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Investigation of Hepatotoxicity after a neuroleptic overdose

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ملخــص

من بين جميع أعضاء جسم الإنسان، يؤدي الكبد أكبر عدد من الوظائف. تعد أنشطته المتعددة مهمة ولها تأثيرات على جميع أجهزة الجسم، بما في ذلك الجهاز العصبي. كما أن معظم المواد التي نتناولها يتم استقلابها في الكبد. يحدث فشل الكبد عندما تتضرر أجزاء كبيرة منه بشكل يتعذر إصلاحه ولا يعود قادرًا على اداء وظيفته بالشكل اللازم. تعد إصابة الكبد الناتجة عن الادوية من رابع أهم أسباب الوفاة ، ومن بين هذه الأخيرة؛ مضادات الذهان المفيدة للاضطرابات السلوكية، التي قد تسبب سمية كبدية خاصة في حالة التعرض لخطر الجرعة الزائدة.

في هذه الدراسة، تم الاهتمام بفحص وتقييم التأثيرات السمية في الكبد بعد جرعة زائدة شبه مزمنة (45 يومًا) وحادة (72 ساعة) من دواء مضاد للذهان "الكلوربرومازين" عند جرذان ويستار. 4 محاور للتجريب منها دراسة التغيرات العامة للجسم، تحديد التحاليل البيوكيميائية (تحليل الدم)؛ تقييم المستقلبات وتقييم معاملات الإجهاد التأكسدي على مستويات مختلفة (الميتوكوندريا والخلية).

تظهر نتائج العلاج شبه المزمن لدينا زيادة في الدهون، البروتينات، TG، MDA، GSH، Mitochondrial GSH، GPx، CAT، الفوسفاتاز القلوي، البيليروبين الكلي، البيليروبين المباشر و (LDH) وانخفاض وزن الجسم، MDA، ASAT، ALAT، الألبومين (من ناحية أخرى تظهر نتائج العلاج الحاد زيادة في ALAT)، Albumin، TG، الدهون، البروتينات، MDA، Mitochondrial MDA، GSH، Mitochondrial GSH، GPx؛ CAT؛ الفوسفاتيز القلوي؛ البيليروبين المباشر؛ إجمالي البيليروبين ؛ (LDH وانخفاض) وزن الجسم ؛ ASAT والميتوكوندريا (GPx).

تشير هذه النتائج إلى أن الجرعة الزائدة من "الكلوربرومازين" تؤدي إلى تسمم الكبد ويمكن أن تسبب إصابته. وقد يكون ذلك جراء تفاعل هذا الدواء مع العديد من العضيات الخلوية مثل الميتوكوندريا

الكلمات المفتاحية: مضادات الذهان، الكلوربرومازين، فئران ويستار، السمية الكبدية، الإجهاد التأكسدي، الميتوكوندريا.

Résumé

Parmi les organes les plus importants du corps humain ; le foie qui remplit le plus grand nombre de fonctions. Ces multiples activités sont importantes et ont des répercussions sur tous les systèmes du corps, y compris le système nerveux. C'est aussi dans le foie que la plupart des substances que nous ingérons sont métabolisées, y compris les médicaments. L'insuffisance hépatique se produit lorsque de grandes parties du foie sont endommagées au point de ne plus pouvoir fonctionner. Les lésions hépatiques médicamenteuses (DILI): (médicaments induites des lésions hépatiques) sont la quatrième cause la plus importante de maladie du foie, parmi ces médicaments, les neuroleptiques qui sont bénéfiques pour la gestion des troubles du comportement, mais ils peuvent encore causer des effets indésirables ou aggraver certaines conditions telles que l'hépatotoxicité surtout si le patient n'a pas respecté la dose prescrite de sorte qu'il tombe dans le danger de surdosage . Dans cette étude, nous nous sommes intéressés à l'évaluation des effets hépatotoxiques après un surdosage sub-chronique (45 jours) et un autre aiguë (72 heures) d'un médicament neuroleptique « Chlorpromazine » dans le foie des rats *rattus Wistar*. Notre étude a été planifiée en 4 axes d'expérimentation ; étude des changements généraux du corps ; détermination des paramètres biochimiques (analyses sanguine) ; évaluation des métabolites (proteines et lipides) ; et une évaluation des paramètres du stress oxydatif au niveau (mitochondriale et cellulaire).Nos résultats de traitement sub-chronique montrent une augmentation de (lipides; protéines; TG; MDA mitochondriale; GSH; GSH mitochondriale; GPx; CAT ;Phosphatase alcaline; Bilirubine totale . Bilirubine directe et LDH). Et une diminution de (poids corporel ; MDA ; ASAT ; ALAT ; Albumine) ; En revanche, les résultats du traitement aiguë montrent une augmentation de (ALAT ; Albumine ; TG ; Lipides ; Protéines ; MDA ; MDA mitochondriale ; GSH ; GSH mitochondriale ; GPx ; CAT ; Phosphatase alcaline ; Bilirubine directe ; Bilirubine totale ; LDH) et une diminution de (poids corporel ; ASAT et GPx Mitochondriale).

Ces résultats signifient que la surdose de « Chlorpromazine » entraîne une hépatotoxicité et peut causer des lésions hépatiques. Le CZP peut interagir avec divers organites cellulaires tels que mitochondries entraînant un dysfonctionnement des hépatocytes .

Mots clés *: neuroleptiques,* Chlorpromazine*, rats Wistar, Hépatotoxicité, Stress oxydant ,Mitochondrie.*

Abstract

Among all of the organs in the human body, the liver performs the greatest number of functions. The liver's multiple activities are important and have impacts on all body systems, including the nervous system. It is also in the liver that most of the substances that we ingest are metabolized, including drugs. Liver failure occurs when large parts of the liver become damaged beyond repair, and the liver is no longer able to function. Drug-induced liver injury (DILI) is the 4th most important cause of liver disease ,among these drugs, the neuroleptics which are beneficial for the management of behavioral disorders, but they still can cause adverse effects or aggravate some conditions such as hepatotoxicity especially if the patient did not respect the prescribed dose so that he falls in the danger of overdose . In this study we were interested in the investigation and evaluation of hepatotoxic effects after a sub-chronic (45 days) and an acute (72hours) overdosing of a neuroleptic drug "Chlorpromazine " in the liver of *rattus Wistar* rats .our study was planned in 4 axes of experimentation ; study of general body changes ; determination of biochemical parameters (blood analysis) ;evaluation of metabolites; and evaluation of oxidative stress parameters at different levels (mitochondrial and cellular).our sub-chronic treatment results shows an increase of (lipids; proteins ; TG; Mitochondrial MDA ; GSH ; Mitochondrial GSH ; GPx ;CAT ;Alkaline Phosphatase ; Totale Bilirubine . Direct Bilirubine and LDH). And a decrease of (body weight ; MDA ; ASAT ; ALAT; Albumine)In the other hand the acute treatment results shows an increase of (ALAT ; Albumine ; TG; Lipids ; Proteins; MDA ; Mitochondrial MDA ; GSH ; Mitochondrial GSH; GPx ; CAT ; Alkaline Phosphatase ; Direct Bilirubine ; Totale Bilirubine ; LDH) and a decrease of (body weight ; ASAT and Mitochondrial GPx)

These results signifies that the overdose of "Chlorpromazine" leads to hepatotoxicity and can cause liver injury .CZP may interact with various cellular organelles such as mitochondria leading to hepatocyte dysfunction .

Keywords: *neuroleptics, Chlorpromazine, Wistar rats, Hepatotoxicity, Oxidative stress, Mitochondri*

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Dedication

This work is dedicates to my beloved parents, who have been my source of inspiration and gave me strength when i thought of giving up, who continually provide their moral , spiritual , emotional and financial support . Their belief in me has kept my spirits and motivation high during this process

To my Dad **“Ali”** : you are my reason of live, thank you for every moment you spent with me and every word of encouragement May ALLAH bless you ..

To my mom **“khadidja”** you are my light, Source of tenderness ,I love you so much may allah bless you and give you health, long life and happiness….

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To my brothers : **Naim** , **Yacine** and my twin soulmate **Akrem** You are my 3 stars, thank you for lightening the path of my studies and for being by my side in all times

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To my flowers and my reason of happiness : **Imen, Kawther ,Amina, Abir, Asma , Zeineb , sara** and **my sweetheart zara**

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I dedicate this simple work to my teachers and friends as well, and I would like to express my gratitude to them ; big thanks to the Future Doctor **Benkhdhir Karim , saker hichem and Dr. Ben Aicha Brahim** for their support and advices .

To my roses **:souhila , loulou , Hiba , wided , Achouak , Noura ,dorsaf , Nourhan ,chaima** And especially to my sweethearts **, Hadjer ,Roumaissa and amina** who was by my side in all times , I love you so much my flowers

Rayane

• Liste of abreviations

ATP : Adenosine 5′-triphosphate
AUC: Air Under Curve
BBC : Bleu Brillant de Coumassie
BSA : Bis Triméthylsilyl Acétamide
CAT : Catalase
CNS : Central Nervous System
CPZ: Chlorpromazine
CYP : Cytochrome
D2 : Dopamine receptor
DNA : Deoxyribonucleic Acid
DTNB :5.5.dithio-bis-2-nitrobenzoic acid
ERN : Espèces réactives de l'azote
FGAs: First Generation Antipsycotics
GPx : Glutathion peroxydase
Gpx: Glutathion Peroxydase
GSH : Glutathion SH
GSTs : Glutathione S Transferase
IM: Short-Acting Intramuscular Injection
LAI: Long-Acting Injectable
LDH : Lactate Dehydrogenase
MDA : Malondialdehyd
NRS : Nitrogen reactive species
ODT: Orally Dissolving Tablet
ROS : Reactive oxygen species
rpm : Revolution per minute
SOD : Superoxyd dismutase
Tab: Tablet
TBA : Tetrabutyalammonium
TCA: Trichloroacetic acid

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Second chapter: Hepatotoxicity and Oxidative Stress

PART 1: Liver and Hepatotoxicity

PART2: Oxidative Stress and Antioxidants

Experimental part

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INTRODUCTION

Introduction

The global prevalence of schizophrenia is estimated at 1%, which represents about 23 million people affected by this disease worldwide according to the WHO **(Jaeschke *et al*.,2021)** Schizophrenia is a serious mental illness that affects how a person thinks, feels, and behaves ; People with schizophrenia may seem like they have lost touch with reality ,they interpret reality abnormally. among Psychotic symptoms (Hallucinations, Delusions, Thought disorder ,Movement disorder and Negative symptoms include loss of motivation, loss of interest or enjoyment in daily activities, difficulty showing emotions...) (**Vuppalanchi et *al*.,2010) .** There are currently several therapeutic strategies and neuroleptics, or more commonly known as antipsychotics which forms the reference drug class in the management of this pathology **(Biour et *al*.,2004) ;** it exists tow classes ; Typical antipsychotics, also known as traditional or first-generation antipsychotics and Atypical antipsychotics, also known as second generation antipsychotics. Both types act in à similar way by blocking receptors in the dopamine pathways **(Gahr et *al*.,2013)** Chlorpromazine was one of the first antipsychotic drugs discovered to be effective in the treatment of schizophrenia during the 1950s. It still remains one of the most commonly used and inexpensive treatments even today **(Winton, 2017).** However, it also has serious side effects, such as blurred vision, a dry mouth, tremors or uncontrollable shaking, depression, muscle stiffness and restlessness. As we know this disease requires long-term treatment so it causes accumulation of the drug and its metabolites , in addition to that, it affects the mind and behavior, which leads to mental disorder, and here lies the problem ;It is possible that the patient does not respect the prescribed doses due to forgetfulness and that he takes excessive doses of the CZP so he falls in the danger of overdose .which leads to sever health problems such as hepatotoxicity Because the liver is responsible for concentrating and metabolizing the majority of medications **(Biour et Salem, 2004)** .Drug-induced liver injury (DILI) is the 4th most important cause of liver disease , it is one of the most common causes of death in the world , The drugs used in psychiatry and neurology are the second most important group of drugs implicated in hepatotoxicity, after anti-infectious drugs . The hepatic reserve is reduced in patients with cirrhosis or chronic hepatic failure, and when DILI occurs in such patients, it can be more severe **(Goria et Marre, 2018).** Therefore, high-risk drugs should be contraindicated in cases of pre-existing liver disease (**Chalasani et *al*.,2014)** .

Chlorpromazine is one of drugs induced liver injury(DILI) it may causes hepatotoxicity at long-term treatment ; its most common manifestation related side effects is cholestasis, which

results from blocked bile secretion. **(Lammert, 2010)** it can be predictable, usually dose-dependent, or in some cases only seen in a small number of patients treated, This is called idiosyncratic hepatotoxicity **(Smith, 2006).**

From what we have just developed, the following question arises: what are the hepatotoxic effects induced by an overdose of neuroleptics?

The objective of this study is the investigation and evaluation of hepatotoxic effects of a neuroleptic "Chlorpromazine " after a sub-chronic (45 days) and an acute (72hours) overdose treatment ,for comparison purposes. The experimental study was conducted in rats of the *rattus Wistar* strain; In the laboratory of toxicology in CHIKH LARBI TEBESSI university –Tebessa-.

CHAPTER I

1- Generality

1.1- Definition of neuroleptics

Neuroleptics, also known as antipsychotic medications, are medications that block dopamine receptors in the nervous system. They are mainly prescribed to manage mental illnesses, such as schizophrenia and bipolar disorder, as well as psychosis. Psychosis describes loss of touch with reality, with specific symptoms such as difficulty concentrating, hallucinating, and engaging in movements without a purpose **(Alex et Pehek, 2007)**

The first neuroleptic or differently named (antipsychotic) chlorpromazine was introduced in 1955 by French psychiatrists Pierre Deniker and Jean Delay. Since then, antipsychotics have been the cornerstone treatment for psychotic disorders, and considered the single greatest advance in the treatment of mental disorders. **(Farde et Wiesel, 1988)**

1.2- Classification

Antipsychotic medicines are classified as "typical" or "atypical". Typical antipsychotics, also known as traditional or first-generation antipsychotics, include haloperidol and chlorpromazine. Atypical antipsychotics, also known as second generation antipsychotics, include quetiapine, risperidone and olanzapine. Both types of antipsychotics act in à similar way by blocking receptors in the dopamine pathway, but atypical antipsychotics are less likely to 'cause the extrapyramidal adverse effects associated with the older typical antipsychotics. However, atypical antipsychotics, along with typical antipsychotics, are associated with serious adverse effects, such as diabetes. melitus, stroke and cardiac death **(Ray et *al.*,2009).**

1.2.1- typical and atypical neuroleptics

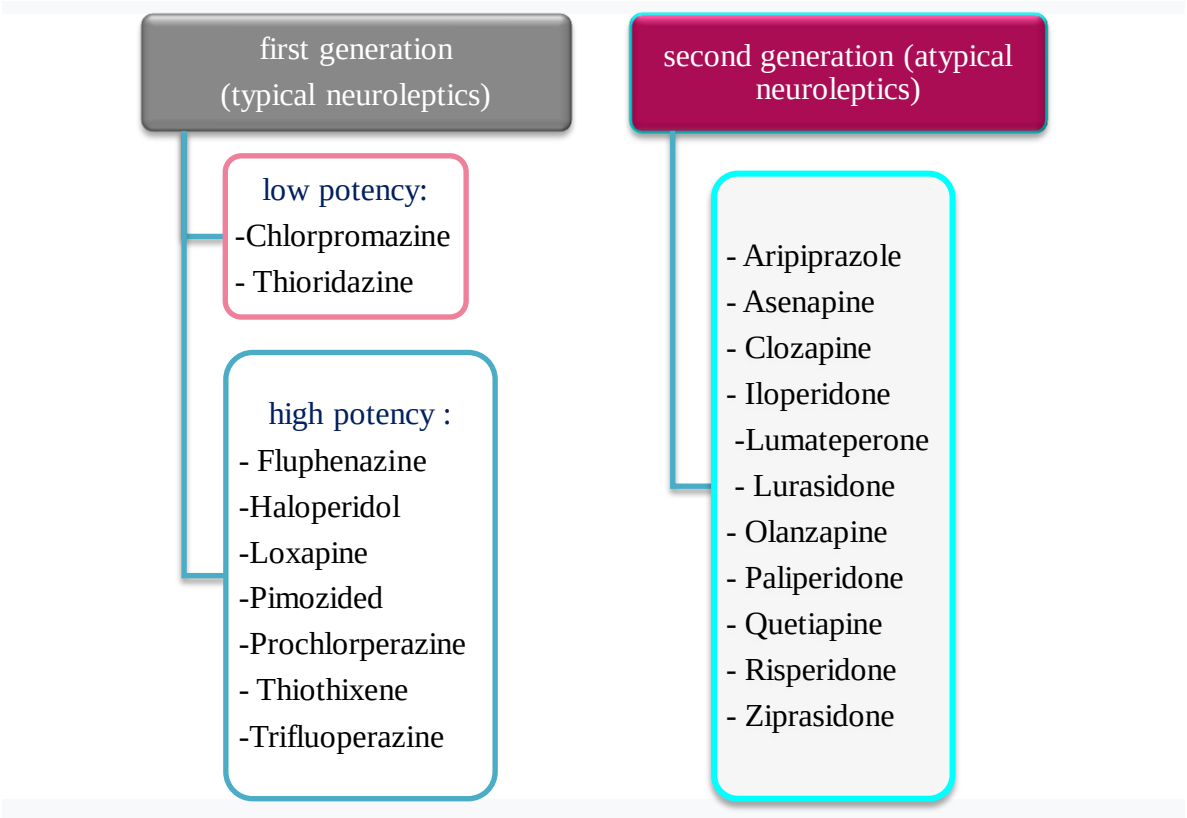


Fig.01: examples of typical and atypical neuroleptics

1.3 - Structural and physicochemical propreties

The first-used class of efficacious antipsychotic agents is Phenothiazine

- **Structural Properties**

Phenothiazine or bioisosteric heterocycle a connector alkyl (side) chain terminated by an aliphatic 3E-amine function **(Rellay, 1999)** they share the same three-ring structure with different side chains joined at the nitrogen atom of the middle ring.

The activity of the group can be affected by substitutions at position 2 or 10,they are categorized into three subclasses based on substitutions at position 10: aliphatic, piperidine, and piperazine phenothiazines **(Stahl, 2008) .**

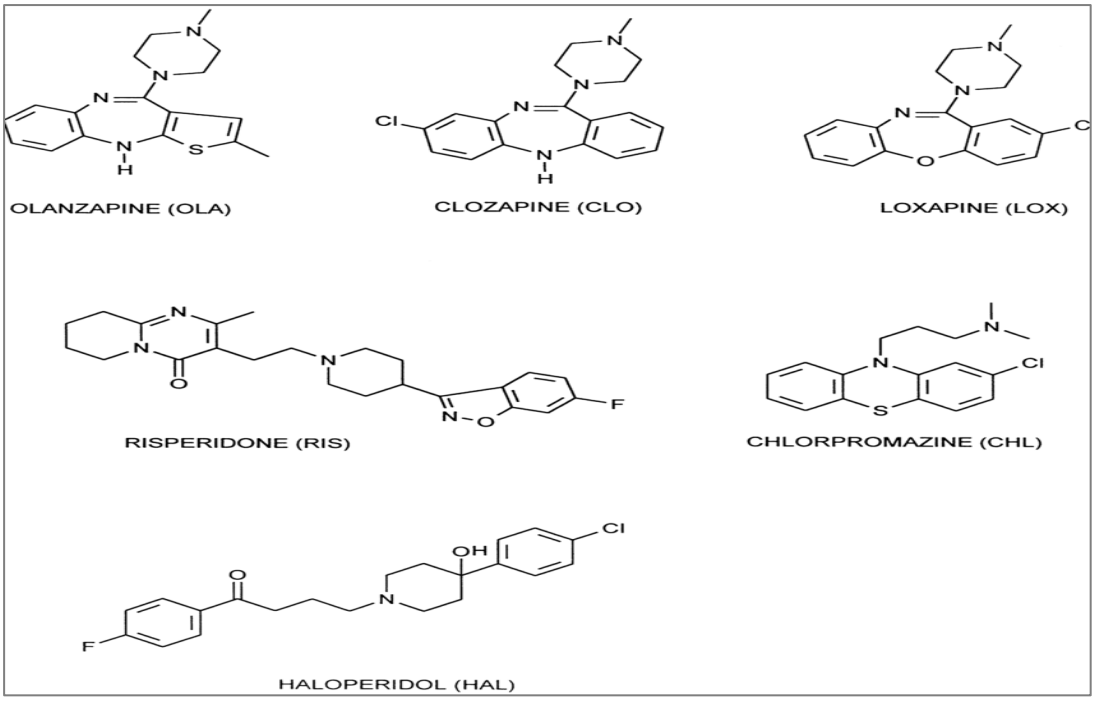


Fig. 02: chemical structures of most used neuroleptics **(Liqchrom et Rel, 2000)**

Phenothiazine Compounds: Three subclasses **(Sadock B, 2009 ; Creese I et *al*., 1976)**

- Aliphatic derivatives (e.g., chlorpromazine (Thorazine))
- Piperidine derivatives (e.g., thioridazine (Mellaril) am): relatively less potent
- Piperazine derivatives (e.g., fluphenazine (Prolixin)): relatively more potent.

- **Physicochemical Properties**

The phenothiazine heterocycle confers a high degree of lipophilicity on these antipsychotics which is balanced (solubility) by the cationized (at physiologic pH) amine function

- The phenothiazines possess two potentially basic functional groups:
- The N10-amine which is very weakly basic (pKb >10) because of the electronwithdrawin effects of the 2 benzene rings attached to it and is not appreciably cationized at phys. pH,
- The side chain tertiary amine function which confers strong organic basicity on the antipsychotic phenothiazines **(Rilley, 1999)**

1.4-Doses / overdoses and half-life of neuroleptics

Antipsychotics usually reach steady state in about three to five half-lives. Thus, steady state levels for chlorpromazine, haloperidol, and most other FGAs are reached in about 3 to 5 days since their half-lives are about 24 hours **(Sadock B et *al.*,2009).**

Table 01: This table represents doses and half -ife of the most used neuroleptics of both first and second generations **(Lieberman J et *al.*,2005).**

Agent (neuroleptic)	Initial oral dose range (mg/day)	Usual oral dose range (mg/day)	Usual maximum oral dose (mg/day)	Formulation	Half-life after oral administration (hours)
Haloperidol	2 to 10	2 to 20	30	Tab, IM, LAI, oral solution	20
Loxapine	20	20 to 80	100	Capsule Oral solution and IM injection	6 to 8
Clozapine	12.5 to 25	150 to 600	900	Tab, ODT, oral suspension	12
Thiothixene	5 to 10	10 to 20	30	Capsule	34
Risperidone	1 to 2	2 to 6	8	Tab, ODT, LAI, oral solution	20
Paliperidone	6	6 to 12	12	ER tab, LAI	23
Olanzapine	5 to 10	10 to 20	30	Tab, ODT, IM, LAI	23
Aripiprazole	5 to 10	10 to 20	30	Tab, ODT, LAI, oral solution	75 to 94

Tab: tablet; ODT: orally dissolving tablet ; LAI: long-acting injectable IV: intravenous; IM: short-acting intramuscular injection

- **Overdose of neuroleptics**

Antipsychotic overdose produces a gamut of manifestations that affect multiple organ systems. The most serious toxicity involves the cardiovascular system and the central nervous system. All typical and atypical antipsychotics cause sedation, which is pronounced in overdose. The most common cardiovascular effects that occur after atypical antipsychotic overdose are tachycardia, mild hypotension; other clinical syndromes in overdose include neuroleptic malignant syndrome (NMS) and antimuscarinic delirium. Seizures may be observed. No antidotes exist for these poisonings, but they most often do well with supportive care **(Alicia B et Richard, 2012)**

1.3- Neuroleptics Efficacy

All antipsychotics are considered equally effective. Rationale for determining which medication to use is based on side effect profile. Primary mechanism of action is Post synaptic blockade of the D-2 receptor. Newer agents e.g., Clozaril Have significant activity at the D-1 receptor; Risperdal and Zyprexa have significant 5-HT2 activity **(Essali et *al*., 1997; Freeman et *al*.,2009)**

- **Pharmacokinetics**

Among the various chemical classes of neuroleptics, the butyrophenones, the phenothiazines, and the thioxanthenes have similar pharmacokinetics, and show larger inter-individual variations in plasma drug levels after oral than after intramuscular doses. Compared to the "classical" neuroleptics, the more water-soluble benzamide derivatives have significantly shorter elimination half-lives, and show smaller inter-patient variations in plasma level **(Dahl, 1990).**

- **Absorption**

Most of the antipsychotics are very lipophilic and cross lipoidal membranes freely **(Javaid, 1994).** They are well absorbed after oral administration but can undergo an extensive "first-pass" effect that may result in large interindividual differences in the bioavailability (10-70%) of these drugs. Since many of the antipsychotics are highly lipophilic compounds, bioavailability can be increased 4 to 10 times after intramuscular (IM) administration. **(Curry,1991)** for example, reported the kinetics of CPZ after various routes of administration in one individual. The area under the plasma-level curve (AUC) per unit of the dose after IV and IM administration were similar, whereas after oral administration the AUC was only 10%

of that achieved after IV administration. CPZ appeared in the systemic circulation within 30 minutes after oral administration and peak-plasma levels were obtained within 2 to 4 hours. By contrast, peak-plasma concentrations were reached within 30 minutes after IM administration.**(Clark et Kaul, 1976).**

- **Distribution**

Most of the antipsychotics are highly bound to plasma proteins (90-99% bound), they are extensively distributed because of their highly lipophilic character. The passage across membranes is primarily by passive diffusion, and hence, highly vascularized tissues, such as brain, lungs and liver, accumulate antipsychotic drugs. Although tissue concentrations may exceed plasma concentrations, there is a rapid exchange between plasma and tis- sues with a high blood supply. **(Curry, 1991).**

- **Metabolism**

As most antipsychotic medications undergo extensive first-pass metabolism, drug-metabolizing enzymes may play an important role in patient response to antipsychotic treatment by determining the proportion of the drug that reaches the systemic circulation and is available to act on its targets in the brain. The cytochrome P450 (CYP) enzymes are the major family of drug-metabolizing enzymes that influence antipsychotic metabolism**.(Guengerich F, 2008)** Antipsychotic drugs are metabolized primarily by CYP1A2, CYP2D6, and CYP3A4, with CYP2C19 playing an important role in clozapine metabolism, as well as the metabolism of many antidepressants **(Hiemke et *al*.,2011).**

- **Elimination**

Only negligible amounts of the unchanged drug are excreted by the kidneys. Phenothiazines, thioxanthenes, and their metabolites are excreted in the urine and the faeces. **(Grunder et *al*., 2009).**

2.2- Pharmacodynamics

Relevance to side effects first generation antipsychotics, as exemplified by chlorpromazine, have been structurally modified to produce drugs with greater affinity for dopamine receptors while retaining some of their activity on other receptor systems (e.g., on alpha1 adrenoceptors, 5-HT2 receptors and histamine1 receptors). In the non-phenothiazine series, a high degree of specificity for the D2 receptors has been achieved with sulpiride and pimozide, with haloperidol showing antagonistic effects on the 5-HT2 and alpha1

adrenoceptors in addition to its selectivity for D2 receptors. The cis-(Z) isomers of the thioxanthines are potent neuroleptics that, in addition to their selectivity for D2 receptors, also show antagonistic effects on D1, 5-HT2 and alpha1 adrenergic receptors; cis(Z)- flupenthixol has a greater effect on D1 receptors than cis-(Z)-clopenthixol. It should be emphasized that the effect of such drugs on 5-HT2 receptors is weak **(Stahl, 2008 ; Sadock B, 2009)**

Table 02: Receptor Affinity Profile of FGAs. **(Taylor et *al*., 2011).**

Drugs	D2 activity	5HT2 Activity	Alpha-1 adrenergic Activity	Antihistamine Activity
Thioridazine	Very high	Very high	Very high	Very high
Haloperidol	Very high	Moderate	Low	Low
Perphenazine	Very high	Very high	Moderate	High
Thiothixene	Very high	Moderate	Low	High
Loxapine	Very high	Very high	Very high	Very high
Fluphenazine	Very high	Moderate	Low	Moderate
Trifluoperazine	Very high	High	Moderate	Moderate

2.3- Mode of action

The therapeutic action of an antipsychotic occurs when 65% to 85% of brain dopamine (D2) receptors are occupied. **(Farde et Wiesel, 1988)** When more than 80% of the dopamine (D2) receptors are occupied, hyperprolactinemia and parkinsonism can result. In addition to percentage occupancy, the duration of time that the antipsychotic drug stays attached to the D2 receptor impacts the degree of extrapyramidal symptoms (EPS). **(Kapur et Seeman, 2000) .** Antipsychotic drugs such as haloperidol and chlorpromazine tend to block dopamine D2 receptors in the dopaminergic pathways of the brain. This means that dopamine released in these pathways has less effect. Excess release of dopamine in the mesolimbic pathway has been linked to psychotic experiences. Decreased dopamine release in the prefrontal cortex, and excess dopamine release in other pathways, are associated with psychotic episodes in schizophrenia and bipolar disorder**. (Pickar et *al*., 1990 ; Liemburg et *al*.,2012).**

- **Effects of Antipsychotics on the Four Dopamine Pathways**

- **Nigrostriatal Pathway: Extrapyramidal Symptoms**

In which fibres originate from the A9 region of the pars compacta and project rostrally to become widely distributed in the caudate nucleus and the putamen. One of the major functions of dopamine in nigrostratal pathway is movements. Antagonism of D2 receptors in the nigrostriatal pathway is associated with increased risk of extrapyramidal symptoms **(Taylor et *al*.,2011 ; Sadock et *al*.,2009).**

- **Tuberoinfundibular Pathway: Hyperprolactinemia**

This originates in the arcuate nucleus of the hypothalamus and projects to the median eminence. Dopamine acts as prolactin-inhibiting factor or it synonyms with prolactin inhibiting factor in tuberoinfundibular pathways. D2 blockade in this path way increases prolactin levels by promoting its release in the pituitary gland causing hyperprolactinemia **(Schatzberg et *al*., 2010).**

- **Mesocortical Pathway**

In which the dopaminergic fibers also arise from the A10 region (the ventral tegmental area) and project to the frontal cortex and septohippocampal regions. Schizophrenia pathophysiology suggests that a dysfunction of mesocortical pathway is associated with cognitive impairments and disturbances of emotions and affect (negative symptoms). Blockade of the mesocortical pathway by high doses of first-generation antipsychotics can induce secondary negative symptoms and cognitive effects **(American Psychiatric Association, 1997).**

- **Mesolimbic Pathway: Antipsychotic Effects**

Where the dopaminergic projections originate in the ventral tegmental area, the A10 region, and then spread to the amygdala, pyriform cortex, lateral septal nuclei and the nucleus accumbens. Over activity of mesolimbic dopamine pathway is thought to be involved in the pathophysiology of positive symptoms of schizophrenia. Blockade of D2 receptors in the mesolimbic pathway has been proposed as a possible mechanism of antipsychotic action of first-generation agents **(Creese et *al*.,1976).**

3- Toxicity of neuroleptics

Antipsychotics cause toxicity by blocking select potassium and sodium channels, as well as cause toxicity via alpha-1 adrenergic blockade and anticholinergic effects .Various organs are affected but primarily the cardiovascular and central nervous system **(Haddad, 2002).**

3.1- Cardiotoxicity

- **Hypotension**: caused by peripheral alpha-1 adrenergic blockad **(Chyka et *al*.,2005).**
- Particularly with clozapine, risperidone, but can occur in all antipsychotic medication
- **Tachycardia**: caused by a compensatory response to decrease vasomotor tone and muscarinic receptor antagonism **(Burns, 2021).**
- **Sudden cardiac death3** : Incidence of sudden cardiac death for patients taking antipsychotics is about twice that of general population **(Ray et *al* .,2009).** Olanzapine has also been reported to be associated with peripheral edema and pericardial effusion **(Arslan et *al*.,2019).**

3.2-Neurotoxicity

Anticholinergic toxicity: findings of flushed skin, dry mucous membranes, mydriasis, altered mental status, and/or fevers are caused by blockade of muscarinic receptors.

Antipsychotics with a high H_1-histamine blockade profile (for example, clozapine, olanzapine, quetiapine) produce more severe central nervous system (CNS) depression **(Burns, 2001).**

Antipsychotics with a high muscarinic blockade profile will produce both central and peripheral anticholinergic findings (for example, clozapine and olanzapine) **(Kapur et Mamo, 2003).**

Extrapyramidal side effects such as neuroleptic malignant syndrome, acute dystonia, akathisia, tardive dyskinesia, and parkinsonian syndromes may occur with acute and chronic use of antipsychotics Mediated by D_2 receptor antagonism.

Atypical antipsychotics are less likely to produce extrapyramidal effects because of lower affinity to D_2 receptors **(Seeman, 2002).**

3.3- Hepatotoxicity

Antipsychotics can induce liver injury by means of three main mechanisms: Hepatocellular, cholestatic and steatosis. The risk of hepatotoxicity with chlorpromazine is well established **(Aronson, 2009).** The main mechanism by which phenothiazines induce cholestatic disease remains unclear. The existence of eosinophilia and rash during its early onset and that there is not a dose relationship for its toxicity reveal that the mechanism could be some type of hypersensitivity. Nevertheless, some authors have indicated that its toxicity might be related to an idiosyncratic metabolic reaction that depends on individual sensitivity The bile duct can be the most affected, and as a consequence, a severe ductopenic syndrome can occur. **(Selim et Kaplowitz, 1999).**

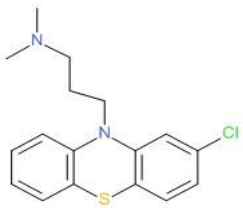
II. Chlorpromazine

1-Definition

Chlorpromazine is a phenothiazine antipsychotic used to treat nausea, vomiting, preoperative anxiety, schizophrenia, bipolar disorder, and severe behavioral problems **;**chlorpromazine's antipsychotic actions are thought to be due to long-term adaptation by the brain to blocking dopamine receptors. It has several other actions and therapeutic uses, including as an antiemetic and in the treatment of intractable hiccup **(Oades, 2000)**

1.1- Physico chemical propreties

Table 03: structure and molecular formula of CZP

Drug	Molecular formula	Structure
Chlorpromazine	C17-H19-Cl-N2-S	

1.2- Use of chlorpromazine

Chlorpromazine is used For the treatment of schizophrenia; to control nausea and vomiting; for relief of restlessness and apprehension before surgery; for acute intermittent porphyria; as an adjunct in the treatment of tetanus; to control the manifestations of the manic

type of manic-depressive illness; for relief of intractable hiccups; for the treatment of severe behavioral problems in children (1 to 12 years of age) marked by combativeness and/or explosive hyperexcitable behavior (out of proportion to immediate provocations), and in the short-term treatment of hyperactive children who show excessive motor activity with accompanying conduct disorders consisting of some or all of the following symptoms: impulsivity, difficulty sustaining attention, aggressivity, mood lability, and poor frustration tolerance**.(Roth et Craigo, 1994).**

1.3- Dose and overdose

- **Half-life~ 30 hours**

Dosage is dependent on the indication for chlorpromazine and is most commonly administered either orally or intramuscularly (IM). When used for the treatment of psychosis, adults typically ingest 50-300 mg/day in oral tablet or an IM injection of 25-50 mg/day. **(Seeman, 2010)** Nausea and vomiting will take 10-25 mg every 4-6 hours orally or 25-50 mg IM every 3-4 hours Prescribed dosing for childhood schizophrenia and autism vary widely. It is valuable for a physical therapist to understand the dosing schedule of a patient's medication. For chlorpromazine, the route of administration may have differing side effects. Inconsistent administration could also alter a patient's mental state**. (Cosi et Koek, 2001)**

2- Pharmacokinetics

The pharmacokinetics of chlorpromazine is also not fully understood and varies per the individual and route of administration. However, the kidneys excrete approximately 43-65% of the daily dose within 24 hours There are 5 clinically important metabolites, 4 of which are biologically active. The elimination half-life is thought to be a series of phases, with the early phase in 2-3 hours, an intermediate phase of 15 hours, and a late phase of up to 60 days A lingering half-life could prolong the effects of this medication in a person's body. Because of this, the physical therapist should be aware that side effects may linger after the dose is administered**.(Meyer, 2011)**

2.1-Absorption

Peak plasma concentrations occur on average 2-3 hours (range 1.5-8 hours) after an oral dose **(Midha et *al.*,1989 ; Yeung et *al.*,1993) .** After intramuscular injection chlorpromazine is slowly absorbed from the injection site, with the peak plasma

concentration occurring 6-24 hours after administration **(Dahl et Strandjord, 1977)** The oral bioavailability of chlorpromazine is about 30% that of intramuscular doses and about 10% that of intravenous doses as a result of pre-systemic metabolism. Readily absorbed from the GI tract. Bioavailability varies due to first-pass metabolism by the liver.

2.2-Distribution

Chlorpromazine is highly lipid soluble and is 98% bound to plasma proteins (**Dollery, 1991).** It is extensively distributed throughout the body and has a mean volume of distribution of 17 L/kg **(Yeung et *al.*,1993).**

- Volume of distribution :20 L/kg
- Protein binding > 90% to plasma proteins, primarily albumin

2.3- metabolism

Extensively metabolized in the liver and kidneys. It is extensively metabolized by cytochrome P450 isozymes CYP2D6 (major pathway), CYP1A2 and CYP3A4. Approximately 10 to 12 major metabolite have been identified. Hydroxylation at positions 3 and 7 of the phenothiazine nucleus and the N-dimethylaminopropyl side chain undergoes demethylation and is also metabolized to an N-oxide. In urine, 20% of chlopromazine and its metabolites are excreted unconjugated in the urine as unchanged drug, demonomethylchlorpromazine, dedimethylchlorpromazine, their sulfoxide metabolites, and chlorpromazine-N-oxide. The remaining 80% consists of conjugated metabolites, principally O-glucuronides and small amounts of ethereal sulfates of the mono- and dihydroxy-derivatives of chlorpromazine and their sulfoxide metabolites. The major metabolites are the monoglucuronide of N-dedimethylchlorpromazine and 7-hydroxychlorpromazine. Approximately 37% of the administered dose of chlorpromazine is excreted in urine. **(Weinreb et *al.*,1978) .**

2.4- Elimination

Excretion is primarily via the kidneys with less than 1% of a dose excreted as unchanged drug in the urine, and 20-70% as conjugated or unconjugated metabolites 5-6% of a dose is excreted in faeces via biliary elimination Some metabolites can still be detected up to 18 months afterdiscontinuation of long-term therapy **(Dollery, 1991).**

3-Pharmacodynamics

Chlorpromazine is a psychotropic agent indicated for the treatment of schizophrenia. It also exerts sedative and antiemetic activity. Chlorpromazine has actions at all levels of the central nervous system-primarily at subcortical levels-as well as on multiple organ systems. Chlorpromazine has strong antiadrenergic and weaker peripheral anticholinergic activity; ganglionic blocking action is relatively slight. It also possesses slight antihistaminic and antiserotonin activity **(Chalasani et Fontana, 2008).**

3.1-mode of action

Chlorpromazine acts as an antagonist (blocking agent) on different postsysnaptic receptors -on dopaminergic-receptors (subtypes D1, D2, D3 and D4 - different antipsychotic properties on productive and unproductive symptoms), on serotonergic-receptors (5-HT1 and 5-HT2, with anxiolytic, antidepressive and antiaggressive properties as well as an attenuation of extrapypramidal side-effects, but also leading to weight gain, fall in blood pressure, sedation and ejaculation difficulties), on histaminergic-receptors (H1-receptors, sedation, antiemesis, vertigo, fall in blood pressure and weight gain), alpha1/alpha2-receptors (antisympathomimetic properties, lowering of blood pressure, reflex tachycardia, vertigo, sedation, hypersalivation and incontinence as well as sexual dysfunction, but may also attenuate pseudoparkinsonism - controversial) and finally on muscarinic (cholinergic) M1/M2-receptors (causing anticholinergic symptoms like dry mouth, blurred vision, obstipation, difficulty/inability to urinate, sinus tachycardia, ECG-changes and loss of memory, but the anticholinergic action may attenuate extrapyramidal side-effects). Additionally, Chlorpromazine is a weak presynaptic inhibitor of Dopamine reuptake, which may lead to (mild) antidepressive and antiparkinsonian effects. This action could also account for psychomotor agitation and amplification of psychosis **(Veijola et *al*.,2014).**

4- Toxicity

The extrapyramidal, anticholinergic, sedative, and hypotensive features of toxicity result from the blockade of dopaminergic, muscarinic, histaminic, and alpha adrenergic receptors respectively.The cardiotoxic effects of phenothiazines in overdose are similar to that of the tricyclic antidepressants. **(Ellenhorn, 1997).** Cardiac arrhythmia and apparent 'sudden death' have been associated with therapeutic doses of chlorpromazine, the sudden cardiovascular collapse being attributed to ventricular dysrhythmia **(Fowler et *al*.,1976; Hollister et Kosec, 1965).**

4.1-hepatotoxicity

The clinically apparent liver injury due to chlorpromazine is likely due to hypersensitivity, based upon the clinical features of a short latency period, fever, eosinophilia, and rapid recurrence upon reexposure. Chlorpromazine is extensively metabolized by the liver via sulfoxidation and oxidation, and some instances of serum aminotransferase elevations as well as more clinically apparent liver injury may be caused by production of a toxic intermediate of its metabolism. Chlorpromazine therapy can also cause weight gain, and some instances of liver test abnormalities during therapy may due to nonalcoholic fatty liver disease.**(Larry et Ripault, 2013).**

CHAPTER II

I. Liver and Hepatotoxicity

1.1- Anatomy

1.1.1- Human's liver

The liver is located in the upper right-hand portion of the abdominal cavity, beneath the diaphragm, and on top of the stomach, right kidney, and intestines.

Shaped like a cone, the liver is a dark reddish-brown organ that weighs about 3 pounds.

There are 2 distinct sources that supply blood to the liver, including the following:

- Oxygenated blood flows in from the hepatic artery
- Nutrient-rich blood flows in from the hepatic portal vein

The liver holds about one pint (13%) of the body's blood supply at any given moment. The liver consists of 2 main lobes. Both are made up of 8 segments that consist of 1,000 lobules (small lobes). These lobules are connected to small ducts (tubes) that connect with larger ducts to form the common hepatic duct. The common hepatic duct transports the bile made by the liver cells to the gallbladder and duodenum (the first part of the small intestine) via the common bile duct**.(johns H, 2019).**

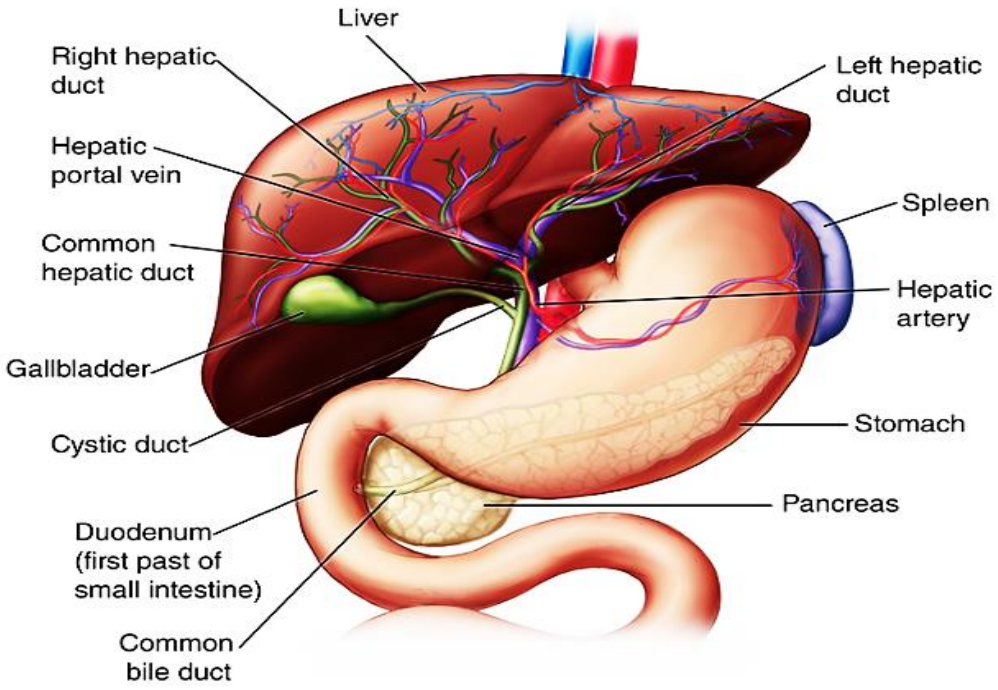


Fig.03: anatomy of the liver **(johnsH, 2019).**

1.1.2- Rat's liver

The liver of the rat is located in the intrathoracic part of the abdominal cavity, with the exception of ventral part of the left hepatic lobe which is located on the ventral abdominal wall in the regioxiphoidea, somewhat left to the median plane. On the right of the intrathoracic part of the abdominal cavity the liver is situated between the diaphragm, descending duodenum, jejunum and right kidney, and on the left between the diaphragm, stomach, jejunum and dorsal end of the spleen. The parietal surface of the liver was strongly convex and it is in contact with diaphragm.(**Nešić et *al*.,2020)**

The rat livers had four lobes (left, middle, right, and caudate). The left and middle lobes formed a single lobe but the middle lobe had a deep notch to which the round ligament attached. The right lobe was split into two sub-lobes and the caudate lobe was divided into the paracaval portion and the Spiegel lobe, which was split into two sub-lobes. The left, right, and caudate lobes had one primary portal branch, whereas the middle lobe had two portal branches. The left and the right sub- and caudate lobes had one large hepatic vein each, whereas three large hepatic veins were observed in the middle lobe. Based on the ramifying patterns of the portal and hepatic veins, the rat middle lobe possessed left and right hepatic components and a main portal fissure. The following rat hepatic lobes were equivalent to the following human liver segments: the left lobe to segment II; the middle lobe to segments III, IV, V, and VIII; and the right lobe to segments VI and VII. The fundamental structures of rat and human livers were similar, and the findings demonstrated a new interpretation of the anatomy of the human liver **(Kimitaka et *al*.,1999)**

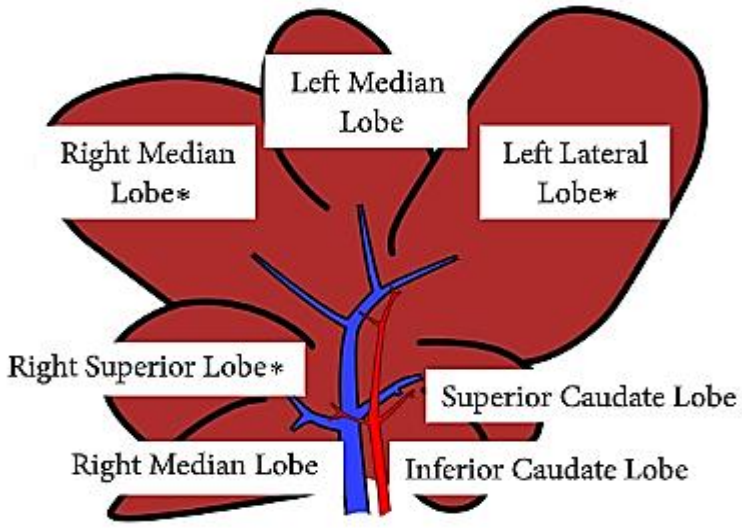


Fig.04: rat's liver anatomy; representation of hepatic lobes **(Giuseppina et *al*.,2019)**

1.2- Liver histology

Histologically speaking, the liver has a complex microscopic structure, that can be viewed from several different angles. Physiologically speaking, the liver also performs many essential functions.**. (Adrian R et Uruj Z, 2022)**

The liver consists of the following major histological components**:**

- Lobule
- hepatocytes
- Kupffer cells
- Parenchyma : which is represented by hepatocytes
- Stroma :
- Sinusoids: which are capillaries travelling between hepatocytes
- Spaces of Disse: (perisinusoidal spaces), which are located between the hepatocytes and the sinusoids. (**UrujZ, 2022)**

1.3- Liver functions

The liver is responsible for an array of functions that help support metabolism, immunity, digestion, detoxification, vitamin storage among other functions **(Arjun, & *al*.2022)**

Represented in the following figure :

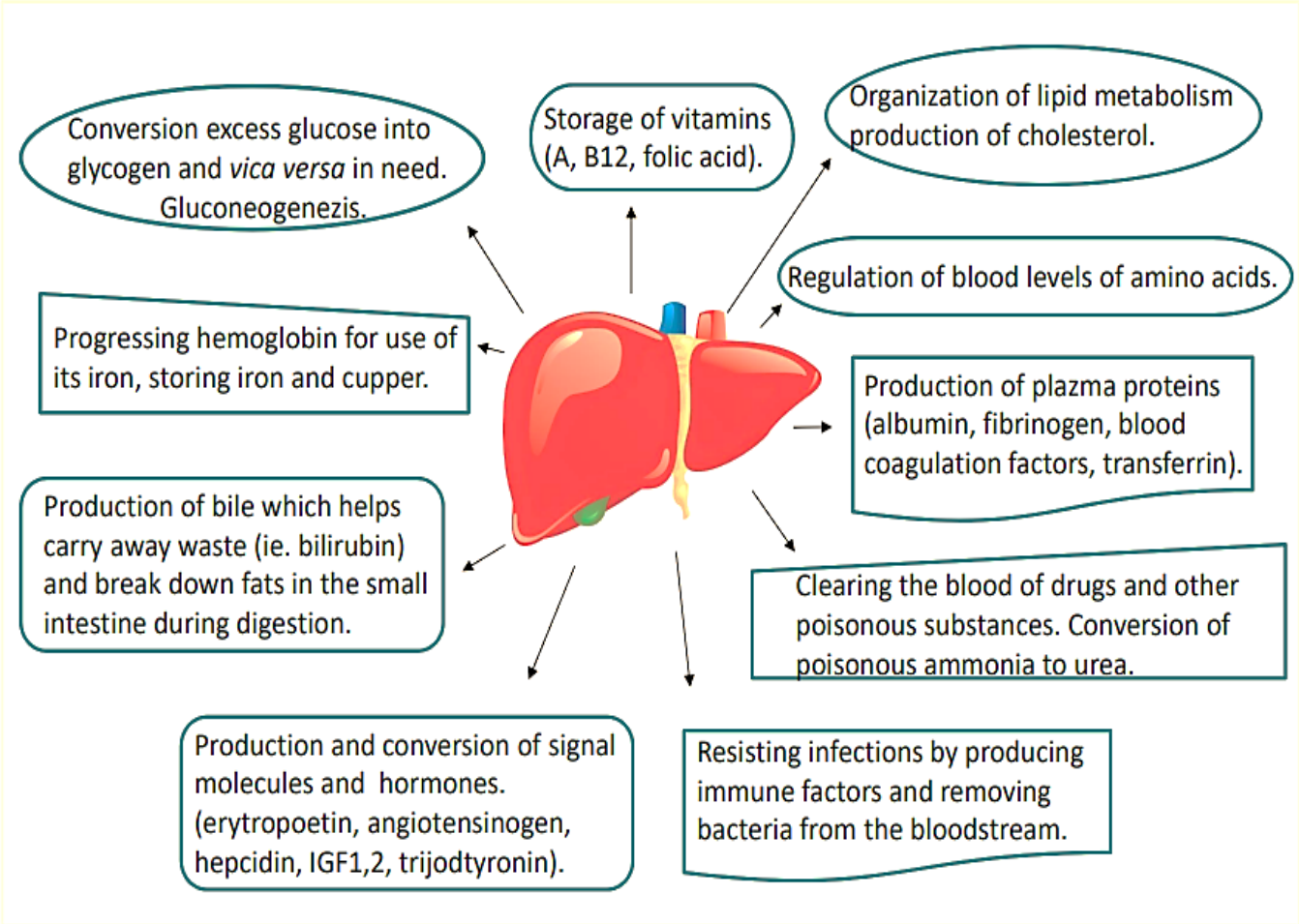


Fig.05: most liver functions **(Anatomy and Physiology, 2018)**

1.4- Liver Pathology

There are many types of liver disease, which can be caused by infections, inherited conditions, obesity and misuse of alcohol. Over time, liver disease may lead to scarring and more serious complications. Early treatment can help heal the damage and prevent liver failure **(American Liver Foundation, 2021).**

- **Liver Hepatitis** inflammation (swelling) of the liver as the result of a viral infection or liver damage caused by exposing to harmful substances such as alcohol. There are several different types of hepatitis from A to E. Some types will pass without any serious problems, while others can be long-lasting (chronic) and cause scarring of the liver (cirrhosis), loss of liver function and, in some cases, liver cancer.**(Wiegand, 2013)**
- **Liver Fibrosis**a process that inflamed liver starts to scar under no treatment. (Scar tissue is a kind of fibrous tissue.) Scar tissue can keep blood from flowing through the liver. As more scar tissue builds up, the liver may not work as well as it once did. If liver disease is diagnosed and treated successfully at this stage, there's still a chance that liver can heal itself over time**.(Kang, 2017)**
- **Liver Cirrhosis**a stage that the liver may become too seriously scarred to be reversed. Cirrhosis can lead to a number of complications, accompanied by a series of symptoms including bleed or bruise easily, jaundice, skin itch, insulin resistance, and type-2 diabetes.
- **Liver Failure** It means that the liver is losing or has lost all of its function. The first symptoms of liver failure are often nausea, loss of appetite, fatigue, and diarrhea. As time progresses, confusion, disorienting, and drowsiness gradually appear...**(Goldberg, 2013).**

2- Hepatotoxicity

Hepatotoxicity induced by drugs or toxins can be grouped into two types: intrinsic reactions (less common) and idiosyncratic reactions (more common) **(Ramachandran et Fisher, 2015)** Intrinsic reactions are predictable, dose-dependent and reproducible in animal models; injury is produced trough toxic metabolites of drugs such as free radicals (generating lipid peroxidation), electrophilic molecules (formation of covalent bonds with hepatic proteins) or active oxygen molecules (generating peroxidation as well). Idiosyncratic reactions are not predictable, not dose-dependent and not reproducible in animals; there are many drugs capable of causing this type of reaction **(Lee, 1999)** The underlying mechanism of the idiosyncratic reaction may be a genetic polymorphism of the cytochrome P450 (CYP450) system, responsible for the drugs hepatic biotransformation. There are two types of idiosyncratic reactions: immune (characterized by hypersensitivity-type reaction) and metabolic (related to metabolism of substances).**(Fisher et *al*.,2015 ;Adams, 2010**).

2.1- Mechanisms of hepatotoxicity

The hepatocytes, cholangiocytes, Kupffer cells, ductal and endothelial cells are involved in the mechanisms by which drugs cause hepatotoxicity **(Grattagliano et *al*.,2009)** having direct effects on cellular organelles such as mitochondria, endoplasmic reticulum, cytoskeleton, microtubules or nucleus.The drug metabolites generated in the liver through biotransformation can cause hepatic damage because formation of toxic or reactive substances such as electrophilic chemicals or free radicals **(Kaplowitz, 2011)** and thus an unchain a variety of chemical reactions may happen. These mechanisms can either generate necrosis or apoptosis or both. The following are some of the main mechanisms of liver injury **(Cullen, 2005)**.

- **Mitochondrial dysfunction**: may be generated by the disruption of β-oxidation of lipids and oxidative energy production within the hepatocytes. Mitochondrial membrane permeabilization can lead to apoptosis, a rupture in mitochondrial membrane can lead to ATP depletion and subsequent necrosis, and an abnormal function can also lead to fat accumulation, so steatosis can be present **(GuManautou. 2013)**
- **Immune response:** is attributed to the formation of new antigens, this give origin to the idiosyncratic hepatotoxicity. Moreover, it can be accompanied by presence of inflammatory cells such as neutrophils and lymphocytes.

- **Oxidative stress**: is produced by ATP depletion accompanied by increase in intracellular calcium concentration, it can generate necrosis **(Grattagliano et *al.*, 2009)**
- **Lipid peroxidation**: is generated by the interaction between free radicals and fatty acids in membrane, the subsequent reaction may produce electrophilic metabolites generating DNA damage **(Pessayre et *al.*,2010).**

II. Oxidative Stress

- **Generalities about oxidative stress**

Living beings have evolved over the past two billon years through adaptation, to an increasing atmospheric oxygen concentration, by both taking advantage of oxygen activating function and developing a complex control network. In these regards, potentially damaging species (reactive oxygen, nitrogen and chlorine species) arise as by-products of metabolism and also work as physiological mediators and signalling molecules. Oxidative stress may be an important factor in numerous pathological conditions (**Vertuani, et *al.*,2004)**

3- Definition of Oxidative stress

The oxidative stress is an abnormal circumstance that crosses our cells or tissues due to the endogenous or exogenous production of oxygenated free radicals that exceed their antioxidant capacities. Therefore, oxidative stress is an imbalance of the pro-oxidant and anti-oxidant balance; it is related to a mitochondrial hyperproduction of reactive oxygen species and a decrease in antioxidant defenses **(favier, 2006 ; Zbadi et *al.*,2018).**

This imbalance results an oxidative damage of all the cellular components: lipids with disturbances of the cellular membranes, proteins with the alteration of the receptors and enzymes, nucleic acids with a risk of mutation and cancerization .**(Sergent et *al.*, 2000).**

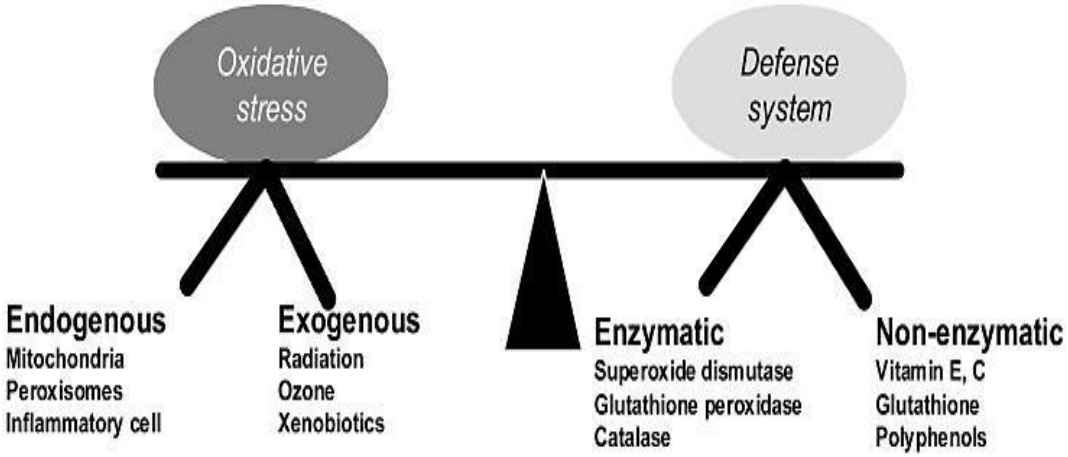


Fig.06: The balance of the oxidative stress and defence system**(Garait, 2006)**

3.1- Free radicals

The formation of free radicals is a normal result of the aerobic metabolism inthe human being. The free radicals are chemical species, molecules, atoms, they are "free". containing one or more single electrons at the level of the top layer; they are very reactive **(Goudable et Favier, 1997).**

Free radicals can be formed by three methods **(Chavane et Melinkeri, 2013)**.

- Addition of a free electron to a non-radical:(NR + e-⟶ R˙)
- Loss of an electron to a non-radical:(NR - e- ⟶ R˙)
- Homolytic splitting of a covalent bond :(A-B ⟶ A˙+B)

3.2- Type of free radicals

There are two main families of reactive species reactive oxygen species (ROS) and nitrogen reactive species (NRS).**(Sies, 1991)**

- **Reactive oxygen species (ROS)**

The mitochondria is the main source of (ROS), they are molecules containing oxygen, , but whose reactivity is much higher than that of the oxygen molecule **(Morel et Barouki ,1999 ; Derlé et *al*,2018)** Oxygen can undergo successive stages of reduction leading to the formation of ROS.

ROS are produced at the beginning of the reduction or oxidation of singlet oxygen 1'O2 to superoxide anion O2.-. And it will trigger a cascade of reactions that give the active oxygen

derivatives. This production of ROS is done in the presence of metals, organic molecules or enzymes (oxidases or complexes of the respiratory chain) **(Barouki, 2006).**

Reactive oxygen species (ROS) are essential cellular messengers important for homeostasis **(Ederlé et *al*,2018)**

- **Reactive nitrogen species (RNS)**

Reactive nitrogen species (RNS), radical or non-radical, are derivatives of oxygen metabolism synthesized by the vascular cell types, they are ensured by different enzymatic systems "NADPH oxidase, NO synthase, the respiratory chain ". The ERNs are derived from the activity of the enzyme NO -synthase which releases the nitric oxide (NO.).The latter will give several ERN derivatives. It is therefore mainly nitric oxide is very diffusible, capable of damaging many organic molecules (**Baudin et *al*, 2006 ; Migdale et *al*.,2011).**

3.3- sources of free radicals production

The different sources of free radical production are represented in **Table 03.**

They are classified into two categories:

- Endogenous sources: free radicals are produced by reactions in the body **(Belkheiri , 2010).**
- Exogenous sources: living beings are exposed daily to pollutants (cigarette smoke, ultraviolet rays, radiation ...) caused the production offree radicals **(Favier, 2003).**

Table04: Main sources of free radical production **(Belkheiri, 2010 ; Favier, 2003).**

Endogenous sources	-Production of free radicals during oxidative respiration -Phagocytic cells -Xanthine/Xanthine oxidase system
Exogenous sources	- Radiation - Transition metals - Pesticides -Drugs...

The production of these pro-oxidant species is normal at low concentration and is accompanied by an important physiological role. At high concentrations, their effects become

deleterious for cells, tissues and various physiological functions**. (Berger, 2006 ; Fontaine, 2007).**

4- Effects of free radicals on the body

4.1- Lipid peroxidation

Lipid peroxidation is the oxidation of mono or polyunsaturated lipids in the presence of oxygen. It is a radical process of chain reaction caused problems at the cell level.

Lipid peroxidation markers can be used in the context of human pathologies such as liver diseases, to monitor states of redox imbalance. These markers include hydroperoxides, aldehydes, oxysterole, and isoprostane**(Domnique, 2020).**

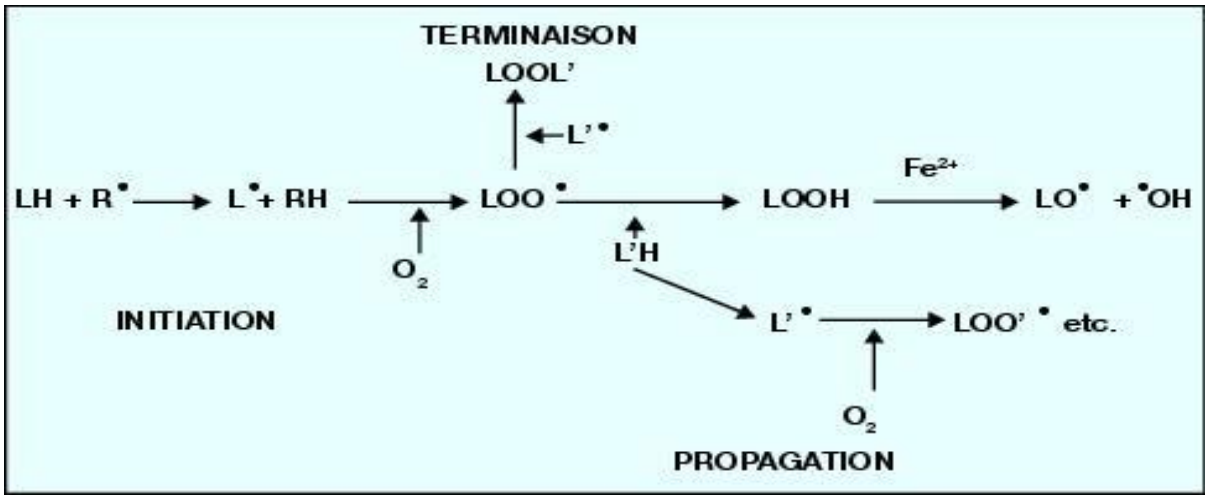


Fig.07: steps of lipid peroxidation **(Amira et Desmon.2020).**

4.1.1- Malondialdehyde MDA

MDA is used as a marker of lipid peroxidation in studies of oxidative stress and redox signaling, it exists in two forms, the first is free and the second is bound with proteins, lipoproteins, nucleic acids and amino acids **(Verma et *al.*, 2019 ; Domnique, 2020)**

4.2- Protein oxidation

Oxygenated free radicals induce modifications in the primary, secondary and tertiary structures of proteins by the formation of carbonylated protein derivatives via several mechanisms including fragmentation and oxidation of amino acids **(Aurousseau,2004 ;Baudin, 2006).**

4.3- DNA Oxidation

ROS can react with the guanine (G) base of DNA, to transform it into 8-hydroxy-2'deoxyguanosine (8-OH2DG) which is capable of inducing specific mutations in DNA that can lead to the development of cancer **(Collins et *al.*, 1997 ; Gasmi., 2018)**

5- Antioxidant system

The body has a number of defence complexes antioxidant, there are two categories: endogenous and exogenous, that is to say the enzymatic antioxidant system and non enzymatic antioxidant system which are summarized in **table 05 (Defreing et pincemail, 2008 ; marielle et *al.*,2020).**

Table 05: enzymatic and non-enzymatic systems **(Pastre,2005)**

Enzymatic antioxidant system (endogenous)	Non enzymatic antioxidant system (exogenous)
- Superoxide dismutase - Glutathione peroxidase - Catalase - Lipases, proteases, endonucleases - (eliminate oxidized molecules) - Albumin, ferritin	- Vitamin E. Vitamin C - Essential oils - Taurine - Carotenoids (lycopene, lutein, etc.) - Polyphenols - Minerals and trace elements

5.1- Enzymatic antioxydant system

5.1.1- superoxide dimustase(SOD)

SOD are intracellular enzymes, they represent the first line of defense **(Nicco et Batteux,2017)** Which play their role of biological protection, they catalyse the dismutation of oxygen and hydrogen peroxide by oxidation and cyclic reduction **(Azadmanesh et Borgstahl, 2018).**

There are three different types of location SOD:

- Copper-Zinc-SOD (Cu-Zn-SOD) SOD1, cytoplasmic.
- Manganese-SOD (Mn-SOD) SOD2, mitochondrial.

- Zinc-SOD (EC-SOD) SOD3, extracellular **(Nicco et Batteux, 2017)**

SODs catalyze the disproportionation of superoxide:

$$2\ HO_2 \rightarrow O_2 + H_2O_2$$

In this way, O^-_2 is converted into two less damaging species.

The pathway by which SOD-catalyzed dismutation of superoxide may be written, for Cu,Zn SOD, with the following reactions:

Cu^{2+}-SOD + $O^-_2 \rightarrow Cu^+$-SOD + O_2 (reduction of copper; oxidation of superoxide)

Cu^+-SOD + O^-_2 + $2H^+ \rightarrow Cu^{2+}$-SOD + H_2O_2 (oxidation of copper; reduction of superoxide)

The general form, applicable to all the different metal-coordinated forms of SOD, can be written as follows:

$M^{(n+1)+}$-SOD + $O^-_2 \rightarrow M^{n+}$-SOD + O_2

M^{n+}-SOD + O^-_2 + $2H^+ \rightarrow M^{(n+1)+}$-SOD + H_2O_2

5.1.2- Glutathione peroxidase (GPx)

This is the second line of enzymatic defense, prevents the formation of free radicals, in mammals. It is a selenium enzyme present in cytosol and mitochondria. It can reduce both H2O2 to H2O and organic hydroperoxides(ROOH) alcohol (ROH) **(Favier,2003 ;GasmiS,2018)**

GSH represents the reduced monomer of glutathione, and GS-SG represents the oxidized glutathione disulfide. GPx greatly promotes and accelerates the following reaction:

2 GSH + H2O2 GS–SG + 2 H2O

This eventually forms a bridge between two cysteines, called the cystine bridge or disulfide bridge and two water molecules . This helps to combat organic peroxides:

2 GSH + R-OOH GS-SG + ROH + H2O **(The Journal of Physical Chemistry A, 2016)**

5.1.3- Catalase (CAT)

Catalases are enzymes that prevent oxidative damage in cells by degrading hydrogen peroxide into water and oxygen with high efficiency **(Mercedes et *al.*, 2009)**

Catalase occurs primarily in peroxisomes, lysosomes and mitochondria. It Neutralizes hydrogen peroxide (H2O2) in water and molecular oxygen **(Casetta et *al.*, 2005)**

2 H2O2 → O2 + 2 H2O.

5.1.4- Glutathione S-Transferases(GSTs)

Glutathione S-transferases (GSTs) represent a family of enzymes that play an important role in the detoxification of electrophilic compounds. The function of GSTs is their activity to catalyze conjugation reactions between glutathione and harmful substances to decrease their reactivity with intracellular macromolecules. **(Moffarts, 2007).**

GSTs complement the action of glutathione peroxidases (GSH-Px) in the second line of antioxidant enzymatic defense **(Fatmi, 2013).**

5.2- The Non-Enzymatic Antioxidant System

5.2.1- Glutathion(GSH)

Reduced glutathione (GSH) is a tripeptide characterized by the presence of a sulfidryl group, it is responsible for the reduction of free radicals **(Gardès A et *al*., 2003)**, the reaction:

GSH +OH° GS° + H2O & GSH +R° GS° + RH

Glutathione can also react with Fe3+ and Cu2+ ions and thus limit their participation in the generation of free radicals through Fenton's reaction:

GSH + $Fe3^{+}$ GS°+ $Fe2^{+}$ + H+

GSH + Cu2+ GS° + $Fe2^{+}$ + H+

It is mainly present in reduced form (GSH) **(Haleng et *al*., 2007)**. Under physiological conditions, its oxidizing form (GSSG)is in very low concentration, and the ratio GSH / GSSG is an excellent marker of lipid oxidation and helps to determine the importance of stress **(Boussekine, 2014).**

6- Link between oxidative stress and liver diseases

Oxidative stress is importantly involved in the pathophysiology of various liver diseases. The redox state participates on the course of the inflammatory, metabolic and proliferative liver diseases. The main sources of the reactive oxygen species (ROS) are represented by the mitochondria and cytochrome P450 enzymes in the hepatocyte, Kupffer cells and neutrophils. Cells are provided with efficient molecular strategies to strictly control the intracellular ROS level and to maintain the balance between oxidant and antioxidant molecules. Hepatocyte's proteins, lipids and DNA are among the cellular structures to be affected primarily by ROS and reactive nitrogen species (RNS). This process disrupts at cellular and molecular level the structure-function relationship on liver cells at different sites **(Jonathan et *al.*, 2016).**

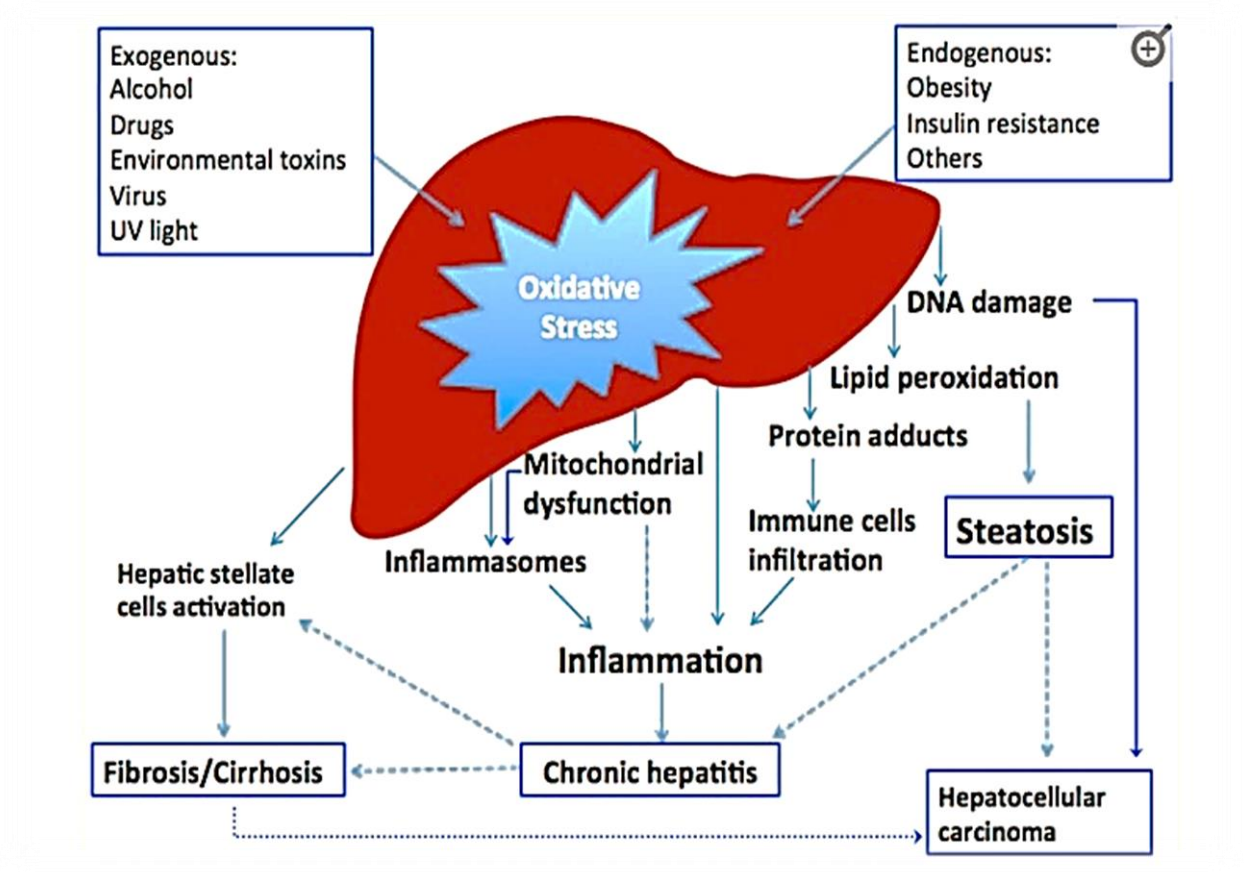


Fig.08: The general mechanism of oxidative stress induced by various factors on liver disease. **(Shali et *al.*, 2015)**

EXPERIMENTAL PARTE

1. Materials and methods

The interest of our scientific research is the detection of the toxicity and hepatotoxic effects of a neuroleptic " Chlorpromazine " in the liver of wistar rats. The experiments carried out in this study were accomplished in the animal's house then in the laboratory of toxicology Department of Applied Biology. University of -LARBI TEBESSI -Tebessa

1.1- Materials

1.1.1-Chemical material

We used in our experimentation a drug " Chlorpromazine " 100mg of the phenothyazine family coming from an authorized pharmacy from a neurologist doctor

1.1.2- Animal material

(biological model) The biological model used in this experiment is the male rats of the *rattus wistar* strain in the number of 18 coming from the Pasteur Institute of Algiers, aged between 06 to 08 weeks are adults. They weigh approximately (120g_250g), they are small mammals most frequently used in scientific experimental researches .

1.2- Methods (experimental protocol)

1.2.1- Animal maintenance

We distributed rats into three groups and putted them in "03" batches, each batch contains 6 rats. They underwent an adaptation period of 15 days in the animal's house of the Biology Department, Faculty of Nature and Life Sciences, University of Tebessa. The ambient temperature was 22±2°C and the natural photoperiod 12/12h, the rats was placed in cages which are lined with weight chips, the cages was cleaned every two days at the end of treatment, the rodents were fed with a concentrate of croquettes which constitute (corn-Tx, soy-P, S, F-lime-salt-phosphate-CMV VL).

1.2.2- Choice of the doses

We used a molecule (drug).The dose of this drug "Chlorpromazine " was 50 mg /kg we chose this dose from the previous studies **(satoshi et *al*.,2014)**. This is the high dose that can affect the liver of rat after .

The distribution and treatment of experimental rodents are illustrated in the following table:

Table06: the method of distribution and treatment dose for 03 days and 45 days

Batch 01 (control)	Batche 02 (CZP .Chronic)	Batche 03 (CZP .Acute)
Gavaged with water 0.5ml/day for 45 days	treated with (CPZ) 50mg/kg for 45 days	treated with (CPZ) 50mg/kg for 3 days

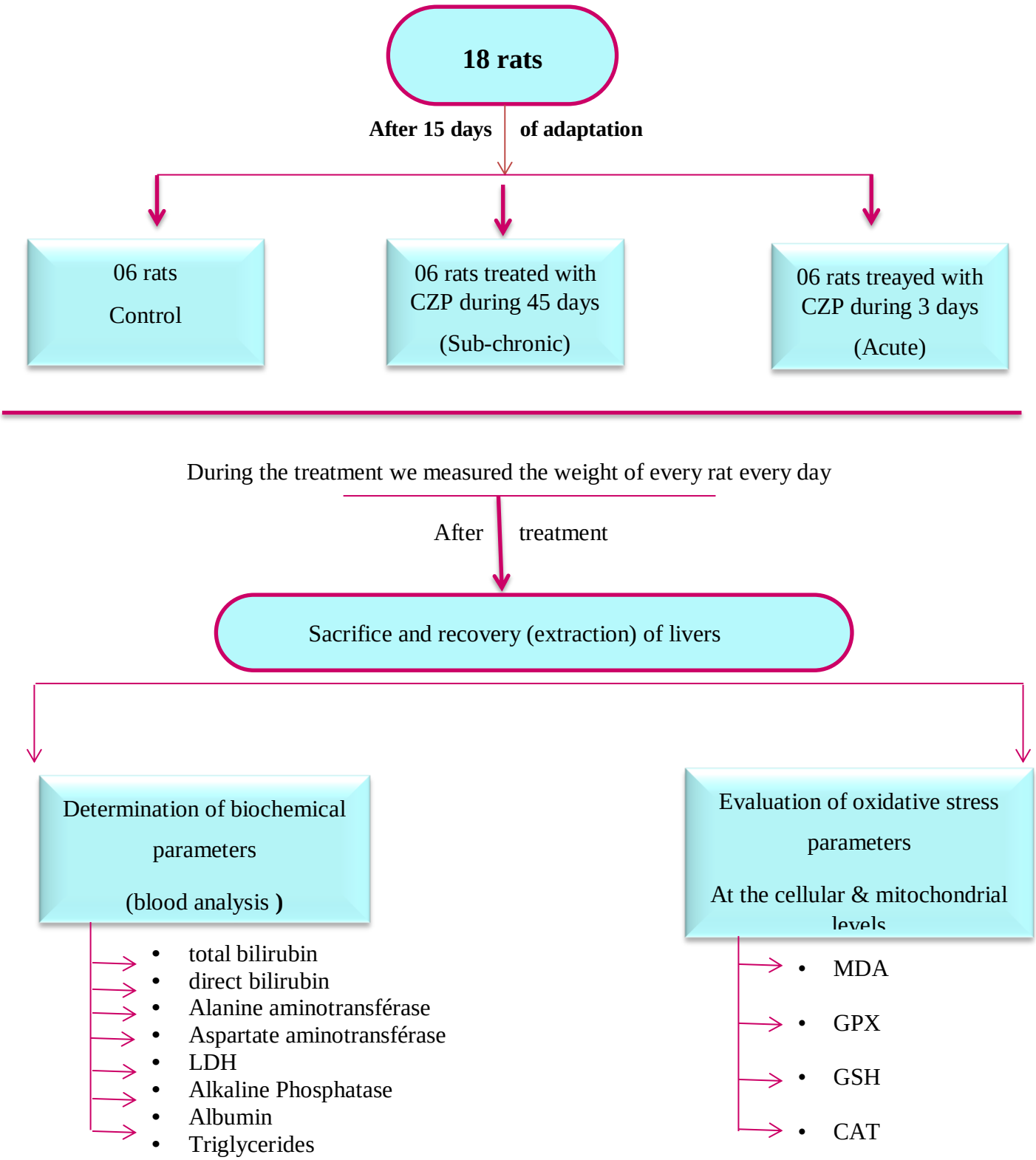


Fig.08: summary diagram of the exerimental protocol

1.2.3- Evaluation of the hepato toxicity induced by " Chlorpromazine "

After 3 days then 45 days of treatment with neuroleptic “Chlorpromazine” We have done tests to evaluate and detect the toxicity of this drug, the tests are as follows:

- Weight measurement
- Blood analysis (biochemical parameters)
- Tests of oxidative stress parameters

1.2.3.1- Weight measurement

We weighed the rats during the treatment (45days) to evaluate the body changes

1.2.3.2- Determination of biochemical parameters

Our tests were done in the private laboratory of medical analysis "Hannabal” laboratory-Tebessa- . The tests are:

- Total bilirubin
- Direct bilirubin
- Alanine aminotransférase
- Aspartate aminotransférase
- LDH
- Alkaline Phosphatase
- Albumin
- Triglycerides

1.2.3.3- Evaluation of oxidative stress parameters

- **Preparation of the homogenates**

300 mg rat liver from different study groups was used. After tissue homogenization in TBS (Tris 50 mM, NaCl 150m M,pH 7.4); then centrifugation of the cell suspension (9000 holes/min, 4°C during 15 min), then the obtained supernatant is aliquoted in eppendorfs tubes then preserved in-20°C until the oxidative stress parameters are determined.

- **Preparation of mitochondrial suspensions**

Mitochondria are extracted using the method described by **(Rustin et *al.*, 1994).** is a purification by differential centrifugation. Briefly, after decapitation of the rats, livers are quickly removed and immersed in TSE buffer (10mM tris, 250mM sucrose, 0.1mM EDTA, pH 7.2 to 4C°). in 3.5ml of TSE, allowing the destruction of cells and the release of mitochondria. The recovered homogenate is centrifuged at 10000t/min for 10min allowing removal of large cellular debris. The recovered pellet is centrifuged a second times at 10000t/min for 10min. The supernatants from both centrifugations are recovered and centrifuged at 14000t/min at 4C° for 10min. The resulting pellet is resuspendu in 1ml of the TSE and centrifuged at 14000t/min for 10min. The base from the latter centrifugation is resuspendu in 1ml of buffer TS (250mM sucrose, 50mM tris, pH 7.2 to 20 C°) and centrifuged for 10min at 14000t/min. The final base consists of the mitochondria, and is recovered in 500µl TS buffer to obtain fresh mitochondrial suspension of which a fraction will be used directly in the ass24essment of structural integrity and functional mitochondria, the rest is kept at -20°C for further assays.

- **Preparation of metabolite's homogenate**

The exctractive and metabolite assays were carried out using the process of **(Shibko et *al.*,1996),** the control and treated echontillons are ground with a crusher in trichloacetic acid (TCA) at 20%(1ml of culture in 1ml of TCA) after a first centrifugation (5000 rpm for 10 min) the first obtained supernatant is discarded and 1 ml of the ether/chloroform mixture (1v/1v) is added to the obtebu base and after a second centrifugation (5000 rpm for 10 min) the supernatant 2 and base 2 are obtained; Supernatant 2 will be used for lipid determination and the base dissolved in NaOH (0.1 N) will be used for protein determination

- **Determination of glutathione (GSH)**

Glutathione assay is performed using the (**Weekbeker et Cory, 1988)** method. principle of this assay is based on the measurement of the optical absorbance of 2-nitro-5-acid mercapturic acid resulting from the reduction of 5,5 dithio-bis-2-nitrobenzoic acid (DTNB) by glutathione (-SH) groups. For this a deproteinization is carried out to keep only the glutathione-specific (-SH) groups. The experimental procedure for glutathione determination is as follows:

- Take 0.8 ml of the homogenate.
- 0.2 ml of the salicylic acid solution (0.25%).
- Shake and leave for 15 minutes in an ice bath.
- Centrifuge at 1000 rpm for 5 min.
- Take 0.5 ml of the supernatant.
- Add 1 ml of Tris buffer, pH 9.6.
- Mix and add 0.025 ml of 5,5 dithio-bis-2-nitrobenzoic acid (DTNB)
- Leave for 5 min at room temperature and read optical densities at 412 nm

- **Malondialdehyde (MDA) determination**

MDA can be detected by a colorimetric reaction to thiobarbituric acid (TBA). Detection of MDA from degradation of 3- or 4-polyunsaturated fatty acids double peroxidized bonds, constitutes a very sensitive method for determining a lipoperoxidation in vitro. MDA is determined using the (**Esterbauer et *al.*,1992)** method. The principle of this assay is based on the condensation of MDA in acid medium and hot with thiobarbituric acid, to form a pigment (pink). This chromogen can therefore be measured by absorption spectrophotometry at 530 nm The experimental procedure for dosing is as follows:

- Take 375 µl of the homogenate (supernatant).
- Add 150 µl of the TBS buffer solution (Tris 50 mM, NaCl 150 mM pH 7.4).
- Add 375 µl of TCA-BHT solution (TCA 20%, BHT 1%)
- Vortexer and centrifuge at 1000 rpm for 10 min.
- Take 400 µl from the supernatant.
- Add 80 µl of HCl 0.6 M.
- Add 320 µl of the Tris-TBA solution (Tris 26 mM, TBA 120mM).
- Mix and incubate in a double boiler at 80°C for 10 minutes.

The optical density was recorded at λ = 530 nm. The absorbance is directly proportional to the amount of MDA formed, thus giving an accurate assessment of peroxidized lipids.

- **Determination of the enzyme activity of glutathione peroxidase (GPx)**

The enzyme activity of glutathione peroxidase (GPx) was measured by the **Flohe and Gunzler (1984)**. This method is based on peroxide reduction hydrogen (H_2O_2) in the presence of reduced glutathione (GSH), the latter is transformed into (GSSG) under the influence of GPx according to the following reaction: $H_2O_2 + 2GSH \longrightarrow GSSG + 2H_2O$

For that, we have preceded the following steps:

- Take 0.2 ml of the homogenate (supernatant).
- Add 0.4 ml GSH (0.1 mM).
- Add 0.2 ml of the TBS buffer solution (Tris 50 mM, NaCl 150 mM pH 7.4).
- Incubate in a double boiler at 25°C for 5 min.
- Add 0.2ml of H_2O_2 (1.3 mM) to initiate the reaction, leave on for 10 minutes.
- Add 1 ml TCA (1%) to stop the reaction.
- Put the mixture in the ice for 30 minutes.
- Centrifuge for 10 minutes at 3000 rpm.
- Take 0.48 ml of the supernatant.
- Add 2.2 ml of the TBS buffer solution.
- Add 0.32 ml DTNB (1.0 mM)
- Mix and after 5 minutes read the optical densities at 412 nm.

- **Dtermination of Catalase activity (CAT)**

The spectrophotometric determination of catalase activity (CAT) is performed using the **Cakmak and Horst (1991)** method. The absorbance decay is recorded for three minutes by a spectrophotometer with a wavelength of 240nm and a molar linear extinction coefficient ε=39400 µM-1. cm-1.L for a final volume of 3ml, the reaction mixture contains:

- 100µl of the raw enzymatic extract,
- 50 µl hydrogen peroxide H_2O_2 at 0.3%
- 2850µl phosphate buffer (50mM, pH 7.2).

The calibration of the apparatus is done in the absence of the enzyme extract. The reaction is triggered by the addition of hydrogen peroxide.

- **Evaluation of metabolites**
- **Lipid determination**

Tissue lipids are evaluated by the method **(Goldsworthy et *al.*, 1972),** 200µl of homogenate is used in 5ml of 20% trichloroacetic acid (TCA), crushed and filtered this mixture; and directly applied a centrifugation at 5000t/min for 10min. The base is kept in a tube containing 1ml of the Ether/Chlorophorme mixture, and after centrifuging this mixture at 5000t/min for 10min, 100µl of the supernatant is taken, to which 1ml of sulphuric acid is added and after stirring the tubes in a 100°C water bath for 10min. After cooling, we take again by means of a 200µl micropipette of the extract to which we add 2.5ml of the 85% sulphophosphovanillinic mixture (0.38g vanillin+195ml orthphosphoric acid+55ml H2O) and leave this mixture 30min in the darkness, reading at a wavelength of 530nm

- **Protein Determination**

The method used for the determination of proteins is that of Bradford (1976), which uses BSA as a standard; on the same sample used for the determination of lipids, the base is recovered from the second centrifugation to which 1 ml NaOH (0.1N) has been added. and agitate energetically for the dissolution of proteins. Then, using a micropipette, a volume of 100µl is taken and 4 ml of the BBC (Bleu Brillant de Coumassie) reagent is added (50 mg BBC +50 ml of orthophosphoric acid at 85% and 500 ml is completed with distilled water). Thus a blue color develops and we pass directly the samples for reading at a wavelength 595nm

- **Statistical analysis**

The results obtained were expressed by the average of six replicates (mean standard deviation), and to better visualize using the Excel 2013 office to represent these results in the form of graphs and histograms. The statistical analysis was performed using the prism graph software. The significance of the difference between the control batche and the treated batches is verified using the ANOVA 1 test and the comparison result as follows:

- $p > 0.05$ = the difference is not significant
- (*) $0.05 > P > 0.01$ = the difference is significant
- (**) $0.01 > P > 0.001$ = the difference is highly significant

- (***) P < 0.001 = the difference is very highly significant.

RESULTS

2.1- Effect of CZP on weight gain

The weight gain results are shown in **Fig.10** The statistical study reveals a non-significant reduction in the weight of rats treated with CZP for 45 days and CZPa for 72 hours compared to controls

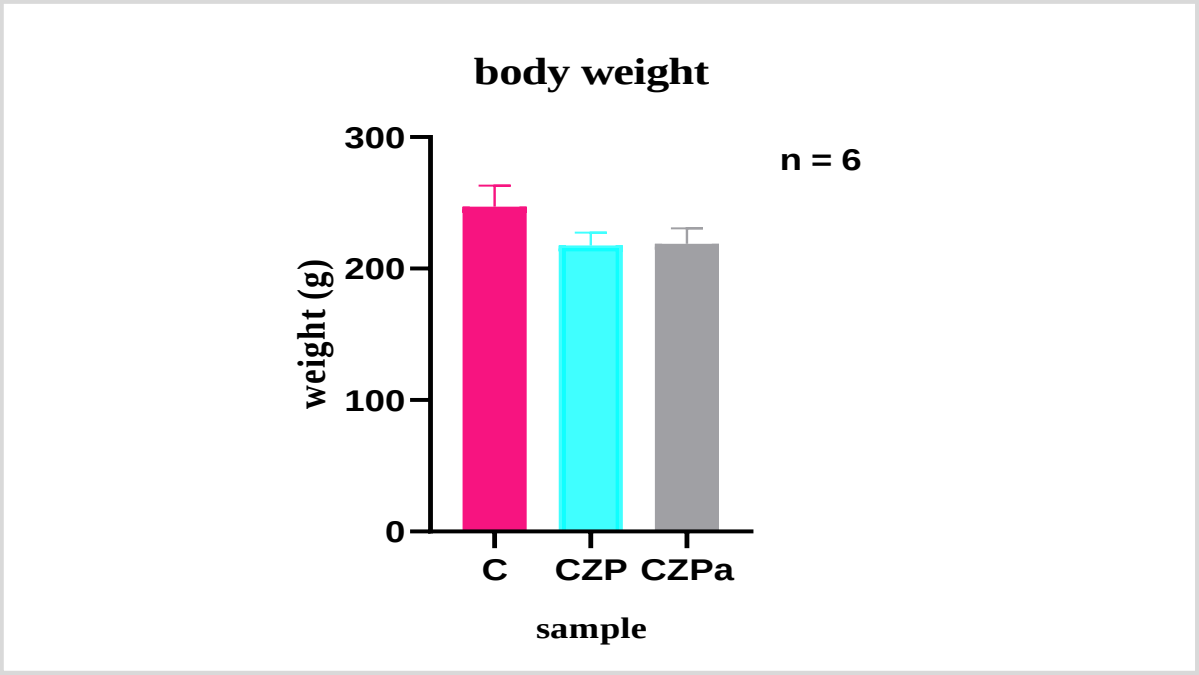


Figure10: Effect of CPZ on weight gain of treated rats after 45 days and 72 hours. Values are expressed as an average ± for all batches of rats (n=6): non-significant difference compared to controls ($p \geq 0.05$)

Table 07: changes in weight gain of rats treated for 45 days and 72 hours by CZP

Settings		Group of treated rats	
	C	CPZ	CPZa
Body weights	247.2 ± 12.36	217.5 ±12.79	218.8 ±12.87

2.2- Effect of CZP on Biochemical parameters (blood analysis)

2.2.1- Effect of CZP on ASAT /TGO

The ASAT results are shown in **Figure 11** The statistical study reveals a significant decreasing in the TGO of rats treated with CZP for 45 days and reveals a significant increasing for the rats treated with CZPa for 72h.

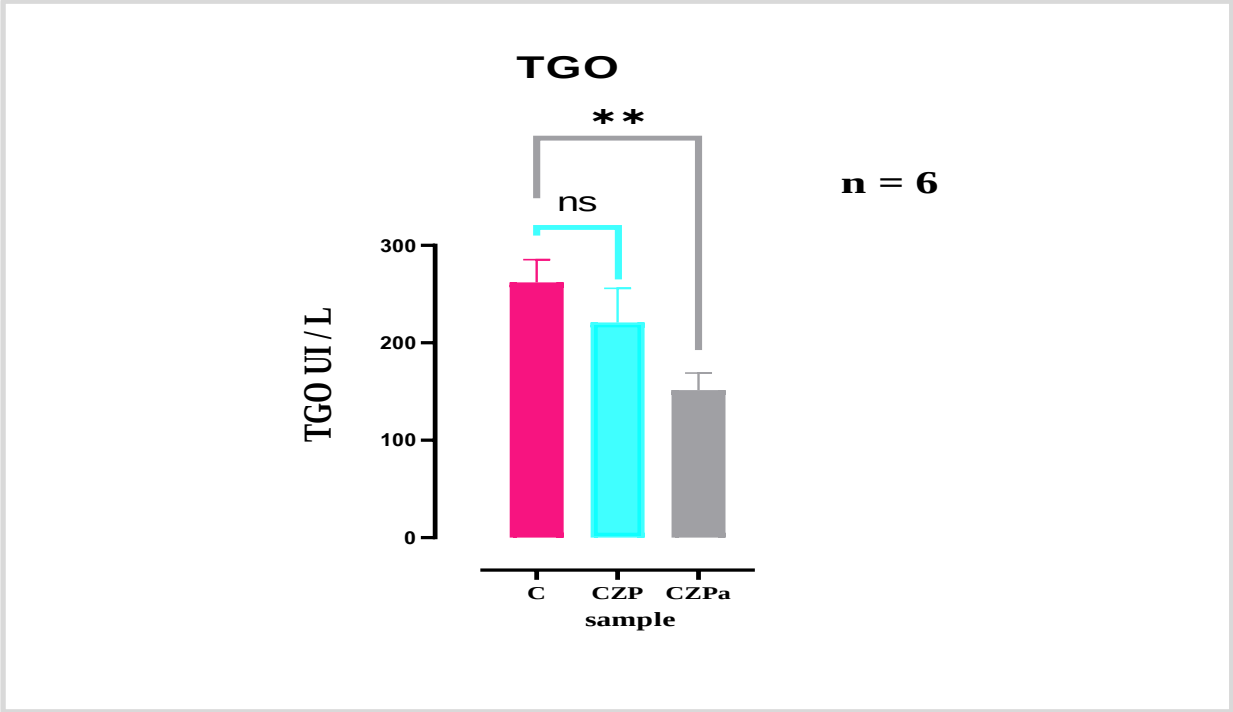


Fig.11: effect of CPZ on TGP in treated rats after 45 days and 72 hours. Values are expressed as an average ± for all batches of rats (n=6): (*) 0.05 > P > 0.01 = the difference is significant,

Table 08: changes in TGP levels in the liver of rats treated for 45 days and 72 hours by CZP

Settings		Group of treated rats	
	C	CPZ	CPZa
TGP/ASAT	107.6 ±23.46	62.57±18.63	151.7±11.50

2.2.2- Effect of CZP on ALAT

The ALAT results are shown in **Figure 12** ; The statistical study reveals a non-significant decreasing in the TGO of rats treated with CZP for 45 days and reveals a highly significant decreasing for the rats treated with CZPa for 72h.

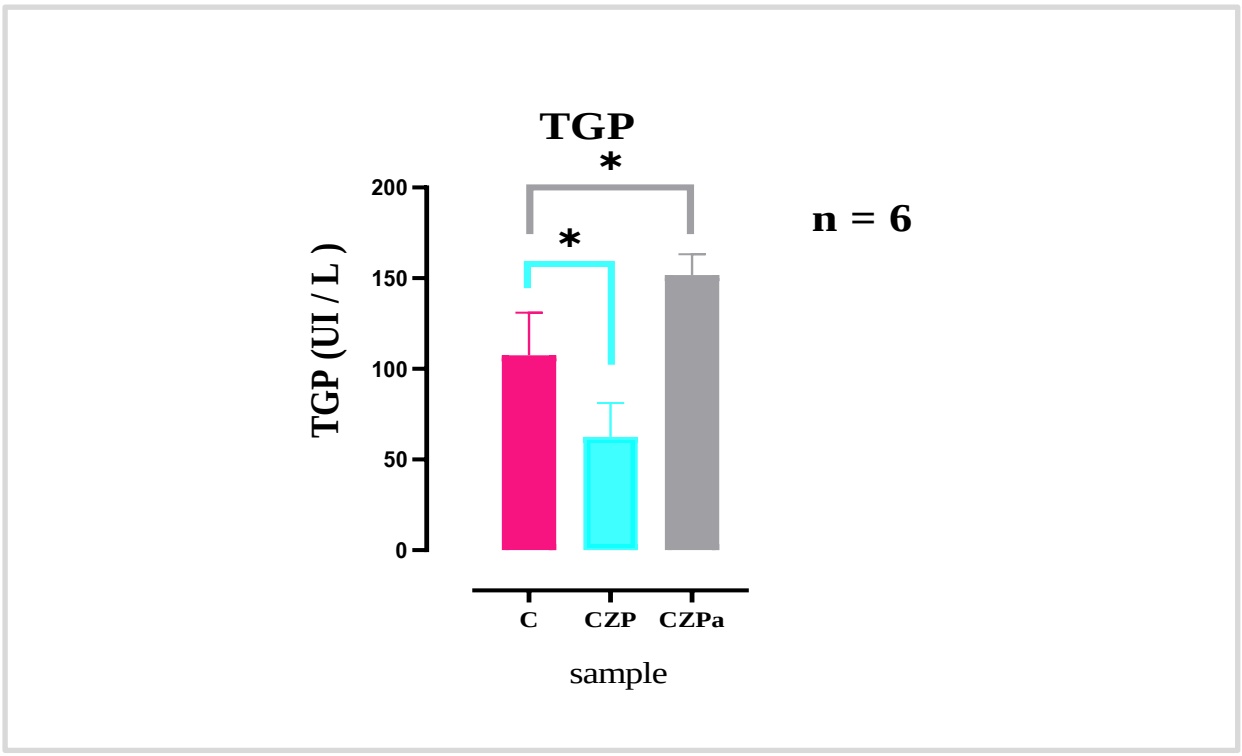


Fig.12: effect of CPZ on TGO in treated rats after 45 days and 72 hours. Values are expressed as an average ± for all batches of rats (n=6): non-significant difference compared to controls ($p \geq 0.05$), (**) $0.01 > P > 0.001$ = the difference is highly significant

Table 09: changes in TGO levels in the liver of rats treated for 45 days and 72 hours by CZP

Settings		Group of treated rats	
	C	CPZ	CPZa
TGO/ALAT	262.2±23.14	221.1±34.82	151.7 ±17.39

2.2.3- Effect of CZP on Totale bilirubine

The Total bilirubin results are shown in **Figure13** The statistical study reveals a not significant increasing in the total bilirubin of rats treated with CZP for 45 days and reveals a highly significant increasing for the rats treated with CZPa for 72h

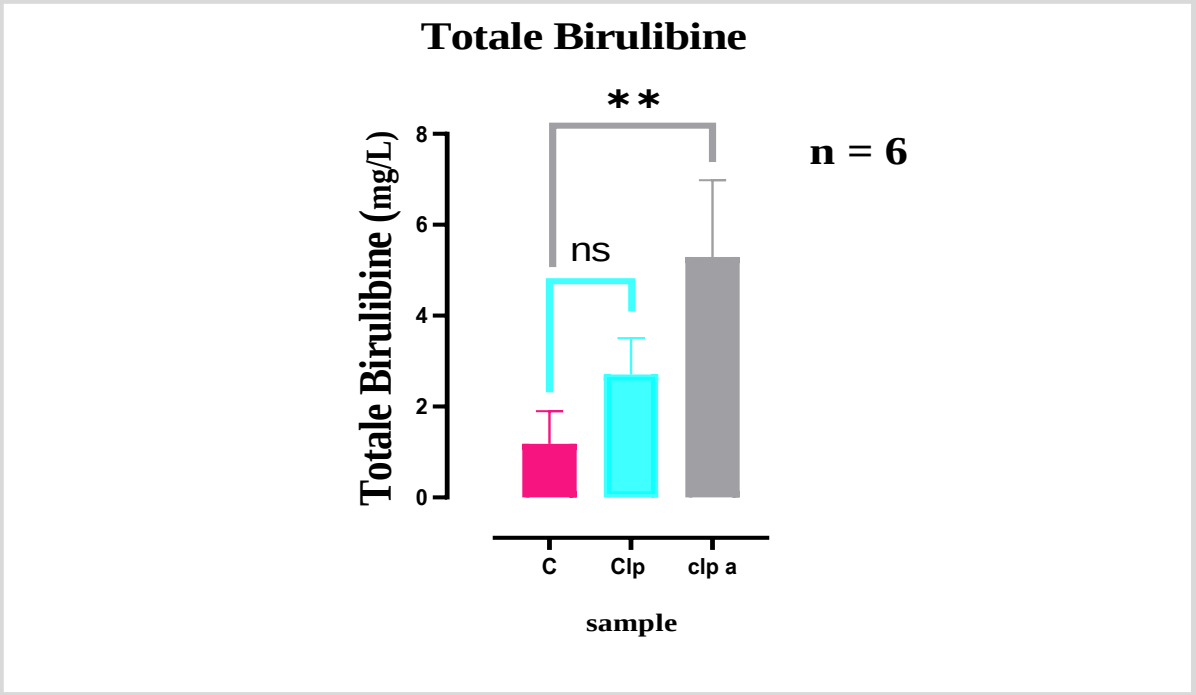


Fig13: effect of CPZ on Total bilirubin in treated rats after 45 days and 72 hours. Values are expressed as an average ± for all batches of rats (n=6): p > 0.05 = the difference is not significant, (**) 0.01 > P > 0.001 = the difference is highly significant

Table10: change in Total Billirubin levels in the liver of rats treated for 45 days and 72 hours by CZP

Settings	Group of treated rats		
	C	CPZ	CPZa
Total bilirubin	1.180±0.7202	2.710±0.7957	5.290±1.695

2.2.4- Effect of CZP on Direct bilirubine

The Direct bilirubin results are shown in **Figure 14**; The statistical study reveals a not significant increasing in the total bilirubin of rats treated with CZP for 45 days and reveals a highly significant increasing for the rats treated with CZPa for 72h

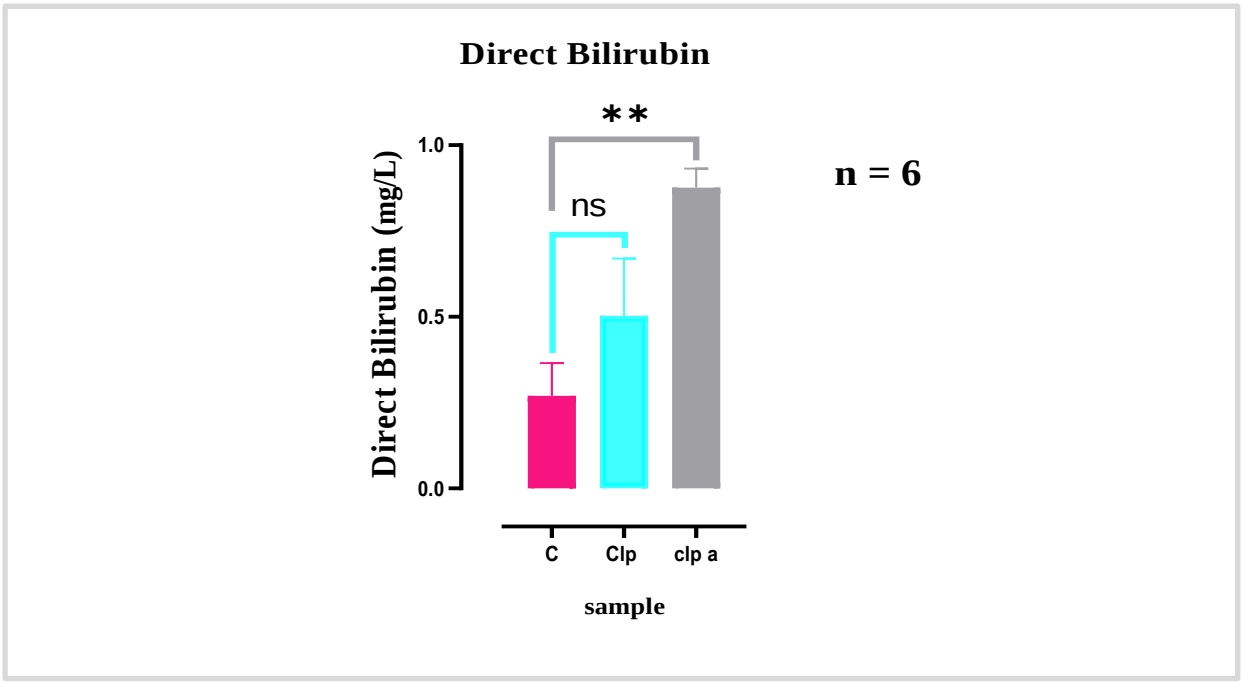


Fig. 14: effect of CPZ on Direct bilirubin in treated rats after 45 days and 72 hours. Values are expressed as an average ± for all batches of rats (n=6): p > 0.05 = the difference is not significant, (**) 0.01 > P > 0.001 = the difference is highly significant

Table 11: change in Direct Billirubin levels in the liver of rats treated for 45 days and 72 hours by CZP

Settings	Group of treated rats		
	C	CPZ	CPZa
Direct bilirubin	0.2700±0.0953	0.5033±0.1665	0.8767±0.0550

2.2.5- Effect of CZP on Triglycerids

The triglycerids results are shown in **Figure 15** The statistical study reveals a significant increasing in the triglycerides of rats treated with CZP for 45 days and reveals a not significant increasing for the rats treated with CZPa for72h

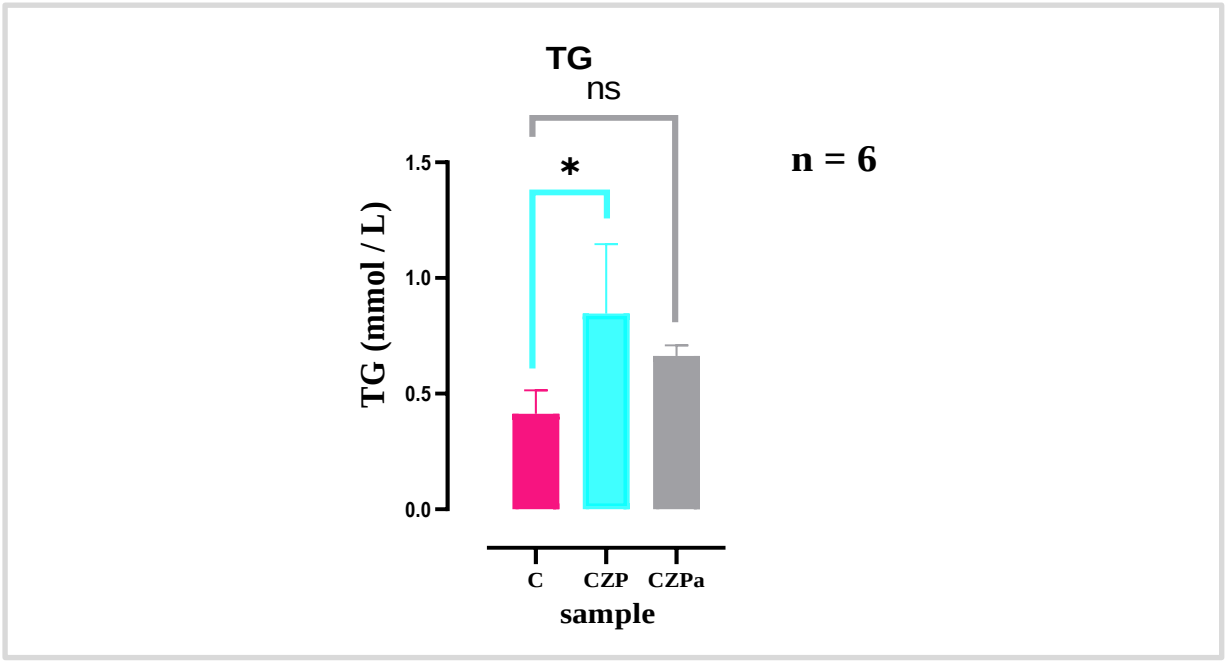


Fig.15: effect of CPZ on triglycerids in treated rats after 45 days and 72 hours. Values are expressed as an average ± for all batches of rats (n=6): non-significant difference compared to controls (p≥0.05), (*) 0.05 > P > 0.01 = the difference is significant

Table 12: change in TG levels in the liver of rats treated for 45 days and 72 hours by CZP

settings	Group of treated rats		
	C	CPZ	CPZa
Triglycerids	0.4133±0.1007	0.8467±0.3001	0.6633±0.04509

2.2.6- Effect of CZP on Alkaline Phosphatase

The Alkalin phosphates results are shown in **Figure16** The statistical study reveals a highly significant increasing in the Alkalin phosphates of rats treated with CZP for 45 days and reveals a very highly significant increasing for the rats treated with CZPa for 72h

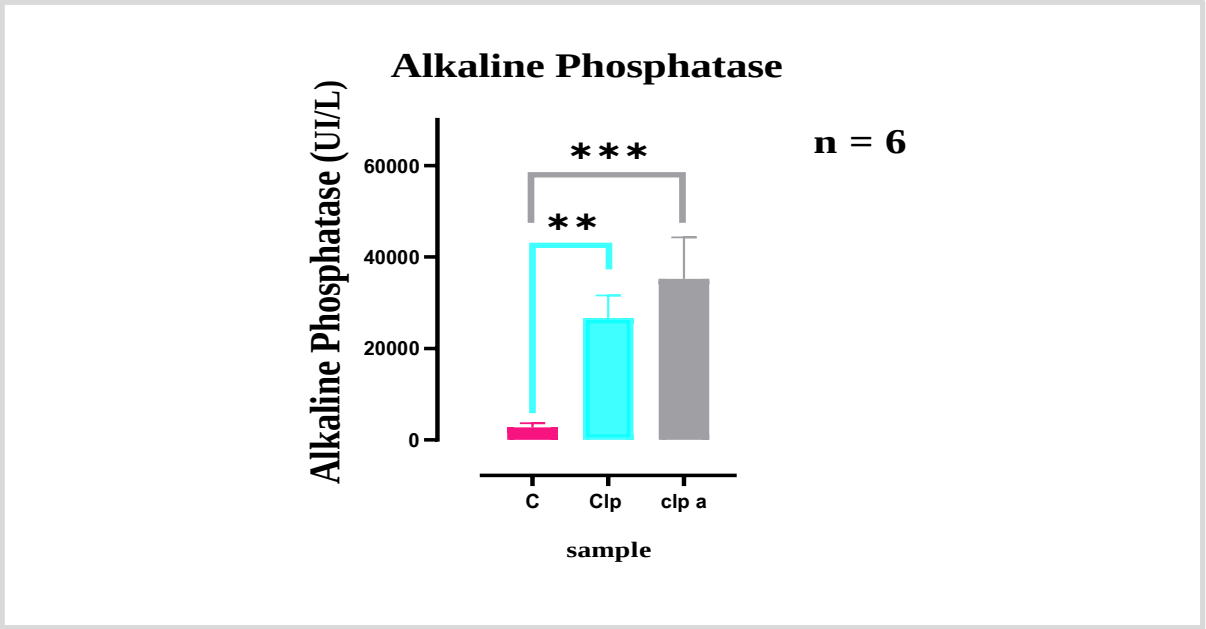


Fig.16: effect of CPZ on Alkalin phosphates in treated rats after 45 days and 72 hours. Values are expressed as an average ± for all batches of rats (n=6): - (**) 0.01> P > 0.001 = the difference is highly significant, (***) P < 0.001 = the difference is very highly significant.

Table 13: changeS in Alkalin phosphates levels in the liver of in rats treated for 45 days and 72 hours by CZP

Settings	Group of treated rats		
	C	CPZ	CPZa
Alkalin phosphates	209.07±28.02	26667±4908	35243±9075

2.2.7- Effect of CZP on LDH

The LDH results are shown in **Figure 17** .The statistical study reveals a not significant increasing in the HDL of rats treated with CZP for 45 days and 72h

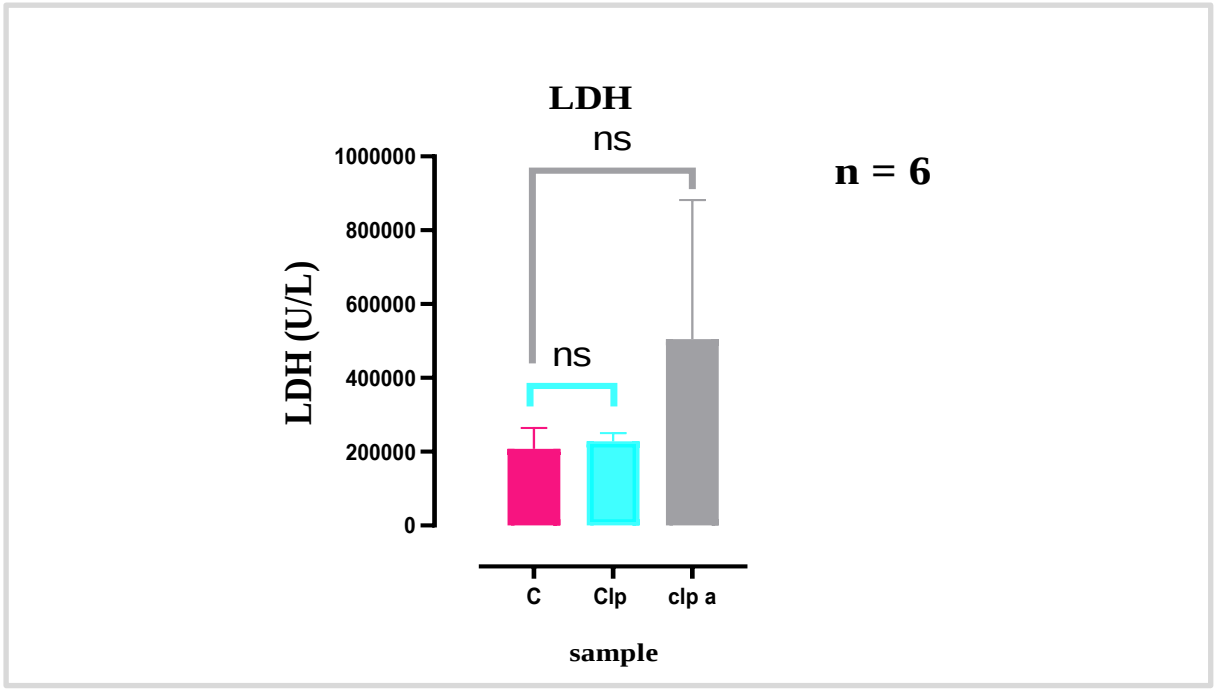


Fig.17: effect of CPZ on LDH in treated rats after 45 days and 72 hours. Values are expressed as an average ± for all batches of rats (n=6): p > 0.05 = the difference is not significant

Table 14: changeS in LDH levels in the liver of rats treated for 45 days and 72 hours by CZP

settings	Group of treated rats		
	C	CPZ	CPZa
HDL	207900±56800	227800±22851	504657±377350

2.2.8-Effect of CZP on Albumin

The Albumin results are shown in **Figure 18** The statistical study reveals a not significant decreasing in the Albumin of rats treated with CZP for 45 days and reveals a very highly significant increasing for the rats treated with CZPa for 72h

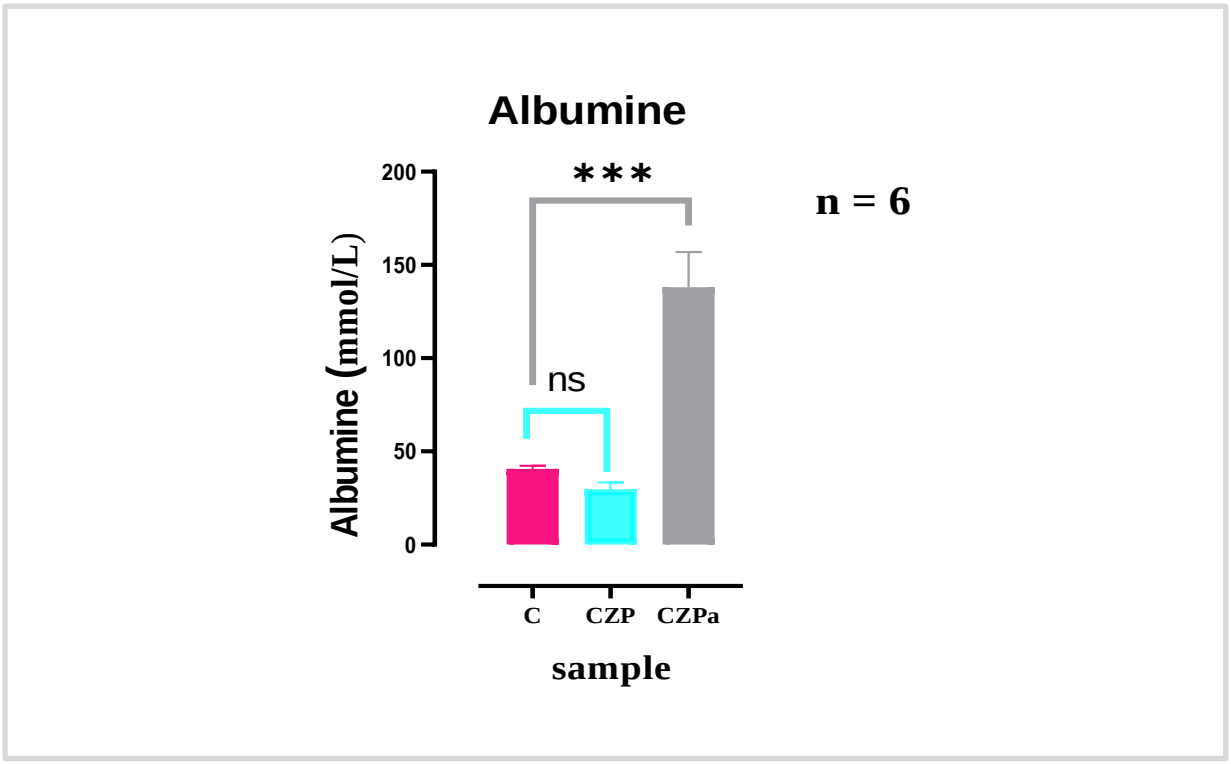


Fig.18: Figure: effect of CPZ on Albumin in treated rats after 45 days and 72 hours. Values are expressed as an average ± for all batches of rats (n=6): non-significant difference compared to controls (p≥0.05), (***) P < 0.001 = the difference is very highly significant.

Table15: changes in Albumin levels in the liver of rats treated for 45 days and 72 hours by CZP

settings	Group of treated rats		
	C	CPZ	CPZa
Albumin	40.61±1.663	29.68±3.750	138.0±18.93

2.3- Effect of CZP on metabolites

2.3.1- Effect of CZP on Proteins

- **Proteins**

The proteins results are shown in **Figure 19** .The statistical study reveals a highly not significant increasing in the proteins of rats treated with CZP for 45 days and 72hours

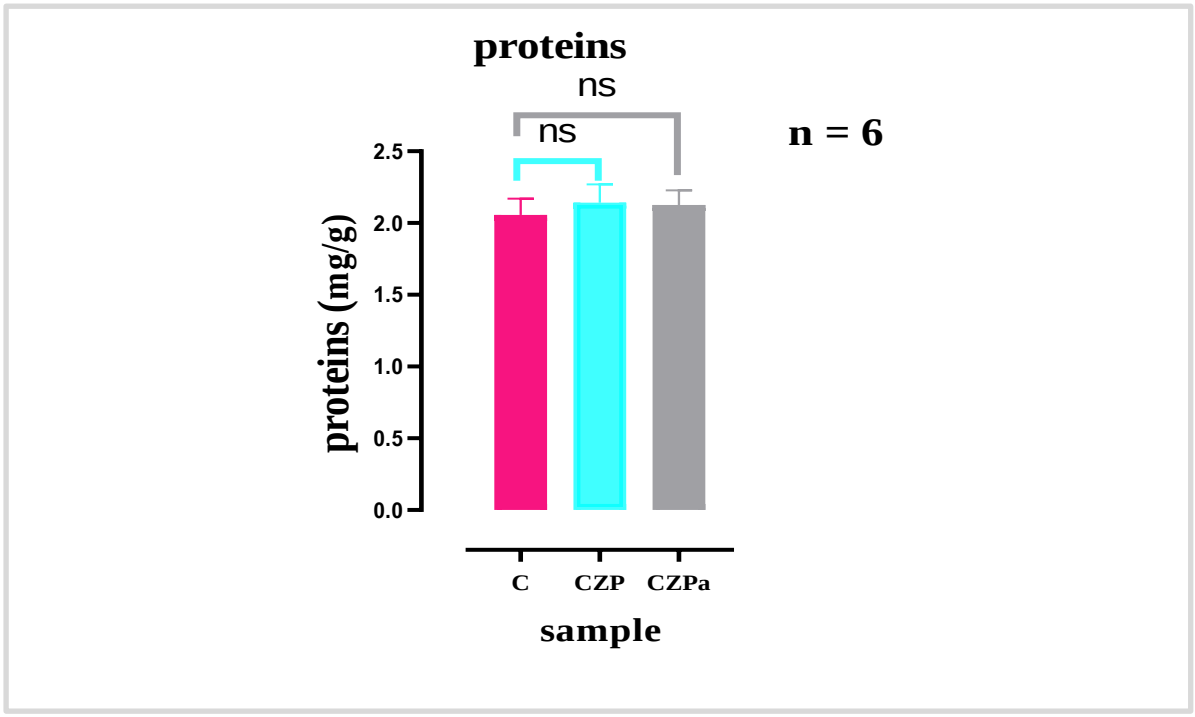


Fig.19: effect of CPZ on proteins in treated rats after 45 days and 72 hours. Values are expressed as an average ± for all batches of rats (n=6): p > 0.05 = the difference is not significant

Table 16 : changes in Proteins levels in the liver of rats treated for 45 days and 72 hours by CZP

settings	Group of treated rats		
	C	CPZ	CPZa
Proteins	2.057±0.1136	2.143±0.1274	2.126±0.1014

2.3.2- Effect of CZP on Lipids

The lipids results are shown in **Figure 20** The statistical study reveals a highly significant increasing in the lipids of rats treated with CZP for 45 days and 72hours

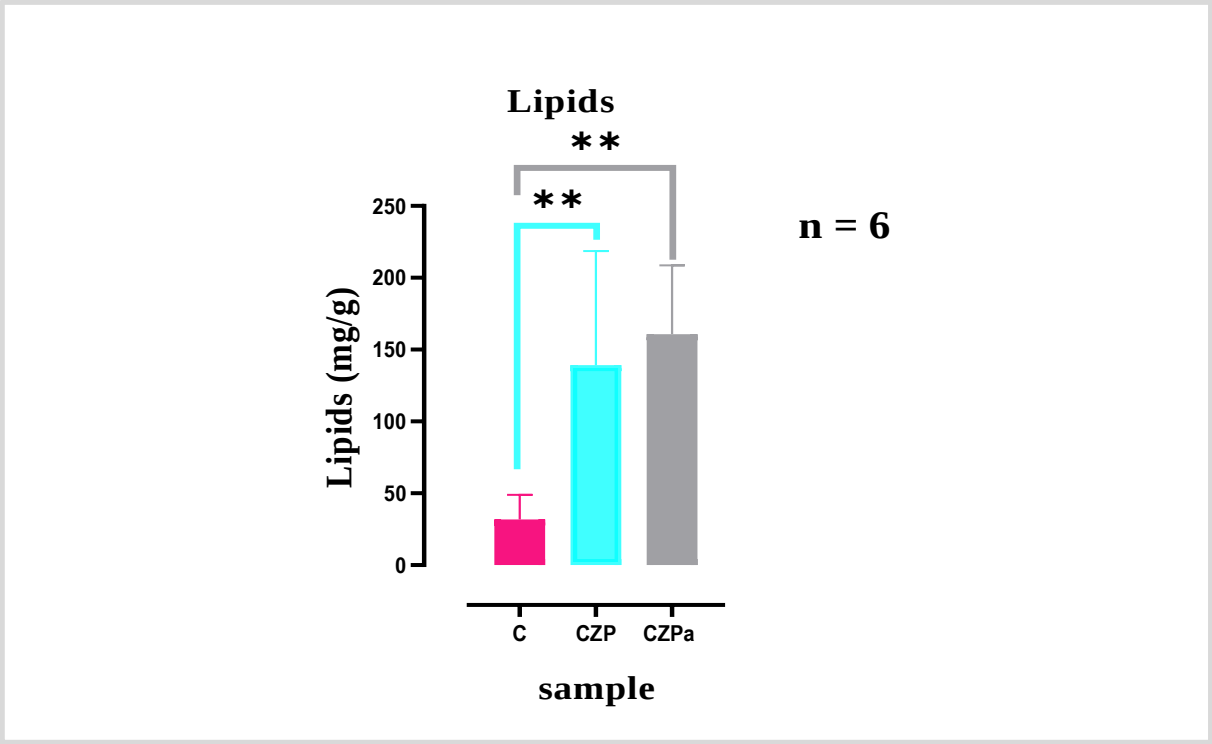


Fig.20: effect of CPZ on lipids in treated rats after 45 days and 72 hours. Values are expressed as an average ± for all batches of rats (n=6): (**) 0.01 > P > 0.001 = the difference is highly significant.

Table 17: changes in lipids levels in the liver of rats treated for 45 days and 72 hours by CZP

settings	Group of treated rats		
	C	CPZ	CPZa
Llipids	31.86±17.17	139.3±79.26	160.7±48.08

2.4- Effect of CZP on oxidative stress activities

2.4.1- Effect of CZP on mitochondrial and cellular MDA

The MDA results are shown in **Figure 21**; The statistical study reveals a significant decreasing in the MDA of rats treated with CZP for 45 days and reveals a very highly significant increasing for the rats treated with CZPa for 72h

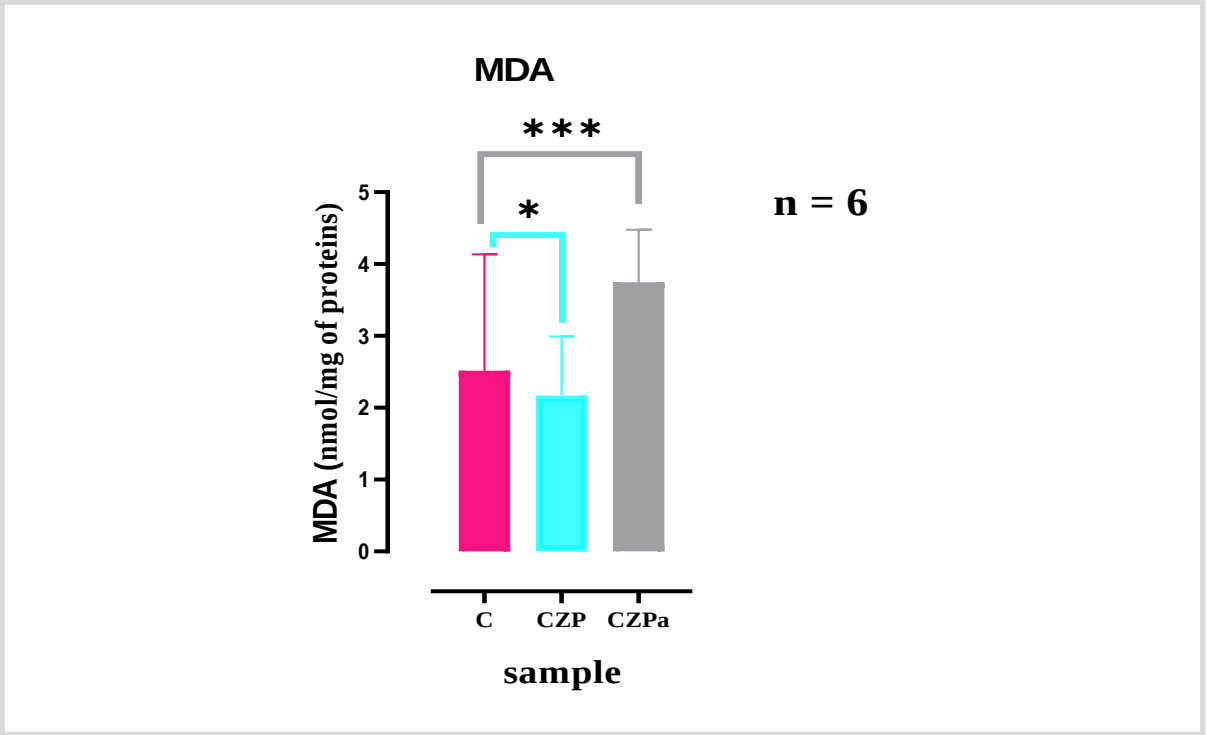


Fig.21: effect of CPZ on MDA in treated rats after 45 days and 72 hours. Values are expressed as an average ± for all batches of rats (n=6): (*) 0.05 > P > 0.01 = the difference is significant, (***) P < 0.001 = the difference is very highly significant.

Table 18: changes in MDA levels in the liver of rats treated for 45 days and 72 hours by CZP

settings	Group of treated rats		
	C	CPZ	CPZa
MDA	1.8400±1.2537	3.383±1.286	5.8533±1.1495

2.4.2. Mitochondrial MDA

The Mitochondrial MDA results are shown in **Figure 22** The statistical study reveals a not significant increasing in the MDA mitochondrial of rats treated with CZP for 45 days and reveals a significant increasing for the rats treated with CZPa for 72h

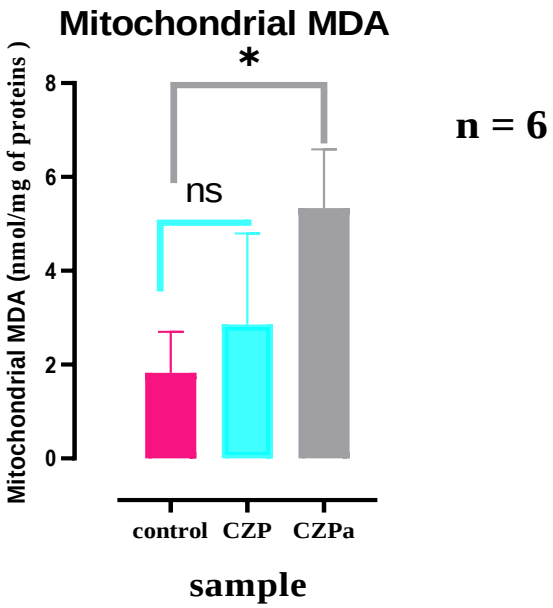


Figure 22: effect of CPZ on mitochondrial MDA in treated rats after 45 days and 72 hours. Values are expressed as an average ± for all batches of rats (n=6): p > 0.05 = the difference is not significant, (*) 0.05 > P > 0.01 = the difference is significant

Table 19: changes in mitochondrial MDA levels in the liver of rats treated for 45 days and 72 hours by CZP

settings	Group of treated rats		
	C	CPZ	CPZa
mitochondrial MDA	2.8500±1.3597	4.4533±3.0269	8.3300±1.9538

2.4.2- Effect of CZP on mitochondrial and cellular GSH

The GSH results are shown in **Figure 23** The statistical study reveals a not significant increasing in the GSH of rats treated with CZP for 45 days and for the rats treated with CZPa for 72h

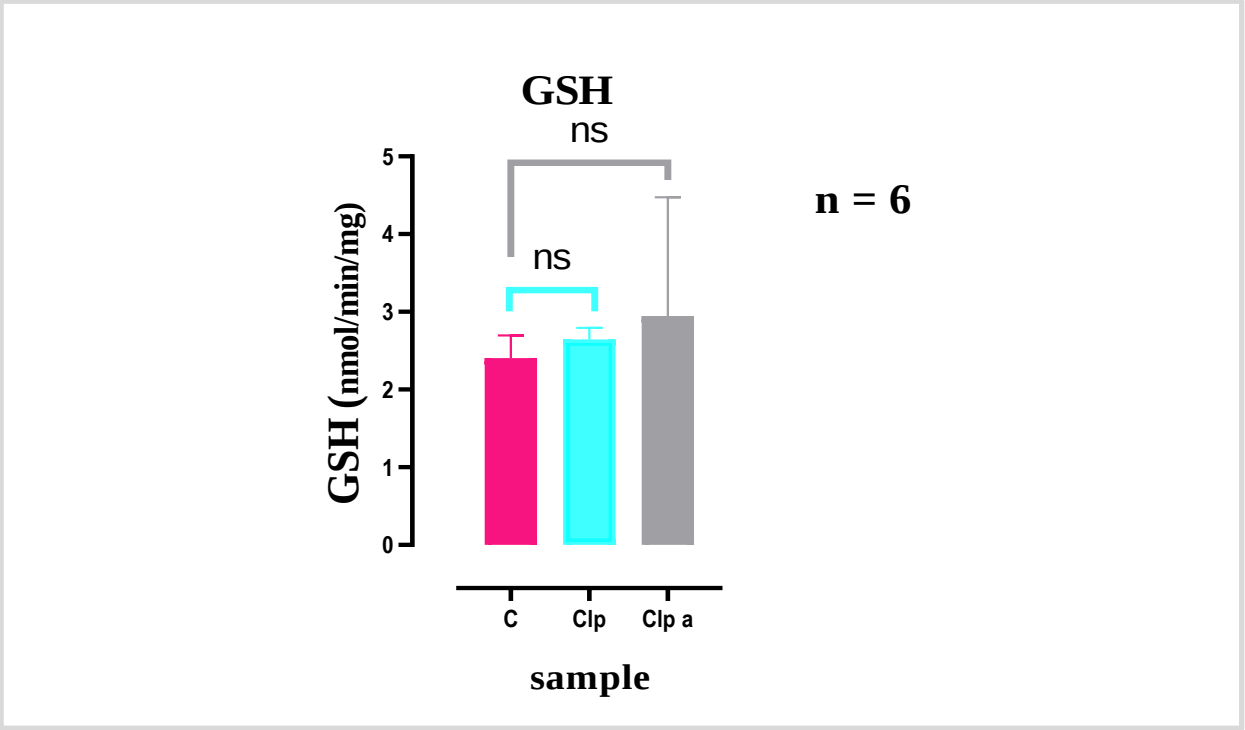


Figure23: effect of CPZ on GSH in treated rats after 45 days and 72 hours. Values are expressed as an average ± for all batches of rats (n=6): p > 0.05 = the difference is not significant

Table 20: change in GSH levels in the liver of rats treated for 45 days and 72 hours by CZP

Settings	Group of treated rats		
	C	CPZ	CPZa
GSH	2.404±0.2924	2.644±0.1512	2.949±1.525

2.4.3. Mitochondrial GSH

The Mitochondrial GSH results are shown in **Figure 24** The statistical study reveals a not significant increasing in the GSH mitochondrial of rats treated with CZP for 45 days and for the rats treated with CZPa for 72h

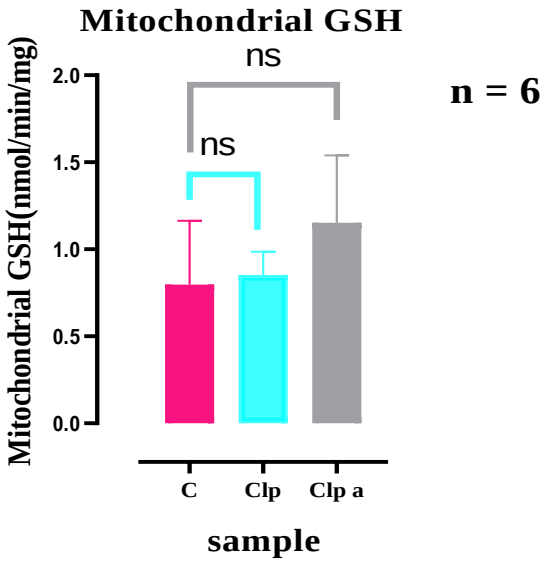


Figure24: effect of CPZ on mitochondrial GSH in treated rats after 45 days and 72 hours. Values are expressed as an average ± for all batches of rats (n=6): p > 0.05 = the difference is not significant

Table 21 : changes in Mitochondrial GSH levels in the liver of rats treated for 45 days and 72 hours by CZP

Settings	Group of treated rats		
	T	CPZ	CPZa
Mitochondrial GSH	0.7965±0.3673	0.8531±0.1334	1.153±0.3869

2.4.3- Effect of CZP on mitochondrial and cellular GPx

The GPx results are shown in Figure 25 ;The statistical study reveals a not significant increasing in the GSH mitochondrial of rats treated with CZP for 45 days and for the rats treated with CZPa for 72h

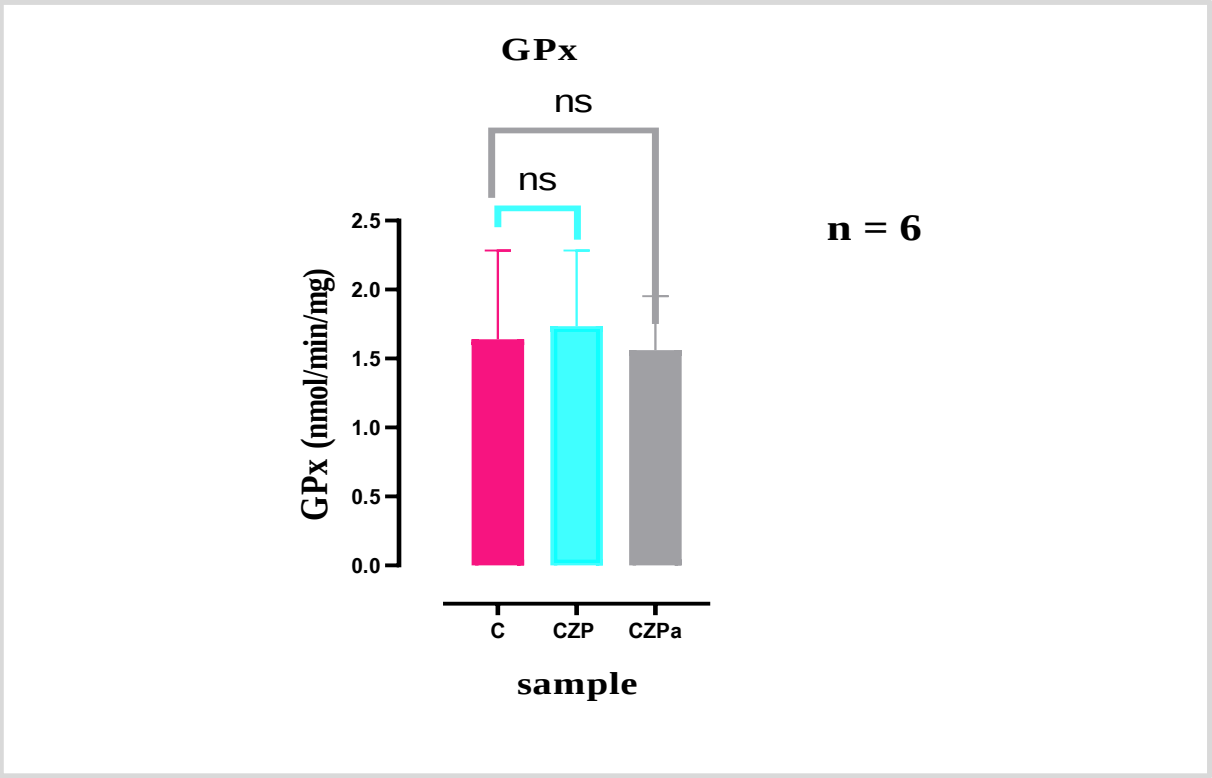


Fig.25: effect of CPZ on GPx in treated rats after 45 days and 72 hours. Values are expressed as an average ± for all batches of rats (n=6): p > 0.05 = the difference is not significant

Table 22: changs in GPx levels in the liver of rats treated for 45 days and 72 hours by CZP

Settings	Group of treated rats		
	C	CPZ	CPZa
GPx	0.69943±0.26353	0.79697±0.25224	0.70972±0.20559

2.4.4. Mitochondrial GPx

The Mitochondrial GPx results are shown in **Figure 26** The statistical study reveals a not significant decreasing in the mitochondria GPx of rats treated with CZP for 45 days and reveals a significant decreasing for the rats treated with CZPa for 72h

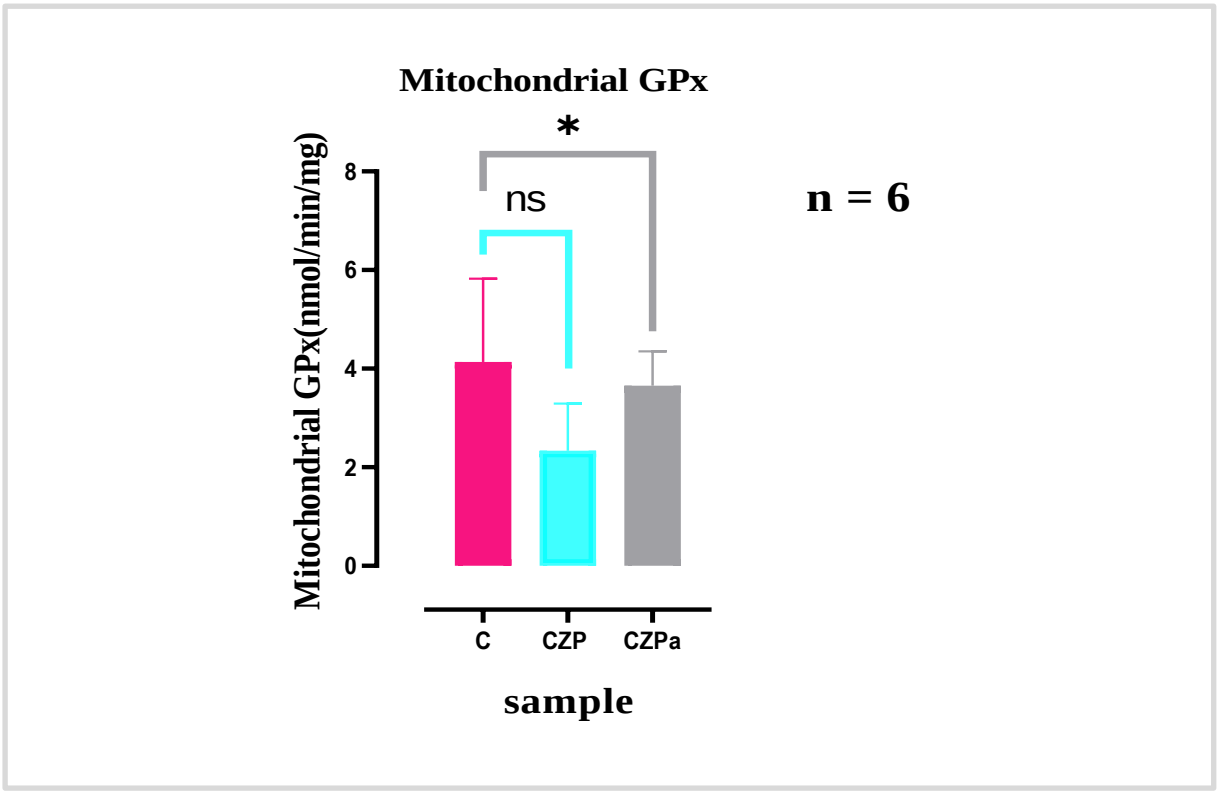


Fig.26: effect of CPZ on Mitochondrial GPx in treated rats after 45 days and 72 hours. Values are expressed as an average ± for all batches of rats (n=6): p > 0.05 = the difference is not significant, (*) 0.05 > P > 0.01 = the difference is significant

Table 23 : changes in Mitochondrial GPx levels in the liver of rats liver treated for 45 days and 72 hours by CZP

Settings	Group of treated rats		
	C	CPZ	CPZa
Mitochondrial GPx	4.134±1.693	2.335±9.569	3.654±0.6970

2.4.4- Effect of CZP on mitochondrial and cellular CAT

The Catalase results are shown in **Figure 27** ; The statistical study reveals a not significant increasing in the catalase of rats treated with CZP for 45 days and for the rats treated with CZPa for 72h

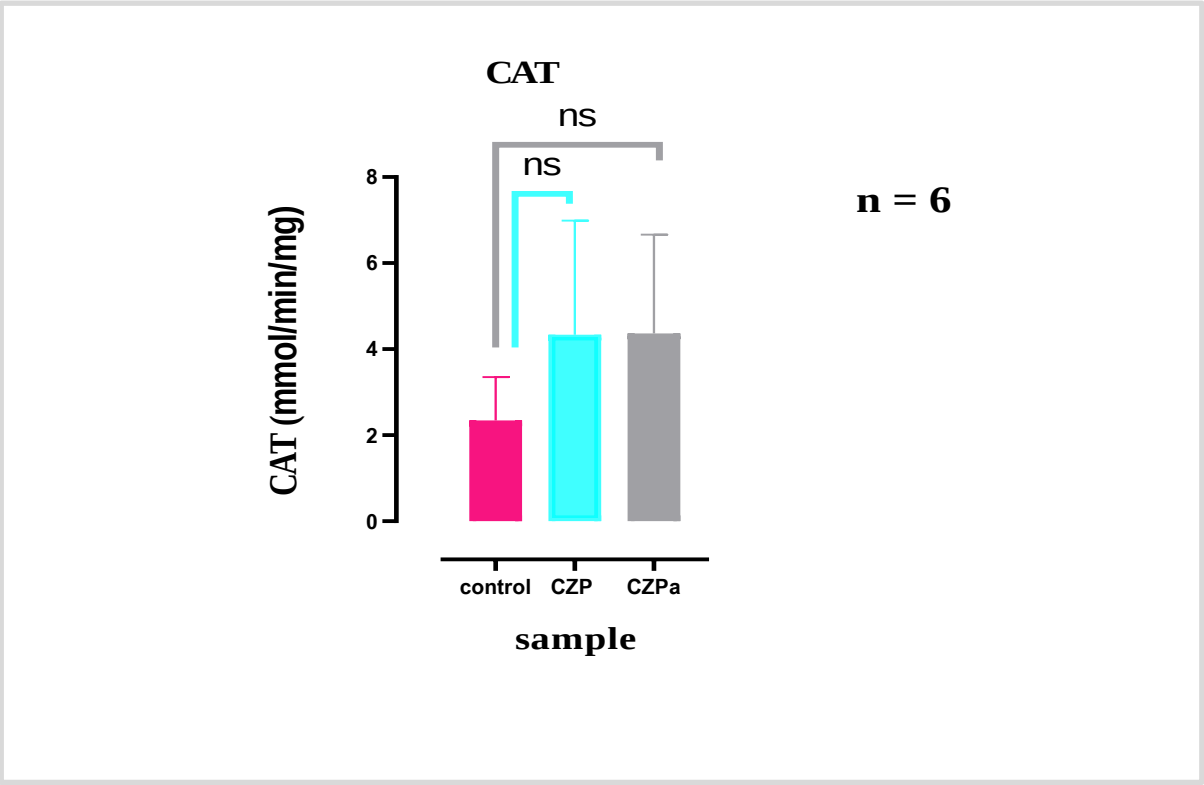


Fig.27: effect of CPZ on Catalase in treated rats after 45 days and 72 hours. Values are expressed as an average ± for all batches of rats (n=6): p > 0.05 = the difference is not significant

Table 24: changes in catalase levels in the liver of rats treated for 45 days and 72 hours by CZP

Settings	Group of treated rats		
	C	CPZ	CPZa
CAT	1.503±1.063	1.884±1.593	3.193±1.455

Mitochondrial CAT

The Mitochondrial Catalase results are shown in **Figure 28:** The statistical study reveals a not significant decreasing in the catalase of rats treated with CZP for 45 days and for the rats treated with CZPa for 72h

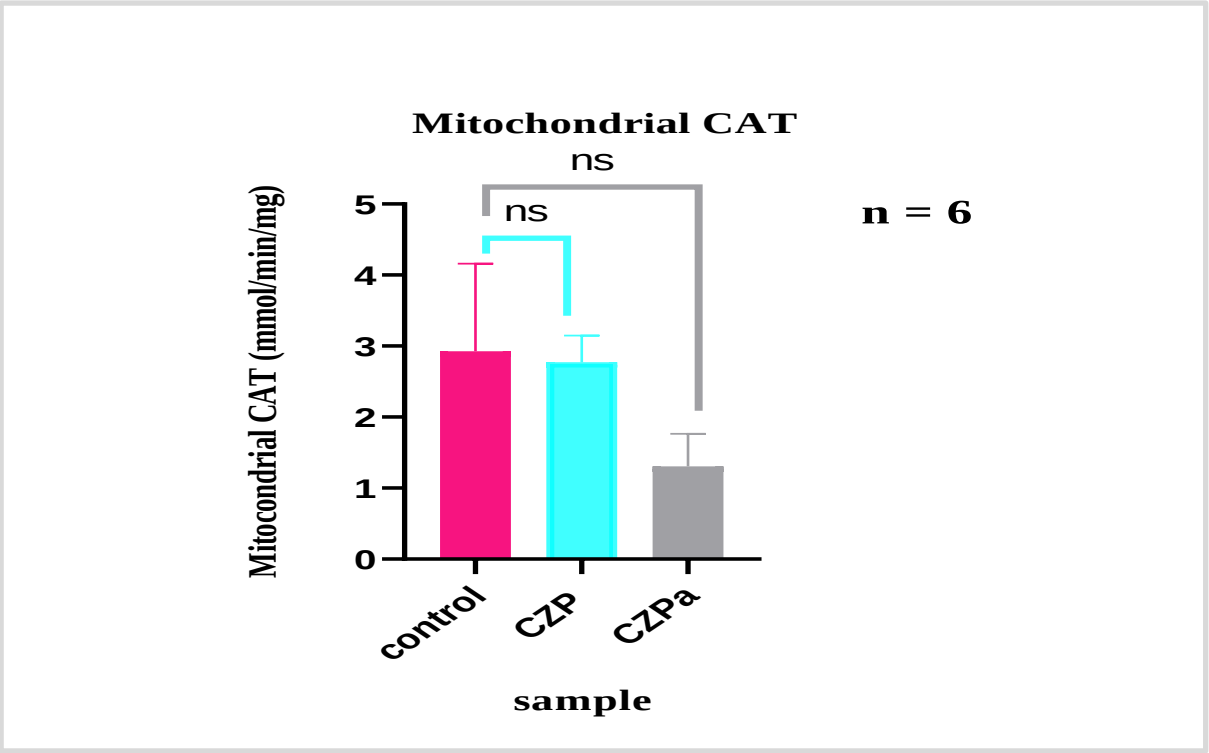


Fig. 28: effect of CPZ on mitochondrial Catalase in treated rats after 45 days and 72 hours. Values are expressed as an average ± for all batches of rats (n=6): p > 0.05 = the difference is not significant

Table 24 : changes in mitochondrial catalase levels in the liver of rats treated for 45 days and 72 hours by CZP

Settings	Group of treated rats		
	C	CPZ	CPZa
Mitochondrial CAT	3.5809±2.1386	2.1953±2.1446	1.0740±1.2457

DISCUSSION

3- Disscussion

Chlorpromazine is one of the phenothiazines widely used in the treatment of psychosis, schizophrenia and bipolar disorders **(Handouei et *al.*, 2019).** the effect with D2 and α1 are associated with side effects such as extrapyramidal side effects (acathesia, late dyskinesia, parkinsonism, acute dystonic reactions) and sympatholytic side effects (hypotension, orthostatic hypotension, reflex tachycardia, dizziness, myosis, sexual dysfunction) **(Varga et *al.*, 2017).**

To evaluate and deepen the liver toxicity in rats exposed to CHLORPROMAZINE (CPZ) at two different times one 72 hours and the other 45 days ; oxidative stress assays , metablolites and some biochemical parameters were performed.

3.1- Effect of CZP on body weight

Weight gain decreases slightly from one week to the next throughout the chlorpromazine treatment period. This decrease is in line with the work of **(Robert et *al.*, 1980)** which is uncertain as to the biological significance of this effect. But generally **(Robert et *al.*,1980)** shows that CPZ causes obesity after a long period of treatment these last results are in agreement with the work of **(Sabina et *al.*,2003 ; Takahiro et *al.*,2006)** which reported antipsychotic drugs induce weight gain and metabolic abnormalities CZP could have more harmful effects on glucose levels and insulin resistance than on other factors, which could be one of the mechanisms responsible for the metabolic syndrome induced by antipsychotic agents.

3.2- Effect of CZP on biochemical parameters and metabolites

The decrease in ASAT and ALAT in rats treated with CZP for 45d, this result is consistent with the work of **(laurence et *al.*, 2002)** it is an indication of the typical stress response in rats. they are tested for this hypothesis by the non-significant increase of LDH and highly significant alkaline phosphatase indicating hepatic tissue lesions, but the increase of TGP for rats treated with CZP for 72h may be caused by gastrointestinal problems **(younossian et *al.*, 2005).** On the other hand, there are other studies that show that the consumption of neuroleptics in the long term causes the increase of TGO and TGP **(Hubertus et *al.*, 2005)** this increase related to the increase in body weight,metabolic and enzymatic problems **(laurence et *al.*, 2002 ; conchillo, 2002)**.

finally, the results are observed at the level of the rats treated in the short term compared to the others because the rats have a very advanced adaptation system **(james et *al.*,2002)**

The non-significant increase in total and direct bilirubin in rats treated with CZP for 45 days is highly significant for rats treated for 72 hours, consistent with the work of **(Vinay et Einar S, 2017)** and it means liver damage can be a diagnostic challenge due to a significant differential diagnosis, the variability in clinical presentation and the lack of serological biomarkers associated with this function There is an insignificant increase in proteins in rats treated with chlorpromazine, these results are in agreement with the work of **(Daniel et *al.*, 1985 ; Amarl et *al.*,1991)** it means that chlorpromazine has a high affinity with ionized calcium, and 40% of calcium is bound to the protein «mainly albumin» that is why we found a very highly significant increase in albumin in rats treated with CZP for 72h same results of **(Hopwel et *al.*,1985 ; Silva et *al.*, 2004).**

The highly significant increase in lipids show that the main intracellular binders for CZP and related drugs are the membrane phospholipid fractions where hydrophobic interactions are mainly involved (**Mary et Bernheim, 1959 ; Jama, 2009)**. Although the non-significant increase in TG was selected to observe the solubility effect of the drugs **(Jean et *al.*, 2019)** the GA released in the blood are then directly usable by the cells; those that are not consumed can bind to Albumin (the main transporter of GA in the blood), that would need more energy ,the GA are derived from triglycerides" therefore the assessment of lipids in liver diseases can be evaluated by measuring: LDH, TG ,Cholesterol.. etc

3.2- Effect of CZP on oxidative stress activities

Malonaldehyde (MDA) is a product of the oxidative damage of lipids, amino acids and DNA, and accumulates with aging and disease. MDA can possibly react with amines to modify proteins into inactivity enzymes and also modify nucleosides to cause mutagenicity ; There was a significant decrease in malonadialdehyde(MDA) in rats treated with CZP for 45days **(Jack et Maurice, 1979 ; Bindoli et *al.*,1987)**. CZP are highly effective inhibitors of thrombin-induced MDA formation, and a very highly significant increase for rats treated for 72h **(Paudel et *al.*, 2010)** this means acute lipid peroxidation,There is a non-significant

increase in mitochondrial MDA in rats treated for 45d and significant increase in rats treated for 72h in accordance with **(Jiangang et *al.*, 2006) ,** accumulation of MDA due to mitochondrial dysfunction,MDA showed dose-dependent inhibition on ADP ratio decreased from protein concentration.

The non-significant increase in GSH and mitochondrial GSH compared to the controls, these results are confirmed by the results of **(Zoltan S et Markus, 2000)** show that effective maintenance of glutathione in endothelial and parenchymal cells is a mechanism to support observed hepatic resistance against intracellular or sinusoidal oxidative stress, but these results disagree with **(Lena et *al.*,2020)** it is observed that chlorpromazine decreases GSH levels in long-term use as indicated by severe liver alterations.

The non-significant increase in CAT in rats treated with CZP regardless of short-term or long-term treatments **(Nevena et *al.*,2015)** these results show that the indication of hepatic oxidative stress may be portable inflammation,focal necrosis of hepatocytes and these results confirm by the increase in GSH that triggers a pro-inflammatory response **(BoWen et Mignyan, 2009).**

The non-significant increase in glutathione peroxidase (GPx) in rats treated with CZP for 45 days and 72 hours, these results in agreement with the work of **(Hussain et *al.*, 2008 ; Djenovec et *al.*, 2017)** mean that the enzymatic activities to trap the ROS levels produced as a result of the accumulation of CZP in the liver. Increased antioxidant enzymes improve the antioxidant potential of organs to reduce oxidative stress. The non-significant decrease in mitochondrial GPx in CZP-treated rats (45 d) and significant decrease in CZPa-treated rats (72 h) **(Bai et Cederbaum, 2001 ; AnJie Jhou et *al.*, 2021)**. the mitochondrial respiratory chain is the main source of ROS, which can be reduced by intracellular antioxidant enzymes such as SOD, GPx and CAT these antioxidants are often unable to cope with the large amounts of ROS produced, this inefficiency of antioxidants is even more serious in mitochondria can lead to cell death by necrosis or apoptosis .

finally the role of CAT and GPx in mitochondria is very important in the protection of cells against oxidative lesions.

CONCLUSION

Conclusion

In our study we have evaluated the hepatotoxicity induced by a neuroleptic of first generation "Chlorpromazine" in *rattus Wistar* rats , which we has made it possible to reach interesting results.

From the obtained results it is concluded: that The sub-chronic treatment with CZP at a dose of 50 mg/kg/day (overdose) for a period of 45 days ; And the acute treatment at the same dose for a period of 72 hours induced deferential hepatotoxic damages so it leads to liver injury .

Our sub-chronic treatment affects rats body at deferent levels ; it results an increase of (lipids; proteins ; TG; Mitochondrial MDA ; GSH ; Mitochondrial GSH ; GPx ;CAT ;Alkaline Phosphatase ; Totale Bilirubine . Direct Bilirubine and LDH). And a decrease of (body weight ; MDA ; ASAT ; ALAT; Albumine) ; In the other hand the acute treatment results shows an increase of (ALAT ; Albumine ; TG; Lipids ; Proteins; MDA ; Mitochondrial MDA ; GSH ; Mitochondrial GSH; GPx ; CAT ; Alkaline Phosphatase ; Direct Bilirubine ; Totale Bilirubine ; LDH) and a decrease of (body weight ; ASAT and Mitochondrial GPx)

These results signifies that the overdose of “Chlorpromazine ”leads to hepatotoxicity and can cause liver injury .CZP may interact with various cellular organelles such as mitochondria leading to hepatocyte dysfunction .

In addition to that We have noticed that the acute treatment induced high significant changes of most parameters ; In contrast the sub-chronic treatment induced lower changes which signifies that the rats were adapted to the dose in the long term treatment

Our study results also a significant and high significant changes in the mitochondrial level Which means that the first cellular organelle may be touched is mitochondria.

CZP is beneficial for the treatment of behavour disorder and deferent neurologic problems ; But it still has side effects especialy when the patient falls in the danger of overdose

Bibiolgraphic References

1. Adams D, Ju C, Ramaiah S, Uetrecht J, Jaeschke H. (2010). Mechanisms of immune-mediated liver injury. Toxicological Sciences :307-321
2. Agid O, Kapur S , Arenovich T, Zipursky R. (2003). Delayed-onset hypothesis of antipsychotic action: a hypothesis tested and rejected. Archives of general psychiatry: 1228-1235.
3. American Psychiatric Association (1997) . Practice guidelines for the treatment of patients with schizophrenia. Am J Psych : 154.
4. Aronson JK.(2009). Meyler's Side Effects of Psychiatric Drugs. Oxford Elsevier.
5. Bindolia M , RigobelloB , Cavallinib .(1987). Decrease of serum malondialdehyde in patients treated with chlorpromazine.Clinica chimica acta:229_232
6. Biour M, Salem CB, Chazouillères O, Grangé J-D, Serfati L, Poupon R.(2004) Hépatotoxicité des médicaments 14e mise à jour du fichier bibliographique des atteintes hépatiques et des médicaments responsables. Gastroentérologie Clinique et Biologique :720–59.
7. Bo Wena,, Mingyan Zhou .(2009). Metabolic activation of the phenothiazine antipsychotics chlorpromazine and thioridazine to electrophilic iminoquinone species in human liver microsomes and recombinant P450s. Chemico-Biological Interactions :220_226
8. Borlido C., Remington G. (2016). Switching from 2 antipsychotics to 1 antipsychotic in schizophrenia: a randomized, double-blind, placebo-controlled study. The Journal of clinical psychiatry :14-20
9. Buckley NA, Sanders P.(2000). Cardiovascular adverse effects of antipsychotic drugs. :215-28.
10. Burns M . (2001) .The pharmacology and toxicology of atypical antipsychotic agents. J Toxicol Clin Toxicol :1-14
11. Campbell D.(2001). The management of acute dystonic reactions. AustraPresc: 24:19-20.
12. Cannon ., Tyrone D.(2015). Progressive Reduction in Cortical Thickness as Psychosis Develops: A Multisite Longitudinal Neuroimaging Study of Youth at Elevated Clinical Risk.Biological psychiatry : 147–157.
13. Cosi C, Koek W.(2001). Agonist, antagonist, and inverse agonist properties of antipsychotics at human recombinant 5-HT(1A) receptors expressed in HeLa cells. Eur J Pharmacol. 55-62.
14. Creese I (1976) . Dopamine receptor binding predicts clinical and pharmacologic potencies of anti-schizophrenic drugs. Science.;192-481.
15. Cullen J(2005) .Mechanistic classification of liver injury. Toxicologic Pathology.: 6-8
16. Curry SH. (2005) . Chlorpromazine: Pharmacokinetics, plasma levels, and clinical response, in, Burrows GD, Norman TR , Psychotropic Drugs: Plasma Concentruiion and Clinical Response. New York: Marcel Dekker : 243-286.

17. Daniel R ,Thomas J. Lukas and D. Martin Watterson .(1985). Drug-protein interactions: binding of chlorpromazine to calmodulin, calmodulin fragments, and related calcium binding proteins.Biochemistry :144-155
18. Dejanovic B , Lavrnja I, Ninkovic M, Stojanovic I, Djuric A , Dilber S, Stevanovic I . (2017). Effects of agmatine on chlorpromazine toxicity in the liver of Wistar rats. the possible role of oxidant/antioxidant imbalance :17-27
19. Emsley R. (2017). On discontinuing treatment in schizophrenia: a clinical conundrum.
20. Essali M .(1997). Clozapine vstypical neuroleptic medication for schizophrenia.: 223-255
21. Farde L., Wiesel M. D .(1988). with Antipsychotic Drugs. Arch Gen Psychiatry, 45, 71-76.
22. Fisher K, Vuppalanchi R, Saxena R.(2015). Drug-induced liver injury. Archives of Pathology & Laboratory Medicine : 876-887
23. Freeman M,. (2009) .The American Psychiatric Publishing Textbook of Psychopharmacology : 697.
24. Gahr M, Freudenmann R, Connemann B, Hiemke C, Schönfeldt-Lecuona C. (2013). Agomelatine and hepatotoxicity: implications of cumulated data derived from spontaneous reports of adverse drug reactions. Pharmacopsychiatry: 214–220
25. García-Cortés M, Andrade R, Lucena M, González-Grande R, Camargo R, Fernández-Bonilla .(2005) . Hepatotoxicidad secundaria a fármacos de uso común. Gastroenterología y Hepatología: 461-472
26. Grattagliano I, Bonfrate L, Diogo C, Wang H, Wang D, Portincasa P.(2009). Biochemical mechanisms in drug-induced liver injury: Certainties and doubts. World Journal of Gastroenterology: 4865-4876.
27. Guengerich FP.(2008). Cytochrome P450 and chemical toxicology. Chem Res Toxicol : 70–83.
28. Haddad PM, Anderson IM. (2002) .Antipsychotic-related QTc prolongation, torsade de pointes and sudden death. Drugs:16-49-71.
29. Harrow M, Jobe T .(2007). Factors involved in outcome and recovery in schizophrenia patients not on antipsychotic medications: a 15-year multifollow-up study. The Journal of nervous and mental disease: 406-414.
30. Hedges D., Jeppson K., Whitehead,P. (2003). Antipsychotic medication and seizures: a review. Drugs Today (Barc) : 551-557.
31. Hendouei N., Saghafi, F., Shadfar F., Hosseinimehr S . (2019). Molecular mechanisms of anti-psychotic drugs for improvement of cancer treatment. Pharmacol:128_130
32. Hiemke C., Baumann P. (2011). AGNP consensus guidelines for therapeutic drug monitoring in psychiatry , Pharmacopsychiatry :195–235.
33. Howden C, Birnie G, Brodie M. (2015) . Drug metabolism in liver
34. Hsing L .(2021). Chlorpromazine, an antipsychotic agent, induces G2/M phase arrest and apoptosis via regulation of mediated autophagy pathways in human oral cancer .Biochemical pharmacology:184

35. Hubertus Himmerich, Christian Kaufmann, Andreas Schuld, Thomas Pollm acher .(2005) .Elevation of liver enzyme levels during psychopharmacological treatment is associated with weight gain. Journal of Psychiatric Research :35-42
36. Human M , Platelet .(2008). Moecular pharmacology:171-180
37. Insel T. Post by Former Director Thomas Insel .(2013). Antipsychotics: Taking the Long View.
38. Jaeschke K. (2021). Global estimates of service coverage for severe mental disorders: findings from the WHO Mental Health Atlas. Glob Ment Health
39. James M. Brady, Nathan J. Cherrington, Dylan P. Hartley, Susan C. Buist, Ning Li and Curtis D. Klaassen .(2002). Tissue Distribution and Chemical Induction of Multiple Drug Resistance Genes in Rats.Drugs metabolism and disposition :838_844
40. Janicak P.(2001). Principles and Practice of Psychopharmacology: 322-325
41. Javaid, J. (1994). Clinical Pharmacokinetics of Antipsychotics. The Journal of Clinical Pharmacology : 286–295.
42. Jeand Baloch , Muhammad Farhan Sohail , Hafiz Shaib Sarwar , Maria Hassan Kiani , Gul Majid Khan , Sarwat Jahan , Muhammad Rafay , Muhammad Tausif Chaudhry , Masoom Yasinzai and Gul Shahnaz .(2019). Self-Nanoemulsifying Drug Delivery System for Improved Oral Bioavailability of Chlorpromazine: In Vitro and In Vivo Evaluation.Medicina:13
43. Jiangang L, HongxiangGaoZhiLiu, ChangshengLiu ,MingyongMiao ,JiankangLiu .(2006) .Malonaldehyde acts as a mitochondrial toxin: Inhibitory effects on respiratory function and enzyme activities in isolated rat liver mitochondria.life science:1466_1472
44. Jingxiang B ,Arthur C .(2001). Mitochondrial Catalase and Oxidative Injury .Biological signal receptor:189_199
45. Johns Hopkins Diabetes Guide - Antipsychotics
46. Kanahara N, Yamanaka H, Shiko Y, Kawasaki Y, Masaomi I. (2021). The effects of cumulative antipsychotic dose on brain structures in patients with schizophrenia: Observational study of multiple CT scans over a long-term clinical course. Psychiatry Research: 111-122.
47. Kaplowitz N. Drug-induced liver injury. Clinical infectious diseases. (2011) :44-48
48. Kapur S, Mamo D. (2003) a century of antipsychotics and still a central role for dopamine D2 receptors. Prog Neuropsychopharmacol Biol Psychiatry: 81-90
49. Kapur S. (2009). The dopamine hypothesis of schizophrenia: Version III-the final common pathway. Schizophrenia Bull:549-562.
50. Kapur S., Seeman P. (2000). Antipsychotic agents differ in how fast they come off the dopamine D2 receptors. Implications for atypical antipsychotic action. Journal of Psychiatry and Neuroscience :161.
51. Kenes M., Hamblin E., Tumuluri. S., Guillamondegui ., O. (2016). Syndrome of Inappropriate Antidiuretic Hormone in a Patient Receiving High-Dose Haloperidol and Quetiapine Therapy. The Journal of neuropsychiatry and clinical neurosciences :29-30.

52. Kishimoto T, Nitta M, Borenstein M, Kane JM.(2013). The journal of clinical psychiatry: Long-acting injectable versus oral antipsychotics in schizophrenia: a systematic review and meta-analysis of mirror-image studies. Physicians Postgraduate Press : 74-957.
53. KP Paudel,S Kumar,SK Meur,A Kumaresan .(2010). Ascorbic Acid, Catalase and Chlorpromazine Reduce Cryopreservation-induced Damages to Crossbred Bull Spermatozoa.Reproduction in domestic animals: 111-117
54. Lee W. (1995). Drug induced hepatotoxicity. The New England Journal of Medicine
55. Leucht S. (2013). Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: A multiple-treatments meta-analysis. The Lancet 2013:951-962.
56. Lieberman J , Stroup T. (2011). The NIMH-CATIE Schizophrenia Study. American Journal of Psychiatry : 770-775.
57. Martí L, Olmo J, Tosca J, Ornia E, Serra M, Rodríguez F.(2005). Clinical evaluation of drug-induced hepatitis. Revista Espanola De Enfermedades Digestivas.: 258-265
58. Mary L& Bernheim C .(1959) . Antioxidant Effect of Chlorpromazine.experimental biology and medicine: 152-166
59. Merlis S. (1976), Pharmacokinetics of Psychoactive Drugs. New York, Spectrum: 191-197.
60. Meyer JM, Brunton LL, Chabner BA, Knollman BA . (2011). the pharmacological basis of therapeutics : 417-562.
61. Miller D. (2004). Atypical antipsychotics: sleep, sedation, and efficacy. Primary care companion to the Journal of clinical psychiatry: 3-6
62. Murray R., Di Forti M. (2018). Increasing expectations and knowledge require a more subtle use of prophylactic antipsychotics. World Psychiatry : 161.
63. Murray, R. M., Quattrone, D., Natesan, S., van Os, J., Nordentoft, M., Howes, O., Taylor, D. (2016). Should psychiatrists be more cautious about the long-term prophylactic use of antipsychotics. The British Journal of Psychiatry : 361-365.
64. Nevena Todorović , Nada Tomanović , Peter Gass , Dragana Filipović .(2015). Olanzapine modulation of hepatic oxidative stress and inflammation in socially isolated rats. Pharmaceutical Sciences :94-102
65. Pessayre D, Mansouri A, Berson A, Fromenty B.(2010). Mitochondrial involvement in drug-induced liver injury. Adverse Drug Reactions, Handbook of Experimental Pharmacology:311-365
66. Pharmacopsychiatry (2013) : 214-220 .
67. Pickar D, Litman RE, Konicki PE, Wolkowitz OM, Breier A .(1990). Neurochemical and Neural Mechanisms of Positive and Negative Symptoms in Schizophrenia". Modern Problems of Pharmacopsychiatry. Modern Trends in Pharmacopsychiatry. 24: 124–151.
68. Ramachandran R, Kakar S. (2009) . Histological patterns in drug-induced liver disease. Journal of Clinical Pathology: 481-492
69. Ray WA, Chung CP, Murray KT, Hall K, Stein CM. (2009). Atypical antipsychotic drugs and the risk of sudden cardiac death:205 .

70. Ray, Wayne A.(2009).Atypical antipsychotic drugs and the risk of sudden cardiac death. New England Journal of Medicine : 225-235.
71. Sabina A, Sreenivasa M, Bhaskara M .(2003). From chlorpromazine to clozapine antipsychotic adverse effects and the clinician's Dilemma.The Canadian journal of psychiatry : 1-10.
72. Saxena R, Kleiner D, Hoofnagle J. (2010). Duloxetine hepatotoxicity:a case-series from the drug-induced liver injury network. AlimentPharmacol Ther 1174-1183
73. Schatzberg AF and Nemeroff, C. (2010). The American Psychiatric Publishing Textbook of Psychopharmacology. American Psychiatric Publishing.
74. Schatzberg AF., Nemeroff C.(2009) The American Psychiatric Publishing Textbook of Psychopharmacology. 4th ed. American Psychiatric Publishing.
75. Schuster D, Laggner C, Langer T. (2005). Why drugs fail--a study onside effects in new chemical entities: 23_35
76. Scigliano G.,Ronchetti G. (2013). Antipsychotic-induced metabolic and cardiovascular side effects in schizophrenia: a novel mechanistic hypothesis. CNS drugs : 249-257.
77. Seeman P.(2002). Atypical antipsychotics: mechanism of action. Can J Psychiatry :27-38
78. Selim K, Kaplowitz N.(1999) .Hepatotoxicity of psychotropic drugs. Hepatology :1347-1351.
79. Selim K, Kaplowitz N.(1999). Hepatotoxicity of psychotropic drugs. Hepatology:1347–1351.
80. Shiovitz T., Welke T., Tigel P ., Anand R., Hartman R., Sramek,. J. (1996). Cholinergic rebound and rapid onset psychosis following abrupt clozapine withdrawal. Schizophrenia bulletin : 591.
81. Stahl SM. Stahlâ .(2008). Essential Psychopharmacology: Neuroscientific Basis and Practical Applications. 3rdedn. New York: Cambrigde University Press.
82. Takeuchi H., Thiyanavadivel S., Agid O., Remington G. (2017). Gradual vs. wait-and-gradual discontinuation in antipsychotic switching: A meta-analysis. Schizophrenia research : 4-8.
83. Taylor, David. (2009). Schizophrenia. The Maudsley Prescribing Guidelines in Psychiatry, Twelfth Edition .16
84. Tiihonen J, Mittendorfer-Rutz E, Majak M, Mehtälä J, Hoti F, Jedenius E, Enkusson D, Leval A, Sermon J, Tanskanen A, Taipale H.(2017). Real-World Effectiveness of Antipsychotic Treatments in a Nationwide Cohort of 29 823 Patients With Schizophrenia. JAMA Psychiatry :686–693.
85. Tiihonen J., Taipale H., Mehtälä J., Vattulainen P., Correll C. U., Tanskanen A. (2019). Association of antipsychotic polypharmacy vs monotherapy with psychiatric rehospitalization among adults with schizophrenia. JAMA psychiatry : 499-507.
86. Tiihonen J., Tanskanen A.,Taipale, H. (2018). 20-year nationwide follow-up study on discontinuation of antipsychotic treatment in first-episode schizophrenia. American Journal of Psychiatry, appi-ajp.

87. Vasan S., Abdijadid S. (2017). Atypical antipsychotic agents. In StatPearls. StatPearls Publishing.
88. Veijola, Juha . (2014). Longitudinal changes in total brain volume in schizophrenia: relation to symptom severity, cognition and antipsychotic medication: 9-7
89. Voineskos A , Mulsant B , Dickie E , Neufeld N, Rothschild A. , Whyte E , Flint A. . .(2020). Effects of Antipsychotic Medication on Brain Structure in Patients With Major Depressive Disorder and Psychotic Features: Neuroimaging Findings in the Context of a Randomized Placebo-Controlled Clinical Trial. JAMA psychiatry.
90. Wang P., Schneeweiss S., Avorn J., Fischer M., Mogun H., Solomon, D., Brookhart, M. (2005). Risk of death in elderly users of conventional vs. atypical antipsychotic medications. New England Journal of Medicine : 2335-2341.
91. Weinreb M, Kraus V, Krausová, Hudcová. (1978). Negligible hepatotoxicity of chlorpromazine in long-term therapy: 206 -280.
92. Winton-Brown T, Elanjithara T, Power P., Coentre R., Blanco-Polaina, P., McGuire P. (2017) . Five-fold increased risk of relapse following breaks in antipsychotic treatment of first episode psychosis. Schizophrenia research, : 50-56.
93. Wunderink L, Nieboer R, Wiersma D, Sytema S, Nienhuis F .(2013). recovery in Remitted First-Episode Psychosis at 7 Years of Follow-up of an Early Dose Reduction/Discontinuation or Maintenance Treatment .
94. Wunderink L, Nieboer RM, Wiersma D, Sytema S, Nienhuis FJ .(2013). Recovery in Remitted First-Episode Psychosis at 7 Years of Follow-up of an Early Dose Reduction/Discontinuation or Maintenance Treatment StrategyLong-term Follow-up of a 2-Year Randomized Clinical Trial. JAMA Psychiatry :913–920.
95. Younossian T. Rochat J , Ketterer J, Wacker P , Janssens .(2005). High hepatotoxicity of pyrazinamide andethambutol for treatment of latent tuberculosis.:462_464
96. Zoltán Spolarics and Markus Meyenhofer. (2000) .Augmented resistance to oxidative stress in fatty rat livers induced by a short-term sucrose-rich diet. Biochimica et BiophysicAct (BBA) - Molecular and Cell Biology of Lipids :190-200

ANNEXES

Annexes

Annexes

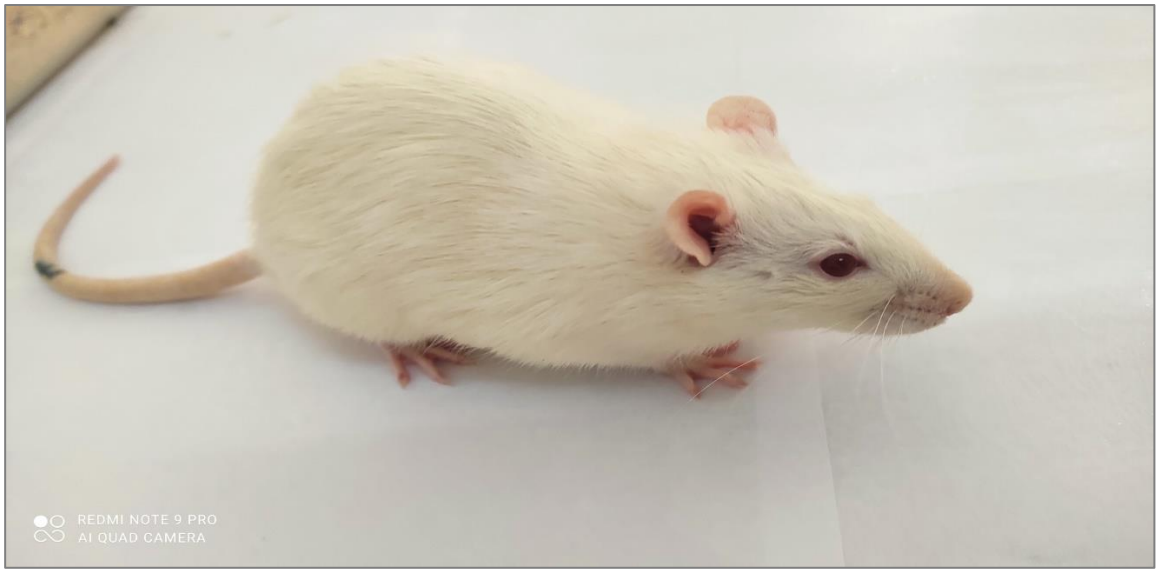
I. Used Materials and machines

- Distilled water.
- TCA (Trichloro acetic).
- Sulphuric acid.
- Orthophosphoric acid (85%).
- Vanillin.
- BBC (Brilliant Blue of Coomassie).
- Ether.
- Chloroform.
- Ethanol (95%).
- Sodium phosphate dibasic.
- SSA (Sulfosalicyl acid).
- Monobasic sodium phosphate.
- Tris.
- HC1.
- NaOH.
- Absolute methanol.
- EDTA (Ethylene diamine acid tetracetic).
- DTNB (5-5'-dithio-bis-2-acidnitrobenoic).
- Centrifuge (SELECTA).
- Analytical balance
- Precision balance (KERN).
- Etuve (HERAEUS).
- pH meter.
- Magnetic stirrer (WITEG).
- Dissection material.
- Centrifuge sigma
- Refrigerator.
- Bain marie (MEMMERT).
- Vortex agitator (THERMOS).
- Spectrophotometer (UV mini 1240, SHIMADZU).
- Mortar + Pestle (Manual crusher).
- Pisstte.
- Watch glass.
- Spatula.
- magnetic. Bar
- Micropipettes (10µl to 5000µl).
- Graduated pipettes.
- Test tubes.
- eppondorf tubes for the sigma centrifuges.
- Tanks for spectrophotometry (plastic and quartz).
- Wattman Paper No. 01.
- Becher.
- Erlenmeyers.
- Funnels.
- Graduated test pieces.

Annexes

II. Details about Sacrifice and dissection of rats

Annex: batche include a goupe of 6 rats



Annex : experimental rat of the *rattus Wistar* stain

Annexes

Annex: dissection of the rat and extracyion of organ

Annexes

III. Biochemical parameters protocols : (Totale and direct bilirubine ; ASAT ; ALATA)

The determination of bilirubin was performed by the colorimetric method (according to the Spinreact data sheet).

- **Principle:** Bilirubin reacts with diazotized sulfanilic acid at acidic pH to produce azobilirubin. This reaction is instantaneous with direct bilirubin (conjugated bilirubin), but with total bilirubin (unconjugated bilirubin) it is indirect and requires solubilization by dimethylsulfoxide (DMSO). (In the absence of DMSO, only direct bilirubin reacts). The intensity of the staining is proportional to the concentration of bilirubin in the sample (**Malloy *and al.*, 1937 ; Kaplan *and al.*, 1984).**

Reagent 1: 30 mmol/l Total bilirubin Sulphanilic acid. 50 mmol/l Hydrochloric acid. 7 mmol/l Dimethyl sulfoxide (DMSO)

Reagent 2: 29 mmol/l Sodium nitrite.

MO:

Reagent 1: 1.5 ml total white

Reagent 2: 50 ul

Sample/calibration: 100ul

Mix and read the optical density after 5 minutes incubation at 555 nm wavelength.

Principle: The albumin reacts with bromocresol green (BCG), to form a colored complex. The pH of the medium is maintained at 4.2 by the buffer. After incubation, the absorbance of the mixture is measured at 628 nm (Doimas 1971)

Reagents: 0.14 g/l Bromocresol green,75 mmol/l Succinate buffer pH,35 7 ml/l Brige

Mix, incubate for 5 min at 370 C. Read the optical densities against white at 628 nm.

Annexes

- **Dosage of Aspartate aminotransferase**

We have used boxes (Spinreact) to perform this dosage.

Principle: Aspartate aminotransferase (AST) also known as glutamate oxaloacetate (GEO) catalyzes the reversible transfer of an amino group from the α-ketoglutarate aspartate forming glutamate and oxaloacetate. Oxaloacetate is reduced to malate by malate dehydrogenase (MDH) and NADH,H+ (Reitman 1957; Murray 1984)

Reagents:

80 mmol/L Tris pH 7.8, 200 mmol/L L- Aspartate, 0.18 mmol/L NADH, 800 U/L Lactate dehhydrogenase (LDH), 600 U/L Malate dehhydrogenase (MDH), 12 mmol/L α-ceftoglutarate

Mix, incubate for one minute. Read at 340 the initial absorbance and start the stopwatch simultaneously. Read again after 1, 2 and 3 minutes

- **Dosage of Alanine aminotransferase**

We used boxes (Spinreact) to perform this dosage.

Principle: The principle is presented according to the following reaction:

The decrease in NADH concentration is directly proportional to the enzymatic activity of alanine aminotransferase in the sample (Reitman 1957; Murray 1984).Reagents

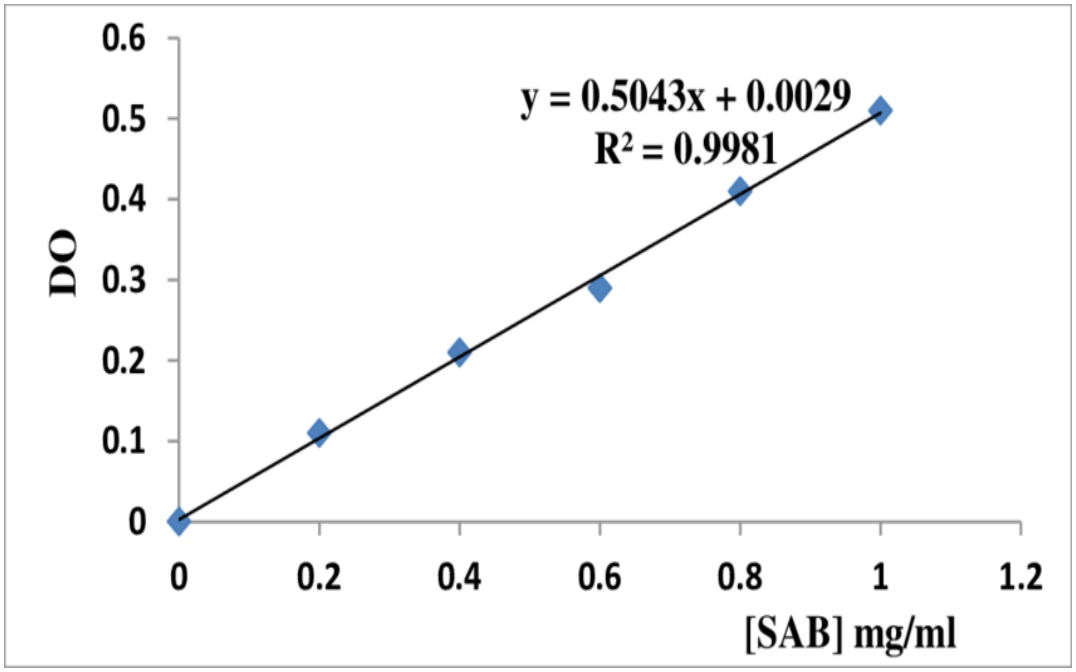
100 mmol/L Tris pH 7.8 , 500 mmol/L L- alanine, 0.18 mmol/L NADH,1200 U/L Lactate dehhydrogenase (LDH),15 mmol/L Oxoglutarate

Mix, incubate for one minute at room temperature and read the initial absorbance

340 nm. Read again after 1, 2 and 3 minutes. Average absorbances

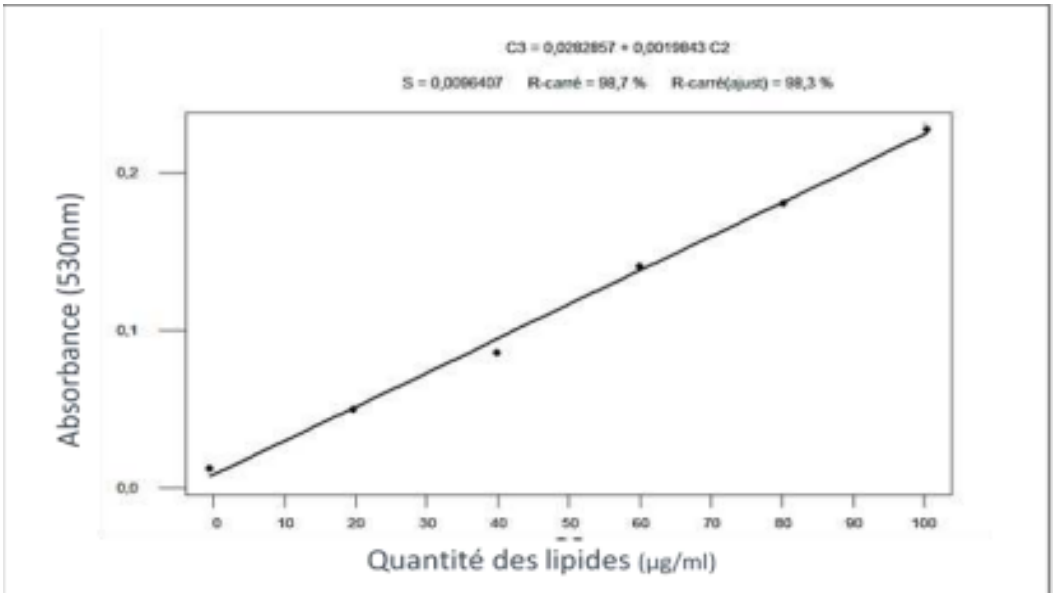
per minute (Abs/min) for use in calculations.

IV. Calibration curves



Annex: calibration curve of proteins

Annexes



Annex: calibration curve of lipids