ or frequently referred to hepatotoxicity and ALP was also considered as one of the biomarkers predicting probable toxicity of the liver (Unuofin et al., 2018; Moussaoui et al., 2020). In addition, negative reactions and deleterious effects on the activity of these hepatic biomarkers were not observed as also previously reported by Moussaoui et al., 2020 for the medicinal plant *Withania frutescens* hydro-alcoholic extract leaves. The level of direct bilirubin was not changed in both groups. The glucose level in treated rats was found to be significantly decreased in treated rats compared to untreated rats. This effects may be due probably to the hypoglycemic property possessed by the administered aqueous extract which was beneficial in time for the treatment of diabetes type 2 mellitus (Nsaka et al., 2012; Cimanga et al., 2015).

A significant decrease of total cholesterol and low density lipoproteins (LDL) as well as significant increase of high density lipoproteins (HDL)w were observed in treated rats compared to untreated group.

Table 5: Effects of aqueous Cc-1 extract from *C. citratus* on biochemical parameters of treated Wistar rats at highest oral dose of 5000 mg/kg bw.

Parameters	Negative control	Cc-1:5000 mg/kg bw	Reference values
Albumin	3.50±0.05	3.82±0.06	3.8-4.8
ALP (IU/L)	117.32±0.07	128.52±0.04	50.8-128
ALT (IU/L)	37.63±0.04	40.02±0.06	17.5-45
AST (IU/l)	127.30±0.08	137.62±0.05	45.3-143
Creatinine (mg/dl)	0.55±0.03	0.60±0.05	0.20-0.80
Direct bilirubin (mg/dl)	0.04±0.01	0.04±0.00	0.03-0.05
Glucose (mg/dl	167.5±0.05	151.21±0.04	148-208
HDL-cholesterol (mg/dl)	65.53±0.04	68.21±0.03	> 35
LDL-cholesterol	56.32±0.03	52.85±0.36	< 130
Total bilirubin (mg/dl)	0.44±0.10	0.48.30±0.07	0.20-0.70
Total cholesterol (mg/dl)	116.32±0.06	114.83±0.04	41-126

Total proteins (g/dl)	7.50±0.05	7.61±0.04	5.7-7.6
Triglycerides (mg/dl)	86.35±0.03	93.68±0.04	20-144

Table 6: Effects of aqueous Cc-1 extract from *L. multifolia* leaves on biochemical parameters of treated Wistar rats at highest oral dose of 5000 mg/kg bw.

Parameters	Negative control	Lm-1:5000 mg/kg bw	Reference values
Albumin	3.57±0.05	3.92±0.03	3.8-4.8
ALP (IU/L)	115.32±0.05	122.52±0.04	50.8-128
ALT (IU/L)	38.63±0.04	41.02±0.06	17.5-45
AST (IU/l)	129.30±0.05	135.62±0.05	45.3-143
Creatinine (mg/dl)	0.55±0.03	0.61±0.04	0.20-0.80
Direct bilirubin (mg/dl)	0.04±0.01	0.04±0.00	0.03-0.05
Glucose (mg/dl	170.5±0.05	168.51±0.04	148-208
HDL-cholesterol (mg/dl)	63.53±0.03	66.21±0.08	> 35
LDL-cholesterol	67.32±0.08	72.85±0.06	< 130
Total bilirubin (mg/dl)	0.46±0.10	0.50.30±0.07	0.20-0.70
Total cholesterol (mg/dl)	120.32±0.06	124.83±0.05	41-126
Total proteins (g/dl)	7.55±0.05	7.71±0.07	5.7-7.6
Triglycerides (mg/dl)	95.35±0.03	98.68±0.05	20-144

These effects might be attributed to the hypolidemic property of the administered aqueous Cc-1 and Lm-1 extracts and in some times, to the increase of the secretion of thyroid hormones T3 and T4 which degraded cholesterol. It was also necessary for the gestion of some degenerative diseases such as cardiovascular diseases, was correlated with risk factors contributing to the death of patients affected of diabetes mellitus type 2. (Akpanabiattu et al., 2013; Cimanga et al., 2015; https://wikipedia.org/wiki/Thyroid_hormones, 2021).

Total bilirubin and triglycerides knew significant increase of their level in treated rat group compared to untreated group with significant difference ($p < 0.05$) while an increase of total proteins level was observed in treated rat groups compared to untreated group suggesting a supplement apport of an exterior supply, but no significant difference was not observed in comparison both groups ($p > 0.05$).

All reported hematological and biochemicam levels of different evaluated parameters in the present study were comparable to other values reported previously in other studies (Oloro et al., 2016; Loha et al., 2019; Gosh et al., 2019; Cimanga et al., 2020) and were in limits of previously reported reference values (http://www.over.com/sites/defaut/files/resoources/rm_rn_r.Wi, 2003; https://lab.dgsm.edea.edu/dlam/files/view/das/diagnostic-lak, 2023).

3.6 Effects of aqueous Cc-1 and Lm-1 extracts of electrolytes and vital organ weights

3.6.1 Effects of aqueous Cc--1 extract from *C. citratus* leaves on electrolytes

Table 5 reported the influence of aqueous Cc-1 and Lm-1 extracts on electrolytes levels. Results indicate that the oral separately administration of these both aqueous in treated rats caused significant increase of all electrolytes levels (Table 8). And all reported values in the present study were found in the limit of reference values (Cimanga et al., 2021). The increase levels of these electrolytes in treated rats compared to negative control may be due to a supplement apport from the administration both selected aqueous extracts.

Chloride was important electrolyte in the maintenance of the cation/anion. It is essential for the control of proper hydratation, osmotic pressure and acid/base equilibre. Low chloride level was found in burns, excessive vomiting, intestinal Addisonian crisis, nephritis, metabolic acidosis, etc. Elevated serum chloride level may be occur in dehydratation, hyperventilation, congestive heart, etc (Imo et al., 2019).

Potassium ion was a major cation in the intracellular fluid and only about 10% was extracellular. Variations in serum potassium level were known to have serious health complications, particularly excess of this element in blood can occur in cases of renal failure that loosed the ability to excrete this mineral. Multiple hydratation can produce hyperkalemia having as consequences muscle weakness, hyptonia and cardiac arrhythmia that can lead to heart failure and can predispose to cardiac arrest (Enemor et al., 2013; Cimanga et al., 2021). To have high level of K (hyperkalemia), was often associated with the occurring or presence of renal failure; dehydratation shock and renal insufficiency. While decreased K level (hypokalemia)

Table 7: Electrolyte levels of treated rats with aqueous Cc-1 extract form C. citartus leaves at 5000 mg/kg bw.

Electrolytes	Negative control	Cc-1: 5000mg/kg bw	Reference values
Calcium	9.75±0.05	12.25±0.05	5.3-13
Chloride	96.65±0.06	108±0.04	95-110
Inorganic phosphorus	8.62±0.06	9.51±0.06	3.4-11
Iron	7.58±0.05	9.25±0.01	-
Potassium	4.5±0.5	5.16±0.05	3.5-5.5
Sodium	135.63±0.06	138.56±0.05	43-156
Sulphate	63.28±0.03	65.12±0.05	-
Bicarbonate	28.13±0.06	30.25±0.06	24-31

Table 8: Electrolyte levels of treated rats with aqueous *L. multifolia* leaves extract at 5000 mg/kg bw.

Electrolytes	Negative control	Lm-1: 5000mg/kg bw	Reference values
Calcium	9.85±0.03	12.47±0.05	5.3-13
Chloride	96.50±0.06	106.56±0.2	95-110
Inorganic phosphorus	8.41±0.02	9.1 1±0.05	3.4-11
Iron	7.88±0.05	9.0 5±0.07	-
Potassium	4.1±0.1	5.56±0.07	3.5-5.5
Sodium	145.63±0.06	148.55±0.08	43-156
Sulphate	60.28±0.03	64.42±0.01	-
Bicarbonate	26.13±0.05	29.685±0.02	24-31

Was mainly associated with malnutrition, negative nitrogen balance, gastro-intestinal fluid losses and hyperactivity of the adrenal cortex and other liquids in human body (Imo et al., 2019).

Bicarbonate was estimated to monitor that acidity of the blood and body fluids, and results may indicate that that acidity of the medium was maintained. Its content was a significant indicator of electrolytes dispersion and anion deficit. Alteration of bicarbonate associated to carbone dioxide (CO_2) dissolved in plasms was characteristic of acid-base imbalance, which can be due to renal tubular acidosis, hyperkalemic acidosis, renal failure and ketoacidosis (Imo et al., 2019).

Moreover, electrolytes like K, Na, Cl^- and HCO^{3-} were among other parameters that were useful in the determination of kidney and liver functions and the increasing or decreasing level of any of them may be an indicator of kidney and liver problems (Amagon et al., 2020; Cimanga et al., 2021). The reported levels of selected electrolytes in the present study were comparable to other reported in various previously studies (Abubakar and Sule, 2010; Enemor et al, 2013; Amagon et al., 2020, Cimanga et al., 2021).

3.6.2 Effects of aqueous Cc-1 and Lm-1 extracts from *C. citratus* and *L. multifolia* leaves on vital organ weights

Results showed that the oral administration of aqueous Cc-1 and Lm-1 extracts at highest oral dose of 5000 mg/kg bw significantly increase all vital organ of treated rats compared to negative control with significant difference ($p < 0.05$). The presence particularly of tannins in aqueous

Table 9a: Influence of administered aqueous Cc-1 extract on vital weights.

Organs	Negative control	Cc-1: 5000 mg/kg bw
Brain	3.55±0.04	4.17±0.05
Heart	0.90±0.05	0.96±0.06
Kidneys	2.46±0.04	2.51±0.05
Liver	3.35±0.05	3.63±0.07
Pancreas	1.66±0.07	1.78±0.04
Spleen	0.71±0.04	0.78±0.06
Testicles	9.55±0.05	11.3±0.07
Ovaries	0.27±0.05	0.32±0.06

Table 9b: Influence of administered aqueous *L. multifolia* Lm-1 extract on vital weights.

Organs	Negative control	Cc-1: 5000 mg/kg bw
Brain	3.7 5±0.02	4.28±0.05
Heart	0.93±0.05	0.96±0.06
Kidneys	2.44±0.04	2.56±0.05
Liver	3.45±0.04	3.65±0.07
Pancreas	1.66±0.07	1.68±0.05

Spleen	0.75±0.06	0.78±0.04
Testicles	9.58±0.04	10.7±0.06
Ovaries	0.28±0.01	0.30±0.06

Cc-1 and Lm-1 extract having astringent property, could account sensibly in the increasing weight of these vital organ weight as also reported previously by Maina et al., 2013 for aqueous extract of *Leptadenia hastate* and by Cimanga et al., 2021 for aqueous extract of *Morinda morindoides* leaves. The oral administration, of aqueous Cc-1 and Lm-1 extracts in treated rat groups did not affect seriously the organ weights, organ-to-body weight ration and there was no change in color and form of these organ keeping their architecture normal compared to negative control. Our results were in good agreement with other studies for the effects of medicinal plants studied on vital organ weights (Unuofin et al., 2018; Cimanga et al., 2021).

4. CONCLUSION

The present study reported for the first time antioxidant activity of extracts and fractions from *Raphia sese* dried fruits *in vitro* as well acute and subacute toxicity of its aqueous extract in vivo. Results revealed that as tested samples from this plant part studies exerted good antioxidant activity and can be used for long time to prevent the occurring of some degenerative diseases like cardiovascular diseases. In addition the administration of aqueous extract in rats at highest oral dose of 5000 mg/kg bw, had not significant effect on hematological and biochemical parameters of treated animals. It did not induced mortality of treated rats since its lethal dose was estimated to be greater than 5000 mg/kg bw and the extract was considered as practically non-toxic and safe *per os*. It also not have significant influence on vital organ weights of treated animals and increased significantly the levels of some analyzed electrolytes.

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