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Evaluation of anti inflammatory activity *Chamaeleo Chamaeleon*'s skin against nickel toxicity in *wistar rats*

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Dedication

This work is dedicated

To my beloved Parents who always

been a source of Inspiration, encouragement and stamina

To my sister Douaa and my brother Djamel Abd Nasser

To all my friends, especially Afaf

And also to my

Self!!

Achouak

Dedication

My big and first thanks go to ALLAH for his blessing and his kindness to me, I am grateful to ALLAH for facilitating my way to reach all my dreams.

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Afaf

Abstract

Saharan culture is very rich in theory which stops us to research on the use of chamaeleon dry skin. This investigation was aimed to study the effect of ethanolic extract of the *C.Chamaeleon*dry skin, Twenty male albino Wistar rats, weighed 180±15 g were divided into 4 groups of 5 animals each (n = 5);Group 1: normal control, Group 2:rats exposed to nickel (20mg / kg) and received normal saline, Group 3: rats exposed to nickel (20mg / kg)was treated with ethanolic extract of *C.Chamaeleon* dry skin (300mg/kg), Group 4: rats exposed to nickel (20mg / kg)was treated with ethanolic extract of *C.Chamaeleon* dry skin (400mg/kg) for 7 days.

Various parameters as hematological, biochemical markers were estimated, In this study, the toxicity test showedan increase in relative liver weight, The results also showed notable changes inbiochemical parameters characterized by a significant increase in theserum concentration of glucose, urea and creatinine serum.However, the activities of transaminases (TGO, TGP) and C-Reactive Proteinserum have shown an increasein rats contaminated with nickel in compared to control. Treatment with *C.Chamaeleon* almost normalizes the parameters biochemical and hematological parameters.

After the extraction processes, the yield of the extraction was (20.78%).Results of physicochemical and biochemical analysis revealed that the dry skin contains respectively 92.74% of dry matter, 77% of ash, 0.99% of protein, 8.3% of carbohydrates and 2.73% of lipid. The results of antioxidant activity showed that theIC50 values of the skin dry was 46.02 mg/ml.FRAP value 4.43±0.09 (mg equivalent of ascorbic acid /g of extract). The ethanolic extract did not show any activity against the bacterial strains. However the results of haemolysis test showed highest haemolytic effect (48.65%) at the concentration of 1000µg/ml. The study reveals anti-inflammatoryproperty was observed in ethanolic extract of *C.Chamaeleon* dry skin in groups treated with nickel.

Key Words: Nickel, inflammation, *Chemaeleon Chamaeleon*, skin, Biological activiy.

الملخص

الثقافة الصحراوية غنية جدًا من الناحية النظرية والتي استوقفتنا للبحث حول استخدام الجلد الجاف للحرباء. يهدف هذا البحث إلى دراسة تأثير المستخلص الإيثانولي للجلد الجاف ل *C. Chameleon*. عشرون ذكرا من الفئران ، وزنها 180 ± 15 جم. في 4 مجموعات من 5 حيوانات لكل منها (ن = 5) ؛ المجموعة 1: الشاهد ، المجموعة 2: الفئران المعرضة للنيكل (20 ملغ / كغ) والمحلول الملحي العادي ، المجموعة 3: الفئران المعرضة للنيكل (20 ملغ / كغ) تم معالجتها بمستخلص إيثانولي من الجلد الجاف *C. Chameleon*(300 ملغ / كغ.) ، المجموعة 4: تم علاج الفئران المعرضة للنيكل (20 ملغ / كغ) بمستخلص إيثانولي من الجلد الجاف *C. Chameleon* (400 ملغ / كغ) لمدة 7 أيام.

خلال هاته الدراسة، تم تقدير المعايير البيولوجية للفئران التجريبية. أظهر الاختبار زيادة في الوزن النسبي للكبد ، وأظهرت النتائج أيضًا تغيرات ملحوظة في المعايير البيوكيميائية تتميز بزيادة كبيرة في تركيز مصل الجلوكوز واليوريا والكرياتينين في الدم. ومع ذلك ، أظهرت أنشطة الترانساميناسات (TGO, TGP) ، ومصل البروتين التفاعليC زيادة في الفئران المعالجة بالنيكل مقارنة بالشاهد. العلاج بالحرباء يكاد يعيد المعايير البيوكيميائية والدموية إلى طبيعتها. بعد عمليات الاستخلاص، كان ناتج الاستخلاص (20.78٪). أظهرت نتائج التحليل الفيزيائي الكيميائي والكيميائي الحيوي أن الجلد الجاف يحتوي على التوالي على 92.74٪ من المادة الجافة و 7٪ من الرماد و 0.99٪ من البروتين و 8.3٪ من الكربوهيدرات و 2.73٪ من الدهون. أظهرت نتائج النشاط المضاد للأكسدة أن قيم IC50 للبشرة الجافة كانت 46.02 ملغم / مل. قيمة ± 4.43 FRAP 0.09 (مكافئ ملغم من حمض الأسكوربيك / غم من المستخلص).

لم يظهر مستخلص الإيثانول أي نشاط ضد السلالات البكتيرية. ومع ذلك، أظهرت نتائج اختبار انحلال الدم أن أعلى تأثير كان (48.65 ٪) بتركيز 1000 ميكروغرام / مل. كشفت الدراسة عن وجود خاصية مضادة للالتهاب في خلاصة الإيثانول للبشرة الجافة *C. Chameleon*في مجموعات تمت معالجتها مع نيكل.

الكلمات المفتاحية: نيكل ، التهاب ، *Chamaeleo Chameleon* ، جلد ، نشاطية بيولوجية

Abbreviation list

C%: Percentage of ash

Ca^{2+}: calcium

CaSR : The calcium-sensing receptor

CAT: catalase

COX-1: cyclooxygenase 1

COX-2: cyclooxygenase-2

CRP: C-reactive protein

DMSO: Dimethylsulfoxide

DNA: Deoxyribonucleic acid

DPPH: 1,1-diphenyl-2-picryhydrazyl

EDTA: ethylenediaminetetraacetate

FNS: Hematological analysis.

GOT: Glutamic Oxaloacetate Transaminase.

GPT: Glutamic Pyruvate Transaminase.

GRA: Granulocyte

GSH: Reduced Glutathion

GSH-Px: glutathione peroxidase

H2O2: Hydrogen peroxide.

HCT: haematocrit

HGB: haemoglobin

IC50: Concentration of inhibition 50% of DPPH radical

Ig : immunoglobulin

IL-10 : interleukins 10

IL-1β : interleukins 1β

IL-6 : interleukins 6

IVIG : intravenous immunoglobulin

LYM: lymphocyte

MH: Mueller Hinton agar

NaCl: Sodium Chloride.

NSAIDs: non-steroidal anti-inflammatory drugs

OM%: Percentage of organic matter

RBC: red blood cell

RBCs: circulating red blood cells

RNS: reactive nitrogen species

ROS: reactive oxygen species

SAIDs: steroidal anti-inflammatory drugs

SOD: Superoxide dismutase.

TBS: Tris Buffer Saline.

TCA: Trichloroacetic acid.

TNF α: tumor necrosis factor

TRIS: hydroxymethyl aminomethane

UV: Ultraviolet

WBC: White Blood Cells

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Summary

Introduction

Introduction

The World Health Organization (WHO) estimates that as many as 80% of the world's more than six billion people rely primarily on animal and plant-based medicines. Traditional human populations have a broad natural pharmacopoeia consisting of wild plant and animal species. Ingredients sourced from wild plants and animals are not only used in traditional medicines, but are also increasingly valued as raw materials in the preparation of modern medicines and herbal preparations **(RÔMULO et *al*., 2005).**

Human use of animals is an integral part of many cultures. Reptiles and human societies have interacted for millennia. Reptiles are among the species most utilized in popular medicine **(SANTOS et *al*., 2012)** and their role in practices and beliefs related to the treatment and/or prevention of diseases has been reported by different traditional communities worldwide. Variety of sources shows that humans have exploited the eggs, meat, blood, oil, shell, skin, bones, and other parts of the reptiles to provide raw materials for food, toolmaking, ornaments, medicines **(ALVES et *al*., 2009).** Lizards are also the group of reptiles whose flesh is believed to have curative properties **(BHOWMIK et *al*., 2015).**

Laboratory studies about the chemical and biological properties of animal products, which would validate their effectiveness in the treatment of diseases and consequently their relevance to human health, are surprisingly scarce and still preliminary **(FLÁVIO et *al*., 2012).**

Heavy metals are significant environmental pollutants and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons. The most commonly found heavy metals in waste water include arsenic, cadmium, chromium, copper, lead, nickel, and zinc, all of which cause risks for human health and the environment **(JAISHANKAR et *al*., 2014).**

Nickel (Ni) metal has become an object of great interest because of its widely distribution in environmental occurrence. In other words, it is used in a wide variety of applications including metallurgical processes. High quantity of nickel has been reported to show various toxicities such as pulmonary, renal and cardiovascular effects (**KECHRID & BOUHALIT, 2018).**

In the region of EL-OUED, south-east Algeria, some lizards such as *Chamaeleo Chamaeleon* is anchored in the remedies of the natives. This reptile is consumed by Souafa for treating tonsillitis. The consumption of *Chamaeleo Chamaeleon* in the region of EL-OUED will then be due to the scarcity of drugs in the old days.

In light of these data, the aim of our work is based on the realization of two following complementary aspects:

The first part: is to focus on the study of physico chemical and biochemical analysis of *C.Chmaeleon* dry skin.

The second part: is to investigate the anti-inflammatory effect of ethanolic extract of *C. Chamaeleon* dry skin against experimental inflammation induced by nickel in rats and study its effect on some hematological, biochemical markers.

First part

Theory part

Chapter I

Nickel

1. Definition

Nickel was firstly isolated by Axel Fredrik Cronstedt, a Swedish chemist in 1751. It is sturdy white metal with magnetostrictive and ferromagnetic properties**(RIZVI et *al.*, 2019)**. Nickel exists in nature mainly in the form of sulfide,oxide, and silicate minerals, High quantity of nickel has been reported to show various toxicities such as pulmonary, renal and cardiovascular effects**(BOUHALIT & KECHRID, 2018).**

2. Elemental properties of Nickel:

Nickel has the properties presented in the following table;

Table 02: Some physicochemical characteristics of the nickel **(GENCHI et *al.*, 2020)**

Chemical symbol	Ni
Atomic number	28
Atomic weight	58.6934
Density	8908 kg/m3
Melting point	1455 C°
Boiling point	2913 C°
Electronic configuration	[Ar] 3d84s2
Isotopes	5 stable; 19 unstable

3. Sources of exposure

Nickel belongs to the ferromagnetic elements, and it is naturally present in the earth crust usually in combination with oxygen and sulfur as oxides and sulfides. In combination with other elements, nickel may be present in the soil, meteorites and emitted from volcanoes. About eight billion tons of nickel are present in the sea**(GENCHI et *al.*, 2020).**

Nickel at non-dangerous concentrations is one of the essential microelements for protein metabolism and regulation of hormones in the human body. However, exposure to nickel at beyond the allowable concentration is associated with different kinds of chronic and acute disorders in humans, such as gene toxicity, neurotoxicity, hepatotoxicity, nephrotoxicity, damage to the kidneys and lungs, shortness of breath, chest pain, skin dermatitis, gastrointestinal disorders, vomiting, diarrhea, inhibition of oxidative enzyme activity, and increased risk of cancer**(ISLAM et *al*., 2019).**

The permissible nickel concentration in wastewater and drinking water, as suggested by the World Health Organization, should not exceed 900 and 0.02 mg/l, respectively **(ISLAM et *al*., 2019).**

4. Metabolism and Absorption of nickel:

When nickel enters the body it is distributed to all organs, but mostly in the kidney, bone, and lungs. If nickel enters the body with contaminated air it is retained in the lungs. Nickel, which enters the blood stream, is excreted in the urine, and if it is entered by the food it is excreted in the faces **(KUMAR & TRIVEDI, 2016).**

Urinary excretion is the major route for the elimination of absorbed nickel. Fecal excretion primarily reflects the nickel that is unabsorbed from the diet and passes through the gut. Nickel is poorly absorbed by the body. Less than 10% is absorbed in the gastrointestinal tract. In short and long-term studies of animal administered various soluble nickel salts orally, nickel was found primarily in the kidneys **(Das et *al*., 2008).**

The relative tissue concentrations were kidneys > lungs > liver > heart > testes. The normal ranges of nickel concentrations in body fluids or tissues (serum, blood, lung and kidney) are not significantly influenced by age, sex, or pregnancy **(KUMAR & TRIVEDI, 2016).**

5. Nickel toxicities:

5.1 Carcinogenicity:

The carcinogenic potential of nickel compounds depends on its solubility in water. Insoluble nickel compounds are known to be more potent carcinogens. The critical processes involved in the carcinogenic action of nickel compounds are oxidative stress, genomic DNA damage, epigenetic changes, and altered regulation of gene expression **(RIZVI et *al*., 2019).**

5.2 Haematotoxicity:

Regarding to oral exposure, the use of nickel sulphate in rats, show that intraperitoneal administration of the compound significantly decreased the erythrocyte count, haematocrit value (PCV %), and haemoglobin concentration when compared with untreated controls. The authors also reported a significant increase in clotting time, followed by decrease in platelet and leukocyte count after nickel treatment in rats. Such a decrease could result in nickel-induced anaemia **(DAS et *al*., 2007).**

5.3 Toxicities in some specific peripheral tissues:

a) Kidneys:

Inflammation in the bronchioles, alveolar congestion, alveolar cell hyperplasia, and sometimes congestion in the lumen was also noticed **(GUPTA et *al*., 2006)**. Following oral exposure to nickel contaminated drinking water, a transient increase in urine albumin was reported in electroplating workers **(SUNDERMAN et *al*., 1988).** Increased levels of nickel in urine were significantly linked to urinary β2- microglobulin levels in nickel refinery workers **(SUNDERMAN & HORAK, 1981**).

b) Liver:

One report described decreased hepatic and renal transaminase activities after nickel treatment in rats, which was found more deleterious in a protein-restricted dietary regimen**(DAS & BUCHNER, 2007).** Das et *al*(2001). Showed, after the nickel treatment of rats, a significant rise in hepatic lipid peroxides and a decrease in antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities and in the hepatic glutathione concentration **(Das et *al*., 2008).**

6. Mechanism:

Experimental studies have shown that nickel-induced carcinogenicity involves multiple molecular mechanisms. The proposed mechanisms suggest the following chain of events nickel compounds enter the cell, activate the receptor CaSR, triggering intracellular Ca^{2+} mobilization and induction of the calcium and hypoxia-inducible factor pathways. Nickel enters the nucleus, directly binds to DNA and reacts with H_2O_2 to form reactive nickel-oxygen complexes, resulting in the oxidation of thymine and cytosine residues.The oxidative stress generated severely damages DNA and inhibits DNA repair pathways. Nickel compounds also induce indirect damage through inflammation by stimulating polymorphonuclear leukocytes to produce ROS **(PATRIARCA et *al*., 1997).**

7. Oxidative stress

The cumulative production of reactive oxygen species/ reactive nitrogen species ROS/RNS through either endogenous or exogenous insults is termed oxidative stress and is common for many types of cancer cell that are linked with altered redox regulation of cellular signalling pathways. Oxidative stress induces a cellular redox imbalance which has been found to be present in various cancer cells compared with normal cells **(VALKO et *al*., 2006).**

8. Antioxidant defence against nickel toxicity:

Suitable mechanisms are present in human body so that steady state concentration of potentially toxic oxygen derived free radicals is kept in check under normal physiological condition by body's intrinsic antioxidant defence system. But enhanced generation of these reactive oxygen species (ROS) can overwhelm cell's intrinsic antioxidant defences and result in a condition known as oxidative stress **(DAS et *al*., 2006)**

Exogenous antioxidants are intimately involved in the prevention of cellular damage by interacting with free radicals and by terminating the chain reaction. In rats, increased lipid peroxide formation and decreased levels of glutathione, SOD, CAT, GSH-Px activities as well as ascorbic acid depletion have been found in the most active metabolic tissues of the body, namely, liver and kidney. Additionally, a decrease in antioxidant enzymes suggests an interaction with the accumulated free radicals and active amino acids of the enzymes, leading to functional impairment and tissue damage **(RODRIGUEZ et *al*., 1991).**

Chapter II

Biological activities

1. Anti-inflammatory activity:

1.1. Definition:

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Inflammation is not a synonym for infection, even in cases where inflammation is caused by infection **(MOHINI et *al*., 2012)**. Redness, warmth, swelling and pain are the classic clinical features of inflammation **(CHEN et *al*., 2016).**

1.2. Causes:

The causes of the inflammatory reaction are multiple and represent the exogenous agents. These causes determine cell and tissue damage that will trigger inflammation:

- Infection: contamination by microorganisms (bacteria, viruses, parasites, fungi).
- Physical agents: trauma, heat, cold, radiation.
- Chemical agents: caustics, toxins, venoms.
- Foreign bodies: exogenous or endogenous **(GHALEM, 2014).**

1.3. Types:

Inflammation can be classified as either acute or chronic inflammation **(ANOSIKE et *al*., 2012).**

a. Acute inflammation:

Is the initial response of the body to injurious stimuli and is achieved by increased movement of plasma and leukocytes from the blood into the injured tissues. The process of acute inflammation is initiated by cellsalready present in the tissues. This is characterized by marked vascular changes, including vasodilatation and increased capillary permeability which are induced bythe actions of the various inflammatory mediators **(ANOSIKE et*al*., 2012).**

b. Chronic inflammation:

Is a prolonged inflammatory response that leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissues from the inflammatory process **(ANOSIKE et *al*., 2012).**

1.4. Anti-inflammatory treatment

Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and steroidal anti-inflammatory drugs (SAIDs) such as dexamethasone have been widely used to combat inflammation **(CHEN et *al.*, 2016)**. Non-steroidal anti-inflammatory drugs (NSAIDs) are drugs with as antipyretic, anti-inflammatory, and analgesic agents. NSAIDs work by inhibiting the cyclooxygenase enzymes **(BENKHALED, 2018).**

The cyclo-oxygenase enzymes catalyze the formation of prostaglandins and thromboxanes **(KESSEL et *al.*, 2014).** Prostaglandins produced by COX-1 play mainly a physiological role (in particular gastric protection), while those produced by COX-2 are mainly produced under inflammatory conditions **(TRABSA, 2015).**

Steroids are potent anti-inflammatory agents that wok by acting on a number of intercellular inflammatory mediators **(KESSEL et *al.*, 2014).**

2. Anti-bacterial activity

Infections that are induced by bacteria are treated with drugs called antibiotics or anti-bacterial. Antibiotics can be described as a compound that functions to either stop bacteria from growing (bacteriostatic agents) or by killing them entirely (bactericidal agents). The effectiveness of these compounds against the survival of bacteria stems from their ability to block critical bacterial cellular processes **(Qaddoori, 2016).**

Natural products have been a rich source of compounds in antibiotic drug discovery with most antibiotic drugs being derived from a natural product. The use of animal resources constitutes an important therapeutic alternative for many populations, and they have been cited being used against illnesses apparently caused by pathogenic microorganisms **(OLIVEIRA et *al.*, 2014).**

3. Haemolytic activity

Haemolysis is an important parameter for measuring the quality of RBC. Haemolysis is caused by disruption of the RBC membrane and the subsequent release of haemoglobin (HBG).The extent of haemolysis is often defined as the percentage of free haemoglobin in relation to the haematocrit (HCT) **(EIMAN et *al.*, 2014).**

3.1. Types:

The immune-mediated destruction of circulating red blood cells (RBCs) is described by two distinct mechanisms: one is the intravascular destruction of RBCs by complement lysis, which is initiated by antibodies that are often, but not exclusively, of immunoglobulin (Ig) M class **(FLEGEL, 2015).**

The second mechanism is extravascular destruction by immune cells, which recognize IgG and complement bound to RBC. If an intravenous immunoglobulin (IVIG) product is free of IgM, intravascular haemolysis should be rare. There is, however, evidence that IgG, purposefully in high abundance in IVIG products, can induce intravascular haemolysis **(FLEGEL, 2015).**

3.2. Detection of haemolysis

Haemolysis is the release of intracellular components from erythrocytes, thrombocytes and leukocytes into the extracellular fluid, i.e., the plasma or serum. Haemolysis is visible as red coloration of plasma or serum after centrifugation of the sample. A report in the literature on the concentration of free haemoglobin (HBG), which is visible as red coloration in the plasma or serum varies between 100 and 300 mg/l **(BABIKER et *al*., 2013).**

4. Antioxidant activity:

4.1. Antioxidants:

An antioxidant is a molecule which has the ability to prevent or slow the oxidation of macromolecules. The role of antioxidants is to lower or terminate these chain reactions by removing free radicals or inhibiting other oxidation reactions by being oxidized themselves **(ADWAS et *al*., 2019)**. Antioxidant compounds act through several chemical mechanisms: hydrogen atom transfer (HAT), single electron transfer (SET) **(SANTOS et *al*., 2019).**

Natural antioxidants are synthesized in human body through metabolic process or are supplemented from other natural sources, and their activity very much depends upon their physical and chemical properties and mechanism of action. This can be further divided into two categories, i.e., enzymatic antioxidants and non-enzymatic antioxidants **(MAMTA et *al*., 2014).**

4.2. Mechanism of Antioxidants:

The mechanisms which followed by antioxidant defence are:

- ✓ Blocking of free radicals production
- ✓ Oxidants Scavenging
- ✓ The converting toxic free radicals into less toxic substances
- ✓ Blocking the production of secondary toxic metabolites and mediators of inflammation 5) Blocking of the chain propagation of the secondary oxidants
- ✓ Repairing the injured molecules
- ✓ Initiation and enhancing the endogenous antioxidant defence system.

All of these defence mechanisms act hand by hand for protection of the body from oxidative stress. The antioxidant systems in the human body consist of powerful non-enzymatic and enzymatic antioxidants **(ADWAS et *al*., 2019).**

Chapter III

Chamaeleon Chamaeleon

1. Definition:

Chameleons, from the Greek khamaileôn "lion that crawls on the ground", are insectivores and vertebrate animals belonging to the class of Reptiles (from the Latin reptilis «crawling» **(BOURDAIN, 2006)**. It is the only chameleon encountered in Western Sahara and on the northern edge of the Sahara **(TRAPE, 2012)**.

- **Synonyms**

- *Lacerta Chamaeleo* **(LINNE, 1758)**
- *Chamaelao Parisiensium* **(LAURENTI, 1768)**
- *Chamaeleo Vulgaris* **(DAUBIN, 1802)**
- *Chamaeleo Cinereus* **(BÖUTTGER, 1874)**
- *Chamaeleo Chamaeleo* **(MERTENS & MULLER, 1928).**

- **Common names**

French: Caméléon commun

Arabic: Buya, Hirbaya, Tata, Oum el bouya, Hirbaa

English: Common chameleon **(MOUANE, 2010)**

2. Classification:

The reptile class is made up of more than 8200 species and is divided into four orders (rhynchocephalics, crocodilians, chelonians and squamates) **(GUILLON, 2010).**

Chamaeleo Chamaeleon are part of the order of Squamates (from the Latin squama "tortoiseshell"), of the sub-order of Sauriens (from the Greek sauros, "lizard") and of the family of Chameleonidae sometimes called Cameleonidae or Chamaeleonidae **(BOURDAIN, 2006).**

Table 01: Classification of *Chamaeleo Chamaeleon* **(GUILLON, 2010)**

Kingdom	Animalia
Phylum	Chordata
Sub-Phylum	Vertebrata
Classe	Reptilia
Sub-Class	Lepidosauria
Order	Squamata
Sub-Ordre	Sauria
Infra-order	Iguania
Family	Chamaeleonidae
Sub-famille	Chamaeleoninae
Genus	*Chamaeleo*
Species	*Chamaeleo Chamaeleon*

3. Morphology:

The chameleon is a species that measures 30 cm **(MAMOU, 2011).** The head of the chameleons is pyramidal, wider at the level of the eyeballs and pointed towards the mouth .The helmet is the part of the head behind the eyes **(BOURDAIN, 2006).**

Two bony ridges (canthi laterales) lie on the head and they are usually covered with enlarged tuber scales. One part of the helmet up to the eyes, and the other part of the eyes up to the end of the mouth. Each of these bone ridges are divided into several parts. So on each side we have a rostral crest (canthus rostralis), an orbital crest (canthus supraorbitalis) and a lateral crest (canthus lateralisproprius) **(BOURDAIN, 2006)**.

Figure 01: *Chamaeleo Chamaeleon***(MAMOU, 2011)**

- **Tail:** in "real chameleons" (chameleons belonging to the genera *Chamaeleo*), the tail is longer than the head and body. It is prehensile and acts like a fifth member by grabbing the objects around it. At rest, it is normally rolled up under the cloaca and deploys as soon as the animal has to grip. When moving ashore, the tail is held back or upward in a semi-curved "S" spiral **(BOURDAIN, 2006).**

  The chameleon with itsprehensile tail particularly illustrates this point. It deploys it to capture the surrounding elements **(SAVEY, 2007).**

Figure 02: Prehensile tail of the Chameleon (*Chamaeleo Chamaeleon*) **(BOURDAIN, 2006)**

- **Blood:** The volume of blood in the chameleon represents around 3 percent of body weight **(BOURDAIN, 2006).** All reptile blood cells are nucleated including erythrocytes and thrombocytes **(SAVEY, 2007).**
- **Scales:** Chameleons, like all Reptiles, have keratinous thickenings on the entire surface of the body which form the scales. The scales are produced by the basal layer of the epidermis **(BOURDAIN, 2006).**
- **Skin:** The skin has the two classic layers encountered in vertebrates; the basal layer of the epidermis produces the scales, made up of keratin, which cover the whole body **(SAVEY, 2007)**; and a deep layer, the dermis. The thickness of the skin varies depending on where it lives. In desert regions, chameleons are protected from desiccation by thick, dry skin **(BOURDAIN, 2006).**

4. Geographic distribution and ecology

Among the 132 species reported in North Africa by **SCHLEICH *et al.* (1996),** 102 are present in Morocco, 92 in Algeria, 62 in Tunisia as well as in Libya and 43 in Egypt. We observe that the number of species decreases from west to east of North Africa **(MOUANE, 2010).**

The common chameleon has a North African distribution that extends to the east India and Sri Lanka via the near and Middle East and Arabia. *Chamaeleo Chamaeleon* is found in the south of Portugal and Spain, Sicily, Malta, Crete, and the north-eastern islands of the Aegean Sea (Chios and Samos); introduced in some of these places, in the Canaries and maybe elsewhere. Outside Europe: South Asia and North Africa (**MAMOU, 2011)**.

Due to their wide geographic distribution, chameleons have a wide variety of habitats. There are chameleons adapted to a desert climate (*Chamaeleo Namaquensis*, Namibia ; *Chamaeleo Chamaeleon* "saharicus", Algeria) **(BOURDAIN, 2006).**

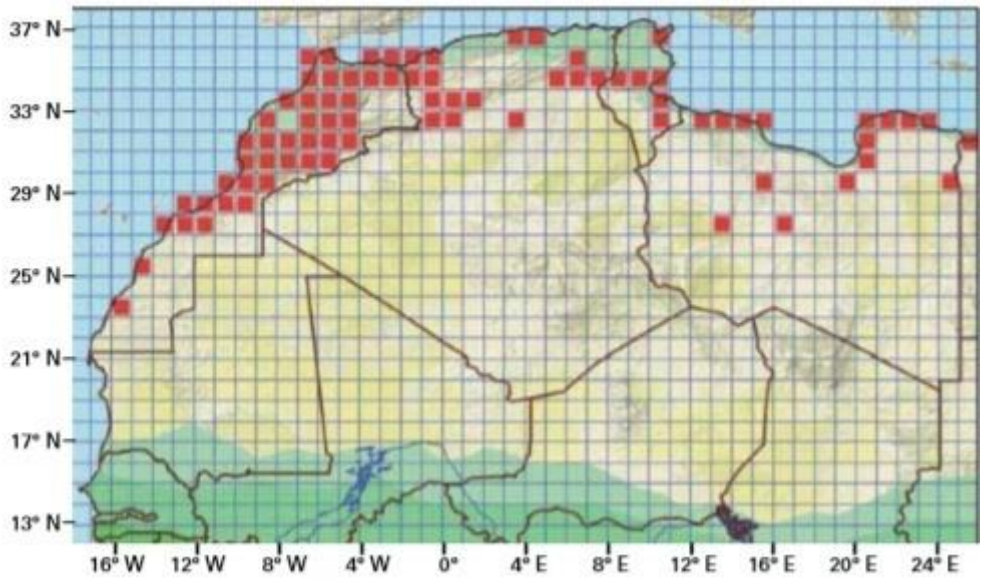

Figure 03: Geographic distribution of *Chamaeleo Chamaeleon* **(TRAPE et al., 2012)**

5. Reptiles' uses

Humans have utilized animals for producing drugs and to treatdiseases and injuries since ancient times. Wild animals and theirproducts constitute ingredients essential in the preparation oftraditional medicines. For many years, ethnobiological studies have been directed more to medicinal plants, but recently, variousinvestigators demonstrated that in culture/nature interaction thereis notable utilization of fauna for medicinal purposes in differenthuman societies **(ALVES & ROSA, 2010).**

Reptiles are among the animal species most often utilized in traditional medicine. **ALVES et *al.*, (2008)** reported that a total of 165 species of reptiles belonging to 104 generaand 30 families are used inpopularmedicine in different countries of theworld.

According to **FERREIRA et *al.*, (2009),** studies with extracts ofreptiles have been conducted with the aim of demonstrating theirantimicrobial and pharmacological activities.

The use of zootherapeutic products is also of importance fromthe point of view of public health, because diseases can be transmittedthrough their medicinal use **(ALVES &ROSA, 2007; FERREIRA et *al.*, 2009).**

Second part

Experimental part

Chapter I

Material & Methods

1. Characterization by investigation

1.1. Objective

In this study, we are interested in carrying out a ethnozoological investigation about the dry skin of *Chamaeleo Chamaeleon* in the region of El-OUED, in order to know:

- The different traditional and medicinal uses.
- The pathologies most treated by this animal.
- The mode of utilization and administration.

1.2. The study area

El-OUED region is located in the South East of Algeria, 600Km from the capitalAlgiers. It is in the northern confines of Erg Oriental (33 ° to 34 ° N and 6 ° to 8 ° E).
It is limited to the east by the immense Tunisian chott El-Djérid, to the north by the chotts Merouane, Melrhir and Rharsa, to the West by the wading of Oued Rhirchotts and to the South by Ouargla.

The climate of the El-Oued region is very hot and dry in summer and cold in winter and characterized by a low rainfall and high temperatures.

1.3. Investigation tools

The therapeutic information (mode of preparation and administration) were collected through questionnaire (in their local language Arabic), herbalists and consumer were interviewed to collect theinformations,diversity of information concerning this people; age class, gender and level of education.

1.4. Conduct of the investigation

In order to obtain informations about the therapeutic use of the skin of *C. Chamaeleon* in traditional medicine; our study was conducted from September 2019 to January 2020 in Souf region. A total of 40 (21male and 19female) people were interviewed to collect thes informations through a questionnaire using the local language **(Annex 01)**.

They were inquired, about the illnesses cured by dry skin of *C.Chamaeleon* and the manner in which the remedies were prepared and administered, including the duration of treatment.

- **Inclusion criteria**

✓ Voluntary persons live in EL-OUED region.

✓ Voluntary persons have knowledge about the therapeutic use of dry skin of *C.Chamaeleon*

- **Exclusion criteria**

⊗ Voluntary persons live in other region.

2. Material

2.1. Skin of *Chamaeleo Chamaeleon*

In this study, the dry skin of *Chamaeleo Chamaeleon* was obtained from the herbalist. Individuals vary in size and weight (19.43±2.20 cm, 37.26±10.18g). These dries skins were cut on sections. The sections of *Chamaeleo Chamaeleon* skin dry were stored at room temperature in airtight containers protected from bright light until the beginning of the experiment.

Figure 04:*Chamaeleo Chamaeleon* dried (Original photo)

2.2.Rats and husbandry condition

In this study, 20 male Wister rats weighting 180±15 g were obtained at the Animal Service of the Pasteur Institute, Algeria. The animals were acclimated for 25 days for adaptation to the conditions of the animal house, with an ambient temperature of (21±2.4) °C. Animals have free access to waterand food by a standard diet **(SOUTHON et *al.*, 1984).**

a. Treatment animals

After a period of adaptation, the animals were divided into four experimental groups of 5 animals eachas follows:

- ✓ **Group 1(T):** were rats served as controls
- ✓ **Group 2 (Ni):** were rats exposed to nickel (20mg / kg weight of rats) for 20 Days.
- ✓ **Group 3 (Ni + 400mg Ext):** were rats exposed to nickel (20mg / kg weight of rats) for 15 Days and then received oral dose of ethanolic extract of *Chamaeleo Chamaeleon* (400mg/Kg) dissolved in distilled water for 7 days.
- ✓ **Group 4 (Ni + 200mg Ext):** were rats exposed to nickel (20mg / kg weight of rats) for 15 Days and then received oral dose of ethanolic extract of *Chamaeleo Chamaeleon* (200mg/Kg) dissolved in distilled water for 7 days.

b. Sacrifice, blood and tissues collection

Animals were sacrificed under slight anesthesia by chloroform (94%) by inhalation; blood samples were collected during theslaughter of animals into EDTA tube to carried FNS and heparin tube. The serum was obtained bycentrifugation for 10 min at 3000 tour/min and used for biochemical analysis assays (glucose, urea, creatinine, GPT, GOT and CRP).

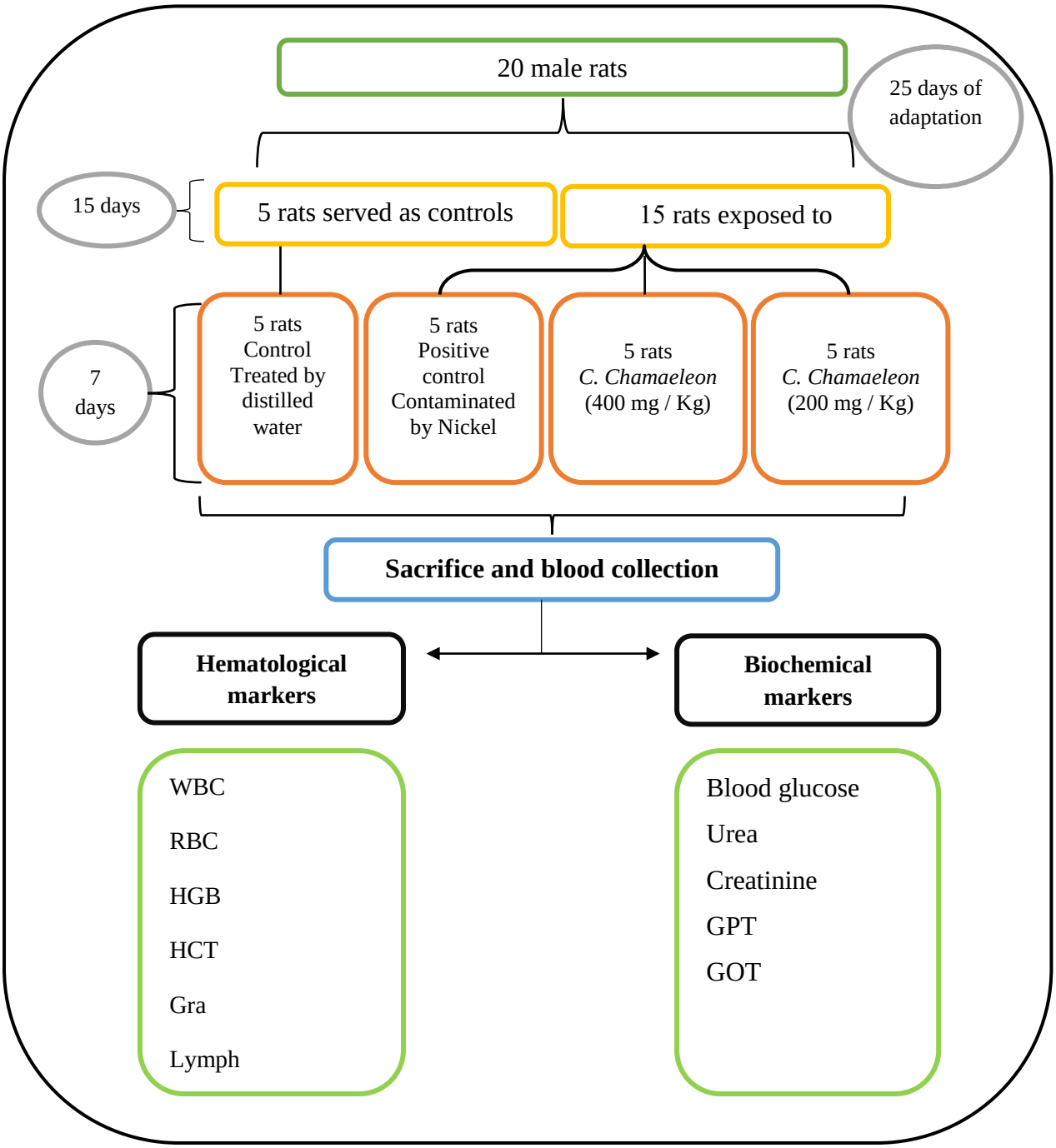

Figure 05: Experimental design of study

3. Methods

3.1. Characterization of dry skin of *Chamaeleo Chamaeleon*

3.1.1. Dry matter

The dry matter content of each sample is determined by drying the sample at 105 °C in a ventilated oven until a constant weight is obtained. The difference in weight corresponds to the loss of humidity and the residue characterizes the dry matter content of the sample **(TOUMI-NESRI, 2018).**

3.1.2. Total ash

The determination of mineral matter (ash) is based on incineration of the sample at a relatively high temperature (400 to 600 ° C) until obtaining white ash **(BENKHALED, 2018)**.

Three samples (Me) are weighed in crucibles in previously tared (Mi). The crucibles are introduced into a muffle furnace at 550 ° C for at least 3 hours until light gray to white ash is obtained. The crucibles are weighed (Mf) after cooling in desiccators **(AOAC, 1990).**

This method allows us to determine both the organic matter (OM %) and ash (C %) content that will be expressed relative to the dry matter. These rates are calculated according to the following formulas:

$$\mathbf{OM\ (\%) = [(Mi - Mf) / Me] \times 100}$$

Me: Mass of test portion

Mi: Initial mass (crucible + sample) before calcination.

Mf: Final mass (crucible + sample) after calcination

$$\mathbf{C\ (\%) = 100 - MO\%}$$

3.1.3. Protein

One gram of tissue was used. After grinding and homogenization of the tissuein 9 mL of TBS (tris 50Mm, Nacl150m M, pH 7.4) and by centrifuge of the tissue suspension (3900rpm / 20min), thenthe supernatant obtained is stored at -20 ° C.

The protein assay was carried out according to the method of **Bradford, (1976).** In a fraction of 100 µl aliquot is added 4 ml of reagent to Commassie Brilliant Blue. This one reveals the presence of proteins by coloring them blue. The absorbance is read at 595 nm against a blank of range.

3.1.4. Total carbohydrates

The extraction of carbohydrates has been carried out according to the method of **SHIBKOet *al.*, (1966).** Weigh 1g of sample sections; then grind it in 9mL of Tris Buffered Saline (TBS) + 1ml of trichloroacetic acid (TCA 20%) and Centrifuge for 10 min at 3000 rpm. Supernatant will be used for the determination of carbohydrates.

The determination of total carbohydrates was carried out according to **DUCHÂTEAU and FLORKIN (1959).** This method uses anthrone as a reagent and a standard glucose solution (1 g/l) as standard.

The method consists in adding to an aliquot fraction of 100 µl of supernatant, 4 ml anthrone reagent and after heating the mixture in a water bath (80 ° C for 10 min) a green color develops, the intensity measured at a wave length of 620 nm is proportional to the concentration of carbohydrates present in the sample.

3.1.5. Lipids

The determination of lipid was carried out according to **FOLCH et *al.*, 1957**. In the first step, lipids were extracted by homogenizing the tissue with 2:1 chloroform-methanol (v/v), and filtering the homogenate.

In the second step, the filtrate, which contained the tissue lipids accompanied by non-lipid substances, was freed from these substances by being placed in contact with water.

Then, 1 gof tissue should be diluted to a volume of 20 ml. The sample is crushed using a mortar. The ground obtained is filtered on degreased filter paper. The filtrate is thus collected in volumetric flasks and adjusted to 20 ml of Folch. 6 ml of filtrate was washed with distilled water (2 times the volume of the filtrate) in order to remove the proteins passed through the filtrate. The solution obtained is centrifuged at 1500 rpm for 10 minutes.

Two phases are obtained: the upper which is eliminated, the other lower, contains essentially all the tissue lipids which is used for the estimation of lipids.

3.2. Ethanolic extract preparation

Fifty nine g of the sections sample were extracted with ethanol using Soxhlet apparatus for 6 h. The ethanol extract of skin *Chamaeleo Chamaeleon* was concentrated by using rotary evaporator at40 °C.for the drying of ethanolic extract using oven.

The extraction yield (%) was calculated as follows:

Extraction yield (%) = (W1/W2) ×100

W1: weight of the extract after evaporating solvent and freeze-drying.

W2: dry weight of the sample **(TRUONG et *al*., 2019)**,

3.3. Ethanolic extract activities

3.3.1. Antioxidant activity

a. Free radical scavenging activity (DPPH)

DPPH: (2, 2-phenyl-1-picryl hydrazyl) is a free radical of purple color which is reduced to DPPH-H (2, 2-phenyl-1-picryl hydrazine), of yellow color, in the presence of donors of protons. The intensity of the coloring is inversely proportional to the anti-free radical activity **(SUSHMA & RANDHIR, 2019).**

The test was performed by mixing 50µl of extract or standard with 1.95ml of DPPH dissolved in methanol (0. 004 %). After shaking, the reaction was placed safe from light during 30 min and the absorbance was read at 517 nm.

The extracts were tested at concentrations ranging (10, 15, 20 and 25 mg/ml) for the ethanolic extract. The synthetic ascorbic acid was used as antioxidant standard at concentrations of (0.4, 0.2, 0.1, 0.06, 0.02, 0.01 and 0.002 mg/ml). Triplicate tests were carried out at each dilution of the standard and the extract. The results are expressed in anti-free radical activity **(SAFFIDINE et *al*., 2015).**

Antiradical activity I (%) = [(Ac - At)/Ac] x 100

Ac: Absorbance of control;

At: Absorbance of the sample

The minimum inhibitory concentration at 50% (IC50) is determined on the graph representing the percentage inhibition of DPPH as a function of the concentrations of the extracts and of the standard **(Sushma & Randhir, 2019).**

b. Ferric reducing/antioxidant power (FRAP)

The ferric ions (Fe3+) reducing antioxidant power (FRAP) method was used to measure the reducing capacity of the plant extracts with a slight modification, which involves the presence of extracts to reduce the ferricyanide complex to the ferrous form **(BAKARI et *al.*, 2018).**

Various concentrations of extracts (10 and 15 mg/ml)and the standard ascorbic acid (0.002-0.5 mg/ml) were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1% w/v). The mixture was incubated at 50°C for 20 min. Then 2.5 ml of trichloroacetic acid (10% w/v) was added to the reaction mixture.

Afterwards, it was centrifuged at 1000 g for 10 min. The upper layer of the solution (2.5 ml) was mixed with deionized water (2.5 ml) and ferric chloride (0.5 ml 0.1% w/v). The absorbance was measured at 700 nm. The reducing power of the extracts was represented as ascorbic acid equivalent (mg AAE/ g of extract) **(LABIAD1 et *al.*, 2017).**

3.3.2. Hemolytic activity

Five milliliters of blood was collected from a healthyindividual in EDTA tube. The blood was centrifuged at 1500 rpm for three minutes in a laboratory centrifuge. Plasma (supernatant) was discarded and the pellet was washed three times with sterile phosphate buffersaline solution (pH 7.2±0.2) by centrifugation at 1500 rpm for 5min. The cells were resuspended in normal saline to 2%.

In vitro haemolytic activity was performed by spectrophotometer method (**YANG et *al.*, 2005**). A volume of 0.5ml of the cell suspension was mixed with 0.5 ml of the extract (50, 100 and 1000 µg/ml concentrations in phosphate buffer saline). The mixtures were incubated for

30min at 37˚C in an incubator. The mixture was centrifuged at 1500 rpm for 10 min in a laboratory centrifuge.

The free hemoglobinin the supernatant was measured in UV-Vis spectrophotometerat 540 nm. Phosphate buffer saline and distilled water were usedas minimal and maximal haemolytic controls. Each experimentwas performed in triplicates at each concentration **(GAURAV et *al.*, 2011)**.The level ofpercentage hemolysis by the extracts wascalculated according to the following formula:

$$\text{Hemolysis \%} = (At - An) / (Ac - An) \times 100$$

At is the absorbance of test sample.

An is absorbance of the control (saline control)

Ac is the absorbance of the control (water control)

3.3.3. Antibacterial activity

a. Bacterialstrains and antibiotics:

Antibacterial activities are carried out on a total of 4 bacterial strains including 3 referenced strains from the Pasteur Institute of Algiers, one strain provided by microbiology laboratory El Mordjan from El OUED.

Table 03: Bacterial strains tested.

Bacterial strains	Code	Gram	Source
Bcillussubtilis	ATCC 6633	+	Institute of Pasteur of Algiers
Pseudomonasaeruginosa	ATCC 9027	-	
Salmonella typhi	ATCC 14028	-	
Staphylococcusaureus	ATCC 6538	+	
Streptococcus spp	/	+	El-Mordjan laboratory

It consists in streaking the surface of the nutrient agar previously poured and solidified in Petri dishes some colonies of the strains stored at 4° C (medium nutrient agar for bacteria).The Petri dishes each containing a strain of bacteria are incubated at 37° C for 24 h **(BOUCHOUKA, 2016).**

Three different antibiotics were used as references.It's about the penicillin 10µg **(P10)**, gentamicin 120 µg **(HLG120)** and vancomycin 30µg **(VA30).**

b. Ethanolic extract, disc and inoculum preparation:

Ethanolic extract of *C. Chamaeleon* are tested on previously selected bacterial strains **(Bouhaddouda, 2016)** Different concentrations (40; 30 and 10 mg / ml) of ethanolic extract were prepared by DMSO **(ABBASSI & TOUIL, 2019).**

In this method we use Whatman paper n ° 3 discs (6 mm) .Then they are put in a test tube and autoclaved and store until use **(BENZEGGOUTA, 2005).**

From a young and pure culture of 18 to 24 hours on isolation medium we prepare abacterial suspension in sterile physiological water (0.9% NaCl) Inoculation should be done in a few minutes after the preparation of the inoculums **(BENKHALED, 2018).**

c. Evaluation of antibacterial activity:

c.1. Principle and inoculation

Antibiotic sensitivity is studied by the method of diffusion in solid medium by the disc method, according to the recommendations of the Society's Antibiotic Committee French Microbiology. This method determines the sensitivity of fast-growing bacteria to a range of antibiotics **(LABIOD, 2016).**

Nineteen ml of MH agar culture medium in super cooling is poured into petri dishes. After solidification, a sterile swab soaked with bacterial suspension freshly prepared is spread on the surface of the agar three times, by turning the box about 60 ° after each application without forgetting to do rotate the swab on itself and finish inoculation by passing the swab over the periphery of the agar in order to have an equal distribution of the inoculum. We let dry the boxes for 15 to 20 min **(BOUHADDOUDA, 2016).**

c.2. Discs application, incubation and reading:

Once the culture medium which contains the microbial suspensions is solidified,we take aseptically using sterile forceps 4 sterile absorbent discs of 6 mm and it is imbibed with 10 µl of three concentrations of the ethanolic extract to test. A prepared disc soaked in DMSO is used as a negative control; then place it on the agar previously prepared (3 boxes for each species). And 3 antibiotic discs (P10, HLG120, VA30**)** are used as positive control (3 petri dishes) **(LABIOD, 2016).**

After these plates was incubated at 37°C for 24-48 hours. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganism and the average of the three measurements in all three replicates was calculated **(SAFFIDINE et *al.*, 2015).**

3.5. Statistical analysis

Our statistical study is carried out by the Minitab software using (Student t test) to comperemeans among our different experimental groups; Differences were considered statically significant at $p < 0.05$.

Chapter II

Results & Discussion

I. Results

1. Characterization of skin of *Chamaeleo Chamaeleon* according to the local population uses

1.1. Description of study population

In our study, the chosen population is characterized by many different characters shown in table (04) (Age, sex, education level). The selected population reaches 40 persons from wilaya of El Oued, after statistical analysis we are obtained the results showed in table below.

Table 04: Description of study population

Characters	Population	
Age	32.4±14.68	
Sex	Men%	52.5%
	Women%	47.5%
Educational level	primary%	10%
	medium%	17.50%
	High school %	12.50%
	High education%	20%
	Other %	40%

1.1.1. Type of knowledge

The results of tribal affiliation consist of large numbers of consumers (74.42%) in all population study (figure 06), in the same population it has been found sellers (13.95%) and others (11.63%).

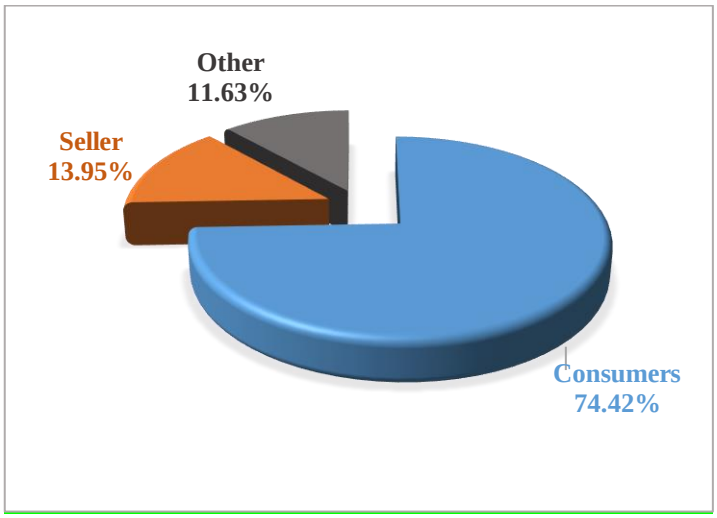

Figure 06: Distribution of consumers and sellers (N = 40)

1.1.2. Therapeutic uses

Concerning the therapeutic uses, the results presented in figure (07) show that the dry skin of *C.Chamaeleon* is mostly used for Tonsillitis (82.92%) than Rheumatism and Vitiligo (4.87%) and lesser degree used for involuntary urination, stomach pain and thyroid disease (2.43%).

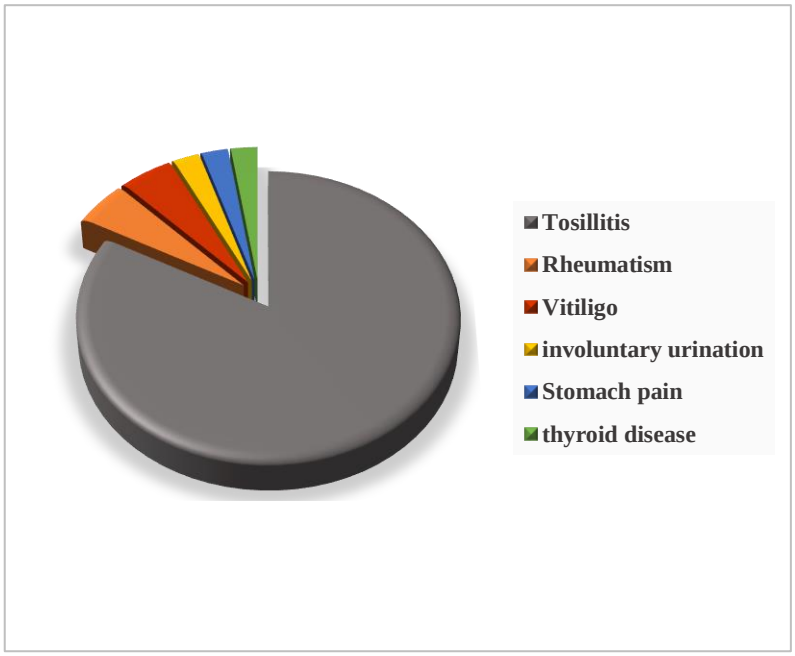

Figure 07: Distribution of questioned persons according to the therapeutic uses (N = 40)

1.1.3. Mode of use

The results presented in figure (08) show that the use under the tongue is most frequent (81.81%); following by the munching (11.36%) than the mode of anointing is the least used (6.81%).

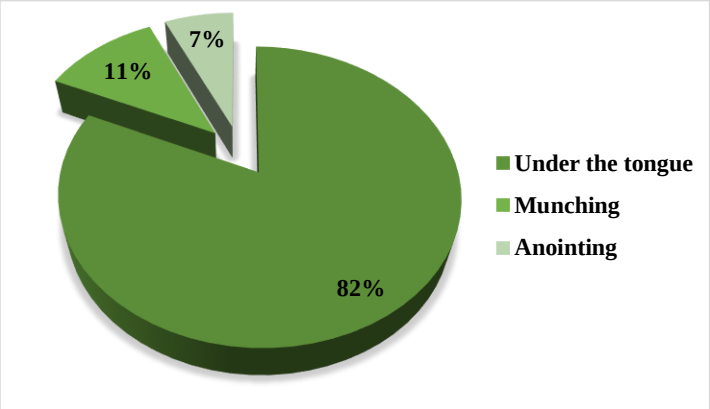

Figure 08 : Distribution of questioned persons according to the mode of use (N = 40)

1.1.4. Additives

The results obtained show that the dry skin of *C.Chmaeleon* prepared with honey is the most used (45.45%) , than in milk and olive oil (18.18%, 36.36%) respectively (figure 09).

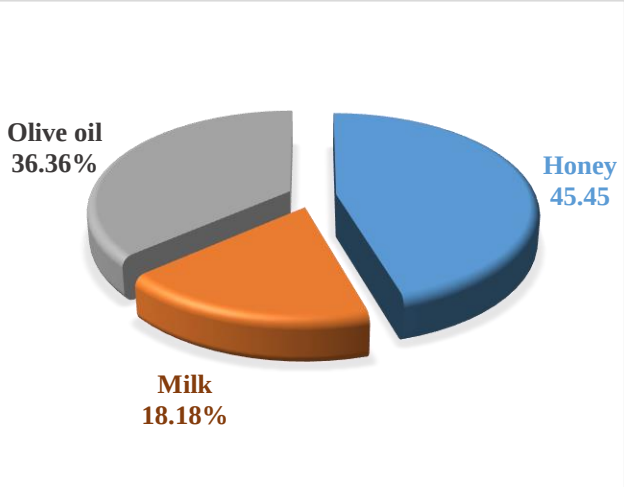

Figure 09: Distribution of questioned persons according to additives used (N = 40)

1.1.5. Duration of treatment

The results presented in figure (10), regarding to the duration of treatment, clearly shows that the most population takes two days of treatment (32.50%); and more than two days is presented by 27.50%. The rest of users takes one night (20%) or one day (20%) of treatment.

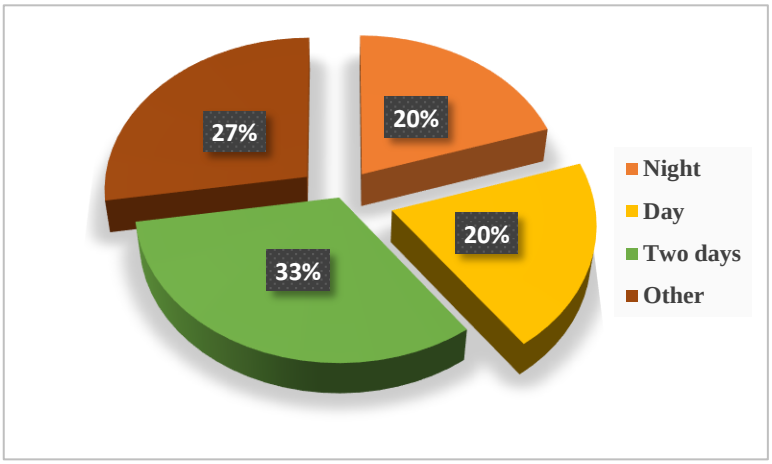

Figure 10: Distribution of questioned persons according to the duration of treatment (N = 40)

2. Experimental characterization of dry skin of *Chamaeleo Chamaeleon*

2.1. Physicochemical and biochemical composition of dried skin of *C. Chamaeleon*

The results of the physicochemical analyzes carried out on dried skin of *C.Chamaeleon* are mentioned in the table below (table 05):

Table 05: Physicochemical and biochemical composition of dry skin of *Chamaeleo Chamaeleon* (g / 100g of dry matter).

Compounds	Dry matter	Organic Matter	Total ash	Proteins	Total carbohydrates	Lipids
Dry skin of *C.Chamaeleon* (%)	92.74±0.61	77±0.03	23± 0.02	0.99± 0.16	8.3±1.4	2.73± 0.41

2.2. Extraction yield

After the extraction process, the extraction yield has been determined using the formula described by **TRUONG et *al*., (2019)**, the results are presented in the table 06.

2.3. *In vitro* assays of *Chamaeleo Chamaeleon*

2.3.1. Antioxidant activity

The evaluation of the antioxidant activity is carried out in two methods:

a) Ferric Reducing Antioxidant Power (FRAP) assay

The results in the table 06 indicate that the FRAP value is 4.43±0.09 (mg equivalent of ascorbic acid /g of extract) in the ethanolic extract of *C.Chamaeleon.*

b) DPPH radicals scavenging activity and IC50 value

It is well known that the antioxidant effect of the extract on DPPH is due to their ability to scavenge the DPPH free radicals by hydrogen donation. Our results (table 06), show that the IC_{50} of the extract (46.02 ± 0.02 mg / ml) is significantly higher than that of ascorbic acid.

Table 06: Extraction yield, values of the DPPH free radical scavenging (IC50) and FRAP assays

Extract	Extraction yield (%)	Antioxidant activity	
		DPPH IC_{50} (mg/mL)	**FRAP** (mg ascorbic acid /g extract).
Dry skin of *C.Chamaeleon*	20.78±0.01	46.02 ± 0.02	4,43±0.09

2.3.2. Antibacterial activity

Antibacterial activity results of ethanolic extract are shown in (table 07). This indicated that the ethanolic extract did not show any activity against three strains (*Bacillus subtilis, Salmonella Typhi* and *Pseudomonas aeruginosa*). The results also exhibited low susceptibility on *Staphylococcus aureus* and *streptococcus sp*. The antibacterial activities registered were weaker than the positive controls used (table 08).

Table 07: Diameter of zones of inhibition (mm) of bacterial growth (using ethanolic extract of *C.Chamaeleon*)

Extract (mg/ml)	Bacteria				
	B. subtilis	*S. Typhi*	*S. sp*	*S. aureus*	*P. aeruginosa*
40	6	6.5±0	7.5±1.3	6.7±0.2	6.5±0.5
30	6	6	7.2±0.2	6.2±0.2	6
10	6	6	7.7±1.2	6.9±0.7	6

Table 08: Diameter of zones of inhibition (mm) of bacterial growth (using antibiotics as positive controls)

positive controls	Bacteria				
	B. subtilis	*S. Typhi*	*S. sp*	*S. aureus*	*P. aeruginosa*
Gentamycin	27.33±2.5	26±1.7	29.33±2	28.66±1.5	31±0
Vancomycin	21.66±1.5	9.6±0.5	19.33±2.3	20.66±1.5	20.66±0.5
Penicillin	6	8±0	6	10.33±0.5	6

2.3.3. Haemolytic activity

In vitro, haemolytic activity on human erythrocytes of various concentrations of ethanolic extract was performed ; the total haemolysis was obtained using distilled water. Haemolytic activity of various concentrations is shown in figure (11); each concentration shows the mean of hemolysis percentage repeated in two experiments.

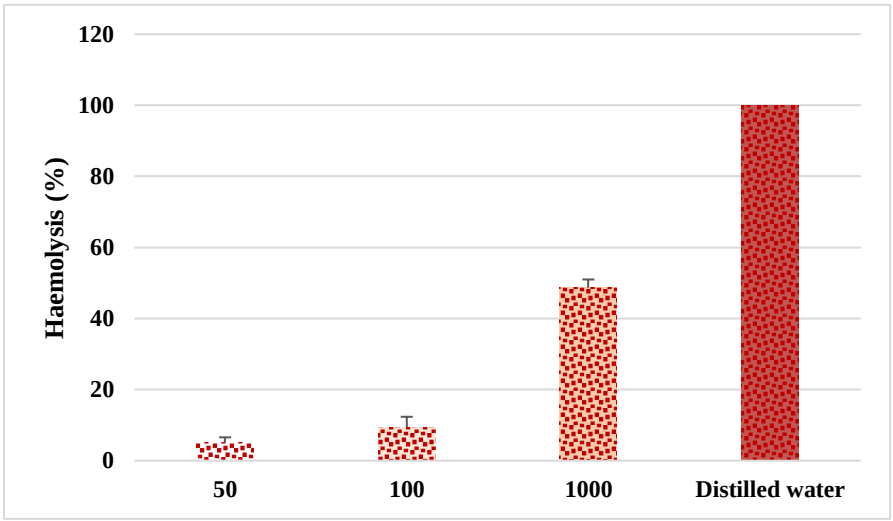

Figure 11: Haemolytic activity of ethanolic extract against human erythrocytes

2.4. *In vivo* assays of *Chamaeleo Chamaeleon*

2.4.1. Study of the relative weight of organs

Concerning the measurements of the relative weights of the organs of the various rats tested; the results are illustrated in the figure (12).

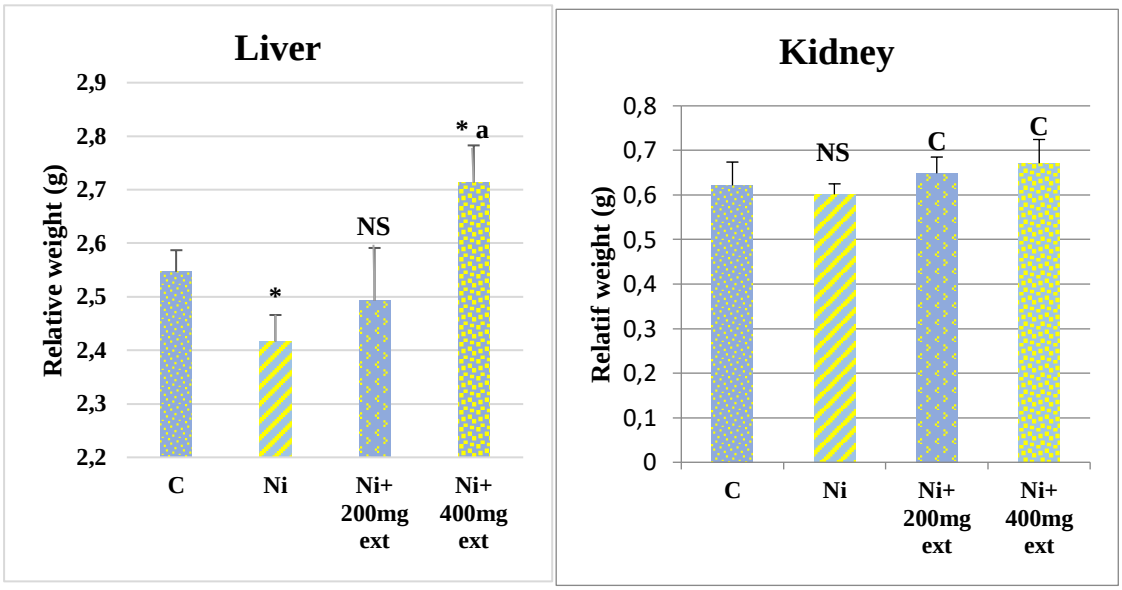

Figure 12: Relative weights of liver and kidney in control group and experimental groups.

Comparison with control group (T): * p <0.05; ** p <0.01; *** p <0.001, comparison with group treated with nickel (Ni): a p <0.05; b p <0.01; vs p <0.001, (n = 5 rats).

2.4.1. Hematological markers

In the present study, the determination of the Hematological parameters, after induction of an inflammation and the use of skin of *Chamaeleo Chamaeleon* as a treatment agent, is represented in the following figure (13).

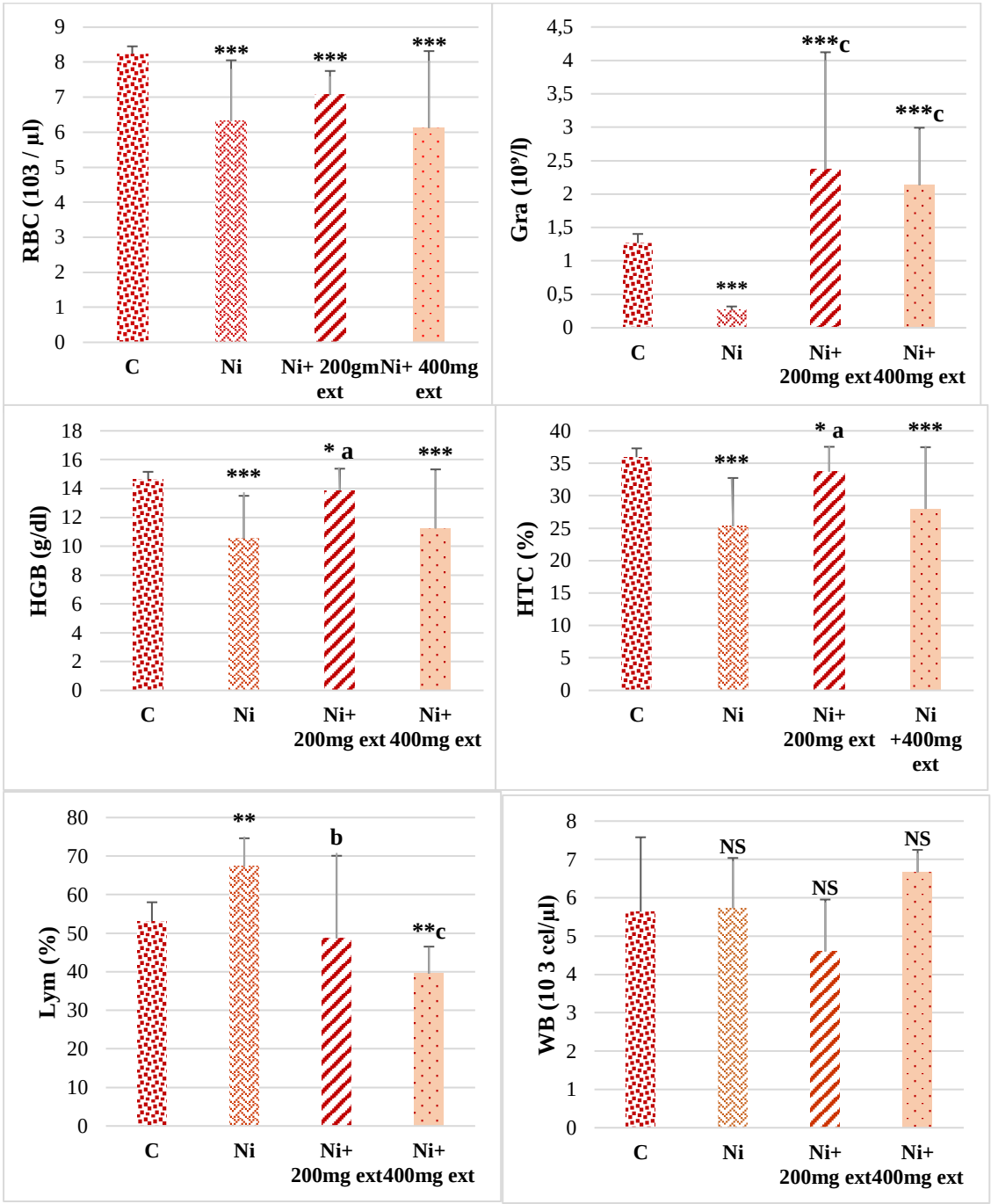

Figure 13: WBC, Lym, Gra, RBC, HGB and HTC levels of control and experimental groups.

Comparison with control group (T): * p <0.05; ** p <0.01; *** p <0.001, comparison with group treated with nickel (Ni): a p <0.05; b p <0.01; vs p <0.001, (n = 5 rats).

2.4.2. Biochemical markers

The results relating to the determination of biochemical markers (Blood glucose, Urea and Creatinine, Transaminases enzymes activities and C - reactive protein) are shown in the following figures (14, 15, 16 and 17).

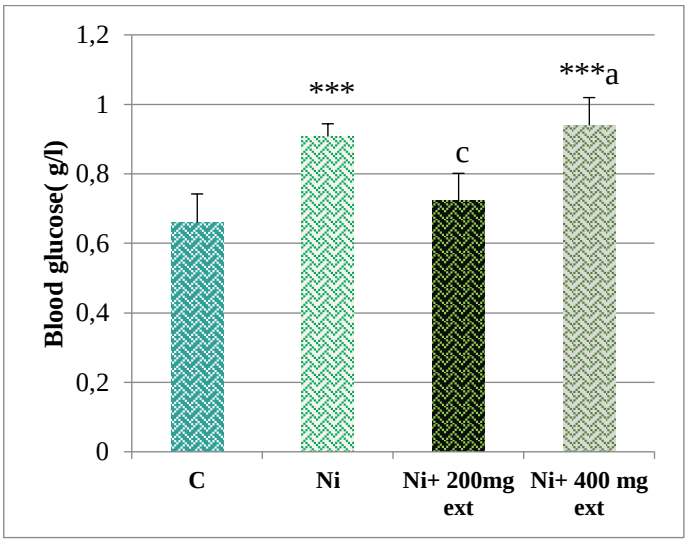

Figure 14: Blood glucose level in the control and experimental groups.

Comparison with control group (T): * p <0.05; ** p <0.01; *** p <0.001, comparison with group treated with nickel (Ni): a p <0.05; b p <0.01; vs p <0.001, (n = 5 rats).

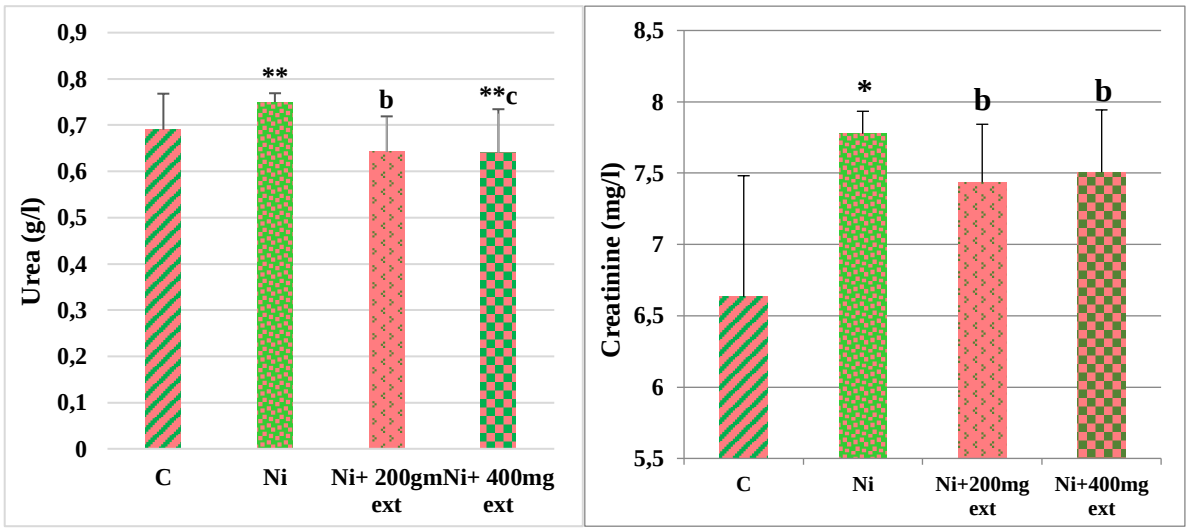

Figure 15: Urea and creatinine levels of control and experimental groups.

Comparison with control group (T): * p <0.05; ** p <0.01; *** p <0.001, comparison with group treated with nickel (Ni): a p <0.05; b p <0.01; vs p <0.001, (n = 5 rats).

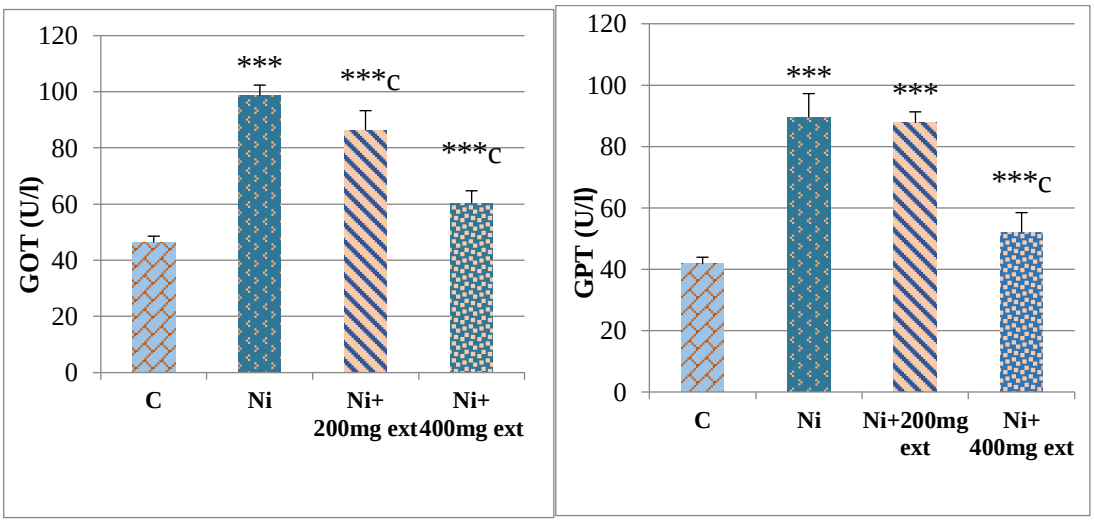

Figure 16: GOT and GPT activities in the control and experimental groups.

Comparison with control group (T): * p <0.05; ** p <0.01; *** p <0.001, comparison with group treated with nickel (Ni): a p <0.05; b p <0.01; vs p <0.001, (n = 5 rats)

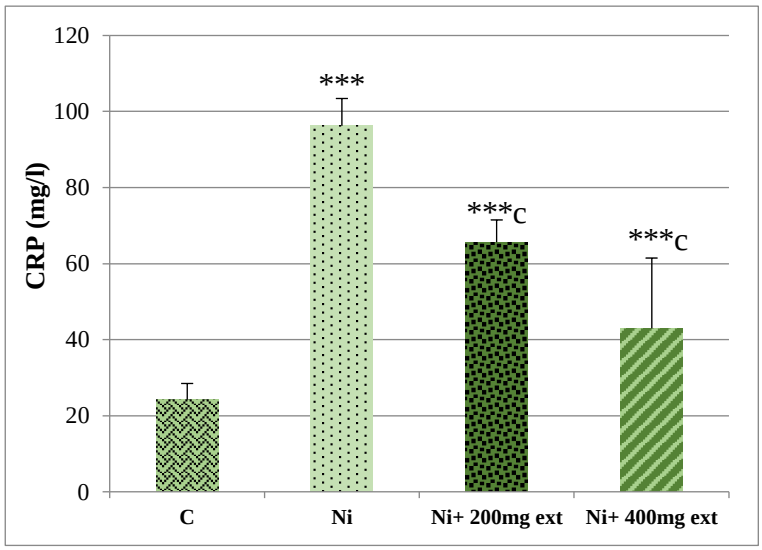

Figure 17: CRP levels of control and experimental group

Comparison with control group (T): * p <0.05; ** p <0.01; *** p <0.001, comparison with group treated with nickel (Ni): a p <0.05; b p <0.01; vs p <0.001,(n = 5 rats).

II. Discussion

The objective of our study is to determine the characteristics compound of *Chamaeleo Chamaeleon*'s dry skin and evaluate the biological activities and the effect of ethanolic extract of the dry skin against inflammation induced by nickel.

1. Characterization of skin of *Chamaeleo Chamaeleon* according to the local population

The respondent is made up of different ages with an average of 32 years coming from various professional origins. The respondents have different ages with an average of 32 years derived from various professional origins. In addition, we found that the population is characterized by the dominance of men (52.5%). This can be explained by the survey site, which is the market that is characterized by the abundance of men .The most of persons questioned are consumers because some of they are sellers in the same time.

Our results about the use of *C.Chamaeleon* skin in rheumatism and stomach pain is in agreement with **SCHMEDA-HIRSCHMANN et al., (2014) and ALVES et *al.*, (2009)** Furthermore, the results support with what is current about the use of *C.Chamaeleon* dry skin for tonsillitis.

Different ways of preparing and administering *C.Chamaeleon* dry skin were recorded. **VATS and THOMAS (2015)** reported that the skin of *Naja siamensis* is powdred and mixed with water. Our results show that the both of milk and honey are added to the preparation in order to improve the taste; especially for children. While the olive oil is added as an ointment.

The difference in the duration of treatment is depending on the immune response of presences or on the severity of the disease.

2. Physicochemical and biochemical characterization

The results of the dry matter of *C.Chamaeleon* dry skin shown are according to results 93.49 % reported by **TOUMI-NESRI, (2018)** about the skink meal. According to **EDES, (2015)**, it is desirable that the water content does not exceed a value of 8%, if we want to avoid microbial damage, when the humidity exceeds 14%, the conditions are conducive to the appearance of mold.

The ash rate makes it possible to judge the richness or the poverty of the meat in mineral element **(STARON, 1982).** The ash content in dry skin of *C.Chamaeleon* is lower than skink meal

39.504 % reported by **TOUMI-NESRI, (2018).** This value may be due to the drying method (filling with salt).

The results obtained in protein content are lower than those of skink meal 46.288% published by **TOUMI-NESRI, (2018).** From a qualitative point of view, the drying process causes physical, mechanical and biochemical changes resulting in denaturation of proteins.

The results show that the dry skin of *C.Chamaeleon* is low in lipid. These results are lower than skink meal 8.39 % mentioned by **TOUMI-NESRI, (2018).** And in agreement with result of Fish meal 3.6 % mentioned by **GUERREIRO &LAURENCE (1992).** The low level of lipid can be due to the addition of salt to the meat causes oxidation of these lipids.

Our results show the quantity of carbohydrate in *C.Chamaeleon* dry skin is around8.3±1.4.This content is higher than those of skink meal 0.024 % recommended by **TOUMI-NESRI, (2018).**

The carbohydrate contents obtained reveal that the glycogen is transformed into lactic acid after the animal's death **(CRAPLET &CRAPLET, 1979).** We can suggest that the absence of antioxidant activity due to the long time after the sacrifice of animal. Also the drying process causes enzymes denaturation.

3. *In vitro C.Chamaeleon* dry skin characterization

3.1. Extraction

In the extraction process, the most important criterion to be evaluated is the extraction yield (quantity in percentage). Extraction yield could be explained by the reasons below:

- Extraction methods include solvent extraction. The selection of the solvent is crucial for solvent extraction. Selectivity, solubility, cost and safety should be considered in selection of solvents **(Zhang et *al*., 2018).** The extraction of hydrophilic compounds uses polar solvents such as ethanol **(SASIDHARAN et *al*., 2010)**
- High temperatures increase the solubility and diffusion. Temperatures that too high, however, may cause the decomposition of thermolabile components **(ZHANG *et al*., 2018).**
- The extraction and drying factor must be considered as important **(NOORASHIKIN, 2014).**

3.2. Antioxidant activity

The DPPH radical scavenging assay has been widely used to evaluate antioxidant properties of compounds such as free radical scavengers or hydrogen donors (**KLOMPONG et**

***al*., 2007).** The anti-oxidant activity results obtained from ethanolic extract of *C.Chamaeleon* dry skin did not accord with the study of **Shams et *al*., (2019)** which show that the ethanol extract of *Uromastyx hardwickii* skin possesses good antioxidant properties in vitro.

The result of FRAP activity demonstrated a similar trend to DPPH radical scavenging activity. Our result did not accord with the study of **SAE-LEAW et *al*., (2015)** who found that the seabass skin exhibited FRAP activity.

Our results have demonstrated that the compounds contained in ethanolic extract could not scavenge DPPH radicals or reduce the ferricyanide complex (Fe^{3+}) to the ferrous form (Fe^{2+}). That may be due to absence of the donors of protons or electrons for free radicals.

3.3. Antibacterial activity

In the present study, we used an ethanolic extract of different doses. Thus, various bacteria and antibiotics were tested. The results obtained show a lack of antibacterial activity against the bacteria tested, with the exception of a weak inhibition in the case of *Staphylococcus aureus* and *streptococcus sp* namely an area of 6.9 ± 0.2 and 7.7 ± 1.2 mm respectfully. This value seems not to be affected by the dose of extract used (10, 30 or 40 mg / ml). On the other hand, the bacteria used show sensitivity towards antibiotic discs (Gentamycin, Vancomycin and Penicillin), with zones of inhibition from 8 to 31 mm.

Our results are close to those obtained by **BAZAZ et *al*., (2015),** which show an almost zero antibacterial effect of lizard skin on a large range of bacteria. Likewise, **SANTOS et *al*., 2012,** shows that the zone of inhibition of the ethanolic extract does not exceed 02 ml against the bacteria tested.

The skin provides a potential avenue of entry for bacteria, fungi and other invaders, this activity is mostly related by bio compounds **(CHANNING, 2006).** Reptiles live in an environment where a myriad of saprophytic and pathogenic microbes flourish putting them in constant direct contact with potential pathogens. Therefore, the skin acts a physical barrier by providing immediate protection from the environment and as a chemical barrier **(BERGSSON et *al*., 2005).**

The antimicrobial effect of animal tissues may be due to the presence of different biochemical groups which act on different mechanisms. This proposed mechanism has been

validated by the observation that antimicrobial peptides work rapidly apparently far too quickly for any process that involves translocation and binding to an intracellular target molecule **(SITARAM et *al.*, 2002).**

According to **FERREIRA et *al*. (2010)**, studies with extracts of reptiles have been conducted with the aim of demonstrating their antimicrobial and pharmacological activities. Various components of the extracts can act as permeabilizers of the cell membrane, increasing the cellular uptake of antibiotics. The interference on bacterial enzyme systems can also be a potential mechanism of action. These mechanisms of action can be involved in the combination of an antibiotic with a natural product extract at a sub-inhibitory concentration added directly to the culture medium **(SANTOS et *al.*, 2012).**

The weak antibacterial activity can be linked to the type of extract used (aqueous or ethanolic). Each solvent is capable of extracting phytochemicals which can probably only be obtained by the use of it, and because the pharmacological activity of an extract of a medicinal plant depends on the extracting solvent. Water is a solvent which preferentially extracts polar compounds such as, for example, di-, tri- and tetra-glycosylated flavonoids **(JONES &KINGHON, 2006).**

Ethanol is a polar solvent and miscible with water. It allows the extraction of polar phytochemicals like lectins, alkaloids, quassinoids, flavones, polyphenols, tannins and saponins. This solvent is also capable of separating micromolecules from macromolecules (proteins and carbohydrates) **(IQBAL et *al.*, 2006).**

3.4. Haemolysis assay

Lysis of erythrocytes was found to be increased with an increase of extract concentration. The effect of ethanolic extract on blood erythrocyte membrane showed a high hemolytic activity, the hemolytic percentage 5.15%, 9.45% and 48.65% were obtained for a dose of 50 µg/ml, 100 µg/ml and 1000 µg/ml respectively. The results showed that the ethanolic extract exhibited highest haemolytic effect (48.65%) at the concentration of 100 µg/ml compared to positive control (distilled water).

Haemolytic assays were performed because compounds possessing potent biological activity may not be useful in pharmacological preparations if they possess haemolytic effect. In addition, these data also may reveal some information about the mechanism of cytotoxicity.

Haemolysis can be induced by several protein toxins from animals **(WEI et *al*., 2013)**. Results show the toxicity result for human erythrocytes treated with ethanolic extract of *C.Chamaeleon* dry skin; this result is in agreement with the study of **Kyriachenko et *al.* (2020)** who showed that the skin secretions of *R. temporaria* and *B. viridis* at the tested concentrations (50 µg/mL) showed the same degree of erythrocyte lysis values (5.15%). while skin secretions of *P. ridibundus*, *B. bombina*,*P. fuscus* and *B. variegata* showed a degree of erythrocyte lysis values (76%, 63%, 66%, 76%) respectively at the same concentration.

The antioxidant compounds GSH, cysteine, and ascorbic acid only minimally reduced the haemolytic activity **(WEI et *al*., 2013).** The result indicates the low antioxidant activity which is inversely proportional with the haemolytic activity. From these results, we deduced that the ethanolic extract induced erythrocyte lysis by inducing pore formation in bilayer lipid membranes rather than causing oxidative damage.

We hypothesize that the hydrophilic pores in erythrocyte cell membranes induced by the salt contains in ethanolic extract caused a colloid osmotic burst that resulted in erythrocyte lysis.

4. *In vivo C.Chamaeleon* dry skin characterization

4.1. Relative weight

Liver relative weights show a significant decrease in group Ni ($p<0.05$), but there is not significant variation of liver relative weight in group treated with 200 mg of extract ($p>0.05$), however the results show a high significant increase in group treated with 400 mg of extract compared to control group ($p<0.05$).

Our results show also in group treated with 400 mg of extract a high significant increase ($p<0.05$) and no significant variation in group treated with 200 mg of extract compared to group Ni ($p>0.05$). As shown the figure (12), the results of kidney relative weight show that there is not significant variation in treatment and nickel groups compared to control group, while, there is a significant variation in groups treatment to Ni group ($p<0.001$).

The findings indicated an increase of relative liver weight of group Ni. This might be as a result of hypertrophy and the selective accumulation of nickel in the liver or nickel can lead to cell death by apoptosis of certain cell lines, due to the accumulation of toxic lipid derivatives such as ceramides. These results are in agreement with **KECHRID et *al.*, (2018)** and **DAS et *al.*, (2008).** Moreover, the treatment with 200 mg of ethanolic extract improved the relative liver weights.

4.2. Hematological parameters

The results show a very high significant decrease in RBC, Gra, HGB and HCT levels ($p<0.001$), with a high significant increase in Lym percentage in groups Ni ($p<0.01$), but there is no difference in WB number, compared to the control group ($p<0.05$), this results show also a significant increase in Gra number in treatment groups compared with control and nickel group ($p<0.001$).

The results of RBC, HGB and HTC indicated a very high significant decrease in group Ni and other treated with 400 mg of extract ($p<0.001$), compared to control group, while there is not significant variation on RBC, HCT and HGB levels in group treated with 400 mg of extract and on RBC in group treated with 200 mg of extract compared to group Ni ($p>0.05$), on the other hand, in group treated with 200 mg of extract; HGB and HCT levels are decreased with signification compared to control group ($p<0.01$), with a significant increase compared to group Ni ($p<0.05$).

For Lym percentage, the results obtained show a significant decrease in groups treated with 200 mg and 400 mg of extract ($p<0.01$, $p<0.001$) respectively compared to group Ni, but there is not significant variation in group treated with 200 mg of extract compared to control group, however the results show a high significant decrease in group treated with 400 mg of extract compared to control group ($p<0.01$). While there is not significant variation of WB number in the group of treatment compared to control group and group Ni ($p>0.05$).

Nickel toxicity causes significant changes in haematological parameters. Our results are in agreement with those of **DAS et *al.*, (2007),** which according to this author decrease in the number of GR, the percentage of HCT and the concentration of HGB, such a decrease could result in nickel-induced anaemia (non regenerative anaemia) arising from injury of haematopoietic stem cells.

The exposure to nickel induced reduction in red blood cells counts, hematocrit and heamoglobin concentration. Thus, it was suggested that nickel may adversely affect the hematopoietic process and bone marrow activity resulting in a reduction of red blood cells and hemoglobin, which is likely due to iron deficiency or chemically induced anemia **(BOUHALI et *al.*,2017).**

An increase of HGB and HCT levels following the treatment by 200 mg of ethanolic extract, it may be due to the reduction of the hematotoxic effect of nickel by the extract. Our

study is in according to **BALAMURUGAN et *al.*,(2007),** they further found that the administration of 80 mg/kg Earthworm pastehas restored the RBC and HGB value.

Nickel induced toxicity might result in significantly decreased of Gra count, authors have associated the cause to hindering of granulopoiesis induced by primary or secondary changes in haematopoietic organs **(Al-Ghanim et *al.*, 2011)** However the treatment with ethanolic extract increase the count of Gra, that may be due to reparation of granulopoisis. Similar findings reported by **Antony et *al*. (2007)** show that Chan Su, has been used in and treatment of infection and granulocytopenia.

Our analysis showed a significant increase in the percentage of level of lymphocytes (%). These results does not agree with those reported by **MAGAYE et *al.*, (2014)**. The signification increase level of lymphocytes (%) that may due to its products range from the neutralization of pathogens with specific antibodies to the activation of macrophages and to direct cytotoxic activity. The results also show a significant decrease in groups treated (200, 400 mg of extract), these results do not support the ones obtained by **CHEN et *al.*, (2015),** who found that *Naja naja atra* Venom increased lymphocyte percentage. Our results suggested that the ethanolic extract is composed of many active components and each of them produces different effects on immune system.

4.3. Biochemical parameters

as shown in the figure (14), the results of blood glucose parameters show a high significant increase in group Ni ($p<0.01$) and the group treated with 400 mg of extract ($p<0.001$) compared to control group, while there is no significant variation ($P>0.05$) in group treated with 200mg of extract. In the other side, the blood glucose was decreased with very high signification ($p<0.001$) in the group treated with 200mg of extract and significant elevation ($p<0.05$) in the group treated with 400mg of extract compared to group Ni.

As for blood glucose level, the results (figure 14) show a very high increase in Ni group compared to control, our findings in according to **DERBAL & KECHRID, (2019),** they further discussed that may be linked with inhibition of insulin secretion from Langerhans' islets and a block of glucose utilization by cells. , or the high glycogen breakdown and new supply of glucose production from other non-carbohydrate sources such as proteins **(DERBAL & KECHRID, 2019).** Nevertheless, it was reduced in group treated with 200 mg of extract, our

results are consisted with earlier study by **BALAMURUGAN et al. (2007),** they reported that blood glucose level was reduced in the 80 mg Earthworm paste/kg treated rats.

In the figure (15), our results show that the creatinine is significantly increased in group Ni ($p<0.05$) compared to control group. No significant deference in the two groups treated with 200mg and 400mg of extract compared to control group ($p>0.05$). Results show a decrease in creatinine level in treatment group compared to group Ni with very high signification ($p<0.001$).

Regarding the urea level the results presented in figure (15), represent a high significant increase in group Ni compared to control group ($p<0.01$). No significant variation of urea level in the group treated with 200mg of extract compared to control group ($P>0.05$), while there is a decrease in the group treated with 400mg of extract with high signification ($p<0.01$). Also, our results obtained show a high significant decrease ($p<0.01$) in the group treated with 200mg of extract compared to group Ni, however a decrease of urea in the group treated with 400mg of extract with a very high signification ($p<0.001$).

Biochemical parameters of kidney function show a significant increase in creatinine level and a high significant increase in urea level in Nickel group compared to control. These results are agreement with the study of **TIKARE et *al.*, (2013)** and **PRASAD et *al.*, (2006).** They showed significantly increased levels of urea and creatinine in nickel treated rats in comparison with untreated control.

The kidney was the major organ of nickel accumulation. The measurements of creatinine, urea, and uric acid are considered a tool for clinical diagnosis of renal dysfunction following acute and chronic oxidative injury these markers are the end products of various metabolic pathways that are excreted in the urine via glomerular filtration whose serum levels are an indicator of renal functions.

So the significant augmentation in serum urea, creatinine upon nickel exposure possibly as a result of cellular damage due to the excess free radical production, this testifies to the installation of renal insufficiency, and may result in a decrease in reabsorption at renal epithelium and disruption of glomerular filtration rate. In other words, the decline in glomerular filtration may be due to a decrease in the number of functional nephrons **(BOUHALIT et *al.*, 2017).**

While in treatment groups the results of biochemical function of kidney appeared a decrease significant in urea and creatinine levels. These results are in agreement with the studies of **WANG & QIN, (2018)** who found that *Najanaja atra* Venom may exert protective effects on nephropathy in rats. Our results also are similarly to **WANG et *al.*, (2015)**; result shown that Cobrotoxin may decrease kidney damage and maintain normal renal function. We could say that ethanolic extract ameliorated renal pathological lesions and may ameliorated glomerular damages.

Our results obtained (figure 16) demonstrate that the group Ni and different treatment groups increased with very high signification compared to control group ($p<0.001$). Although there is a no significant deference ($p>0.05$) of GPT between the group treated with 200mg of extract compared to group Ni, while the group treated with 400mg of extract decreased with a very high signification ($p<0.001$).

Concerning the GOT results obtained show that a very high significant increase ($p<0.001$) in group Ni and treatment groups compared to the control group. In addition a decrease of GOT with very high signification ($p<0.001$) in treatment groups against nickel group.

Increase of both transaminases GOT and GPT have been observed in the serum of rats following Ni treatment, our results are consisted with earlier study reported by **Kechrid et *al.*, (2018)**. The increase could be attributed to the hepatic damage resulting in increased release of functional enzymes from the biomembranes **(Misra et *al.*, 1990)**. According to other mechanisms, an increase in serum GOT and GPT activities is normally indicative of leakage of these enzymes which may be due to tissue damage following nickel toxicity thereby resulting in altered membrane permeability, the latter being necessary condition for the release of these enzymes from the tissue into the plasma **(SIDHU et *al.*, 2004).**

The levels of these transaminases might provide a clear indication on the extent of cytotoxic damage that occurs in various tissues. However the addition of ethanolic axtract restores the altered levels of GOT and GPT. These reports emphasized the hepatocurative efficacy of the extract **(PARI & PRASATH, 2008).**

The results presented in figure (17) clearly show that CRP in group Ni and treatment groups is increased compared to control group with very high signification ($p<0.001$).In contrast, there is a decrease of CRP in treatment groups compared to group Ni with very high signification ($p<0.001$).

CRP is one of the important markers of inflammation. it is synthesized by the hepatocytes. The primary regulators of CRP are the cytokines interleukin (IL)-6 and IL-1β and tumour necrosis factor (TNF)-α. The CRP level appeared a very high increase in Nickel group compared to control this result is in agreement with **Deng et *al.* (2009)** who showed that Concentrations of CRP in the serum were more higher in Nickel group than the control group.

Cytokines synthetized by neutrophils and macrophages stimulate the production of acute-phase proteins, including C-reactive protein (CRP). CRP levels in serum increase in response to IL-6 during infection and inflammation, which appears during the late phase of infection **(KUMAŞ et *al.*, 2016).**

Increased levels of CRP were decreased by ethanolic extract, these decrease may be due to reduce the number of immune cells, following by a decrease of cytokines synthesis, such as our results shown about lymphocytes reduction.

Conclusion

Conclusion

Studies on the valuation of natural products of animal origin are necessary, because, firstly, it is an area that remains little explored in relation to plant substances, and secondly; the medicinal potential of animal derivatives can lead to significant advances in conventional medicine.

Similarly, the desert represents a biotope rich in bioresources with biological properties, notably important therapeutic.

The aim of this study is the evaluation of biological activities, essentially; the anti-inflammatory effect of ethanolic extract of *C.Chamaeleon* dry skin against inflammation induced by nickel.

Physico-chimical and biochemical characterization, show that the dry skin of *C.Chamaeleon* is low in lipids and sugar content , protein and ash content. The *in vitro* study of ethanolic extract of *C.Chamaeleon* appeared a weak antibacterial and antioxidant activity with an importance hemolytic activity.

The *in vivo* study of ethanolic extract of *C.Chamaeleon* dry skin show up their important Anti-inflammatory activity, and appeared that the treatment with ethanolic extract improves relative liver weight, which clearly shows the therapeutic effectiveness against tissue damage to this organ and the absence of the effectsecondary toxic to these target organs.

Treatment with ethanolic extract induces significant restoration of certain hematological and biochemical parameters, which show the therapeutic effect of this ethanolic extract against physiological dysregulation of several biological systems in relation to the parameters studied.

Chamaeleo Chamaeleon is one of the little studied lizards, so, given the importance of these results, it would be interesting to continue the research, taking in considering the following recommendations:

- Detecting the compounds of ethanolic extract.
- Extraction with different types of solvent.
- Compare the biological activities with fresh samples of skin.
- study other biological activities with different doses and extract

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Annexes

Annex 01 : Questionnaire sheet

Name : ….

Age : …

Education level : primary ☐ medium ☐ High school ☐ High education ☐ Other ☐

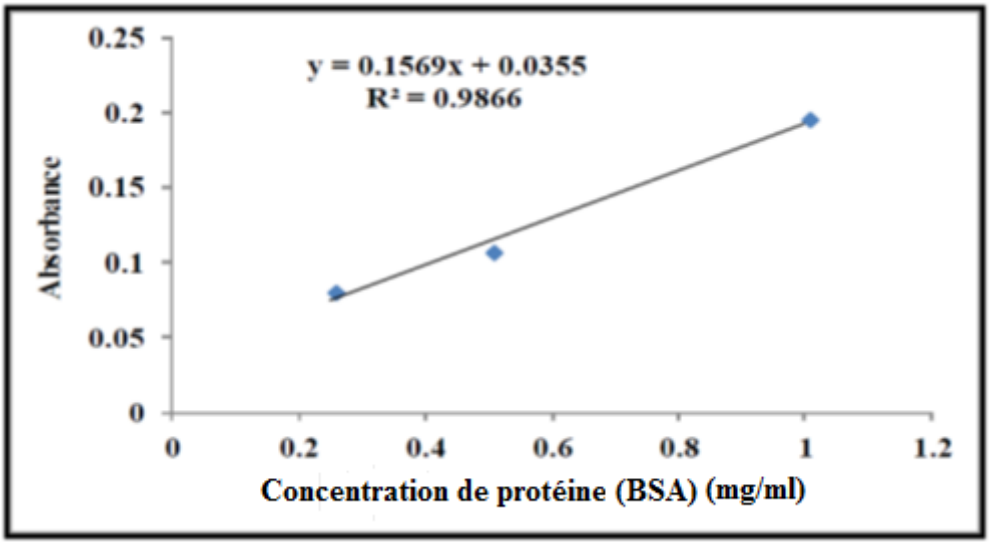
Sex : Women ☐ Men ☐

Chamaeleo Chmaeleon

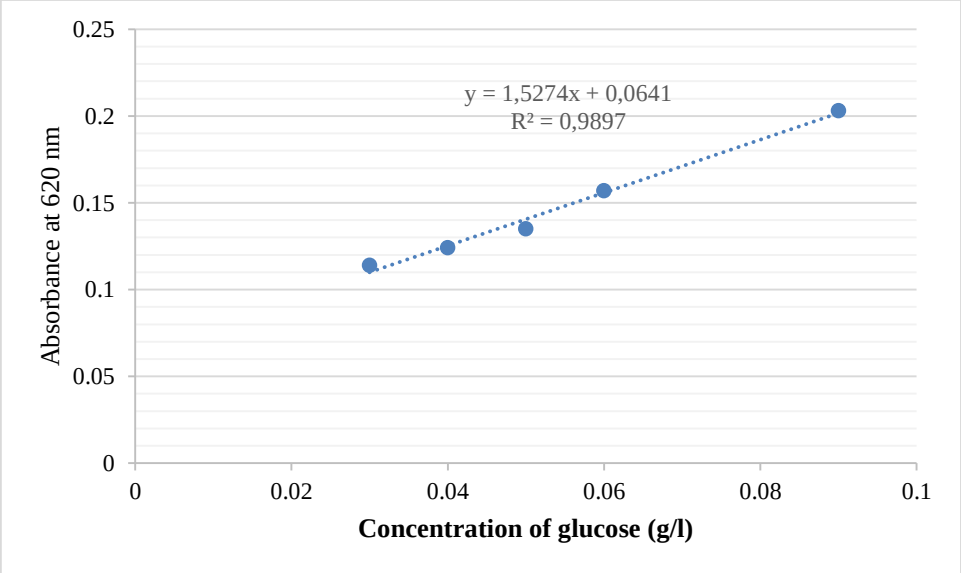
In order to know the multiple uses of *Chamaeleo Chmaeleon* we invite you to answer the following questions

1- You are: consumer ☐ seller. ☐ another ☐
2- Indications: nutritional ☐ medical ☐ Another ☐
3- Manual : ……..
4- Are there any extras : …….
5- Is the disease accompanied with fever : Yes ☐ No ☐
6- Do you take certain medications : Yes ☐ No ☐
7- Frequency of use : Once ☐ Twice ☐ More ☐
8- Duration of treatment : one night ☐ one day ☐ two days. ☐
9- Do you use : all the time ☐ just sometimes ☐
10- Do you know about other uses : ……..

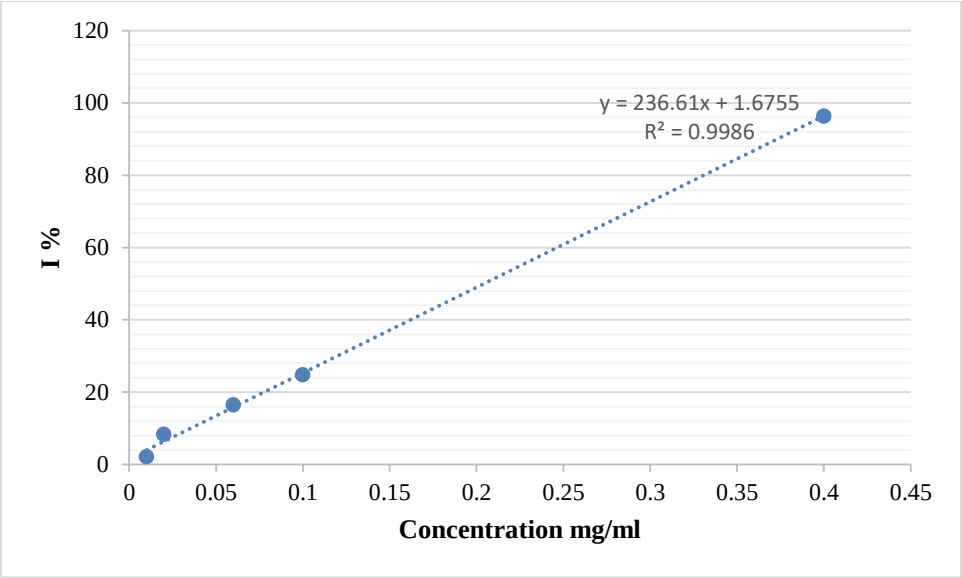
Annex 02 : Calibration curves



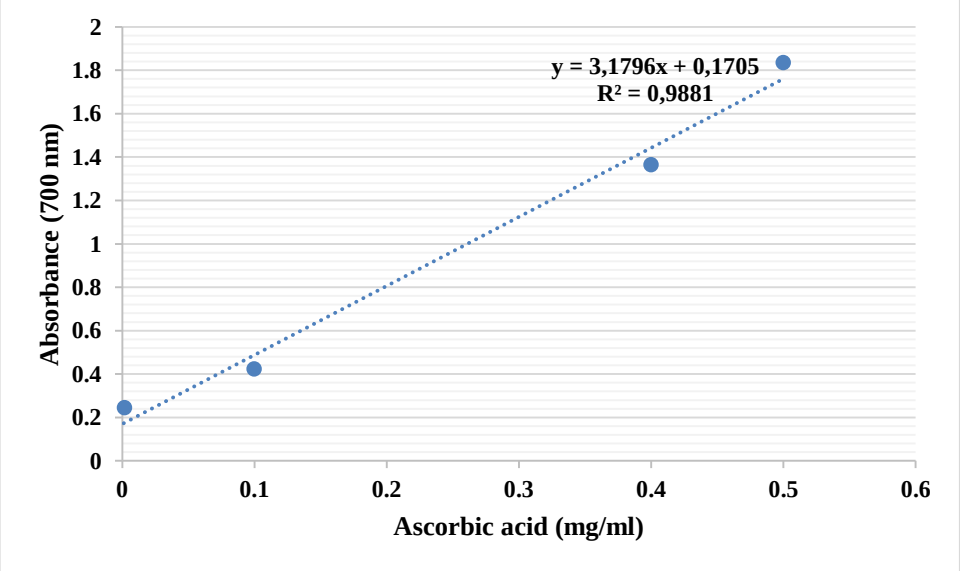
A linear calibration curve of protein



A linear calibration curve of carbohydrates



A linear calibration curve of Ascorbis acid (DPPH assay)



Linear calibration curves of Reducing power assay FRAP

Annex 03: Antibacterial activity pictures