



In Vivo* Cytotoxic Effect of Aqueous Extract of *Ocimum Basilicum

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Abstract

Ocimum basilicum has long been used traditionally to treat cancer, convulsions, epilepsy, gout, nausea, sore throat, toothache and as a first aid treatment for snake and wasp bites. However, little research has been done into its toxicity. We wanted to contribute to the cytotoxic study of the aqueous extract of the leaves of this plant. The aim of the study was to assess the in vivo cytotoxicity of the aqueous extract of *Ocimum basilicum* leaves on Wistar rats. This aromatic herb of interest is linked to the need to seek medication through a therapy that is less toxic and has no side effects. The study was carried out on Wistar rats at a dose of 2000 mg/kg for batch 2; batch 1 being the normal control given distilled water. Liver, kidney and immune functions were investigated by biological analyses (creatinine, ALAT transaminase and blood count) and histopathological examinations. In addition, phytochemical analysis of this plant was carried out to determine the nature of the bio-active molecules present in the plant. In vitro antioxidant activity was measured by DPPH scavenging activity. The administration of the single dose aqueous extract did not cause any deaths. Toxicity showed that the extracts have no effect on kidney, liver and immune function. Analysis of histological sections of the liver, kidneys and spleen indicates good functioning of these organs, thus confirming the biochemical and hematological tests carried out. This safety encourages the use of this aromatic herb in traditional medicine, cooking and cosmetics.

Keywords

Ocimum basilicum, Cytotoxic, Histological sections, Biochemical tests, Haematological tests.

INTRODUCTION

Cytotoxicity is defined as the property of a substance to be toxic to cells, eventually destroying them. In parallel, toxicity assesses the harmfulness of a substance, whether at a single high dose or repeated doses over the long term, which can induce morphological and functional lesions in a living organism^[1,2]. It is also used to determine the degree or harmfulness of a substance and to regulate its use. Several parameters can be used to assess the toxicity of a substance: the method of administration, the dose to be administered, the mortality rate, the variation in weight, the histopathology of organs, and the variation in biochemical and haematological parameters^[3]. Nowadays, plant-based therapies are of great interest, with 70% of people in the Third World finding relief in traditional medicine and 80% of people living in Africa^[4]. Plants are therefore a valuable source of natural products with sometimes unknown therapeutic potential and low cost; hence the need to carry out studies on their possible toxicity in order to highlight their various pharmacological properties and promote their use^[5,6]. *Ocimum basilicum*, a member of the Lamiaceae family, shares its membership with a wide range of plants with diverse biological properties. Among the most renowned members of this family are thyme, mint, oregano, basil, sage and savory^[7]. With more than 200 genera and 7,000 species, the Lamiaceae represent the sixth largest plant family, widely distributed throughout the world and considered relatively easy to grow. Their aromatic character and usefulness as spices make them valuable in the pharmaceutical, food and cosmetics sectors^[7]. Species of the genus *Ocimum* are among the medicinal plants historically best known for their antimicrobial, immunomodulatory, antistress, anti-inflammatory, antiulcer, antidiabetic, hepatoprotective, chemoprotective, antihyperlipidaemic, cardioprotective, antioxidant, radioprotective, memory-enhancing, antiarthritic, antifertility, antihypertensive, anticoagulant, anticataract, vermifuge and antinociceptive properties. *Ocimum basilicum* is one of the most studied species in this genus^[7,8]. Basil is used in traditional medicine in many countries. Basil leaves are used in traditional medicine as a tonic, stimulant, carminative, stomachic, antispasmodic, antiviral and vermifuge, particularly against stomach cramps, diarrhoea, constipation, angina, coughs, kidney dysfunction, bronchitis, lung ailments, rheumatism, inflammation, headaches, hypertension and as a contraceptive^[9]. As an herbal tea, the leaves were recommended against nausea, flatulence and dysentery. Basil essential oil is produced in India, Bulgaria, Pakistan and the Maghreb (Algeria, Morocco, Tunisia and Egypt). It is an essential

oil with a very strong smell and taste, which may have antioxidant properties and is reputed to have antispasmodic, anti-infectious, calming and relaxing properties^[10]. Some authors have recommended it for mental fatigue, colds, spasms, rhinitis and as a first aid treatment for wasp stings and snake bites^[11]. Basil is used in the treatment of certain disorders related to the respiratory tract, including asthma, bronchitis, coughs and gastrointestinal disorders, as well as cardiovascular disease, neurocognitive disorders and metabolic disorders^[12]. The flowers and leaves of *Ocimum basilicum* are consumed in the form of infusions, syrups and decoctions as sudorific, stimulant, carminative, diuretic and febrifuge agents, and are frequently suggested for bronchitis and coughs. Leaf extract has been used in the treatment of wounds, acne and vitiligo^[13]. In addition, the leaves and flowering parts of *Ocimum basilicum* are used as antispasmodic, aromatic, carminative, digestive, galactogenic, stomachic and tonic agents in traditional medicine. Basil polysaccharides have been widely used in the treatment of cancerous diseases in traditional Chinese medicine^[14]. The aim of this study is to assess the *in vivo* cytotoxicity of the aqueous extract of *Ocimum basilicum* leaves on Wistar rats, thereby contributing to our understanding of its effects on health.

MATERIALS AND METHODS

The study was experimental. It took place at the Applied Biology Research Laboratory (LARBA) of the Ecole Polytechnique d'Abomey-Calavi (EPAC), specifically at the Non-Communicable Diseases and Cancer Research Unit (UR-MNTC); in the Experimental and Clinical Biology Unit of the Medical and Pharmaceutical Biotechnology Research Laboratory of the Ecole Nationale Supérieure des Biosciences et Biotechnologies Appliquées of the National University of Science, Technology, Engineering and Mathematics (UBEC/LaBiMeP/LaBEC/ENSBB/UNSTIM) and in the Laboratory of Physiology and Experimental Pharmacology of the Faculty of Science and Technology of the University of Abomey-Calavi (LPPE/FAST/UAC).

Animal material

The experimental study population consisted of 10 female Wistar rats. Their selection was based on the following inclusion criteria: they had to be at least 06 weeks old, show no signs of physical malformation, be nulliparous, non-pregnant and weigh between 150 and 200g. The rats were then acclimatised to laboratory conditions (12 hours of light, 12 hours of darkness, temperature 25°C) for 07 days and then divided into 02 batches of 05 animals. The animals had free access to continuous water in bottles and to food in the form of pellets.

Plant material

The plant material consists of the leaves of *Ocimum basilicum*, an aromatic herb with antihyperglycemic properties that is acclimatised in Benin. The raw material was collected in July 2023 in the south of Benin (Abomey-Calavi market) and its identification was confirmed by a botanical expert. The *Ocimum basilicum* leaves are then cleaned and dried at laboratory temperature to better preserve the heat-sensitive molecules. The dried leaves are then ground into powder using a grinder and stored in airtight containers.

Preparation of extracts

The aqueous extract of *Ocimum basilicum* leaves was obtained by decoction by boiling 01 litres of water containing 100g of dried leaf powder in the dark for 30 min. This was followed by filtration and oven drying (50°C). The extract obtained was weighed and stored in a sterile bottle.

Phytochemical screening

The percentage inhibition of the DPPH radical is calculated according to the following equation:

$$\text{DPPH inhibition (\%)} = (1 - \text{DO}_{\text{essai}} / \text{DO}_{\text{blank}}) \times 100 \times 100.$$

The Cl_{50} , which is the concentration of leaf extract or Quercetin responsible for 50% inhibition of DPPH radicals, is determined on the graph representing the percentage of DPPH inhibition as a function of the concentrations of extracts and Quercetin.

Assessment of acute oral toxicity

The acute toxicity test (TOA) was carried out as recommended in guideline 423 of the Organization for Economic Co-operation and Development for the testing of chemicals [17]. We set up two batches of five rats each: batch 1 (control) and batch 2 (Test: aqueous extract of

Ocimum basilicum leaves). Each batch consisted of five female Wistar rats. Each animal in lot 1 was given a single dose of distilled water by gavage, and the animals in lot 2 were given a single dose of 2000 mg/kg body weight of aqueous extract of *Ocimum basilicum* leaves by gavage. The animals were carefully observed for four (4) hours and then daily for 14 days. They were weighed at the beginning and end of the experiment

Phytochemical screening was carried out according to the method used by N'Guessan et al [15]. The phytochemical compounds sought were mucilages, flavonoids, leuco anthocyanins, anthocyanins, tannins, saponosides, reducing compounds and coumarins.

Determination of anti-free radical activity using the DPPH test

The free radical scavenging activity of the aqueous extract of *Ocimum basilicum* leaves was measured using the 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) test according to the method of Parejo et al, [16]. 200 µg/ml of leaf extract or Quercetin (reference antioxidant) was prepared in methanol. A volume of 2.5 ml of this solution was mixed with 2.5 ml of DPPH (100 µM), also prepared in methanol. After homogenisation, the mixture was incubated at room temperature (25°C) in the dark. After 15 minutes of incubation, the absorbance was read at 517 nm against a "blank" containing only methanol.

and blood was collected by orbital puncture at the beginning of the experiment and again after 14 days [18].

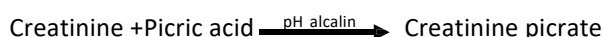
Blood tests

We performed the following blood tests. Creatinine to investigate kidney function. ALAT transaminase to assess liver function. The blood count (NFS) in the blood is carried out to explore immune function [18].

Biochemical tests

-Creatinine measurement

- Type of method: Colorimetric kinetics (Jaffé method).
- Principle: Creatinine forms creatinine picrate in the presence of picric acid in an alkaline medium. The rate of appearance of creatinine picrate, read at 505 nm, is proportional to creatinine concentration.



- Procedure: We took 500 µl of reagent R1 plus 500 µl of reagent R2 in all the tubes and mixed with 100 µl of the standard in the standard tube and 100 µl of each sample in the corresponding tubes. We then automatically read the optical density at 520 nm after adding each sample.

-Determination of ALAT transaminase

- Type of method: Enzymatic kinetics.

- Principle: ALAT catalyses the transamination reaction between Alanine and α- ketoglutarate, leading to the formation of pyruvate and glutamate. Pyruvate, in the presence of NADH, will give lactate and NAD⁺ under the action of lactate dehydrogenase (LDH). The rate at which NADH disappears, measured at 340 nm, is proportional to the enzymatic activity of ALAT.



- Procedure: We took 800 µl of reagent R1 plus 200 µl of reagent R2 and mixed with 100 µl of each sample in the corresponding tubes. The rate at which NADH disappears, measured at 340 nm, is proportional to the enzymatic activity of ALAT.

Haematological tests

The blood count (CBC) was performed using a SYSMEX KX-N21 automated system. This haematological parameter is used to investigate immune function [18].

Histological analysis

Histological analyses are carried out on organs such as the liver, kidneys and spleen. At the end of the experiments for the acute toxicity test (TOA), the animals were dissected. The liver, kidneys and spleen were removed, fixed in a 10% buffered formalin solution and embedded in paraffin. Sections of the samples (5 µm) were mounted on glass slides, deparaffinised and hydrated. For histological analysis, the sections were stained with haematoxylin and eosin (H&E) using a standard protocol [19]. Photographs were taken at 400 X magnification.

Statistical analysis

The raw data from our experiments were entered and recorded using Microsoft Excel 2013. Statistical

analyses and graphs were produced using SigmaPlot software version 14 (year 2017). Our results are presented as mean ± standard variants of the analysis of variance (ANOVA) test. The classic ANOVA test (parametric test) was used for data following a normal distribution and in the event of equality between the variances of the groups being compared. For parameters where at least one of these conditions was not met, we used the non-parametric alternative, the Kruskal-Wallis ANOVA test. These tests allow us to determine whether or not there is a significant difference between the values of the two groups considered. The exposed groups were compared with the control (unexposed) group. Error on the mean (SEM) we used two. The significance threshold was 0.05.

RESULTS

At the end of our study, morphological parameters, variations in body weight, variations in biochemical parameters and histological sections of organs enabled us to assess the acute oral toxicity of the aqueous extract of *Ocimum basilicum* leaves.

Chemical analysis of *Ocimum basilicum* extracts Phytochemical screening

Tableau I: Phytochemical constituents of *Ocimum basilicum* leaves

Chemical compounds	<i>Ocimum basilicum</i>
Mucilages	+
Saponosides	+
Tanins condensés	+
Tanins galliques	+
Anthocyanes	+
Flavonoïdes	+
Leuco anthocyanes	+
Coumarines	+
Composés réducteurs	-
Legend: Absent: (-); Present: (+)	

Ocimum basilicum contains all the desired chemical compounds to varying and greater degrees, with the exception of coumarins. The secondary metabolites with a very pronounced presence are mucilages, saponosides and gall tannins.

Content of total phenolic compounds and total flavonoids in aqueous extracts of *Ocimum basilicum* leaves

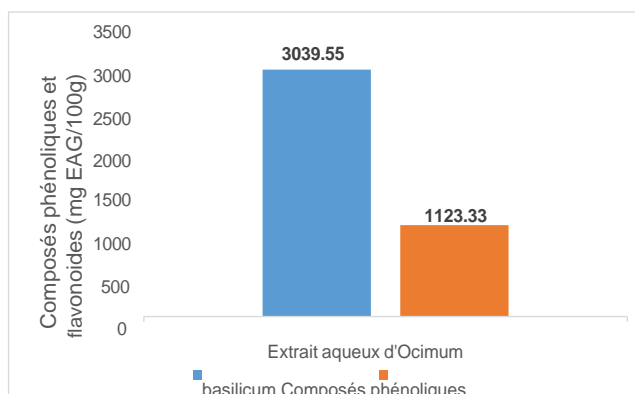


Figure 1: Average content of total phenolic compounds and total flavonoids in mg EAG/100 g in the aqueous extract of *Ocimum basilicum* leaves.

The results are expressed as gallic acid equivalent (GAE mg/100 g). The concentration of flavonoids in the aqueous extract of *Ocimum basilicum* leaves was 1123.33 mg GAE/100 g and its total phenolic compound content was 3039.55 ± 8490.35 mg GAE/100 g.

Determination of antioxidant activity using the DPPH test

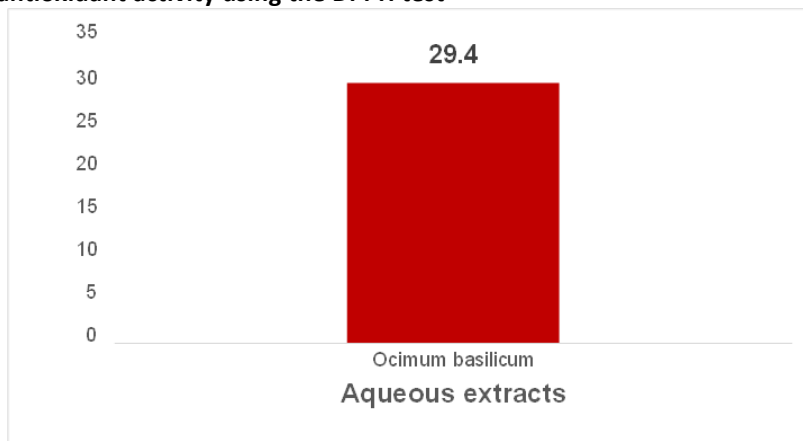


Figure 2: Antioxidant activity of the aqueous extract of *Ocimum basilicum* leaves assessed by the DPPH assay, expressed as % DPPH inhibition.

The results of the DPPH test show that the aqueous extract of *Ocimum basilicum* leaves has a DPPH inhibition % of 29.4%.

Assessment of acute toxicity Haematological parameters

Table II: Blood cell counts of rats at the beginning and end of toxicity tests with aqueous extract of *Ocimum basilicum*.

Parameters	Mean at D0	Mean at D14	P	Difference
NB	7±1	7±2	0.8387	not significant
NR	3.95±2.60	7.68±0.10	0.0046	Significant
HB	15.2±0.17	11.1±4.82	0.2189	not significant
HTE	45.16±0.11	33.3±14.46	0.0017	Significant
VGM	82.17±5.49	43.16±18.34	0.1052	not significant
TCMH	27.67±2.21	14.38±6.1	0.1101	not significant
CCMH	33.65±0.43	24.2±10.39	0.2697	not significant
L	20.66±4.5	23±1.73	0.3356	not significant
N	71±5	68.66±2.3	0.7321	not significant
M	7±1	7±1	P>0.9999	not significant
E	0.7±0.51	1.33±0.57	0.6627	not significant
PLT	638.33±60.33	983±30.41	0.0144	Significant

NB: Number of white cells; NR: Number of red cells; HB: Haemoglobin HTE: Haematocrit; CCMH: Mean corpuscular haemoglobin concentration; VGM: Mean corpuscular volume; TCMH: Mean haemoglobin content platelets N: neutrophil; E: eosinophil; B: basophil; M: macrophage; L: lymphocyte.

At D0, the mean values for red blood cell count, haematocrit and platelets were 3.95 ± 2.60 , 45.16 ± 0.11 and 638.33 ± 60.33 respectively. At D14, the mean values for red blood cell count, haematocrit and platelets were 7.68 ± 0.10 , 33.3 ± 14.46 and 983 ± 30.41

respectively. There was a significant change in the mean red blood cell count ($p=0.0046$), haematocrit ($p=0.0017$) and platelet count ($p=0.0144$) at D0 and D14 in rats treated with the aqueous extract of *Ocimum basilicum* leaves (Table II).

Physical and biochemical parameters

Tableau III: Physical and biochemical parameters of rats at the beginning and end of toxicity tests with aqueous extract of *Ocimum basilicum*

Parameters	Mean at D0	Mean at D14	P	Difference
Weight	187 ± 30.13	188 ± 31.43	0.6914	not significant
ALAT transaminase	31.66 ± 16.86	34.33 ± 3.21	0.8208	not significant
Creatininemia	7.8 ± 1.55	11.76 ± 2.21	0.0924	not significant

At D0, the mean values for weight, ALAT transaminase and creatinine were 187 ± 30.13 , 31.66 ± 16.86 and 7.8 ± 1.55 respectively. At D14, the mean values for weight, ALT transaminase and creatinine were 188 ± 31.43 , 34.33 ± 3.21 and 11.76 ± 2.21 respectively.

There was no significant variation in the mean values at D0 and D14 for physical and biochemical parameters in rats treated with the aqueous extract of *Ocimum basilicum* leaves (Table III).

Histological analysis

Effects of aqueous extract of *Ocimum basilicum* leaves on liver parenchyma

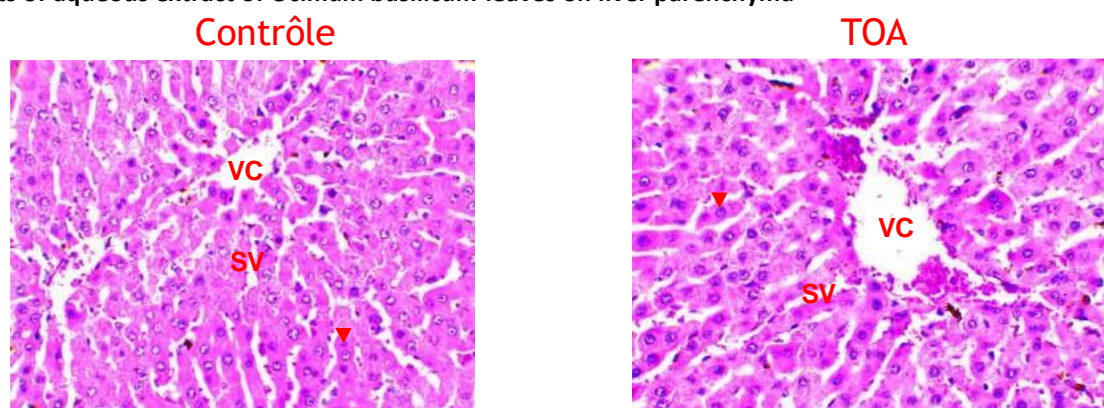


Figure 3: Histological sections of the livers of rats subjected to acute toxicity by aqueous extracts of *Ocimum basilicum*.

The livers of the rats showed no visible atypia with normal parenchyma as in control rats. The hepatocytes (arrows) are organised in laminae

distinguished by clearly visible venous sinusoids (VS) that exchange with the centrilobular vein (CV). Magnification: 400X

Effects of aqueous extract of *Ocimum basilicum* leaves on renal parenchyma

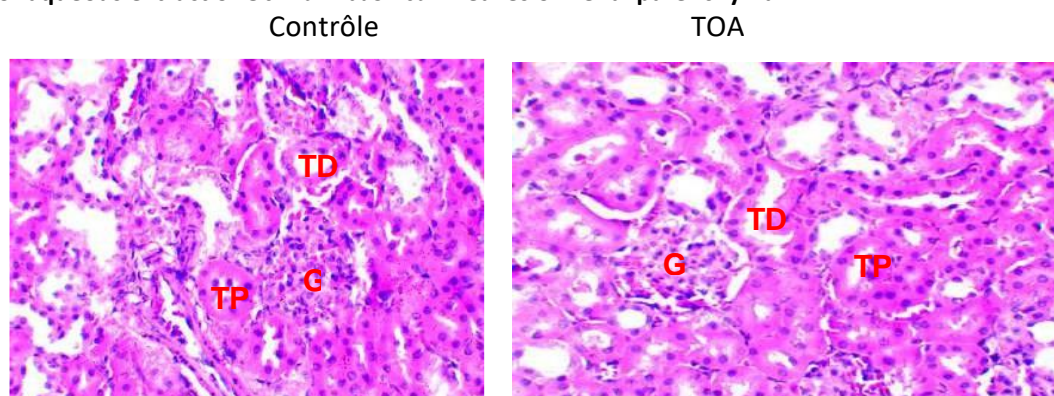


Figure 4: Histological sections of the renal parenchyma of rats subjected to acute toxicity by aqueous extracts of *Ocimum basilicum*.

The renal parenchyma showed no visible atypia and was similar to that of control rats. The glomeruli (G),

proximal tubules (PT) and distal tubules (DT) were clearly visible, as in control rats. Magnification: 400X

Effects of aqueous extract of *Ocimum basilicum* leaves on splenic parenchyma

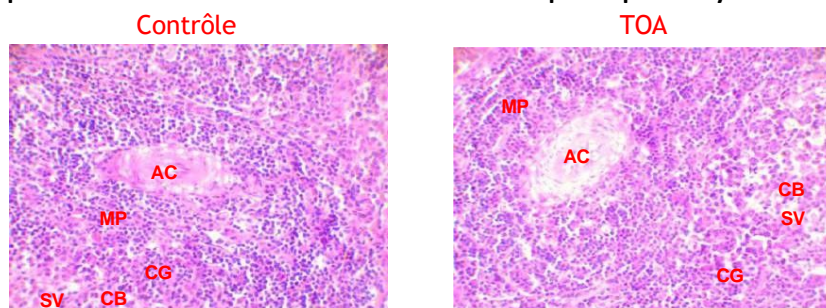


Figure 5: Histological sections of the spleen of rats subjected to acute toxicity by aqueous extracts of *Ocimum basilicum*.

The splenic parenchyma is typical, as in the control rats. The central arteries (CA), periarteriolar mantles (PM) and germinal centres (GC) are clearly visible in the white pulp, as in control rats. The venous sinusoids (VS) are clearly distinguished by Billroth's cords (BC) in the red pulp. Magnification: 400X.

DISCUSSION

The aim of this study was to assess the *in vivo* cytotoxicity of the aqueous extract of *Ocimum basilicum* leaves on Wistar rats. The phytochemical analyses carried out indicate that *Ocimum basilicum* leaves contain, to varying and greater degrees, all the chemical compounds of interest with the exception of coumarins. These phytochemical results are in line with the results found by Kajal and Singh [20]. These leaves are known medicinal properties including cardiac, antibacterial, antioxidant, and hypoglycaemic activities [21]. The concentration of flavonoids in the aqueous extract of *Ocimum basilicum* leaves was 1123.33 mg EAG/100 g and its total phenolic compound content was 3039.55 ± 8490.35 mg EAG/100 g. This indicates that the aqueous extract of *Ocimum basilicum* leaves has a higher content of total phenols (3039.55 ± 8490.3 mg EAG/100 g) than flavonoids (1123.33 mg EAG/100 g). These results are in line with those of Hafiza Sehrish Kiani and colleagues [22]. The results of the DPPH test show that the aqueous extract of *Ocimum basilicum* leaves has a DPPH inhibition % of 29.4%. These results concur with those of Zoran Ilić and colleagues who found that basil has antioxidant activity because its extracts contain appreciable levels of total phenolic content and have a good DPPH radical scavenging capacity [23]. A strong correlation between antioxidant activity and total phenolic content in basil extracts has been described [24]. However, Leija Cengic and colleagues observed DPPH inhibition, up to 90% for the aqueous extract of *Ocimum basilicum* [25]. The variations observed in the antioxidant capacities of these extracts between different studies may be due to several key factors such as differences in the extraction techniques used (which may affect the composition of the extracts),

the specificity of the plant parts examined (leaves, stems, roots, which have distinct phytochemical profiles), variations in the environmental conditions under which the plants were grown (impacting their chemical composition), differences in the analytical methodologies applied to assess antioxidant activity, and the quality and purity of the chemical reagents, particularly the DPPH used in absorbance measurements. The study of the acute toxicity of the aqueous extract of *Ocimum basilicum* in rats showed that these extracts, administered orally, showed no mortality for doses up to the limit dose of 2000 mg/kg PC, in line with OECD standards [26]. The morphological parameters observed were normal for the animals. In addition, no significant differences were observed in order to be beneficial for various the mean weight of the rats after administration of the extracts at this dose. Assessment of renal function by measuring creatinine revealed no significant difference between creatinine levels before and after administration of the aqueous extract of *Ocimum basilicum* leaves at a dose of 2000 mg/kg PC, suggesting normal renal function, in agreement with the results obtained by Goshu and colleagues [27]. ALT levels, indicative of hepatic dysfunction, were comparable before and after administration of the aqueous extract of these leaves, confirming a normal functional state of the liver, as reported in the literature by Maboune and colleagues [28]. Analysis of histological sections of the liver, kidneys and spleen of rats administered the aqueous extract of *Ocimum basilicum* leaves at a dose of 2000 mg/kg PC during the toxicity study confirmed the normal functioning of these organs as revealed by the biochemical and haematological tests. The livers of the rats showed no visible atypia and had normal parenchyma, as did those of the control rats. The hepatocytes were organised into laminae repaired by clearly visible

venous sinusoids that exchanged with the centrilobular vein. The renal parenchyma also showed no visible atypia and was similar to that of control rats, with glomeruli and proximal and distal tubules clearly visible, as in control rats. Finally, the splenic parenchyma was typical as in control rats. The central arteries, periarterial cuffs and germinal centres are clearly visible in the white pulp, as in control rats. The venous sinusoids are clearly distinguished by Billroth's cords in the red pulp, as in control rats. These results are in line with those of Perdroza and colleagues ^[29]. The haematopoietic system is one of the most sensitive targets for toxic compounds and it is therefore mandatory to record any possible alterations resulting from a tested substance ^[30]. With regard to the cytotoxicity of the aqueous extract of *Ocimum basilicum* leaves, in our study a significant variation in the number of red blood cells, haematocrit and platelets was observed in rats given 2000 mg/kg of the aqueous extract of *Ocimum basilicum* leaves. Some authors, such as Zhan and colleagues, reported significant variations in haematological parameters in their study based on the toxicity of the aqueous extract of *Ocimum basilicum* leaves in rats ^[31,32]. These variations indicate that the aqueous extract of *Ocimum basilicum* leaves has a significant impact on the haematopoietic system of rats. This highlights the importance of a detailed assessment of the safety and toxicology of these leaves before considering their use for medicinal purposes. It is crucial to understand that these effects may depend on the dose and the specific physiology of the rats treated.

CONCLUSION

The cytotoxic study showed that there were no deleterious effects on the physical appearance of the animals, and no change in the texture or architecture of the organs studied. Similarly, the aqueous extract of *Ocimum basilicum* leaves had no effect on renal or immune function. Analysis of histological sections of the liver, kidneys and spleen showed that these organs functioned well, confirming the biochemical tests carried out.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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