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Effect of oleaster oil on the toxicity of a food colorant tartrazine

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*In the Name of **ALLAH**, Most Gracious, Most Merciful*

*All the Prays is due to **ALLAH** alone, the Sustainer of all the worlds*

*This work is dedicated to my wonderful family, to my mother "**Karima**" mostly, who was my motivator to complete this dissertation; my dear father "**youssef**", my beautiful sister "**Asma**" and my wonderful brother "**Ahmed**", my grandmother who was never forgotten me in her prayers and my grandfather.All the rest of my family special my maternal aunt "**Hassina**" and her little angels "**Tasnim**" and "**Wassim**" and all my friends…thanks a lot; I appreciate your stand up and support to me.*

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CHAIMA

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*To my mate in the dissertation **AOUISSI CHAIMA***

To my dear teachers.

To anyone who loves me.

To anyone who helped me with a word, an idea or encouragement.

MADJIDA

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ABBREVIATIONS LIST

Abbreviation	Signification
ADI	Acceptable daily intake
ANOVA	Analysis of variance
BBC	Coomassian blue
CAC/GL	Class names and the international numbering system for food additives
CCE	Commission for Environmental Cooperation
CDNB	1-chloro-2,4-dinitrobenzene,
CE	Commission of the European Communities Regulation
CFR	Code of Federal Regulations
COI	European Commission Regulation
EDTA	Ethylenediaminetetraacetic
FAO	Food and Agriculture Organisation of the United Nations
GSSG	Oxidized Glutathione
IA	Acid index
Ip	Peroxide index
Ir	Refractive index
Is	Saponification index
ISO	International Organization for Standardization
JECFA	Expert Committee on Food Additives
KOH	Potassium hydroxide
LEICA RM 2125RTS	Rotary microtome
Mo_8O_{23}	Molybdenum oxides
$Na_2S_2O_3$	Sodium thiosulfate
NAS	National Academy of Science
nD	refractive index scale
NRC	National Research Council

PUFA	Polyunsaturated fatty acids
ROS	Reactive oxygen species
SLEE MTP	Tissue processor manual
SLEEMPS/P1	Compact paraffin embedding center
rpm	Revolutions per minute of rotor
TCM	Traditional Chinese Medicine
Tz	Tartrazine
US	The United States
WHO	World Health Organisation
W_8O_{23}	Tungsten oxides

ABSTRACT

The present study consists of evaluating the toxic effects of the food dye "Tartrazine" on certain vital functions in male white rats "Albino Wistar", and, on the other hand, to test the modulating effect of oleaster oil against these toxic effects induced by this dye.

Initially, in order to determine the antioxidant power of oleaster oil, a preliminary phytochemical study was carried out, initiated by a quantitative analysis of phenolic compounds and followed by an anti-radical test. The results obtained reveal the presence of significant levels of total phenols with high antioxidant power against the DPPH radical.

In addition, our results showed that the administration of "Tartrazine" for 21 days caused alterations in the body, resulting in the emergence of an oxidative stress state, This is revealed by an increase in lipid peroxidation, disturbances in the antioxidant systems; reduced glutathione levels, enzymatic activities of glutathion-S-transferase, catalase and glutathione peroxydase. However, oleaster oil supplementation to the rats administered mitigated some of the toxic effects of the dye.

Histopathological results showed minor damage in the tissues of the kidney, liver.The food colorant Tartrazine caused; renal hemorrhages, the dilation of the renal tubules, as well as the infiltration of hepatic inflammatory cells. While oleaster oil supplementation in rats protected the liver and kidney tissue by reducing these histological changes.

In conclusion, the present study showed that the intake of Tartrazine causes disturbances in the body, which are mitigated by the use of oleaster oil, which has a high antioxidant capacity to reduce oxidative stress caused by the dye.

Keywords: Food dye; Tartrazine; Oxidative stress; Oleaster oil.

RESUME

La présente étude consiste à évaluer d'une part les effets toxiques de colorant alimentaire "Tartrazine "sur certaines fonctions vitales chez les rats blancs mâles «Albino Wistar», et d'autre part, de tester l'effet modulateur de l'huile d'oléastre contre ces effets toxiques provoqué par ce colorant.

Dans un premier temps, et afin de déterminer le pouvoir antioxydant de l'huile d'oléastre, une étude phytochimique préliminaire a été effectuée, initiée par une analyse quantitative des composés phénoliques et suivie par un test anti-radicalaire. Les résultats obtenus ont révèle la présence des taux importants des phénols totaux avec un pouvoir antioxydant élevé contre le radical DPPH.

En outre, nos résultats ont montré que l'administration du "Tartrazine" pendant 21 jours a provoquée des altérations au niveau de l'organisme, traduisant par l'apparition d'un état de stress oxydant, ceci est révélé par une augmentation de la péroxydation lipidique, des perturbations dans les systèmes de défense antioxydants ; taux de glutathion réduit, les activités enzymatiques de la glutathion-S-transférase et la glutathion peroxydase. Cependant, le traitement par l'huile d'oléastre aux rats administrés a atténuée certains effets toxiques de colorant.

Les résultats histopathologiques ont montré des légères dommages dans les tissus des reins, du foie. Le colorant alimentaire Tartrazine provoqué ;des hémorragies rénales, la dilatation des tubules rénaux, ainsi l'infiltration des cellules inflammatoires hépatiques. Tandis l'huile d'oléastre a protégé le tissu hépatique et rénal contre ces altérations histologiques.

En conclusion, la présente étude a montré que la consommation de Tartrazine provoque des perturbations au niveau de l'organisme, qui sont atténuées par l'utilisation de l'huile d'oléastre, que possède potentiel antioxydant capable de contracter le stress oxydant induit par la toxicité de colorant.

Mots-clés : Colorant alimentaire ; Tartrazine ; Stress oxydant ; Huile d'oléastre.

الملخص

تتكون الدراسة الحالية من تقييم التأثيرات السامة لملون الطعام "التارترازين" على بعض الوظائف الحيوية في ذكور الجرذان البيضاء "Albino Wistar"، ومن ناحية أخرى اختبار التأثير التعديلي لزيت الزيتون البري "الزبوش" ضد هذه التأثيرات السامة الناجمة عن هذه الصبغة.

أولاً، من أجل تحديد القوة المضادة للأكسدة لزيت الزيتون، تم إجراء دراسة كيميائية نباتية أولية، بدأت بتحليل كمي للمركبات الفينولية وتلاها اختبار مضاد للجذور. كشفت النتائج التي تم الحصول عليها عن وجود مستويات كبيرة من الفينولات الكلية ذات قوة مضادة للأكسدة عالية ضد جذري DPPH.

بالإضافة إلى ذلك، أظهرت نتائجنا أن تناول "التارترازين" لمدة30 يومًا تسبب في حدوث تغيرات في الجسم، مما أدى إلى ظهور حالة من الإجهاد التأكسدي، ويتجلى ذلك من خلال زيادة بيروكسيد الدهون، واضطرابات في أنظمة الدفاع المضادة للأكسدة؛ انخفاض مستويات الجلوتاثيون، والأنشطة الأنزيمية للجلوتاثيون-S-ترانسفيراز والجلوتاثيون بيروكسيداز. ومع ذلك، فإن زيت الزيتون البري الذي تم إعطاؤه للفئران خفف من بعض التأثيرات السامة للصبغة.

أظهرت النتائج التشريحية المرضية تلفاً طفيفاً في أنسجة الكلى والكبد. تسبب الملون الغذائي التارترازين في حدوث نزيف كلوي وتوسع الأنابيب الكلوية بالإضافة إلى ارتشاح الخلايا الالتهابية الكبدية. في حين أن معالجة الفئران بزيت الزيتون حافظت على أنسجة الكبد والكلى عن طريق تقليل هذه التغيرات النسيجية.

في الختام، أظهرت الدراسة الحالية أن استهلاك التارترازين يسبب اضطرابات في الجسم، والتي يتم تخفيفها باستخدام زيت الزيتون البري، مع وجود قدرة عالية مضادة للأكسدة لتقليص الإجهاد التأكسدي الناجم عن هذه الصبغة.

الكلمات المفتاحية: ملونات غذائية؛ التارترازين؛ الاكسدة؛ زيت الزيتون البري.

BIBLIOGRAPHIC PART

General Introduction

General Introduction

Population growth and changes in lifestyle throughout the past few decades have led to significant modifications in food product composition. The food business also underwent a significant shift as a result of changing dietary preferences and nutritional needs, which included the addition of new food components to food items. **(Martins et *al*., 2018)**

The number of artificial and natural food additives used by the global food business has increased in the last several decades. Furthermore, foods may include poisonous or dangerous ingredients that are overlooked by people. Food colors are one of the many groups of chemicals used in food that are intended to increase food's appeal to customers. Regretfully, it has been reported that a number of them are carcinogenic, genotoxic, and mutagenic. **(Zingue et *al*., 2021)**

Of course, food additives are chemical substances; they are defined by the internationally recognized Codex Alimentarius (Food and Agriculture Organisation of the United Nations/World Health Organisation [FAO/WHO], 2016) as any compound that is purposefully added during the manufacturing, processing, preparation, treatment, packing, packaging, transport, and holding of food in order to fulfill a technological function (including organoleptic). These compounds are not typically consumed as food on their own or as an ingredient in food. Additives or pollutants added to food in order to enhance or preserve its nutritional value are not included in this definition. Industrialized foods use an extensive list and a lot of chemical additives to preserve and/or enhance their biological, physicochemical, rheological, and sensory qualities, including pH, texture, homogeneity, color, flavor, sweetness, crunchiness, overall quality, and stability, Food additives are categorized into 25 groups based on their commercial use; including over 230 distinct chemicals. Certain chemicals exhibit many major actions because of their distinct functions, which vary depending on the food's processing method and additional amounts. **(Martins et *al.*, 2018)**

Natural and synthetic additives are the two main categories into which food additives are often separated. As a result, the primary method used to create natural food additives is purifying the components that come from plant or animal sources. On the other hand, chemically synthesized additives are made from chemical raw materials that may be used to extract and purify organic or inorganic stuff. **(Wu et *al*., 2021)**

Food additives are divided into several functional groups. Section 2 of the Codex Alimentarius Standard CAC/GL 36-1989 has a table including the additive functional groups listed below **(Blekas, 2016)** :

- Acidity regulators are additions that regulate a food's acidity or alkalinity.
- Anticaking agents are additions that lessen food ingredients' propensity to stick together.
- Additives known as antifoaming agents work to stop or lessen foaming
- Antioxidants are additives that help foods last longer on the shelf by preventing oxidation-related degradation.
- Bulking agents are food additions that increase bulk to a product without significantly affecting its energy value.
- Bleaching agents are food additives used to decolorize food (apart from wheat).
- Carbonating agents are additions that give food its carbonation.
- Carriers are additions that make food additives or nutrients easier to handle, apply, or use by dissolving, diluting, dispersing, or in some other way changing their physical makeup without changing the food additive or nutrient's function or having any technical impact.
- Colors are additions that may change or add color to food.
- Color retention agents are additives that maintain, enhance, or stabilize a food's color.
- Emulsifiers are food additives that create or preserve a consistent emulsion of two or more phases.
- Emulsifying salts are additions that reorganize proteins to stop fat from separating while processed food is being made.
- Firming agents are additives that maintain the crispness and firmness of fruit or vegetable tissues, or they can work in conjunction with gelling agents to create or reinforce gels.
- Agents for treating flour, which are substances added to flour or dough to enhance its color or baking quality.
- Enhancers of flavor are additions that improve a food's natural taste and/or smell.
- Additives known as foaming agents enable the formation or maintenance of a homogeneous dispersion of a gaseous phase in a liquid or solid food.
- Gelling agents are additives that provide food texture by causing a gel to develop.

- Glazing agents are additives that give food a glossy look or a protective layer when they are applied to its exterior.
- Humectants are chemicals that counteract the drying impact of an environment to keep food from drying out.
- Packaging gases are substances added to a container before, during, or after food is placed inside of it in order to prevent certain food from happening, including oxidation or spoiling.
- Preservatives are chemicals that help goods last longer on the shelf by preventing microbial decomposition.
- Additives called propellants are used to force food out of a container.
- Raising agents are additions or mixtures of additives that allow gas to escape, increasing the volume of batter or dough.
- Sequestrants are additives that regulate a cation's availability.
- Stabilizers are additives that allow two or more components to remain in a uniform dispersion.
- Sweeteners are additions that give food a sweet flavor instead of sugars (mono- or disaccharides).
- Thickeners are additives that make food more viscous.

The United States (US) Code of Federal Regulations (CFR) divides food additives into two categories: direct or secondary direct food additives and indirect food additives.

There are eight types of direct food additives. Two of these categories are nutrition and flavorings.

There are four types of secondary direct food additives. Two of these groups contain solvents and enzymes.

Food colors are divided into two categories: certified colors and non-certified colors. **(Blekas, 2016)**

In some nations, food additives were categorized and given distinct numbers in order to control them and educate customers. Every authorized additive in Europe is assigned a unique number known as an "E number" according to the European Union's numbering scheme. The Codex Alimentarius Commission has now embraced and expanded this numbered system to globally identify all additives, irrespective of their approval status

(Codex Alimentarius Commission 1989). The additives may be simply categorized into categories with distinct functions using this way **(Long et *al.*, 2021)** (Fig1).

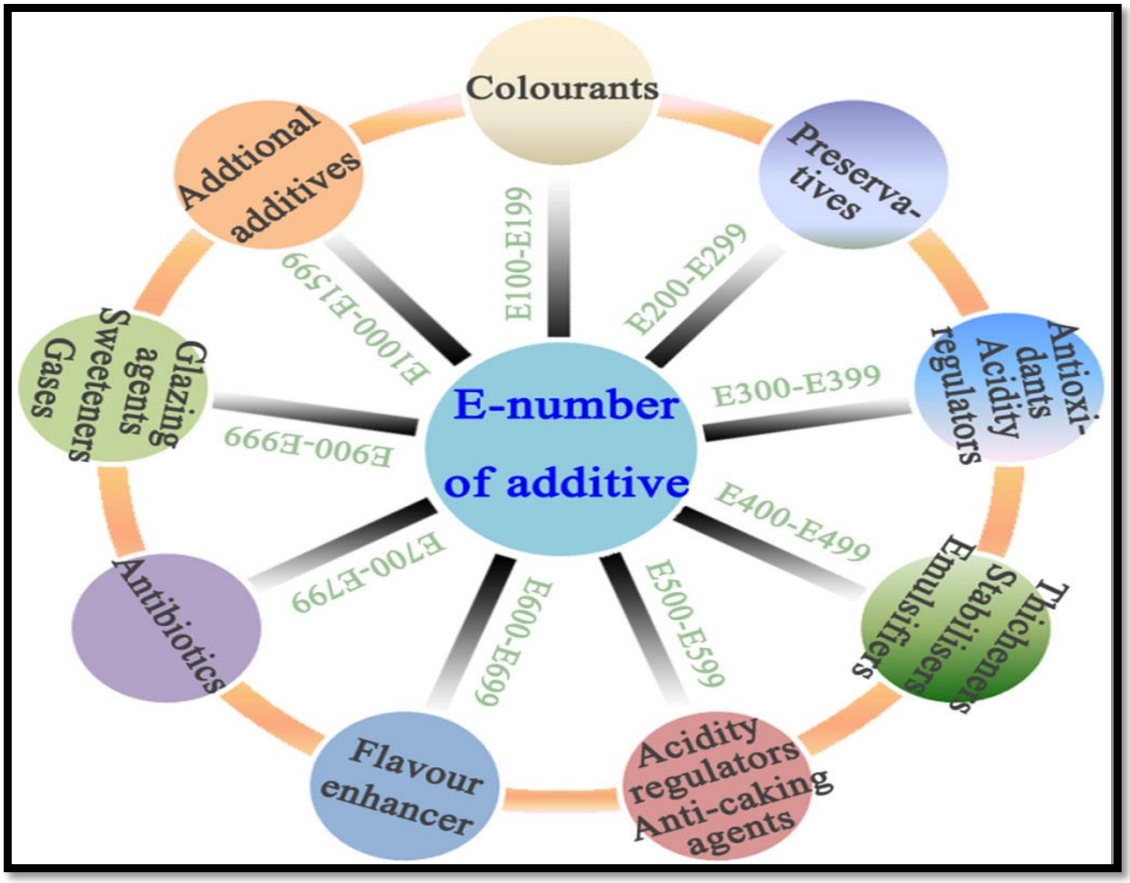


Figure 1. shows the broad grouping of food additives with E-numbers ranging from E100 to E1599. **(Wu et *al.*, 2021)**

Of course, for these food additives a strict food safety policy is necessary when using them since some of the components may be harmful to consumers' health. Research has indicated that a person's cumulative intake of food additives may lead to the development of allergies, diabetes, obesity, and metabolic diseases. Careful observation is also necessary for illegal food additives, which are employed in some businesses to conceal substandard manipulation techniques or the process of deterioration. **(Martins et *al.*, 2018)**

And because color, which is strongly tied to food quality and affects how consumers perceive sweetness and flavor, is the first feature of food that people consider. Furthermore, a variety of elements, including the presence or absence of oxygen, metals, light, oxidation processes, pH, and water activity, might influence undesired color alterations that occur

during the preparation and storage of food products. The food industry heavily employ chemicals that affect color to get around this problem **(Martins et *al*., 2018).**

And historically, Scully explores in 1995 the value of appearance for medieval cooks, who valued color, particularly yellow. The preferred coloring agents were saffron and egg yolk, though gold leaf was sometimes (rarely) employed. Interestingly, the Parisian government forbade the coloring of butter in 1396 ; this further highlights the significance of yellow in an early decree against color adulteration National Academy of Science - National Research Council **(NAS/NRC, 1971)**. Regretfully, not all coloring materials were safe ; medieval chefs discovered that verdigris, or copper oxides, produced a superb shade of green **(Scully, 1995)**. In 1820, Fredrick Accum published *A Treatise on Adulterations of Food and Culinary Poisons* in response to the growing use of toxic colorants and other adulterants. In this work, he condemned various practices such as artificially greening pickles and candies with copper salts and coloring hedge leaves with verdigris to mimic green tea **(Accum, 1820)**. Despite being widely sensationalized, Accum's concerns did not immediately lead to reform **(Hutt & Hutt, 1984)** ; paradoxically, the usage of hazardous colorants increased after Sir William Henry Perkin synthesized mauve in 1856 from the coal-tar derivative methylanaline. The contemporary organic chemical industry was born out of this synthetic purple dye, and the food industry, which was rapidly turning to processed meals to serve a growing industrial middle class, was also spurred to search for and employ synthetic colorants **(Tannahill, 1988)**. Because synthetic dyes have more stable colors, stronger dyes, and uniform hues, the food sector preferred them over colorants derived from plants, animals, or minerals **(NAS/NRC, 1971)**. The French were using the coal tar derivative fuchsine, now known as Magenta I, to color wine just four years after Perkin made his original discovery **(Marmion, 1979)**. By 1886, the US Congress had authorized the use of artificial coloring for butter, and by 1896, for cheese **(Marmion, 1979)**. In the United States, food coloring was done with over 80 different dyes by 1900 **(Noonan, 1972)**.

Food colorings are the most well-known and often used type of food additive. Firstly, a colorant is a type of additive added to food in order to restore colors lost during preparation, improve flavor, or improve appearance **(Coultate & Blackburn, 2018).** Both natural and synthetic colorants are available; natural food colors are defined as naturally occurring pigments derived from plants, animals, or minerals. Conversely, artificial food colors, sometimes referred to as synthetic food colors, are produced chemically. Artificial pigments

offer several benefits over natural pigments, including reduced microbiological contamination, excellent color consistency, great stability, and inexpensive production costs. Due to the benefits of synthetic pigments like tartrazine (E102), which is an azo dye mostly added to soups, sauces, ice creams, ice lollies, sweets, chewing gum, marzipan, jam, jelly, marmalade and mustard yogurt etc. **(Wu et *al*., 2021)**

More than 60% of all dyes are Azo dyes, which are the most widely used kind. Azole dyes make up around 70% of all dyes used in industry. The functional group (-N¼N-) that unites two identical or non-azo alkyl or aryl radicals that are symmetrical or asymmetrical characterizes these molecules. The most significant synthetic colorants that are widely utilized in the production of textiles, prints, and paper are azo dyes. **(Benkhaya, 2020)**.

The look and quality enhancement of food is greatly influenced by artificial azo dyes. Products using Azo dyes are more appealing to consumers, especially kids. **(Hofer & Jenewein, 1997)**. Azo dyes have long been used in the food sector globally, however because of their harmful effects on health, their usage is debatable. **(Eman et al., 2000)**. Certain azo dyes, including quinoline yellow (E104), sunset yellow (E110), and tartrazine (Tz) (E102), are allowed to be used in food items by the European Union. Tz is a water-soluble dye with an orange hue that finds use in a variety of sectors, including the food, cosmetic, textile, pharmaceutical, and medicine industries **(Merinas-Amo et al., 2019)**.

To produce tartrazine one molecule of dioxysuccinic acid and two molecules of p-hydrazinobenzene sulfonic acid are condensed. Condensing one molecule of diethyloxaloacetate with one molecule of p-hydrazinobenzene sulfonic acid is an additional method of preparation. When diazotized sulfonic acid is linked, tartrazine is created. **(Jain et *al*., 2003)**

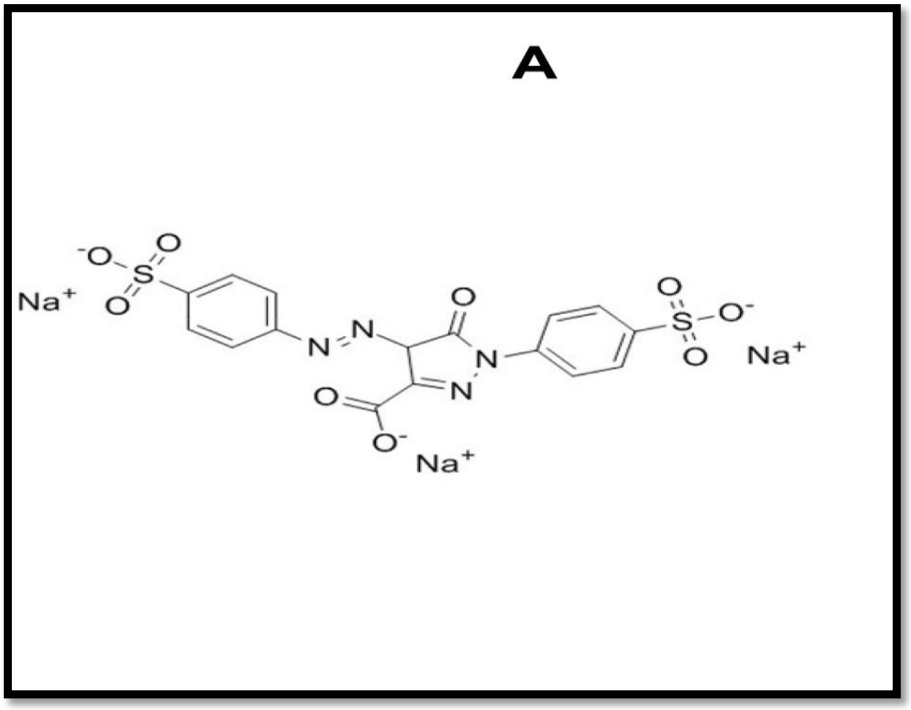


Figure 2. Tartrazine (E102) (trisodium salt of 3-carboxy-5-hydroxy-1-*p*-sulfophenyl-4-*p*-sulfophenylazopyrazole), (A) Molecular structure of tartrazine dye (Tz). **(Nasser et *al.*, 2020)**

Tartrazine's acceptable daily intake (ADI) was set at 7.5 mg/kg/day by The Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1964 ; nevertheless, tartrazine is prohibited or restricted in many other countries. **(Long et al., 2021)**

The amount of data and published literature indicates that tartrazine has a variety of harmful effects on many organs and health systems. Even at modest dosages, food additives, such as the colorant tartrazine, have a deleterious effect on and alter the biochemical indicators in vital organs like the kidney and liver. Because the production of ROS induces hepatic oxidative stress, the risk rises with increasing dosages and regular usage. Children are vulnerable to the negative effects of tartrazine because they often ingest these compounds in beverages, chocolates, gum, chips, and many other goods. Intestinal flora may transform tartrazine into aromatic amines, which can then transform into nitrosamine. ROS are released in this way. Therefore, it is crucial to inform customers about the negative consequences of these food-grade azo dyes. Due to the reduction in regular food consumption, these food additives may have an impact on children's growth and body weight. Tartrazine is one of the azo dyes, which also causes hypersensitivity and allergic responses. **(Kamal & Fawzia, 2018)**

Numerous in vivo investigations using varying dosages of tartrazine have not shown any development of neoplastic alteration in experimental animals or cytotoxic alterations in tissues and organs. **(Khayyat et *al*., 2017)**

In the modern day, medicine is typically understood to be the science and art of healing, encompassing a range of medical procedures developed to cure and prevent disease in humans. Modern medical techniques are used in contemporary medicine to diagnose and cure illnesses and injuries. Plants, animal parts, and minerals were frequently employed ceremonially as magical ingredients in prehistoric traditional medicine, based on spiritualism or mystic abilities. Herbal medicine has evolved into more structured forms in recent years, such as Ayurveda & Unani medicine on the Indian subcontinent and Traditional Chinese Medicine (TCM) in China. **(Mosihuzzaman, 2012)**

Herbs in the form of extracts, natural oils and essential oils are often used as alternative natural treatments. “Oleaster oil” is one of the alternative natural treatments used by the people of Algeria. And Olives (*Olea europaea L*), a plant with many commercially significant cultivars and wild olive genotypes, are mostly found in Mediterranean areas. **(Conceição et *al*., 2002)** The Oleaster tree is a member of the wild olive tree, technically known as *Olea europaea oleaster*, belongs to the family *Oleaceae*, which is characterized by an abundance of olein. Olives produced by olive trees are part of the Mediterranean flora. **(Ozturk et *al*., 2010)**. It is a little leafed tree with quadrangular branches that is quite prickly. **(Beddiar et *al*., 2007)** Oleaster comes in two forms that are physically identical: native and originating from wild offspring of olive trees, sometimes known as "feral." **(Besnard & Bervillé, 2000)**.

It has long been believed that the wild olive tree typifies all Mediterranean trees. Through the conquests and trade that accompanied the rise of the Mediterranean civilizations—the Greeks, Romans, Arabs, and Phoenicians—it has been extensively dispersed. Wild olive populations are limited to a few remote locations inside native Mediterranean woods, where natural processes (wind and birds) may disperse pollen and stones. The majority of other olive varieties that resemble wild ones can be classified as feral varieties (either cultivated olives left to grow wild or olives made from farmed olive pits dispersed by birds). Based on characteristics such as fruit, pit, and leaf forms and colors, tree architecture and phenology (flowering period), more than 2000 Mediterranean variants have

been identified. Cutting or grafting are two vegetative methods of propagating olive varieties. **(Nadine et *al.*, 2014)**

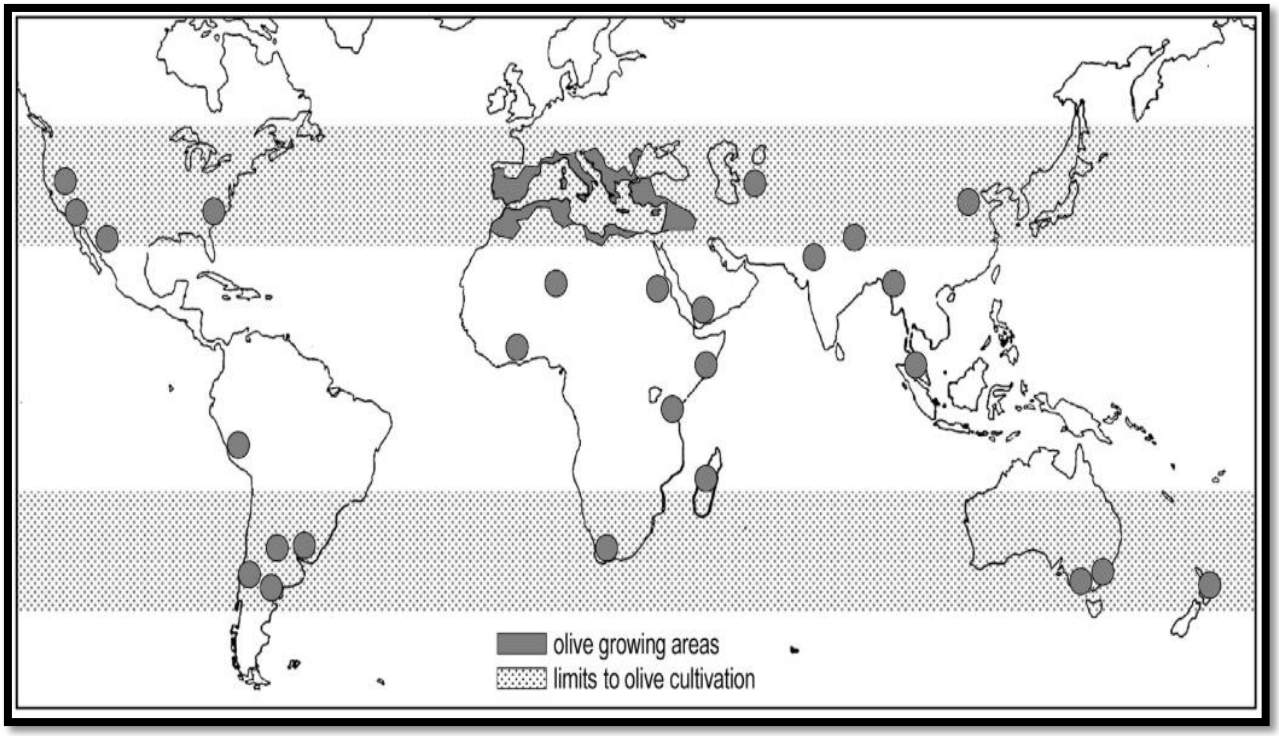


Figure 3. Global distribution of regions that produce *Olea europaea L*. **(Nadine et *al.*, 2014)**

Oleasters have smaller fruits with a less fleshy mesocarp and a higher pit/mesocarp ratio, a longer juvenile stage, comparatively low oil content, spinescent juvenile shoots, and a greater capacity to withstand harsh environments than cultivated olive trees **(Terral and Arnold-Simard, 1996).** The abundance of olive resources sparked a great deal of interest in creating and choosing new olive varieties with exceptional oil quality. **(Bouarroudj et *al.*, 2016).**

Figure 4. *Olea europaea L. ssp.Africana* fruit and leaves.**(https://www.randomharvest.co.za)**

Unlike olive oil, which is employed in a variety of industries, wild olive oil is not a consumer product. But it's still edible. Its application is limited to treatment. **(Sidi Mammar, 2012).**

Although little is known about oleaste oil, several studies have documented the positive effects of different cultivars of wild olive oil on health. According to recent studies, oleaster oil has greater levels of oleic acid and antioxidants than oil derived from cultivars. shown that the plasma lipid profile of healthy individuals has improved as a result of consuming oleaster oil. Additionally, Oleaster oil has been shown to be a useful hair lubricant and an antidote to various toxins**.** Algeria possesses substantial olive resources that are underutilized. The species Olea europea makes up as much as 2.3% of Algeria's total cultivated land. Three zones make up Algerian oléiculture: west, middle (Kabylie), and east. In Bejaia (East center), oleaster inhabits large uncultivated regions. **(Bouarroudj et *al.*, 2016).**

That is why we were interested in undertaking this work, which aims to study the toxic effects of tartrazine on certain vital functions and on oxidative stress in rats, and to test the modulating effect of oleaster oil against the toxics of the tartrazine food color.

In order to present the study conducted in this field, we will first provide a general introduction in which we mention food additives and their classifications, and we will touch

on a historical overview of the use of the color yellow in food. We will also mention tartrazine and its various harms, and oleaste oil and its effectiveness as an antioxidant.

In the second section, which is experimental in nature, the modulatory effect of the oil is assessed in relation to the oxidative stress and toxic effects of tartrazine in vivo at the organ level.The influence of the different treatments was analyzed by:

- Exploring the profile of hepatic, renal and brain oxidative stress: MDA, GSH, GST, GSH-PX and CAT
- The histological study of three target organs (liver and kidneys.)

And finally, we will discuss all the results obtained in this study, and we suggest some perspectives for this research work.

EXPERIMENTAL PART

MATERIAL & METHODS

Material and Methods

1. Vegetable Material

The vegetable material used is oleaster oil obtained from wild olive tree fruits. The name for this tree in colloquial language is "Zebbouch.". All of the fruits were matured and collected in December 2023. The extraction of oil from the wild olive tree was performed using a traditional artisanal approach.

2. Sensory analysis of oleaster oil

The evaluation of the organoleptic characteristics of our oleaster oil sample was carried out at our university by six volunteers. Each taster must smell and then taste the oil.

Sensory analysis focuses on the following characteristics: color, appearance or texture, smell, flavor, and taste.

The following form needs to be completed by each taster:

Taster n°…:

- **Color: ……**
- **Smell: ……..**
- **Appearance/texture: ……**
- **Flavor: ……..**
- **Taste: ……**

3. The analysis of physico-chemical characteristics

3.1. Determination of physical parameters

3.1.1. Refraction index

- **Principle**

A refractometer graduated between nD = 1.3000 and 1.7000 is used for the measurement, which is done at 20°C. The deviation of light as it travels through a liquid is measured with this device. The **ISO 6320, (2000)** methodology is used.

- **Operating Mode**

  - Use distilled water to calibrate the apparatus.

- Use Joseph's paper to clean the refractometer's blade.
- Adjust the chamber circle after a few drops of oleaster oil have been added to the blade divided into two halves: dark and light.
- Compute readings while accounting for the temperature (20 °C) **(Azzouni & Benariba, 2016)**.

3.1.2. Hydrogen potential

- **Principle**

The number of free hydrogen ions (H^+) in the olive oil determines the hydrogen potential (pH), which indicates the acidity or alkalinity of the medium **(Audigie et *al.*, 1984)**.

- **Operating Mode**
  - The pH metre's temperature should be adjusted to suit the surroundings.
  - Always rinse the probe with distilled water, then wipe it.
  - In a beaker, take 100 ml of oleaster oil for analysis.
  - Read the pH after submerging the probe into the fluid **(Azzouni & Benariba, 2016).**

3.1.2. Water content

- **Principle**

It involves heating a certain amount of oil to initiate the evaporation process and continue until the water is eliminated completely **(Benosman et *al.*, 2005)**.

- **Operating Mode**
  - After 30 minutes of drying at 105°C on an incubator, allow the beaker to cool in weigh the empty bowl and use a drier.
  - Weigh out twenty grammes of oleaster oil, then put it in a plate and heat it to 105 degrees Celsius for an hour.
  - After letting it cool in a dryer, weigh it **(Bouassila & Mayouf, 2017)**.

The water content is calculated as follows:

Water content = (m0+m1)-m2/moil.100

Where:

- **m0**: The mass of an empty beaker.
- **m1**: The mass (g) of the beaker with the test sample before heating in the casing.
- **m2**: The mass (g) of the beaker after it has been heated in the incubator for the test.
- **moil**: the sample's mass.

3.1.4. Absorbance by Spectrophotometry

➢ **Principle**

Extinction at 232 nm and 270 nm of a crude fat can be considered as an image of its oxidation state; more the extinction at 232 nm is strong, more olive oil is rich in secondary oxidation product **(CE2568/91).**

➢ **Operating Mode**

- ➢ Oleaster oil sample (10 mg to 20 mg) are diluted in cyclohexane (25 ml) until optical densities (DO) are less than 1.
- ➢ The absorption readings are performed in a quartz cuvette compared to that of the Solvent **(ISO 3656, 2011)**.
- ➢ Specific extinction values at 232 nm and 270 nm are calculated according to this formula:

$$K232 = ((Abs1+Abs2)/2)/P.10 \qquad K270 = ((Abs1+Abs2)/2)/P.10$$

$$\Delta K = K270 - 1/2(K266+K274)$$

Where:

- **K232:** specific extinction at λ = 232 nm.
- **K270:** specific extinction at λ = 270 nm.
- **K274:** specific extinction at λ = 274 nm.
- **K266:** specific extinction at λ = 266 nm.
- **P:** test sample (g).

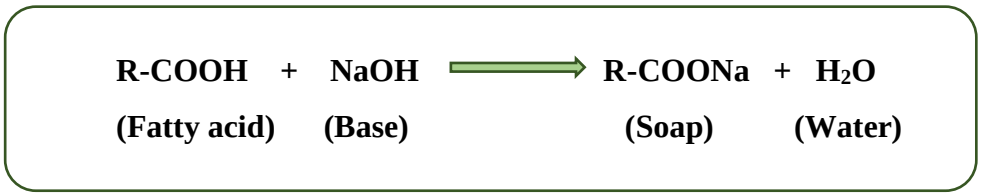
3.2. Determination of chemical parameters

3.2.1. Acid index

- **Principle**

The composition of the oil is an acid-base mixture that corresponds to neutralisation.

According to the reaction:



The acid index (IA) measures how many milligrammes of potassium hydroxide are needed to neutralise one gramme of fat's free acids **(Lion, 1955).**

- **Operating Mode**

The free acidity of every oil has been measured in accordance with the official standard established by the international organization for standardization **(ISO 660, 1996):**

- 1 gram of oleaster oil dissolved in 50 ml of ethanol/chloroform mixture (V/V).
- In order to determine the concentration of the mixture, a solution of potassium hydroxide or sodium (0.1N) in the presence of 0.3 ml of a solution containing 1% phenolphthalene up to twisting of the colored indicator (pink coloring becomes transparent).
- Free acidity was then expressed as a percentage of free oleic acid according to formula:

$$\text{Acidity\%} = (V.C.M).100/10.moil$$

Where:

- **V**: is the volume in ml of the titrated KOH solution used.
- **C**: is the exact concentration, in mol/l of the titrated solution of KOH used.
- **M**: is the molar mass in g/mol of the oleic acid (=282).
- **m**: is the test sample in grammes.

3.2.2. Peroxide Index

An indicator of the quantity of peroxide in a fat sample is the peroxide index **(Lion, 1955).**

- **Principle**

After dissolving the sample in a solution of acetic acid and chloroform, it is exposed to a potassium-iodide solution. In the presence of starch stains, or colored indicators, the released iodine is treated with a sodium thiosulfate solution **(Bouhadira, 2011).**

- **Operating Mode**

Every oil's peroxide index has been established in accordance with the international for standardisation **(ISO 3960, 2007).**

- 1g of oleaster oil is dissolved in 12.2 ml of the acetic acid/chloroform mixture (3V/2V).
- 15 ml of a saturated potassium iodide solution is added to the mixture and placed in the dark for 5 minutes.
- Add 1 ml of a starch solution and 60 ml of distilled water (a purple tint develops).
- The resultant mixture was titrated using a sodium thiosulfate (0,01 N) solution until the violet tint changed to a translucent color.
- A blank test was carried out under the same operating conditions.

The peroxide index is calculated using the following formula:

$$\textbf{IP (m.equ } O_2\textbf{/Kg) = (V-V0). 1000. T/PE}$$

With:

- **T:** Title or normality of sodium thiosulfate solution ($Na_2S_2O_3$).
- **V0:** Volume of sodium thiosulfate used for a blank test (ml).
- **V:** volume of sodium thiosulfate used for testing (ml).
- **PE:** test sample **(Oudin & Baziz, 2017).**

3.2.3. Saponification Index

- **Principle**

The saponification index is the number in milligrammes of KOH (hydroxide of

Potassium) is necessary to convert continuous fatty acids and triglycerides into soap. in a gramme of product. The method of analysis used is the one recommended by the standard **(ISO 3657, 2009).**

- **Operating Mode**
  - Two grammes of oleaster oil were weighed and then added to 25 ml of alcoholic KOH (0.5N).
  - The Erlenmeyer flask was blocked and placed in a boiling Marie bath for 20 minutes following homogenization
  - After cooling, 0.5 ml of phenophthalene was added, and the sample was titrated with HCl (0.5 N) until the pink color completely disappeared.
  - The blank is just composed of 25 ml of alcoholic KOH and phenophthalene **(Lecoq, 1965).**

The saponification, expressed in mg of KOH/g, is equal to:

IS mg of KOH/g = ((V0-V).N/m).56,10

Where:

- **V0**: Volume of HCl required to titrate the blank.
- **V**: HCl volume required to titrate the sample.
- **m**: Test sample in grammes.
- **56,10**: Molar mass, expressed in g/mol of KOH.
- **N**: Normality of KOH solution (0.5N).

4. Extraction and dosages of phenolic compounds

4.1. Extraction of phenolic compounds

In order to extract phenolic compounds, we used **(Pirisi et *al*., 2000).**

- 10 g of oleaster oil and 10 ml of methanol solution (methanol/water 80/20, v/v) are placed in a centrifugal tube.
- sway at the vortex for 10 minutes.
- After centrifugation for 15 minutes at 3800 rpm, the methanol phase is recovered and transferred into a 50-ml volumetric flask.
- the operation is repeated 3 times.
- In a rotavapor at 40 °C, the three phases that were recovered evaporate

to get away from the solvent.

4.2. Dosage of polyphenol compounds

- **Principle**

The ability of total polyphenols to decrease the phosphotungstic and phosphomolybdic acids found in folin-reactive Ciocalteu in tungsten oxides and molybdenum is used to determine their dosage (W_8O_{23} & Mo_8O_{28}). The latter exhibits a blue-

ish coloring at 760 nm, which is correlated with the polyphenol content of the samples **(Singleton et *al*., 1999).**

- **Operating Mode**

Total phenolic compounds were determined using the method recommended by **(Vasquez Roncero et *al*., 1973)**, which uses the Folin-Ciocalteu reagent and gallic acid as standards.

- With vigorous agitation, 500 µl of Folin-Ciocalteu reagent and 450 µl of distilled water were added to a tube containing 50 µl of extract.
- 400 µl of Na_2SO_3 (7.5%) was added after 3 minutes.
- The tubes were incubated at 25 °C and in the dark for 40 minutes.
- The absorption was read at 725 nm against a blank containing methanol instead of the extract.

The content of phenolic compounds of the oil has been determined from the gallic acid calibration curve and the results are expressed in mg gallic acid equivalent per kg of oil (mg EAG / kg oil).

5. DPPH Free Radical Trap Test

- **Principle**

The principle of this test is the ability of the extract to reduce free radical DPPH (2,2-diphenyl-1 picrylhydrazyl) of dark purple color, which is transformed into yellowish coloration (after reduction). This discoloration is measurable by spectrophotometry **(Brand et *al*., 1995)** (Fig 7)

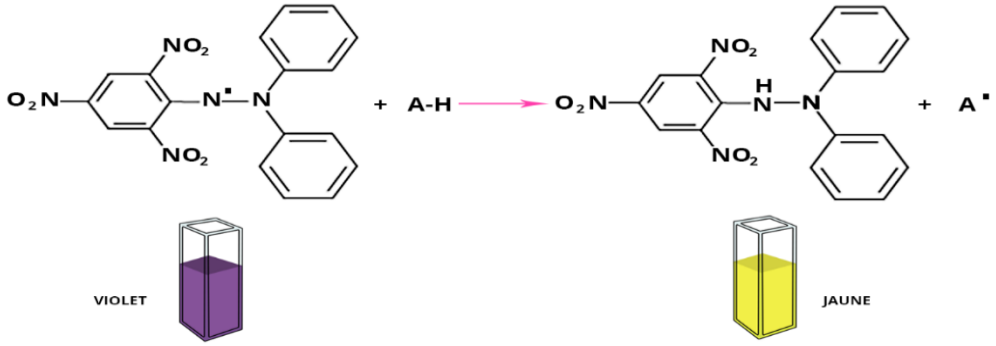


Figure 5. The reduced and unreduced forms of the DPPH molecule **(https://chimactiv.agroparistech.fr/fr/aliments/antioxydant-dpph/theorie).**

- **Operating Mode**

According to the protocol described by **Mansouri et *al*., (2005).**

- To prepare the DPPH solution, dissolve 2.4 mg of DPPH in 100 ml of methanol ($6x10^{-5}$ M).
- 25 µl of the extract or standard solution (ascorbic acid) are added to 975 µl of DPPH.
- The mixture is left in the dark for 30 minutes, and the discoloration of the negative control containing the solution of DPPH and methanol is measured at 517 nm.

Anti-radical activity is estimated by the following equation:

% of anti-radical activity = (Control Abs) – (Sample Abs) / (Control Abs) ×100

For the evaluation of this activity, a range of dilutions ranging from 0 to 2 mg/ml for ascorbic acid and parent extract have been prepared.

The different optical densities allowed an exponential curve to be drawn, which means that there is a proportional relationship between the percentage of reduction of the free radical and the concentration of the extract in the reactive medium.

Calculation of the IC50

This is known as the test sample's IC50 (50% inhibitory concentration) required to have a 50% reduction in DPPH radical.

IC50s are calculated graphically by inhibition percentages based on different concentrations of the tested extract **(Torres et *al*., 2006).**

Note: Ascorbic acid is used as a positive control.

6. In vivo study of tartrazine toxicity

6.1. Discovering tartrazine

Food color Tartarazine (E102), it's common ingredient in many foods and beverages we consume like dumplings and soft drinks.It was obtained from factory and a sweets shop in Guelma.

6.2. Prepare a tartrazine solution

Tartrazine solution used in vivo study was prepared in distilled water with the dose of (300 mg/Kg) **(Abd El-hakam & Farrag, 2022)**.

6.3. Animals

2 to 3 months 24 adult male rats Albino Wistar (180 to 280 g body weight) were live in our animal facility (faculy of natural and life sciences and earth sciences) at a temperature of 22± 2° C and a natural photoperiod.

Rats are placed in grilled plastic cages where they have free access to food and water.

6.4. Treatment of animals

Rats are divided into 4 equal groups (six rats in a group)

Group 1: Control "C": received distilled water.

Group 2: Tartrazine Group"T" (300 mg/Kg) **(Abd El-hakam & Farrag, 2022).**

Group 3: Tartrazine + oleaster oil Group"TO" (300 mg/Kg + 2 ml/Kg respectively) .

Group 4: Oleaster oil Group "O" with a dose of 2 ml/Kg **(Laib & Medbouh, 2016).**

All agents were given orally by intragastric tube for 30 days.

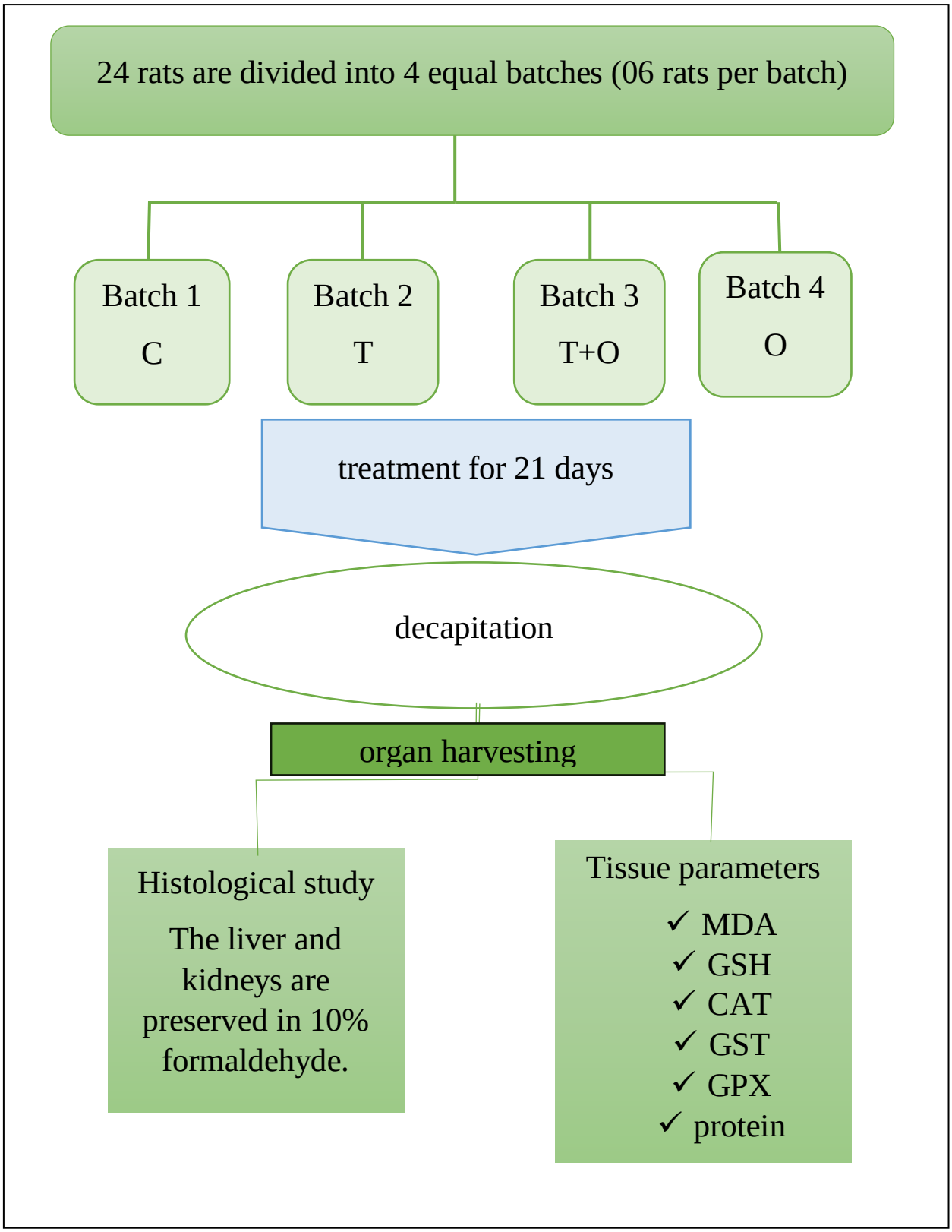


Figure 6. Summary diagram of the experimental protocol.

6.5. Organ harvesting

Rats were sacrificed and were removed certain organs: the kidneys, liver and brain. Three organs were rinsed in a 0.9% sodium chloride (NaCl) solution, a portion of these organs was stored in the freezer to measured oxidative stress parameters, and a second part was preserved in a 10% formalin solution to make the histological sections.

6.6. Assay of oxidative stress parameters at the tissue level

6.6.1. Preparation of homogenization

- **Operating Mode**

➢ 1g each of brain, kidney, and liver from the various study groups was used.The tissues were ground and homogenised in phosphate buffer (0.1M, pH = 7.4), then the cell suspension was centrifuged for 15 minutes at 5000 rpm and 4°C.Once obtained, the supernatant is divided into Eppendorf tubes and kept cold until analysis(GSH, GSH-Px , CAT , GST and protein dosage).

➢ 0.2 g of liver, kidneys, and brain from the different groups studied was used. After grinding and homogenization of the tissues in KCl buffer (1.15%), centrifugation of the cell suspension was carried out (5000 rpm, 4°C, 15 min). The supernatant obtained is aliquoted into Eppendorf tubes and then stored at -20°C until the moment of dosage (MDA).

6.6.2. Dosage of malondialdehyde (MDA)

- **Principle**

Lipid peroxidation in the liver, kidney, and brain is assessed by malondialdehyde (MDA) dosage using the method of **Ohkawa et *al*., (1979)**. MDA is one of the end products of the breakdown of polyunsaturated fatty acids (PUFA) under the influence of free radicals released during stress. One MDA molecule is condensed with two thiobarbituric (TBA) molecules to form a pink-colored complex.

- **Operating Mode**

➢ Add 0.25ml of homogenate, 0.25 ml of 20% trichloroacetic acid (TCA), and 0.5ml of 0.67% thiobarbitric acid (TBA).

- After 15 minutes of heating to 100 °C, the liquid is cooled, and 2 ml of n-butanol is added.
- After 15 minutes of centrifugation at 3,000 rpm, the absorption is determined on the overflow using the spectrophotometer at 532 nm.

The concentration of MDA is deducted from a standard range established under the same conditions with a solution of "1,3,3,3-tetratoxypropane" which gives the MDA after its hydrolysis (Annex)

6.6.3. Reduced glutathione dosage (GSH)

Glutathione dose is determined using the technique of **Weckbeker and Cory, (1988).**

- **Principle**

This dosage is determined by measuring the optical absorption of 2-nitro-5-marcapturic acid, which is produced when glutathione's (-SH) clusters reduce 5.5-dithi-bis-2-nitrobenzoic acid.

- **Operating Mode**

- Take 0.8 ml of homogenate.
- Add 0.2 ml of salicylic acid solution (0.25%).
- Shake and leave for 15 minutes in an ice bath.
- For 5 minutes, centrifuge at 1000 rpm.
- Extract 0.5 ml of the supernatant liquid.
- Add 1 ml of Tris-EDTA buffer, pH 7.4
- Mix and add 0.025 ml of 5.5 dithi-bis-2-nitrobenzoic acid (DTNB) at 0.01M.

After five minutes at room temperature, measure the optical densities against the reactive blank at 412 nm.

The GSH concentration can be obtained using the following formula:

$$GSH\ (n\ mol\ /mg\ protein) = DO.1.1,525/13100.0,8.0,5.mg\ protein$$

- **DO**: Optical density.

- **1:** Total volume of solutions used in deproteinization (0.8 ml homogenate + 0.2 ml salicylic acid).
- **1.525:** Total volume of solutions used in the dosage of GSH at the level of supernatant liquid (0.5 ml supernatant + 1 ml Tris + 0.025 ml DTNB).
- **1300:** Group absorption coefficient (–SH) at 412 nm.
- **0.8:** Volume of homogeneity.
- **0.5:** Volume of overflow.

6.6.4. Enzymatic activity of catalase dosage (CAT)

- **Principle**

The method of **Aebi, (1983)** is used to measure the activity of the CAT enzyme. The following process, which occurs at 25 °C in the presence of the enzyme source, is the basis for the principle, which states that H_2O_2 will vanish:

- **Operating Mode**

➢ 50µl of homogenate and 2.95 ml of an H_2O_2 solution (19 mmol /ml), made in the potassium phosphate buffer (pH 7.4 ,0.1M), are combined in a quartz cuvette.

➢ The change in absorption is followed for one minute at a 15-second interval at a wavelength of 240 nm.

The molar extinction coefficient (ε = 0.043 $mM^{-1}cm^{-1}$) is used to calculate the enzymatic activity, and the findings are reported in µm of H_2O_2 break down/min/mg of protein

6.6.5. Glutathione s-transferase activity dosage (GST)

- **Principle**

The measurement of GST activity is to provide the enzyme with a substrate in general of (CDNB) 1-chloro-2,4-dinitrobenzene, which reacts easily with many forms of GST and glutathione. The combination of these two products leads to the formation of a new molecule that absorbs light at 340 nm.

- **Method**

The method used in this study for dosing GSTs is that of **Habig et *al*., (1974)**. This is to apply the GSTs contained in the homogenate to a mixture (GSH+CDNB) at a temperature of 37°C and a pH of 6.5. The variation in optical density, due to the appearance of the GSH-CDNB complex, is measured for 1 minute for 5 minutes at a wavelength of 340 nm.

- **Operating Mode**

Reagents	Blank (µl)	Assays (µl)
Phosphate buffer (0.1 M, pH 6.5)	850	830
CDNB (0.02M)	50	50
GSH (0.1 M)	100	100
Homogénate	/	20

The optical density value of blank (spontaneous substrate conjugation) was then reduced to the value of each test to measure the variation due solely to enzyme activity.

The GST concentration is obtained by the following formula:

GST(n mol /min/mg protein)= DO sample /min – DO blank/min ÷ 9,6 × mg prot

Where:

- **DO**: optical density of the sample /min.
- **DO/min blank**: optical density of blank /min.
- **9.6**: GSH-CDNB extinction coefficient expressed in Mm cM.

6.6.6. Protein dosage

- **Principle**

The method of **Bradford, (1976)** is used to calculate the concentration of proteins. The proteins react with a colored reagent that contains ethanol, coomassian blue (BBC), and orthophosphoric acid. Proteins' NH_2 groups react with this reagent. The color's intensity corresponds to the proteins' concentration.

- **Operating Mode**

➢ 50 µl of homogenate is added to 2.5 ml of Bradford reagent.

➢ Agitation.

➢ After incubating for 5minutes at room temperature, read the absorbance against a reactive blank at λ = 595 nm

The concentration of proteins is deducted from a standard range established under the same conditions with a solution of "BSA."

6.6.7. Glutathione peroxydase enzyme activity dosage (GSH-Px)

- **Principle**

The method developed by **Flohe and Gunzler, (1984)** was used to evaluate the enzyme activity of glutathione peroxydase (GSH-Px). This method is based on the reduction of hydrogen peroxide (H_2O_2) in the presence of reduced glutathione (GSH); the latter is transformed into GSSG under the influence of GSH-Px according to the following reaction:

$$H_2O_2 + 2GSH \xrightarrow{GSH-Px} GSSH + 2H_2O$$

- **Operating Mode**

➢ Take 0.2 ml of homogenate (surnageant).

➢ Add 0.4 ml of GSH

➢ Add 0.2 ml of TBS buffer solution (Tris 50 mM, NaCl 150 mM, pH 7.4).

➢ incubate for 5 minutes at 25°C in a maria bath.

➢ To start the reaction, add 0.2 ml of H_2O_2 (1.3 mM), and let it act for 10 minutes.

➢ Add 1 ml of TCA (1%) to stop the reaction.

➢ For 30 minutes, place the mixture in the ice.

➢ Centrifuge at 3000 rpm for 10 minutes.

➢ Take 0.48 ml of the supernatant liquid.

➢ Add 2.2 ml of TBS buffer solution.

➢ Add 0.32 ml of DTNB (1.0 mM).

➢ Mix and, after 5 minutes, read the optical densities at 412 nm.

The enzymatic activity of GSH-Px is determined using the next formula:

GSH-Px = (DO sample - DO standard/Do) .(df .0,04/ standard.cp)

- **0.04:** GSH substrate concentration**.**
- **df:** dilution factor

7.Histological study

The histological study was made on pathological anatomy lab of the IBN ZOHR Hospital in Guelma.

The following steps are part of the technique used:

7.1. Fixation of the organs

Fixation is carried out immediately after sampling of the sample to be observed by fixing liquid or by freezing. It allows you to immobilise it and keep it in a state close to the living, respecting the tissue structure.

This is the most important step in producing good histological cuts. The fixing fluids are very numerous; we have used Formol, which is commonly used for the conservation of animal or plant tissues.

7.2. Preparation of cassettes

Before starting the process of dehydration of the parts, the organ in its entirety must be placed in tissues cassettes (LEICA) made of poly acetal with incorporated lid.

7.3. Dehydration of samples

To ensure that the parts are thoroughly dehydrated before being added to the paraffin, the sample must be pre-dehydrated by adding ethanol in place of the water that was previously present. An automatic filled sample preparation system completes these stages.

- Placing cassettes in a tissue processor manuel (the SLEE MTP) guarantees processing speed, flexibility, savings, and environmental protection.
- Easy to use, it includes 12 stations: ten plastic or glass beeches for reagents and processing solvents (xylene, ethanol, and formol) and two aluminium paraffin pots. It can carry up to 240 cassettes (standard: 120).
- The LCD panel allows for complete programming, and this method depends on the tubs' spinning mechanism for nearly 16 hours and 40 minutes.

7.4. The inclusion and realisation of the blocks

The purpose of this operation is to penetrate a semi-hard substance into the parts, such as paraffin, in order to create a consistency and homogeneity favourable for the cutting while respecting the integrity of the tissue and stiffening the sample.

A paraffination station (SLEE MPS/P1) that consists of a heating plate, an inclusion area, and a thermostat tank is used to carry out paraffination.

1. The organ is placed in a stainless-steel mould, and paraffin is added by pushing on the distribution pedal that has already melted at a temperature of T > 65°C in the inclusion region, which takes the shape of a 6-liter main tank. The organ is then fixed with a metal pince by operating on the cooled surface at T = -10°C.
2. Cover the mould with the cover of the cassette and let it cool for a few moments on the refrigerated plate dedicated to the finalisation of inclusion at T = [0 at 20°C] to obtain the blocks.
3. Until the pieces are cut, the blocks are kept cool.

7.5. Organ cuts

For fine histological cuts, the cassettes must first be thickened to remove excess paraffin with a thickness of 10 µm.

1. After thickening, adjust the thickness of the microtome (LEICA RM 2125RTS) between 0.5 and 0.1 µm, and then place the cassette in the right position and orientation with the shaver.
2. The movement restarted, and at each passage of the piece, there are cuts (0.1–0.5 µm thickness) that automatically glue, among others. So we get a piece of cutting tape.

3. The fractional tape is placed on the surface of a blade containing hot water drops heated on a heating plate, or T = 60°C. Leave the cut on the surface of the water for as long as it takes to smooth it out.
4. Before placing the blades in the stove, briefly drain them vertically to remove the excess water, then dry the flat blades on the heating plate (SLEE MPS/P1), and finally, the blades can be placed in blade porters in straight positions, then dried on the drying oven.
5. Usually, a diamond pen was used to mark the edges of each blade.

7.6. Coloration

The dyes used for coloring are specific to moistened materials. For this reason, the blades need to be deparaffinated before being rehydrated in baths with lowering alcohol content.

- **Deparaffination of cuts**: Place the rack in a drying oven (model PANACEA) at T € [80 - 100°C] for 20min.
- **Dehydration of cuts**: Place the rack out of the drying oven directly in a Xylene crystallizer between 10 and 60 s. Rinse in two ethanol baths and tap water to remove the trace of paraffin.
- **Coloring with Mayer**: Immerse the rack in a bath containing Mayer hematoxyline 8min and then rinse under tap water.
- **Eosine dyeing**: plunge the blade rack into the eosine crystallizer for 8 minutes.
- **Dehydration**: perform a 2nd rinse with ethanol, xylene-acetone, Xylene as follows: 4 ethanol crystallizers, 2 xylene-acetone (50% 50%) and 2 Xylene.

7.7. Mount

Apply a drop of EUKITT adhesive solution on the blade, then cover it with a dry, clean cover-object blade by slanting it gradually to allow the solution to cover the cut without trapping the spherical bubbles.

The solution should not overflow, the blade is adhesive and the preparation is ready for microscopic observation.

7.8. Microscope observation

The cuts are observed under an optical microscope.

8.Statistical analysis of the results

The calculations were carried out using the GraphPad software for data analysis and statistical processing (Version 10). The results are represented as: average ± average type deviation, The data were

analysed through the ANOVA variance analysis, and, using the Turkay test, we compared the averages. The differences are considered to be:

- Significant * when ($p < 0.05$).
- Highly significant ** when ($p < 0.01$).
- Very highly significant *** when ($p < 0.001$).
- Non-significatives when $p \geq 0.001, 0.01, 0.05$

With **p**: threshold of significance.

DISCUSSION

Discussion

1. Organoleptic analysis(sensory)

Our examined sample is a virgin oil of dark yellow color and medium appearance that has a good taste and an acceptable smell.

The organoleptic properties of virgin or extra virgin olive oil are related to the variety of fruits, the degree of maturity at the time of harvest, ecological factors, variations in the harvest and storage operations of the fruits, and also to the variability of the processes of extracting and storing the olive oil **(Baba Hamed, 2017).**

This organoleptic test result remains subjective as it requires a professional jury member with experience in tasting oleaster oil.

2. Physico-chemical analysis

First of all, it is necessary to determine the organoleptic characteristics and the physico-chemical composition of oleaster oil (physicochemical indices, polyphenols and anti-radical activity), obtained in a traditional way in Guelma region. The main interest of the analysis of the physico-chemical indices lies in the identification of olive oil through its physical and chemical properties. The geographical origin of the samples including the climate factor and the method of preparation of the oil may have a slight impact on some of its physico-chemical characteristics **(Hilali et *al*., 2005; Gharby et *al*., 2011).**

2.1. Physical Indices

The process of lipid oxidation is affected by pH. Acidic oils have a pH of less than 4. The pH is altered by outdoor storage. This parameter allows the complete elimination of water and volatile products **(CCE, 1991)**

The refraction index is also an important criterion for the purity of the oil (Ollë, 2002), this parameter varies in proportion to the iodine index **(Chéneveau, 1917).** The oleaster oil studied has a value of 1,470 this value is equal to value found by **Bennouar et *al*., (2022).**

The humidity is 0.026%, which is lower(less) than that found by **Bennouar et *al*., (2022)**; this result is sufficient in the COI (2018) (less than 0.2) and CODEX (COI, 2011, CODEX, 1989) standards, which advocate a lack of water in virgin olive oils; these humidities may be caused by hydrolysis and oxidation of oils.

Extinction at 232 nm and 270 nm shows a richness in secondary oxidation products such as: hydroperoxides and ketones **(Arbi Nehdi, 2013)**. Based on our results for specific absorptions (K232, K270), the two recorded values perfectly fit within the limits set by COI,

(2018) for an extra virgin olive oil (K 232 ≤ 2,5; K 270 ≤ 0,22). our values are close to the values of **Bennouar et *al*., (2022).**

2.2. Chemical indices

Acidity allows to control the level of hydrolysis, enzymatic or chemical degradation, of the fatty acid chains of triglycerides **(Abaza et *al*., 2002)**.

Our result (12.90%) is very high compared to COI and CEE standards (<0.80). This value is higher than that found by **Baccouri et *al*., (2007)** on oleaster oil from other regions derived from triphase extraction, which is probably due to the harvest time and the extraction method.

According to **Gutierrez et *al*., (1999)**, as the fruit near the end of its maturation stage, it will see an increase in lipolytic enzyme activity and be more exposed to mechanical damage and pathogenic infections.

Fungal lipase activity and storage conditions may probably the cause of the increase in acidity, which is equal to the acid index divided by two **(Kiritsakis & Markakis, 1984).**

According to research by **Jean-Marc & Mireille, (2003)**, there is no interaction between the quantity of free fatty acids and their oxidation state, so oleic acidity should be utilised as a composition rather than a quality criteria. However, the nutritional value of non-oxidized free fatty acids is nearly equal to that of the same acids when they are glyceridic.

The peroxide index is 65 meqO_2/Kg of oil, which indicates a high level of oxidation. This value is higher than that found by **Bacauri et *al*., (2007)** and by **Bennouar et *al*., (2022)**. who found that the percentage of five varieties of Tunisian olive oil ranged from 2.4 to 7.66 (meqO2/Kg oil). The values are quite high in compared to the norms of COI and CEE commercial standards (CEE, 2005, COI, 2011) (< 20,0).

According to **Kiritsakis & Markakism, (1988),** the main factor causing the deterioration of olive oils during storage is oxidative rancissement. It is caused by the oxidation of unsaturated fatty acids, which results in the creation of compounds having an unpleasant taste and smell.

This high value would be directly linked to production conditions: harvest, storage, and crushing. The differences between samples collected under similar extraction conditions would be due to the diversity in the composition of the oils.

The saponification index is inversely proportional to the length of the chain; the longer the chair length increases, the less the saponisation index will be **(Harper, 1977)**. The saponification index of the oil studied is 201.96 mg KOH/g. This result is higher than that obtained by **Belarbi et *al*., (2011)**, estimated at (162.9 mg KOH/g).

2.3. Dosage of phenolic compounds

2.3.1. Polyphenols content

Polyphenols are recognized as antioxidant compounds, their presence in olive oil is linked to their general properties, improvement of stability, nutritional value and sensory properties **(Servili et *al*., 2004).**

According to the classification proposed by **Montedoro et *al*., (1992)** allowing to distribute varieties according to the content of phenolic compounds, our oleaster oil would be in the category of varieties with medium content of polyphenols (less than 500 mg EAG/kg).

The oleaster oil studied shows a content of 331.42 mg EAG/kg of phenolic compounds close to the result of **Bennouar et *al*., (2022) (**314 mg EAG/kg), and superior to the samples studied by **Bouarroudj et *al*., (2016)** with values between 135,09 à 202 mg EAG/kg.

Several factors can influence the polyphenol content: olive variety, maturity, soil quality as well as the extraction process used and the conservation conditions of the oils (**Ollivier et *al*., 2004).** So there are other factors located by **Laribi, (2015)** like Climate and environmental factors and the harvest period, oil extraction system, extraction condition.

In fact, the research by **Dabbou et *al*., (2011)** indicates that the oleaster oil shows that the olive oil studied seems interesting in terms of minor compounds (polyphenols and volatile compound). The richness of phenolic compounds in olive oil is explained by its proven resistance to critical conditions such as water stress **(Durand & Terral, 2006)**.

As a result. The richness of phenolic compounds in the oleaster is a criterion of quality.

2.3.2. DPPH Free Radical Trap Test

The result of the antioxidant activity of olive oil against the DPPH radical shows a significant inhibitory power (67.47%) but remains a lower power than that of ascorbic acid (98%). The high content of secondary metabolites (polyphenols) in this oil explains this significant action. which hold an anti-oxidants licence. The primary mechanisms by which these antioxidants work as radical-trapping agents, reducing agents, singlet oxygen deactivators, metal chelators, and hydrogen donors are due to the redox characteristics of their hydroxyl groups and the structural interactions between the various functional groups in their compound structure **(Barreca et *al*., 2011; Penyaringan et *al*., 2016).**

3. Effect of tartrazine and oleaster oil on the variation of oxidative stress parameters

3.1. Proteins

Our findings revealed a decrease in protein levels in the liver and brain. This decrease is explained by the fact that most proteins include groups (SH, OH), which react readily with free radicals produced by oxidative stress, causing these proteins to denature, fragment, or lose their characteristics. Primary and Secondary Structures **(Käkelä et *al.*, 1999; Harris *et al.*, 2011).** Furthermore, rats' exposure to environmental stress can affect the metabolism and synthesis of proteins and amino acids in the liver **(Stinson et al., 1992)**. The drop in protein levels following tartrazine force-feeding might possibly be attributable to a fall in dietary protein consumption. **(Pari & Amudha, 2011)** The current investigation, which was conducted to assess the harmful consequence of tartrazine ingestion, found clear evidence of oxidative stress caused by the substance. Endogenous mechanisms and external influences such as medication, chemical, and pollution exposure produce reactive oxidants in biological systems, Where we find that the study prepared by **Adedoja et *al.*, (2021)** proved that the Tartrazine increased oxidative stress markers, MDA, H_2O_2, and chloramine levels in D. melanogaster larvae while decreased antioxidant enzyme activity, TR and GST. Furthermore, tartrazine changed the expression of apoptotic and oxidative stress genes such as HSP27, ILP2, ANCE, and Dcp **.** In contrast, the results revealed that treatment with oleaster oil increased protein activity in rats gavaged with tartrazine. This is because the oil contains bioactive chemicals that are good free radical scavengers. (**Suzuki *et al.*, 2011**). **MDA**

According to **Draper et *al.* (1988) and Valko et *al.* (2005),** MDA is the most prevalent aldehyde produced by lipid peroxidation. This aldehyde may serve as an essential indication of lipid peroxidation **(Favier, 1997; Bonnefont-Rousselot et *al.*, 2001).** Our findings revealed a highly substantial rise in MDA levels in kidney and liver tissue following tartrazine treatment. Our findings support those of **El.Golli and Elbini, (2016),** who discovered that tartrazine contributes to lipid peroxidation in the kidneys and liver, resulting in a modification in antioxidant status..

This change is accompanied by increased lipid peroxidation. This is explained by tartrazine's detrimental effects on the body, as well as the generation of reactive oxygen species and enhanced lipid peroxidation in cells.

Malondialdehyde has been shown to cause membrane component cross-linking and polymerization, as well as mutagenic and genotoxic effects caused by impaired antioxidant defense mechanisms, such as detoxification and scavenging enzymes, or by an increase in lipid peroxidation following the interaction of reactive oxygen species (ROS) and cell membranes. **(Attig *et al.*, 2010; Misra *et al.*, 1990).** In this example, MDA damage was reduced following treatment with the oil, and the rats were rescued with tartrazine. These findings imply that olive oil has an antioxidant effect, which might be attributed to the high concentration of this bioactive molecule and antioxidants that prevent lipid oxidation and modification induced by excessive radioactive material generation. conducted by **Bouarroudj et al. (2016)** This month has high levels of vitamin E, C, and polyphenols, which are powerful antioxidants that include flavonoids, terpenoids, saponosides, tannins, and alcaloïdes. Overall, this is a good approach for ERO. **(Suzuki et *al.*, 2011).**

3.2. Reduced Glutathione (GSH)

GSH plays an important function in the detoxification of free radicals and heavy metals. In this case, the low level of glutathione (GSH) is due to an increase in its consumption rate when free radicals and their products are produced, as it is considered one of the most important non-enzymatic antioxidants and contributes to the removal of free radicals and their products as it transforms from active to non-active form. The active disulfide is Glutathione disulfide, which contains a sulfur group and acts as a good reducing agent by easily blowing hydrogen atoms due to the weak bonds between sulfur and hydrogen SH and the strength of the bonds between carbon and hydrogen CH in free radicals, thus protecting cell membranes from free radical damage. **(Aldaamy & Merza Hamza, 2022)**

Our findings reveal that tartrazine significantly reduces GSH levels in the tissues investigated (liver and kidney).

Our findings in the liver and kidneys are consistent with a recent study, which found that tartrazine intoxication causes oxidative stress and a decrease in the levels of antioxidant defense systems GSH in the kidney and liver.

Indeed, **Amin et *al.*, (2010)** demonstrated a drop in GSH levels in the case of tartrazine-induced toxicity by blocking glutathione synthetase and glutathione reductase, resulting in less GSH production. All of these variables contribute to a significant drop in reduced glutathione (GSH) and an increase in oxidized glutathione (GSSG), resulting in a decrease in the activity of GSH-dependent enzymes.

On the other hand, the results demonstrated that treatment with oleaster oil induces a large rise in glutathione content due to the efficient activation of the antioxidant system against oxidative stress. **(Bennouar et *al*., 2022).** This result is due to high antioxidant potential of oleastre oil.

3.3. Glutathion-s-transferase (GST)

GST activity, an enzyme that catalyzes the conjugation of glutathione (a nucleophilic group - SH) to a wide variety of compounds (bearing electrophilic groups) and is also involved in the transport and elimination of reactive compounds that perform other antioxidant functions, has been widely used as a stress biomarker **(Fitzpatrick et *al*., 1997).** This enzyme is important in xenobiotic detoxification because it has a high capacity to reduce lipid peroxides **(Griffith, 1999; Iscanetal, 2002)** and/or to protect against harmful metabolites produced by macromolecule degradation after exposure to oxidative stress **(Hayes & Pulford, 1995).**

Our findings indicate a decline in GST activity, which can be explaine by the import of GST in the catalysis of the conjugation of substrat electrophiles in order to prevent glutathione from protecting the cell from xenobiotics **(Ferrari et *al*., 2007).** Our results are consistent with this story **El Golli et *al*., (2016)** working on healthy cells and **Diksha et *al*., (2017)** working on healthy cells.

3.4. glutathione peroxydase (GSH-Px)

The results revealed a significant decrease in the activity of the Gpx enzyme in the Tartrazine-treated group compared to the negative control group, which is consistent with our study **(Amin et *al*, 2010)**, in which (Gpx) participates in the process of stimulating the H_2O_2 reduction generated by oxidative stress by GSH, which provides an electron. To scavenge free radicals, the concentration drops, which is owing to its stimulation of GSH to eliminate reactive oxygen species (ROS), as well as the decrease of Gpx as a result of GSH depletion due to increasing oxidative stress. **(Aldaamy & Merza Hamza, 2022)** On the opposite hand, the results showed that treatment with oleaster oil causes a significant increase of Gpx.

3.5. Catalase enzyme activity (CAT)

Catalase (CAT) represents the second step of the enzymatic defense system. It supports hydrogen peroxide previously produced by SODs and water metabolism **(Chakrabarti, 1982).** It plays an important role in protecting the body against damage from oxidative stress

(Cakmak & Horst, 1991). It consists of a transformation of hydrogen peroxide (H_2O_2) into water and molecular oxygen (O_2). According to our results, a highly significant decrease in CAT activity is observed in the kidneys, liver and brain in rats gavaged with tartrazine compared to healthy control rats. This decrease is concomitant with the overproduction of $O_2^{\bullet-}$ and H_2O_2 which may be the origin of the oxidation of major cellular components such as lipids, proteins and DNA, causing their inactivation and subsequent degradation. This result suggests that tartrazine indirectly induced an increase in H_2O_2, therefore that it caused a case of oxidative stress. Indeed, the decrease in CAT activities causes an increase in H_2O_2. This is in agreement with previous studies carried out on rats poisoned with tartrazine. **(Amin et *al.*, 2010)** On the other hand, the results showed that treatment with oleaster oil increases the activity of catalase.

4. Histological analysis

4.1. In the liver

Microscopic observation carried out on topographical staining of histological sections reveals the severe toxic action due to tartrazine at a dose of 300 mg/Kg. This toxicity which results in:

the majority of hepatocytes present the beginnings of water degeneration, the cytoplasm of these dead cells presents a homogeneous eosinophil, this eosinophil is explained by the loss of basophilic RNA from the ribosome of the altered ergoplasm, the disorganization of the mitochondria and the revelation of acidophilic group released by protein lysis, these rays of darker dead cells, with more or less defined contours, are called pyknotic.

This nuclear pyknosis is the result of a chromatin condensation probably importable to the drop in the pH of the medium in anaerobic metabolism and in places, a hyperplasia is found with a sinusoidal narrowing (hepatocytic trabeculae close together) as well as a dilatation of the centrolobar vein and a narrowed door space.

According to the results of the **Aboel-Zahab et *al.*, (1997)** study, the administration of a mixture of synthetic dyes to rats for one month revealed pigmentation of the portal vein and those of Kupffer in the liver and interstitial tissues as well as renal tubular cells.

We notice a convergence in the results between healthy control rats and rats gavaged with oleaster oil؛ We notice an improvement in the level of rats gavaged with tartrazine + oleastr oil this is due to the fact that this oil is an excellent antioxidant in cases of oxidative poisoning

4.2. In the kidenys

This histological analysis of the tissue architecture of the kidneys revealed a narrowing of the Baumann space, a reduction in the lumen of the convoluted tubes, hyperplasia of the cells in some areas and a dilation of the arcuate veins at the corticomedullary junctions. On the cytological level, pyknotic noyons appear as rounded bodies of dark colors, the nuclear envelope ruptures and the abnormal chromatin fragments, we find small particles of dark color, made up of denatured nuclear material which we find in cells is the phenomenon of karyorrhexis. And for rats treated with the oil, we find that they almost similar to the healthy control rats

CONCLUSION

Conclusion

A synthetic food coloring of an azo nature, tartrazine remains widely used in the food and culinary sectors. Although it does not present any proven nutritional benefit, tartrazine is, on the other hand, widely used to improve the appearance of products for marketing purposes, and excess consumption can be harmful to the body and its vital functions.

The use of vegetable oils and medicinal plants is still the most widespread form of medicine throughout the world today, which allows us to remedy everyday problems in a natural way.

This work made it possible to experimentally determine the harmful effects following subchronic or oral consumption of tartrazine in Albino Wistar rats at dose of 300 mg/kg. The study focused on certain organs (liver, kidneys, and brain). and their histology (liver, and kidneys). And on the other hand, to test the modulatory effect of oleaster oil against the toxicity of the food colorant (tartrazine)

In light of the results obtained, it can be concluded that;

- ❖ Oleaster oil contains a composition rich in polyphenols and present in small quantities of flavonoids.
- ❖ Oleaster oil has a very significant anti-radical power, is directly related to its content of secondary metabolites, and depends on all the antioxidant substances, their nature and their quantity.
- ❖ The administration of tartrazine to rats caused;

- Oxidative stress results in a clear increase in the marker of lipid peroxidation (MDA) in the liver, kidneys, and brain, a reduction in the level of GSH, GSH-Px, GST and CAT.
- Histological changes in the liver (hepatic inflammation), in the kidneys (glomerular hemorrhage and the appearance of aggregates of inflammatory cells) These changes are in agreement with the biochemical results observed.

- ❖ Supplementation of oleaster oil to poisoned rats caused;

- A reduction in the level of tissue MDA.

- An improvement in enzymatic antioxidant status such as GSH-Px , CAT and GST and non-enzymatic status such as GSH in the liver, kidney and brain
- Attenuation of histological alterations and rehabilitation of liver and kidney tissues.

These results clearly show that tartrazine exerts its cytotoxic effects as an oxidative stress and this possibly through the formation of oxygenated free radicals. Thus, supplements with oleaster oil make it possible to limit the disturbances caused by tartrazine . These supplements seem to have beneficial effects, by limiting free radical phenomena and repairing oxidative damage, this is explained by their antioxidant power which therefore leads to contracting the state of oxidative stress.

In order to deepen this work, it would be interesting to;

- Evaluate the effects of chronic exposure to tartrazine over a longer period with other doses in order to highlight their toxic effects.
- Conduct this research on other biological models and other treatments.
- Deepen the study using more comprehensive techniques at the subcellular and molecular level including PCR testing, and serology.

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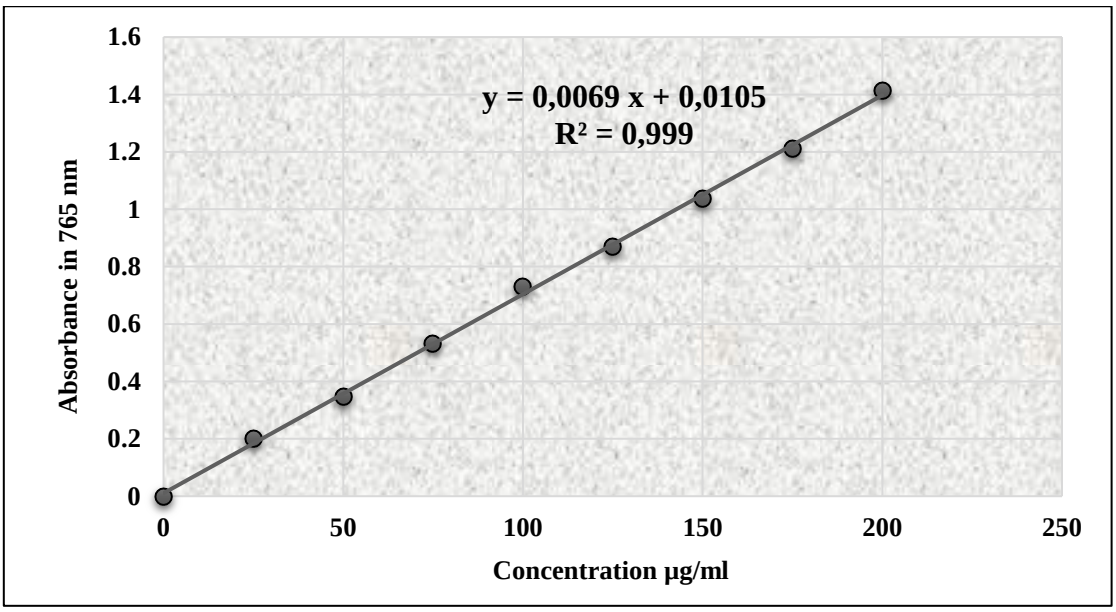
APPENDICES

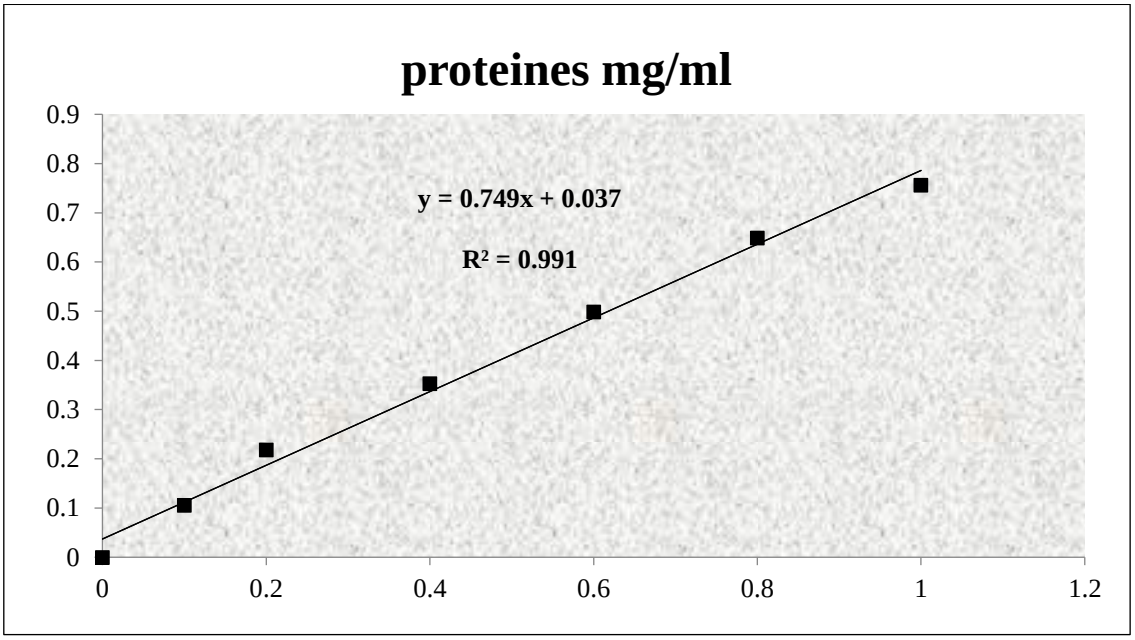
1. Calibration curves

1.1. Gallic acid calibration curve



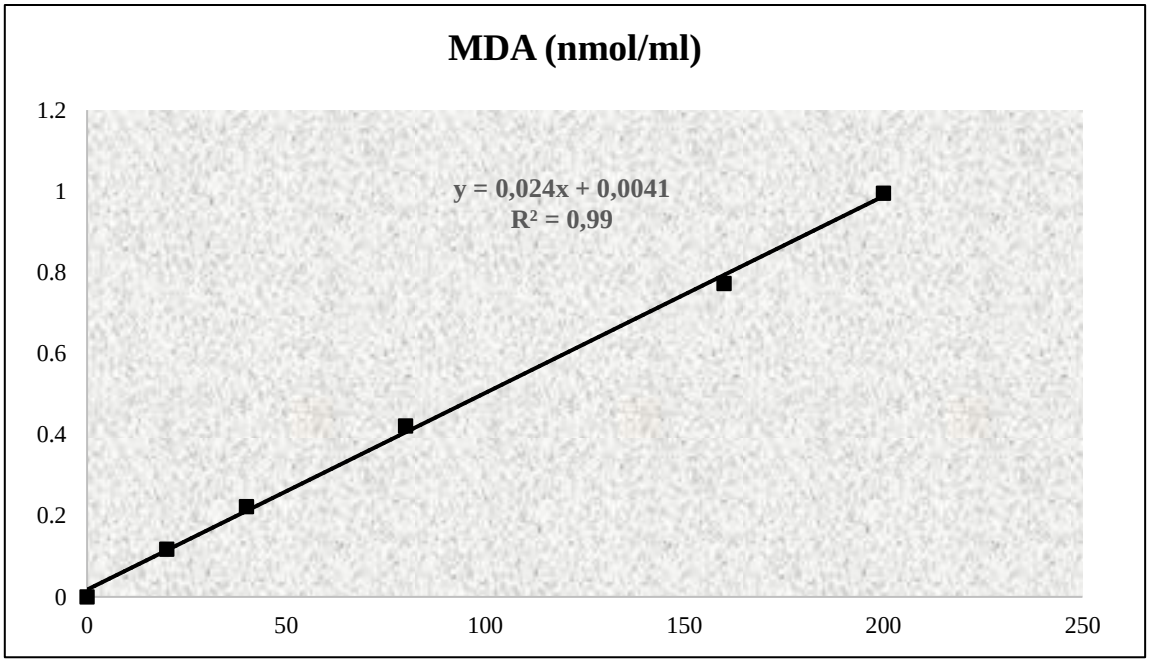
Appendice 1. Gallic acid calibration curve for the determination of total polyphenols

1.2. Protein calibration curve



Appendice 2. Calibration curve for protein assay.

1.3. MDA calibration curve



Appendice 3. MDA calibration curve