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The protective effect of *Ephedra alata (Alenda)* extract on gentamicin induced hepatotoxicity

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To the queen of my heart my superhero mom. I am grateful for your never-ending love and support it was the only thing that kept me going.

To my dad's soul, you're guiding hand on my shoulder will remain with me forever "may we meet again"

To my brothers Abdou & Aymen and my little sister Nouha, you're my company that I shared every aspect of my life with, went through sadness, hardships, happiness, and you filled the void within me with joy.

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This work is dedicated to the dearest people to my heart:

My beloved mother and father for their love and affection.

My sisters and brother: for their care and motivation.

My lovely friend: Ahlem. for her encouragement and

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AYA

Contents

Contents

%: Percentage

°C: Degree celsius

µg /ml: Microgram per milliliter

µl: Microlitre

ALP: Alkaline phosphatase

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

ATP: Adenosine triphosphate

BHA: Butylated hydroxyl anisole

BHT: Butylated hydroxyrotoluene

CAT: Catalase

CO_2: Carbon dioxide

COX2: Cyclooxygenase

COX2: Cycloxygenase-2

DNA: Deoxyribonucleic Acid

DPPH: 2,2-diphenyl-1-picrylhydrazyl

DTNB: 5,5'-dithiobis-2 nitro benzoic acid

ECM: Electronic Content Management

EDTA: Ethylenediamine tetraacetic acid

g: Gram

GPx: Glutathione peroxidase

GSH: Qlutathione

GST: glutathione S-transferase

H_2O_2: Hydrogen peroxide

HCAs: Hydroxycinnamic acids

HCV: Hepatitis C virus

HLA: Human leukocyte antigen

HO-1: Heme-oxygenase 1

IGFs: Insulin binds growth factors

IL-1b: Interleukin-1b

iNOS: Nitric oxide synthase

KCl: Potassium chloride

LDH: Lactate dehydrogenase

MDA: Methylenedioxyamphetamine

mg: Milligram

NAD(P)H: Nicotinamide adenine dinucleotide phosphate

NAFLD: Non-alcoholic fatty liver disease

NASH: Non-alcoholic steatohepatitis

NDGA: Nordihydro guaretic acid

NF-kB: nuclear factor-kB

NQO1: Quinone reductase

Nrf2: Nuclear factor erythroid-2-related factor-2

NSAID: Nonsteroidal anti-inflammatory drugs

OCH_3: Methoxyl

OH: Hydroxyl

PG: Propyl gallate

PUFAs: Polyunsaturated fatty acids

RNA: **Ribonucleic acid**

ROS: Reactive Oxygen Species

SOD: Superoxide dismutase

TBA: Thiobarbituric acid

TBARS: Thiobarbituric Acid Reactive Susbtances

TBHQ: Tertiary butyl hydroquinone

TCA: Trichloroacetic acid

TNB: Thionitrobenzoic acid

TNFαR1: Tumor necrosis factor-α receptor type 1

UV : Ultra-violet

Introduction

Introduction

The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for the overall health and well being (Pandit et al., 2012; Boubekri et al., 2014).

Hepatotoxicity is damage caused by exposure to a drug or non-pharmacological agents. Risk factors include idiosyncrasy, age, gender, alcohol consumption, smoking, concomitant use of other drugs, previous or underlying liver disease, genetic and environmental. Although most lipophilic drugs can cause hepatotoxicity, antibiotics, nonsteroidal anti-inflammatory drugs (NSAIDs) and anticonvulsants are the pharmacological groups which are the most frequent causes (Cano et al., 2017).

One of the most widely used class of drugs are antibiotics. These drugs prevent many problems caused by infections. However, antibiotics have side effects and can damage various body organs including liver, kidney, brain, blood, skin, eyes, mouth, etc. (Najafian et al., 2014).

Gentamicin (GM) is an aminoglycoside with broad bacteriocidal activity against many aerobic gram negative and some aerobic gram positive organisms. GM is the most commonly used aminoglycoside antibiotic and is indicated for moderate-to-severe bacterial infections caused by sensitive agents, primarily gram negative bacteria (Abdel-Raheem et al., 2010).

Like other aminoglycosides, gentamicin is thought to act by binding to bacterial ribosomes and inhibiting protein synthesis (Khan et al., 2011). Its adverse effect on some vital organs in the body, such as kidney, liver, etc., makes its use limited in clinical settings. The main side effects include liver damage, which is one of the major factors of liver inefficiency in a significant number of people taking this medication. Increased production of Reactive Oxygen Species (ROS), which can be seen after the use of gentamicin in cells, is effective in inducing toxic impacts of this drug on the structure and function of tissues (Ogundipe et al., 2021).

Some studies have reported that oxidative stress has role in gentamicin-induced nephrotoxicity. Gentamicin enhanced the production of superoxide anion, hydrogen peroxide and hydroxyl radicals by mitochondria. Free radicals cause peroxidation of phospholipids membrane, DNA strand breakage, protein denaturation. Most significant biological damage of active

metabolites is their reaction with unsaturated lipid and so their peroxidation. This effect induces changes in membrane fluidity, thus the membrane gets permeable even to molecules as large as enzymes (Najafian et al., 2014).

Medicinal plants are considered as rich resources of ingredients which can be used in drug development pharmacopoeial, non-pharmacopoeial or synthetic drugs. A part from that, these plants play a critical role in the development of human cultures around the whole world. Plant is an important source of medicine and plays a key role in world health (Salmerón-Manzano et al., 2020; Garg et al., 2021).

Medicinal plants are a source for a wide variety of natural antioxidants and are used for the treatment of diseases throughout the world. Some of these properties are antimicrobial, anti-cancer, anti-diabetic, anti-atherosclerosis, immunomodulatory, and even reno-protection or hepato-protective effects. Recently, due to beneficial effects of antioxidants, particularly natural antioxidants, in the treatment and prevention of diseases, there has been a considerable interest in finding natural antioxidants from plant sources. The studies on medicinal plants show that most of them possess significant antioxidant activity (Rafieian-Kopaei, 2012).

Recent studies have shown that natural antioxidants obtained from different alternative systems of medicine display a wide range of biological activities. Various alternatives possessing antioxidant properties have been used in order to minimize gentamicin induced oxidative stress in animal models. Many plant extracts have been reported to be effective in ameliorating organ toxicities (Khan et al., 2011; Rodrigues et al., 2014).

Ephedra alata subsp. *alenda*, is a plant belonging to the family *Ephedracea*. It is used for its therapeutic properties, especially to treat respiratory infections (Boubekri et al., 2020; Hadjadj et al., 2020).

This study was designed to investigate whether the crude hydro-methanolic extract of *Ephedra alata* subsp. *alenda* ameliorates the hepato-toxicity induced with gentamicin in rat model.

Bibliographic Review

I-The liver

The liver is the largest organ, accounting for approximately 2.5% of average body weight. It is a unique organ due to its dual blood supply from the portal vein (approximately 75%) and the hepatic artery (approximately 25%). It's a critical organ in the human body that is responsible for an array of functions that help support metabolism, immunity, digestion, detoxification, vitamin storage among other functions. It has an impressive capacity for regeneration (Sherif et al., 2010; Mahadevan and Liang, 2017; Arjun Kalra et al., 2021).

The liver is structurally and functionally heterogeneous and has been considered second only to brain in its complexity. Being the "main extracellular compartment" for adult vertebrates, it has thousands of vital functions including the efficient uptake of amino acids, carbohydrates, bile acids, cholesterol, proteins, lipids and vitamins for storage and metabolism subsequent to release into bile and/or blood, it transforms the toxic substances to elements that the body can eliminate it before their distribution into the blood stream (David et al., 2005; Lorente et al., 2020).

It's a critical hub for numerous physiological processes. These include macronutrient metabolism, blood volume regulation, immune system support, endocrine control of growth signalling pathways, lipid and cholesterol homeostasis, and the breakdown of xenobiotic compounds, including many current drugs. The important roles performed by the liver, not only in the storage and release of nutrients but also in the neutralization and elimination of a variety of toxic substances (Baratta et al., 2009; Lorente et al., 2020).

I-1-General Description of the Liver

The liver weighs approximately 1500 g, and is located in the upper right corner of the abdomen. The organis closely associated with the small intestine, processing the nutrient-enriched venous blood that leaves the digestive tract. The surface of the liver is smooth and dome shaped, where it is related to the concavity of the inferior surface of the diaphragm (figure 1). The liver lies mainly in the right upper quadrant of the abdomen where it is hidden and protected by the thoracic cage and diaphragm. The normal liver lies deep to the ribs 7 – 11 on the right side and crosses the midline towards the left nipple (Lena Sibulesky, 2013; Ozougwu, 2017).

It's divided into 4 lobes: right, left, caudate, and quadrate. The right and left lobes are the largest, while the caudate and quadrate are smaller and located posteriorly. The falciform ligament separates the right and left lobes. Inferior to the falciform ligament is the round

ligament, which protrudes from the liver slightly. The caudate lobe is located superiorly, approximately between the right and left lobes. Adjacent to the caudate lobe is the sulcus for the inferior vena cava. Just inferior to the caudate lobe is the porta hepatis, where the hepatic artery and hepatic portal vein enter the liver. The liver is held on place by a system of mesenteries posteriorly, and is also attached to the diaphragm via the falciform ligament. Most of the liver is covered by visceral peritoneum (figure 2) (Abdel-Misih and Bloomston, 2010; Ozougwu, 2017).

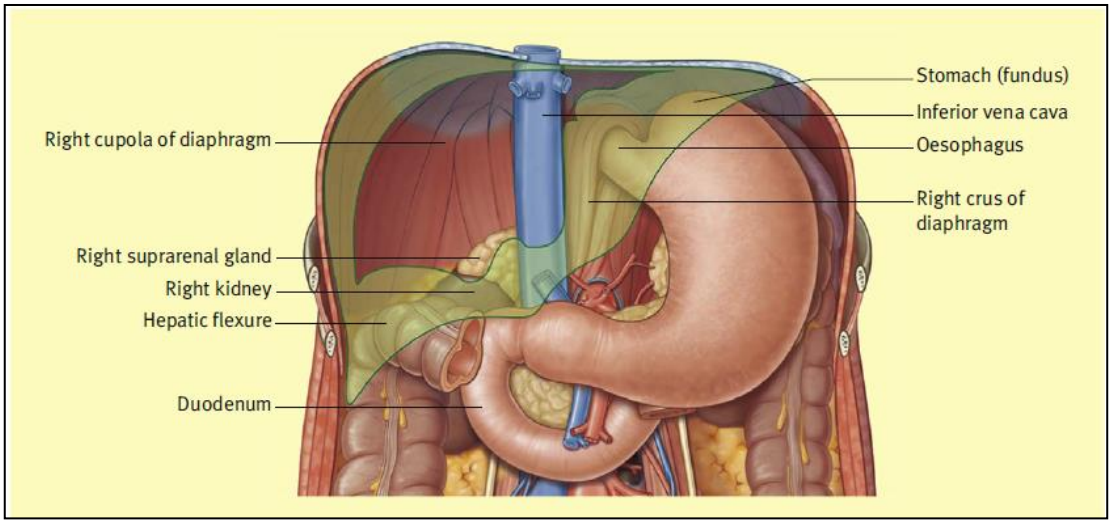


Figure 1: The 'bed' of the liver. The outline of the liver is shaded green. The central bare area is unshaded (Harold Ellis, 2011).

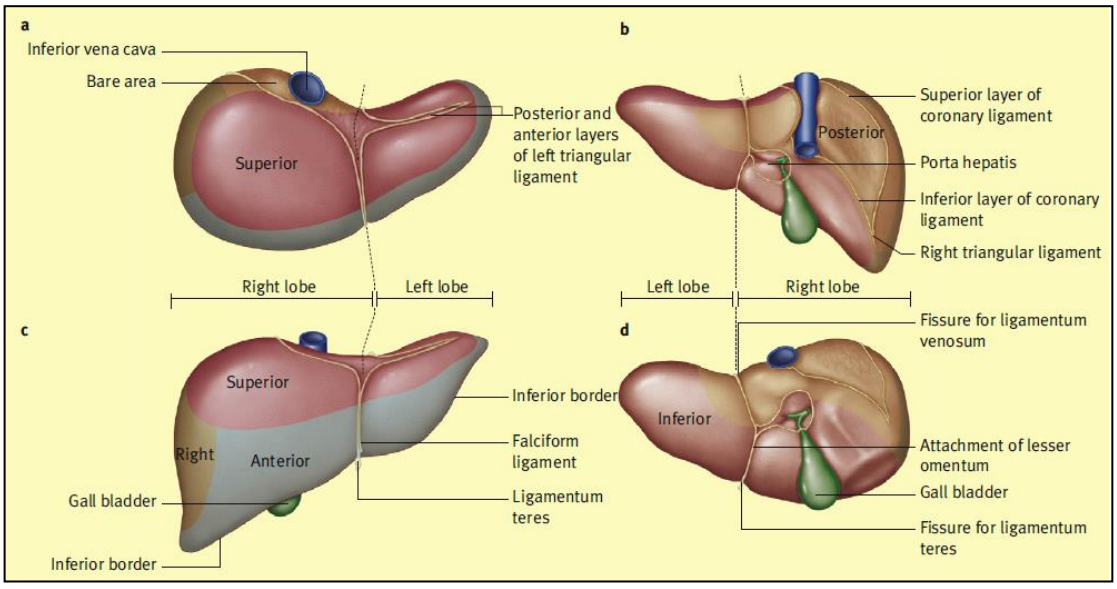


Figure 2: The surface features, ligaments and peritoneal attachments of the liver (Harold Ellis, 2011).

I-2-Histology of the Liver

The basic functional unit of the liver is the liver lobule (figure 3, figure 4). A single lobule is about the size of a sesame seed and is roughly hexagonal in shape. Explained that the primary structures found in a liver lobule include:

- Plates of hepatocytes which forms the bulk of the lobule;
- Portal triads at each corner of hexagon;
- Central vein ;
- Liver sinusoids that run from the central vein to the portal triads;
- Hepatic macrophages (Kupffer cells) ;
- Bile canaliculi ("little canals") – formed between walls of adjacent hepatocytes;
- Space of Disse – a small space between the sinusoids and the hepatocytes (Andersen et al., 2010).

The portal triads consist of three vessels: a hepatic portal arteriole, a hepatic portal venule, and a bile duct. The blood from the arteriole and the venule both flow in the same direction-through the sinusoids toward the central vein, which eventually leads to the hepatic vein andthe inferior vena cava. Secreted bile flows in the opposite direction – through the bile canaliculi away from the central vein, toward the portal triad, and exiting via the bile duct. As blood flows through the sinusoids and the space of disse toward the central vein, nutrients are processed and stored by the hepatocytes, and worn-out blood cells and bacteria are engulfed by the Kupffer cells (Abdel-Misih and Bloomston, 2010; Ozougwu, 2017).

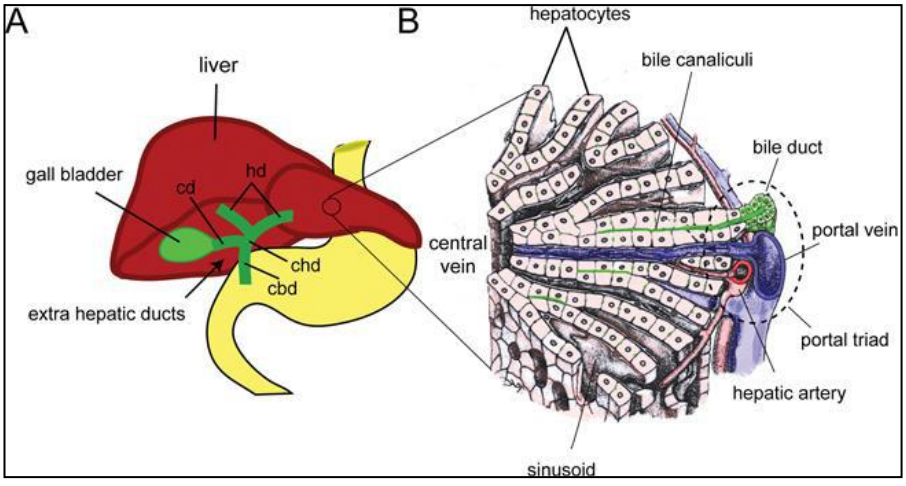


Figure 3: Cellular architecture of the liver *(Ozougwu, 2017)*.

The liver is the first site of passage for venous blood arriving from the intestines via vena porta. The areas around the influx blood vessels are named periportal. The areas surrounding efflux blood vessels are the perivenous. The periportal area is highly complex and consists of a dense matrix containing collagen where afferent blood vessels are found, together with bile ducts, nerve and lymph. Spaces within the matrix contain a variable cell population, such as fibroblasts, hematopoietic cells and inflammatory cells. Also found here are epithelial cells of the bile ducts, endothelial cells of the blood vessels, and smooth muscles of arteries and veins (Andersen et al., 2010; Abshagen et al., 2015).

The liver lobule consists mainly of plates of hepatocytes and sinusoids, with a light matrix of collagen to form a network between the two. Kupffer cells, as well as fat storing stellate cells are found here. These types of cells reside mainly in the tissue space between the hepatocyte and the sinusoids. Terminal bile ductules connect here to the bile cannaliculi between hepatocystic plates. The walls of the hepatic sinusoid are lined by three different cell types: the sinusoidal endothelial cells, kupffer cells and stellate cells. Additionally, pit cells, the liver specific natural killer (NK) T cells are often present in the sinusoidal lumen (Mahadevan, 2014).

The main parenchymal mass is normally that of hepatocytes. In rat, the hepatocytes make up about 60% of liver cell count and the remaining 40 %, non-parenchymal cells only make up for about 6-7% of the liver volume while the remaining volume of approximately 23 % is formed by extracellular spaces. The liver is made up of many different cell types, namely (Ozougwu, 2017):

- Hepatocytes;
- Endothelial cells;
- Kupffer cells (liver resident macrophages) and
- Stellate cells (liver fat storing cells) (figure 4).

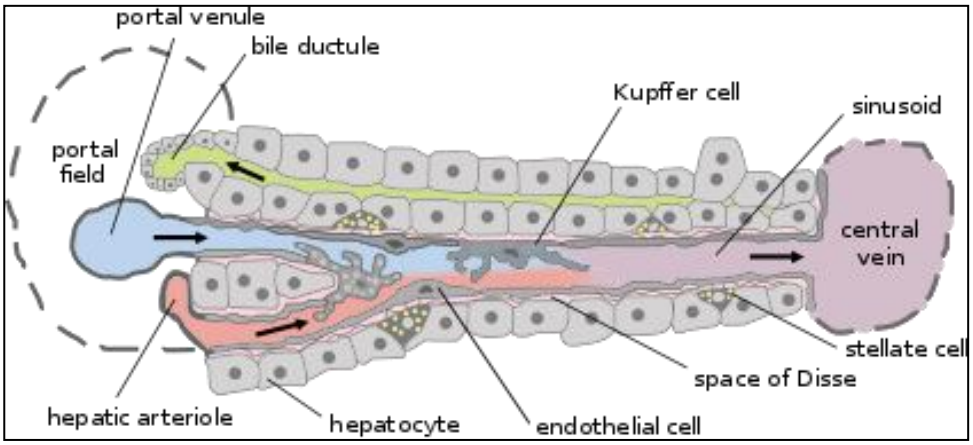


Figure 4: Diagram showing main cell types of Liver: hepatocytes, endothelial cells, Kupffer cells and Stellate cells. Source:https://en.wikipedia.org/wiki/Liver

I-3-Functions of the liver

The liver controls glycolytic and urea metabolism, blood detoxification and cholesterol levels, while supporting the hematopoietic and digestive systems (Gordillo et al., 2015).

I-3-1-Metabolic function

The liver is a central regulator of metabolism; it plays a central role in metabolic homeostasis and is a major site for synthesis, metabolism, storage and redistribution of carbohydrates, proteins and lipids (Lars et al., 2012; Gordillo et al., 2015).

- ***Liver lipid metabolism***

Hepatic lipid and glucose metabolism are closely interrelated with inflammatory, proliferative and apoptotic signaling within the liver. In the liver, these catabolic and anabolic pathways can hardly be separated. They share intermediate metabolites and receptor signaling, and go hand in hand in the pathogenesis (Lars et al., 2012; Nguyen et al., 2008)

- ***Glucose metabolism***

The liver plays a central role in the maintenance of glucose homeostasis. During the fed state it takes up excess amounts of glucose and converts it to glycogen and fatty acids while oxidizing it to CO_2. To maintain normoglycemia during fasting states, the liver releases glucose derived from glycogenolysis and gluconeogenesis into the blood stream (Hijmans et al., 2013).

- ***Protein and amino acid metabolism***

- As a protein synthetic organ, the liver is responsible for 85–90% of circulating protein volume.
- The liver has a high capacity to break down proteins and metabolize the amino acids that comprise them.
- Amino acid metabolism can provide energy for the hepatocyte, but requires disposal of nitrogenous wastes (Liangyou Rui, 2014; Elijah Trefts et al., 2017).

- ***Hormonal metabolism***

The liver synthesizes different hormones or prohormones such as IGFs (Insulin binds growth factors) or angiotensinogen. It inhibits a number of hormones circulating: certain steroids, such as corticosteroids, Oestrogens and progesterones are also metabolized, conjugated and released at the two vascular and biliary poles of the cell. Testosterone and androgens are also reduced and then glucuronides in the hepatocyte, as is thyroxine, which is said to be probably partly destroyed by the liver (Brockman and Laarveld, 1986).

- ***Immune function***

The liver is the main hematopoietic organ during certain stages in fetal development and continues to be a hematopoietic organ even after birth. It can produce all leukocytes lineages from resident hematopoietic stem cells. The portal tract of the liver contains many different cells of hematopoietic origin, as well as hematopoietic stem cells. The liver contains cells involved in adaptive and innate immunity; it plays a role in immunologic surveillance within the hepatic sinusoids (Mehal et al., 2001; Sheth and Bankey 2001).

- ***Storage function***

The liver stores certain vitamins and minerals, including iron and copper, in durations of excessive intake and releases them in durations of need. It can store vitamins B12 and D for several months and vitamin A for several years. Besides vitamins E and K. Iron is stored in the liver as ferritin, an iron protein complex and is released as needed for red blood cell production (Ozougwu, 2017).

- ***Biliary function***

The liver assists intestinal digestion by secreting 700 to 1200 mL of bile per day. Bile is an alkaline, bitter-tasting, yellowish green fluid that contains bile salts, cholesterol, bilirubin, electrolytes and water. It is formed by hepatocytes and secreted into the canaliculi (Ozougwu, 2017).

- ***Vascular and Hematologic Functions***

Because of its extensive vascular network, the liver can store a large volume of blood. The amount stored at any one duration depends on pressure relationships in the arteries and veins (Ozougwu, 2017).

- ***Detoxification function***

The adult liver is the main organ responsible for detoxifying and metabolizing a variety of exogenous and endogenous chemicals, foreign molecules, and hormones to make them less toxic or less biologically active (Butura Angelica, 2008).

I-4-Pathology of the liver

The majority of hepatic disorders are observed pathologically as hepatic cell necrosis, intra-hepatic or extra-hepatic bile duct obstruction (cholestasis), and hepatic atrophy and/or fibrosis. It can be induced by different factors such as viruses, alcohol, toxic bile acids, fatty acids, drugs, and immune response (Wang, 2014).

I-4-1-Alcoholic hepatitis

Alcoholic hepatitis is defined by a set of histological lesions characteristics, but not specific, of alcohol poisoning. Hepatitis lesions alcoholic predominate in the central lobular region and very often associate with other hepatic lesions induced by ethyl alcohol: steatosis, and cirrhosis, these three lesions are often associated (Lucey et al., 2018).

I-4-2-Drug-Induced Hepatitis

Drug-induced hepatic injury is the most frequent reason cited for the withdrawal from the market of an approved drug, and it also accounts for more than 50% of the cases of acute liver failure. The patient's age, sex, and body-mass index affect metabolism and, therefore, outcomes, as do the simultaneous use of other foods and drugs and physiologic changes such as pregnancy and renal or liver disease.

Drug mediated injuries may be prevented by screening methods that can identify aberrant gene polymorphisms or RNA-expression profiles before a patient uses a drug. Pharmacogenetic testing can identify unique cytochrome P-450 alleles that affect drug levels, particularly if the reaction involves multiple steps (Lee, 2003).

I-5-Liver damage

I-5-1-Fibrosis

Hepatic fibrosis is the result of the wound-healing response of the liver to repeated injury (figure 5). After an acute liver injury, parenchymal cells regenerate and replace the necrotic or apoptotic cells. This process is associated with an inflammatory response and a limited deposition of the extracellular matrix. If the hepatic injury persists, then eventually the liver regeneration fails, and hepatocytes are substituted with abundant extracellular matrix, including fibrillar collagen. The main causes of liver fibrosis are hepatitis C virus infection, alcohol abuse, and non-alcoholic steatohepatitis (NASH) (Schuppan and Afdhal, 2008*;* Bataller and Brenner, 2005

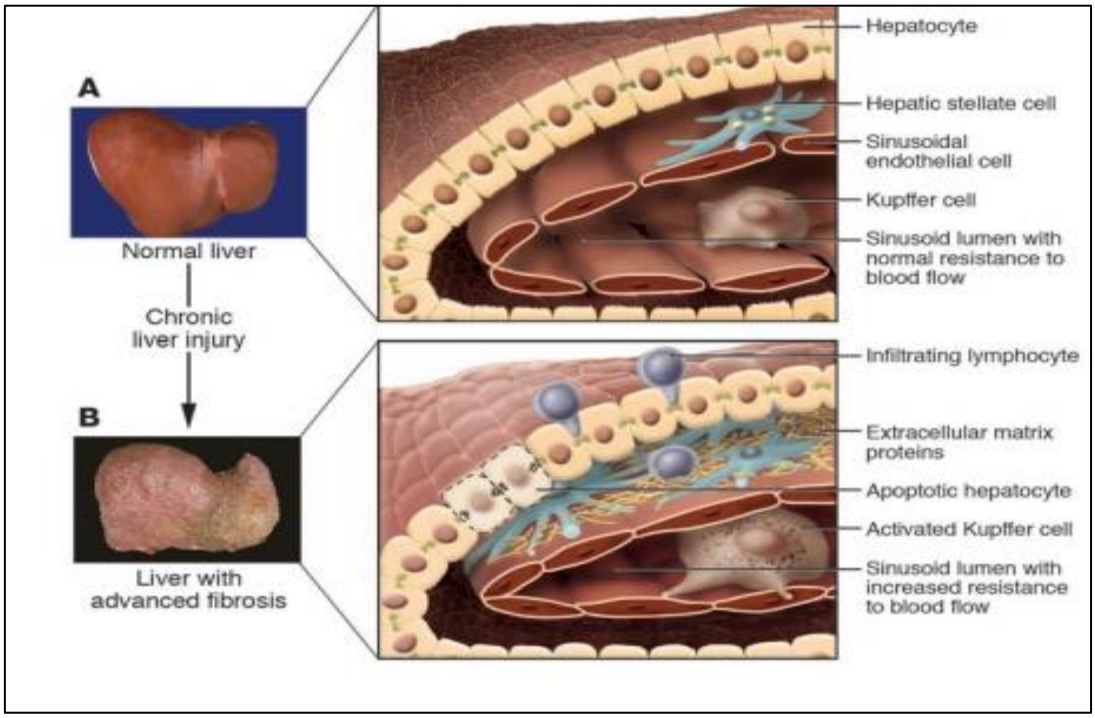


Figure 5: the difference between normal and advanced fibrosis liver (Bataller and Brenner, 2005)

I-5-2-Cirrohosis

Cirrhosis is an advanced stage of liver fibrosis that is accompanied by distortion of the hepatic vasculature. The resultant vascular distortion leads to shunting of the portal and arterial blood supply directly into the hepatic outflow (central veins), compromising exchange between hepatic sinusoids and the adjacent liver parenchyma, hepatocytes. Cirrhosis and its associated vascular distortion are traditionally regarded as irreversible but recent data suggest that cirrhosis regression or even reversal is possible (figure 6) (Schuppan and Afdhal, 2008).

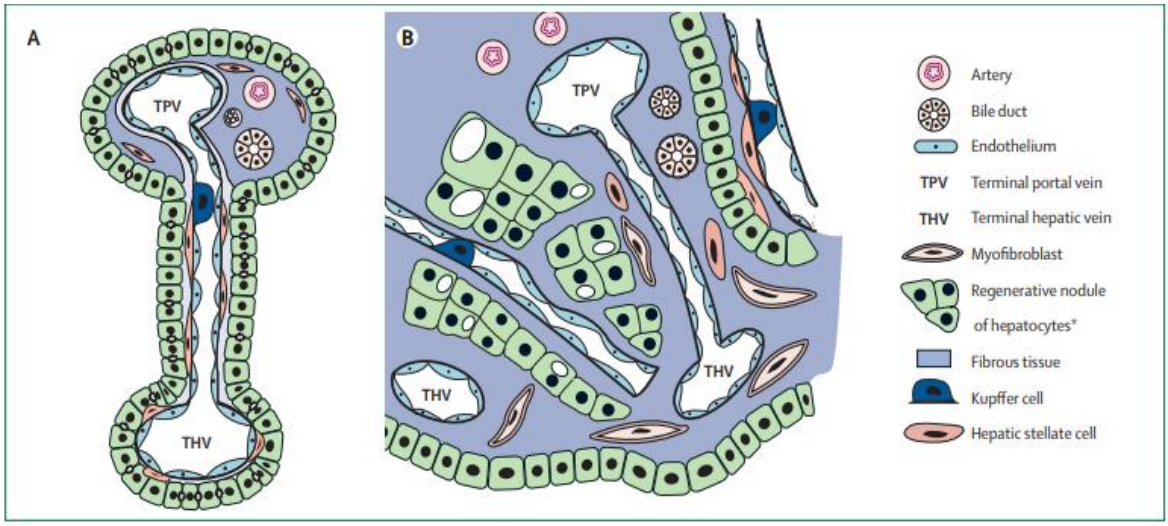


Figure 6: Vascular and architectural alterations in cirrhosis (Schuppan and Afdhal, 2008)
(A) Healthy liver:. (B) Cirrhotic liver

I-5-3-Carcinoma

Hepato-cellular carcinoma is one of the commonest solid organ tumours worldwide, and cirrhosis is a major risk factor for progression, among others its pathogenesis seems to arise from the development of regenerative nodules with small-cell dysplasia through to invasive hepato-cellular carcinoma. Mortality of hepato-cellular carcinoma associated with cirrhosis is rising in most developed countries, whereas mortality from cirrhosis not related to hepato-cellular carcinoma is decreasing (Schuppan and Afdhal, 2008).

I-5-4-Steatosis

Steatosis or non-alcoholic fatty liver disease (NAFLD) is commonly associated with abdominal obesity and metabolic disorders. It is a frequently observed histological lesion defined by the accumulation of fatty acids in the form of triglycerides in the cytoplasm of hepatocytes, most often resulting in large vacuoles pushing back the nucleus to the periphery. Liver steatosis contains more than 5% fat (Lemoine et al., 2012; Brunt, 2007).

I-5-5-Necrosis

Necrosis is a premature death of cells, pathologic process that is often caused by external factors, anti-apoptosis as a strategy is beneficial to liver repair response. Therapeutic options of liver disease depend on the understanding toward pathogenic mechanisms of different etiology (Wang, 2014).

I-5-6-Cholestasis

In cholestatic livers, bile cannot flow from the liver to the duodenum. Toxic bile acids are accumulated in liver or cholestasis. Basic distinctions include an obstructive type of cholestasis where there is a mechanical blockage in the duct system such as gallstone or malignancy, and metabolic types of cholestasis that there are disturbances in bile formation because of genetic defects or side effect of many medications (Wang, 2014).

1-6-Hepatotoxicity associated to drugs

Hepatotoxicity is damage caused by exposure to a drug or non-pharmacological agents. Risk factors include idiosyncrasy, age, gender, alcohol consumption, smoking, concomitant use

of other drugs, previous or underlying liver disease, genetic and environmental. Although most lipophilic drugs can cause hepatotoxicity, antibiotics, nonsteroidal anti-inflammatory drugs (NSAIDs) and anticonvulsants are the pharmacological groups which are the most frequent causes. Types of injuries are classified in:

- Hepatocellular damage is defined as isolated increases of alanine aminotransferase (ALT) to over two times the ULN or an ALT/alkaline phosphatase ratio greater than five. Hy's law defines this type of injury as ALT values greater than three times the ULN.
- Cholestatic damage is defined as isolated increases of alkaline phosphatase to over two times the ULN or a ratio of less than two.
- Mixed damage is defined as ALT and alkaline phosphatase over two times the ULN and a ratio greater tha two, but less than five.

Hepatotoxicity is related to mitochondrial dysfunction, inhibition of cellular respiration or alteration in β oxidation of fatty acids. These result in apoptosis, necrosis, autophagy and, therefore, cell death (figure 7) (Cano et al., 2017; Cano and Amariles, 2018).

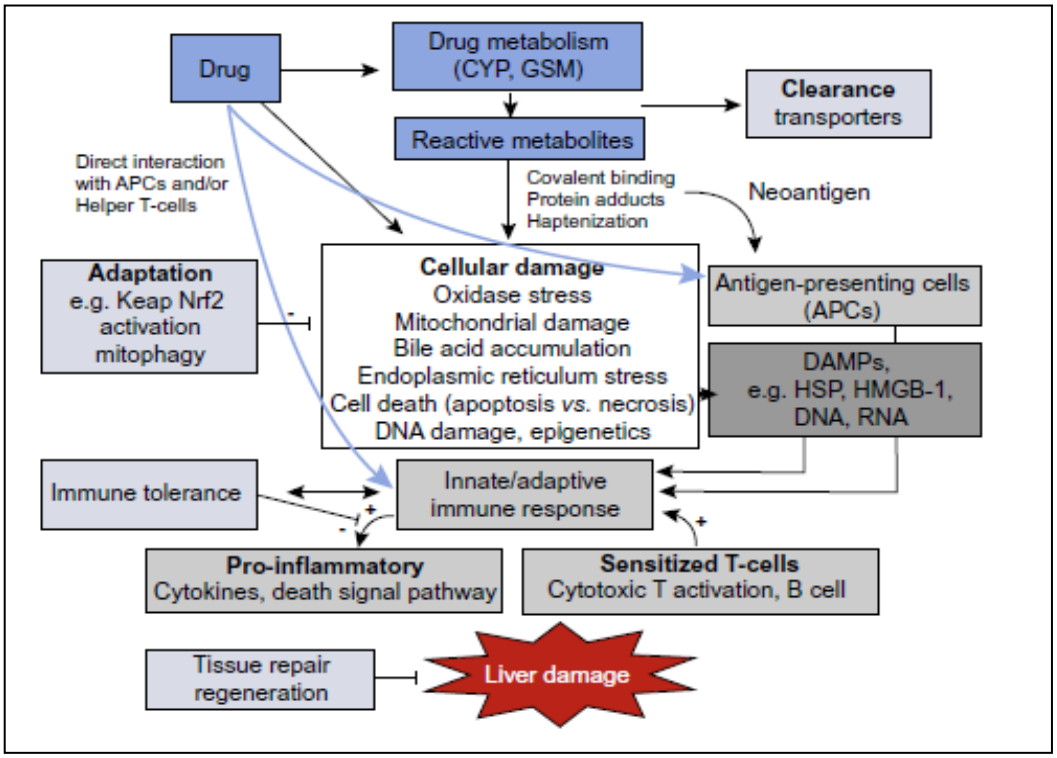


Figure 7: Current mechanistic understanding in the initiation and progression events relevant to idiosyncratic drug-induced liver injury (Chen et al., 2015).

II-Gentamicin: Model of hepatotoxicity

II-1-Aminoglycosides

Aminoglycoside antibiotics (AG) are used in infections treatment (such as ocular, pulmonary, and intestinal infections) produced by Gram-negative bacteria and bacterial endocarditis. Their cationic structure, which depends on the number of amino groups and on their distribution within the molecule, looks like it has an important role in their toxicity, mostly affecting renal (nephrotoxicity) and liver (hepatotoxicity) tissues in which they accumulate. In spite of their undesirable toxic effects, AGs still constitute the only effective therapeutic alternative against germs insensitive to other antibiotics because of synergy with betalactamic antibiotics, little resistance, and low cost (Vicente et al., 2010).

II-2-Gentamicin

Gentamicin (GM) has been discovered in 1963 by Marvin Weinstein's set at Schering Plough through isolated from various species *Micromonospora purpurea*, it is a commonly used as aminoglycoside antibiotic (Drea et al., 2017). Gentamicin is widely used for the treatment of various infectious diseases such as the treatment of a variety of susceptible bacterial infections in poultry, bovines, and equines (Majeed et al., 2018). GM is a powerful antibiotic produced as a mixture of three main components called gentamicin C_1, C_{1a}, and C_2. They differ slightly structurally, and display approximately the same antibiotic activity (figure 8) (Tangy et al., 1984).

The three components of gentamicin C bind to the 30S ribosomal subunit at the same binding site but with different affinities; gentamicin C1a binds to 30S subunits with slightly higher affinity than C2, whereas C1 binds with the lowest affinity compared the others (Yoshizawa et al., 1998).

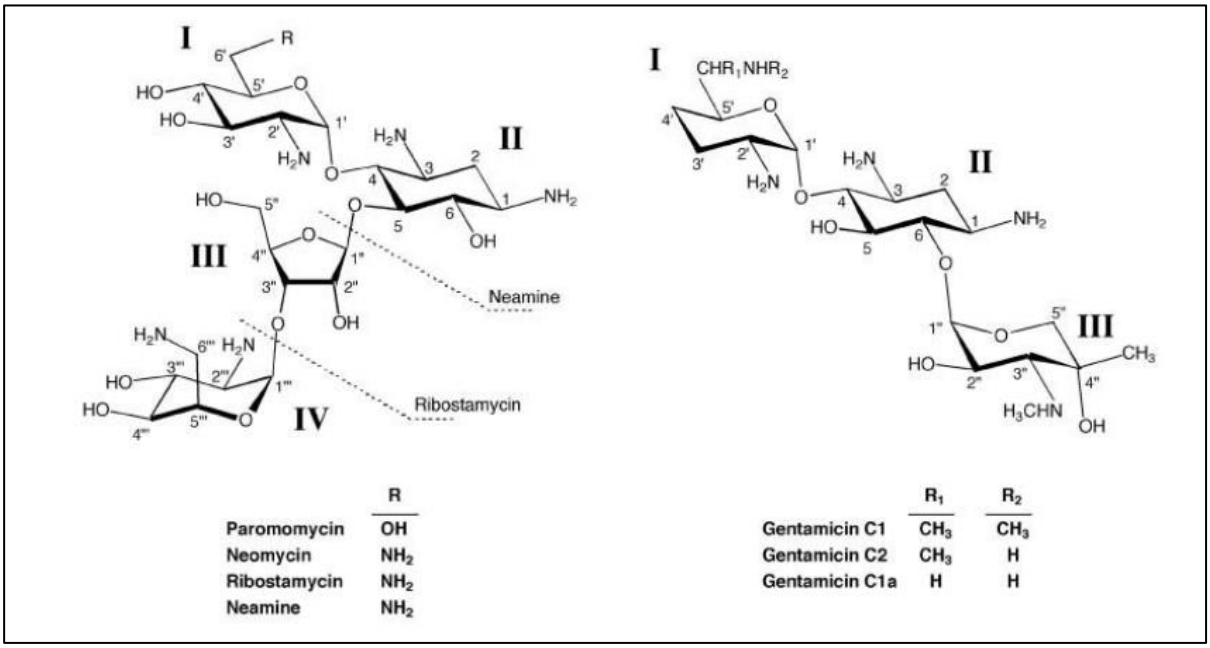


Figure 08: Structures of the aminoglycoside antibiotics that bind in the A site of 16S rRNA. Comparison of the gentamicin components (4–6 ring II–ring I, ring II–ring III linkages) and the neomycin group (4–5 ring II–ring I, ring II–ring III linkages) of aminoglycosides (Yoshizawa et al., 1998)

II-2-1-Gentamicin`s Pharmacokinetic

Gentamicin is rapidly absorbed after intramuscular injection and peak serum concentrations are usually reached within 30 to 90 minutes and are measurable for 6 to 8 hours. The serum half-life of GM is approximately 2 to 3 hours in adults with normal renal function (Ravindra et at, 1994; Gervaix et al., 2017).

The elimination of GM is an important pharmacokinetic consideration because in long term therapy the dose required to maintain a target steady-state serum concentration is determined by the clearance. More than 90% of the elimination of GM is accounted for by renal clearance, mostly via glomerular filtration. Many studies showed that the renal clearance and, therefore, the systemic clearance of gentamicin often declines with age (Triggs et al., 1999).

[2]*II-2-2-Mecanisme of action*

Once aminoglycosides have reached the cytoplasm, their major antibacterial activity is due to the inhibition of protein synthesis. Indeed, aminoglycosides introduce errors in the

sequence of newly elongated peptides and impact all four principal stages of the translation process (initiation, elongation, termination and recycling) (Prokhorova *et al*., 2017).

Gentamicin, an aminoglycoside antibiotic, is bactericidal. GM passes through the gram-negative membrane in an *Oxygen-dependent* active transport. As oxygen is required, this is why aminoglycosides are not effective in anaerobic bacteria. Once in the cytoplasm, GM bind to the 16s rRNA at the 30s ribosomal subunit, disturbing the translation of mRNA and, thus, leading to the formation of truncated or nonfunctional proteins. The mechanism of the bactericidal activity of GM has not been fully elucidated yet, but some propose that truncated proteins are placed at the cell wall, compromising its impermeability, while others also suggest that accumulation of reactive oxygen species, as a consequence of depletion of proteins involved with oxidation-reduction reactions, may lead to bacterial death. Gentamicin, like all aminoglycosides, exhibit concentration-dependent killing. Higher concentrations correlate with greater antimicrobial killing (Pacifici, 2019; Chaves and Tadi, 2021).

II-2-3-Nephrotoxicity

A central aspect of aminoglycoside nephrotoxicity is their tubular cytotoxicity. Treatment of experimental animals with gentamicin results in apoptosis as well as necrosis of tubular epithelial cells. In culture, gentamicin also causes both apoptosis and necrosis of these cells. The phenotype of death might depend on the concentration of the drug, as with other cytotoxic compounds such as cisplatin and H_2O_2. It might also depend on the concurrence of other triggering or predisposing factors, such as the degree of ischemia, on specific points of the renal parenchyma. Apoptosis is an ATP-requiring process (figure 9) (Vicente et al., 2011)

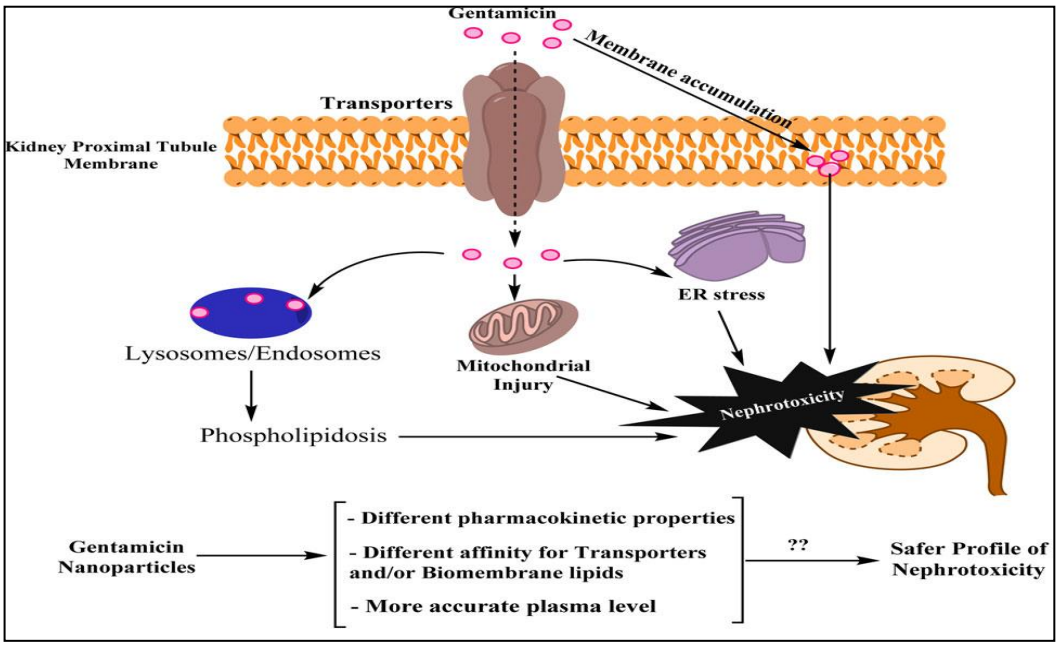


Figure 09: Mechanisms of nephrotoxicity induced by gentamicin as reported in previous investigations. The proposed reasons for safer profile of nephrotoxicity of gentamicin nanoparticles in current investigation are given. ER: endoplasmic reticulum (Jamshidzadeh et al., 2014)

II-2-4-Hepatotoxicity

The pathogenesis of gentamicin-induced liver toxicity is multi-factorial and its mechanism is not fully understood. Among these, oxidative stress is suggested to be a major cause of hepatotoxicity.

Nuclear factor erythroid-2-related factor-2 (Nrf2) is a mark of oxidative stress and functions. In response to reactive oxygen species (ROS), cytosolic Nrf2 is dissociated from Keap1 and translocated into the nucleus leading to the activation of detoxifying enzymes and antioxidant proteins such as glutathione S-transferase (GST), heme-oxygenase 1 (HO-1), NAD(P)H, quinone reductase (NQO1) and glutathione (GSH). Also, oxidative stress leads to the activation of transcription factor like nuclear factor-kB (NF-kB), it plays a major role in the regulation of genes included in inflammatory process like the inducible nitric oxide synthase (iNOS), cycloxygenase-2 (COX2), interleukin-1b (IL-1b) and tumor necrosis factor-a (TNF-a). Excessive ROS production could lead to cell death through the modulation of Bcl2 proteins and caspases (figure 10) (Kahn et al.,2004).

- Gentamicine induce inflamation and apoptosis of liver cells by increasing NF-KB, TNFαR1, COX2 (Phatchawan et al., 2017).
- Treatment with gentamicin damages liver tissues, the intraperitoneal administration of gentamicin produced a significant elevation of AST, ALT and LDH activities (Mahmoud et al., 2014) and also increased serum levels of markers of liver function such as total lipids, phospholipids, triglycerides and total bilirubin (Aziz et al., 2017).
- Gentamicin induced hepatotoxicity, it induces an increase in the oxidative stress and produce free radicals and supresses the non-enzymatic and enzymatic antioxidants in liver, which results in an excess production of reactive oxygen species which affects membrane lipids and deteriorates proteins and nucleic acids. This leads to damage, disfunction and liver toxicity (Elsayed et al., 2016).
- Gentamicin is involved in the formation of free radicals. Mechanism of gentamicin toxicity is based on the formation of cytotoxic metabolite. Gentamicin causes irregular and condensed nuclear materials, organelles deteriorations and Poly (ADP-ribose) polymerase fragmentation and induce apoptosis in hepatic cells (Shrivastava and Mishra, 2018).

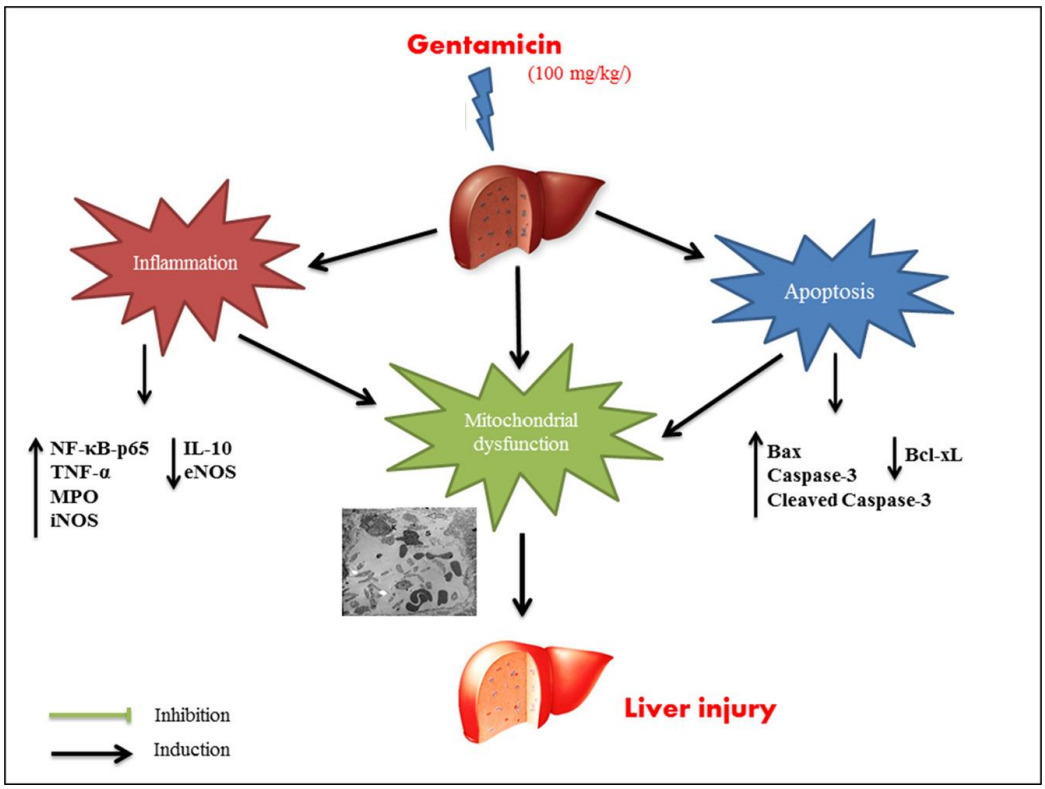


Figure 10: Hepatotoxicity action of Gentamicin (Ali et al., 2020)

III-Polyphenols

Thousands of years ago, plants were the oldest form of medication in traditional medicine in countries around the world used to treat several diseases, and play a central role in human health (Marrelli, 2021). They have started to consider an essential source in treating/preventing a various kind of disease (Haider Mohammed, 2020). Each plant consists of several important ingredients that can be used in medical field, and can be involved in the development of different kind of drugs (Yuan et al., 2016).

Secondary metabolites (SMs) are natural products synthesized mainly by bacteria, fungi and plants. They are molecules of low molecular weight with diverse chemical structures and biological activities (Mosunova and Collemare, 2020). Secondary metabolites are useful natural products that are synthesized through secondary metabolism in plants. The production of some secondary metabolites is linked to the induction of morphological differentiation and appears as cells undergo morphological differentiation and maturation during plant growth. Plant secondary metabolites are important candidates for human nutrition. A number of plant secondary metabolites possess antioxidant properties that act as the first line of defense against oxidative damage induced by different environmental constraints (Bhattacharya, 2019).

Several criteria have been considered for the classification of SM: chemical structure (presence of rings or sugars), composition (containing nitrogen or not), their solubility inorganic solvents or water, and the biosynthetic pathway, the SM in plants can be divided into three large groups: terpenes, phenolic compounds, and alkaloids (Francesca et al., 2019).

- **Terpenes:** are among the most widespread chemically diverse groups of natural products. Terpenes are a unique group of hydrocarbons based natural products whose structures may be derived from isoprene. Terpenes are classified by the number of 5-carbon units. Many of them have pharmacological activity and are used for diseases treatment both in humans and animals (Justin et al., 2014; Tiwari and Rana, 2015).
- **Phenolics:** one of largest group of secondary plants constituents synthesized by fruits, vegetables possessing one or more aromatic rings with one or more hydroxyl groups. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins. Plant phenolics are generally involved in defense against ultraviolet radiation or

aggression by pathogens, parasites and predators, as well as contributing to plants' colors (Dai and Russell, 2010).

- **Alkaloids**: are a large and complex group of cyclic compounds that contain nitrogen. About 2,000 different alkaloids have been isolated, many of alkaloids have biological activity, and some of them are being used as drugs in modern medicine (e.g. morphin, codeine, reserpine, etc.) (Pallardy, 2008).

III-1-Structure of polyphenolic compounds

Phenolic compounds are categorized as flavonoids and non-flavonoids:

- ***Non-flavonoids:***

Compounds with smaller and simpler chemical structures than flavonoids belong to this class. However, there are also non-flavonoids with complex structures and high molar mass. Phenolic acids, coumarins, stilbenes and lignans constitute mainly this group.

- ***Flavonoids:***

Flavonoids constitute the major group of phenolic compounds; they have a C6-C3-C6 skeleton with two aromatic rings connected by a three-carbon link. Their antioxidant activity depends on the presence, number and position of hydroxyl groups in the chemical structure of these compounds. Flavonoids can be divided into subclasses: flavones, isoflavones, flavonols, anthocyanins, proanthocyanidines flavanols and flavanones (Rodríguez et al., 2020).

III-1-1-non-flavonoid polyphenolic compounds

III-1-1-1-Phenolic acid

Phenolic acid is a large family of natural compounds that are widely distributed in plants, with properties that are very important and useful for both plant producers and human health, it's a very strong antioxidant and has antibacterial activity, antivirus, anti-inflammatory and anticancer (Khasan et al., 2019).

Two types of phenolic acids can be distinguished as plant metabolites: hydroxybenzoic acids and hydroxycinnamic acids. Although the basic skeleton is the same, the numbers and positions of the hydroxyl groups on the aromatic rings establish the variety of phenolic acids that can be found in different foods (Herrero and Ibáñez, 2012).

III-1-1-2-Hydroxybenzoic Acids

Hydroxybenzoic acids have a general structure of the C6–C1 type derived directly from benzoic acid. Variations in structure lie in the hydroxylations and methoxylations of the aromatic cycle (Murkovic, 2003).

The salicylic acid, vanillic acid, protocatechuic acid, gallic acid, benzoic acid and ellagic acid categorized under the sub-category of hydroxybenzoic (figure 11) **(**Prabhu et al., 2021).

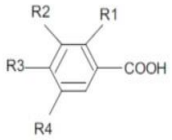
Structure	R1	R2	R3	R4	Acides phénoliques
	H	H	H	H	Acide benzoïque
	H	H	OH	H	Acide p hydroxy benzoïque
	H	OH	OH	H	Acide protocatechique
	H	OCH3	OH	H	Acide vanillique
	H	OH	OH	OH	Acide gallique
	H	OCH3	OH	OCH3	Acide syringique
	OH	H	H	H	Acide salicylique
	OH	H	H	OH	Acide gentisique

Figure 11: Basic structure and main hydroxybenzoic acids (Sarni and Cheynier, 2006).

III-1-1-3-Hydroxycinnamic acids

Hydroxycinnamic acids (HCAs) possess a simple chemical backbone consisting of a phenylpropanoid C6-C3 structure and are the major subgroup of phenolic acids with ubiquitous distribution in the plant kingdom. Caffeic acid, ferulic acid, coumaric acid, sinapinic acid, cinnamic acid are a common example of hydroxycinnamic acid. They exist in all fruit parts, but concentrations are higher in the outer parts of ripe fruit. Because of their wide distribution and high concentration provide them with a key role in the biosynthesis of more complicated phenolic systems (figure 12) (Teixeira et al., 2013; Prabhu et al., 2021).

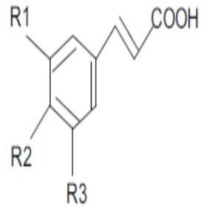
Structure	R1	R2	R3	Acides phénoliques
	H	H	H	Acide cinnamique
	H	OH	H	Acide p coumarique
	OH	OH	H	Acide caféique
	OCH3	OH	H	Acide férulique
	OCH3	OH	OCH3	Acide sinapique

Figure 12: Basic structure and main hydroxycinnamic acids (Sarni and Cheynier, 2006).

III-1-1-4-Stilbenes

Stilbenes are part of a very large group of polyphenols, that of the derivatives of cinnamic acid (phenylpropanoids) they have classical C6–C2–C6 structures with two hydroxyl groups on the A ring and one on the B ring and they exist in plants as aglycones or glycosides, providing protection against bacterial, mold, or fungal invasion. Examples of stilbenes are the phytoalexins resveratrol and piceatannol, a resveratrol metabolite (figure 13) (Martinez and McIntosh, 2017).

Stilbenes have an extraordinary potential for the prevention and treatment of different diseases, including cancer, due to their antioxidant, cell death activation, and anti-inflammatory properties which associate with low toxicity under *in vivo* conditions (Sirerol et al., 2015).

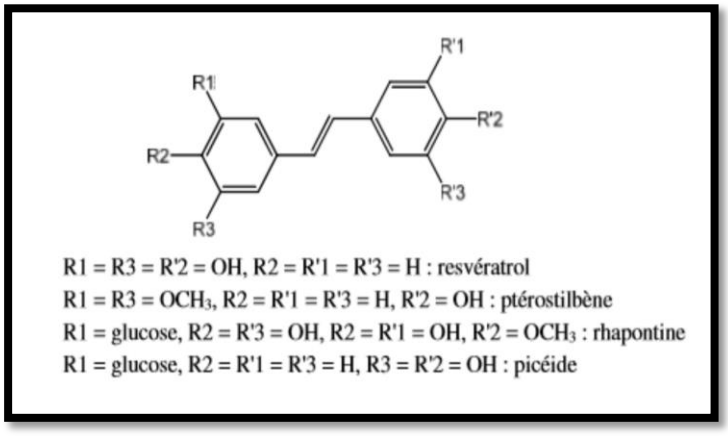


Figure 13: Basic structure of stilbenes and its main types (Perret, 2001).

III-1-1-5-Lignans

The chemistry of lignans has been studied for the past 65 years. They possess very different structural composition and can be found in nature in different parts of the plant, although they can be found in fiber rich foods such as grains, nuts, seeds, vegetables, and drinks such as tea, coffee (figure 14) (Zanellaa et al., 2016).

Lignans are considered important in cancer prevention and treatment, besides other beneficial health effects, e.g., antioxidant, anticarcinogenic, antimutagenic, and anti-estrogenic effects (Teodor et al., 2020).

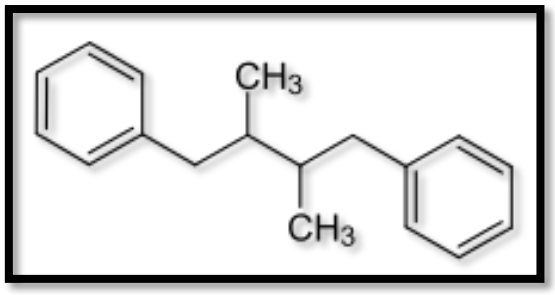


Figure 14: Basic structure of Lignan (Jost and Jost - Tse, 2016).

III-1-1-6-Coumarins

Coumarins are present in different plant organs including leaves, fruits, flowers and roots, but also in the exudates of plants roots. These compounds are biosynthesized from phenylalanine via the shikimic acid and they are generally unsaturated lactones and comprise another class of compounds C6C3 (figure15) (Matos et al., 2015; Stringlis et al., 2019).

Coumarins have been recognized for many years as an important class of pharmacologically active compounds and anti-inflammatory properties, it was shown recently in numerous studies that coumarins play an important role in iron (Fe) homeostasis, oxidative stress response (Siwinska et al., 2021).

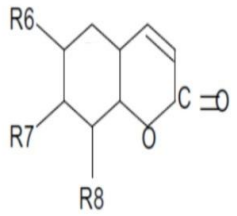
Structure	R6	R7	R8	Acides phénoliques
	H	OH	H	Umbelliférol
	OH	OH	H	Aescultol
	OCH3	OH	H	Scopolétol
	OCH3	OH	OH	Fraxétol
	H	OH	OH	Daphnétol

Figure 15: Basic structure of coumarin and its main type (Macheix et al., 2005).

III-1-1-7-Xanthones

Xanthones are one of the biggest classes of compounds in natural product chemistry. A number of xanthones have been isolated from natural sources of higher plants, fungi, ferns, and lichens (Negi et al., 2013).

Xanthones skeleton is a planar, conjugated ring system composed of carbon 1-4 (aromatic ring A) and carbon 5-8 (aromatic ring B) (figure 16) (Niaz, 2020),

Xanthones constitute an important class of biologically active heterocycles, their synthesis has drawn great attention in the field of medicinal and pharmaceutical chemistry. They possess antiviral, anti-inflammatory antibacterial, antioxidant activities (Bedi et al., 2017).

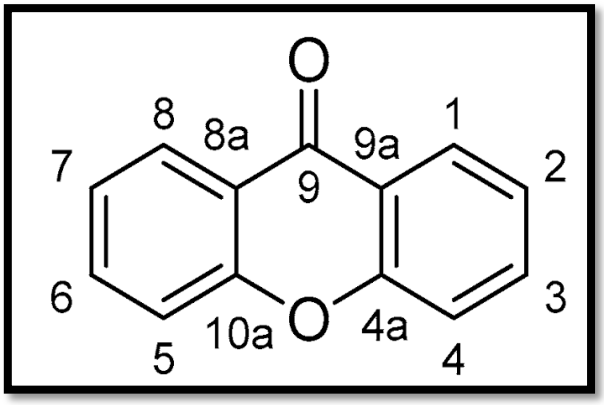


Figure 16: Basic structure of Xanthone (Feng et al., 2020).

III-1-2-Flavonoid polyhenolic compounds

Flavonoids are an important class of natural products; particularly, they belong to a class of plant secondary metabolites having a polyphenolic structure, widely found in fruits, vegetables and certain beverages **(**Panche et al., 2016). The basic chemical structure of flavonoid is a skeleton of diphenyl propane, contain fifteen carbon atoms in their primary nucleus: two six-membered rings linked with a three-carbon unit which may or may not be a part of a third ring. Mainly two benzene rings (ring A and B) are linked together through third heterocyclic oxygen-containing pyrene ring. So, this structure is also referred to as C6-C3-C6 labeled A, B, and C (figure 17) (Karak, 2019).

They have several important functions in plants, such as providing protection against harmful UV radiation or plant pigmentation. In addition, they have antioxidant, antiviral and antibacterial properties. They also regulate gene expression and modulate enzymatic action. The major sub-groups of flavonoids include flavone, flavonol, flavanone, flavanonol, anthocyanidin, flavanol and isoflavone (Kozłowska and Węgierek, 2016; Shan Ku et al., 2020).

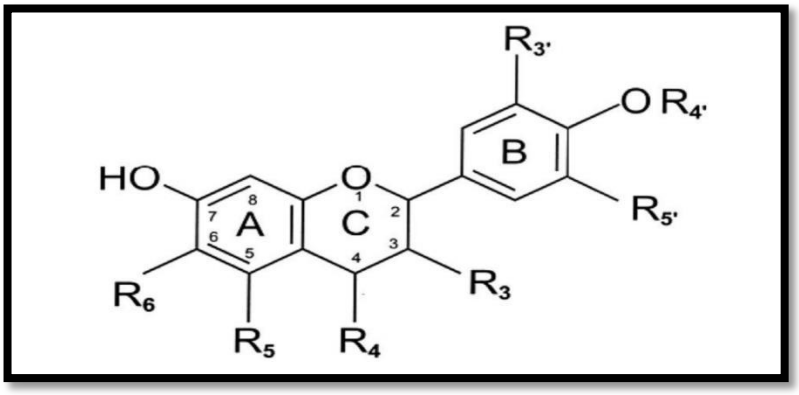
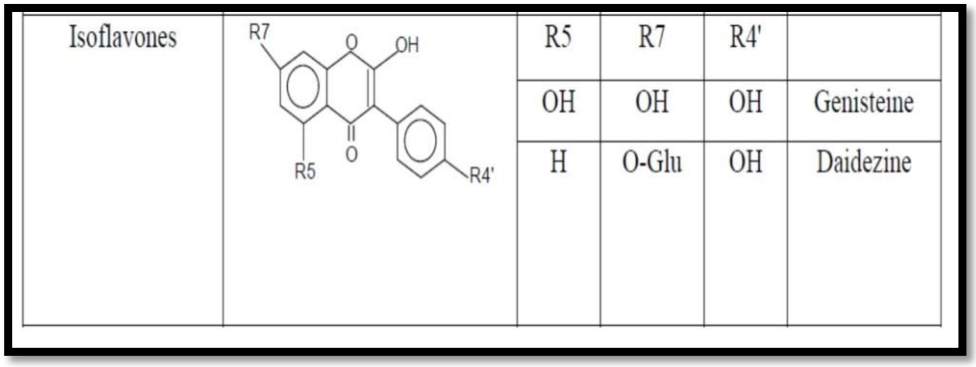


Figure 17: Basic structure of flavonoids (Panche et al., 2016).

III-1-2-1-Isoflavones

Isoflavones are polyphenolic plant-derived compounds acting as phytoestrogens due to their structural similarity to 17-β-estradiol (Bernatoniene et al., 2021), hydroxyl groups in the C_7 and C_4 positions, like estradiol molecule. Isoflavones can bind to estrogen receptors. They are contained almost exclusively in leguminous plants (figure 18) (Pérez-Chabela and Hernández-Alcántara, 2018).

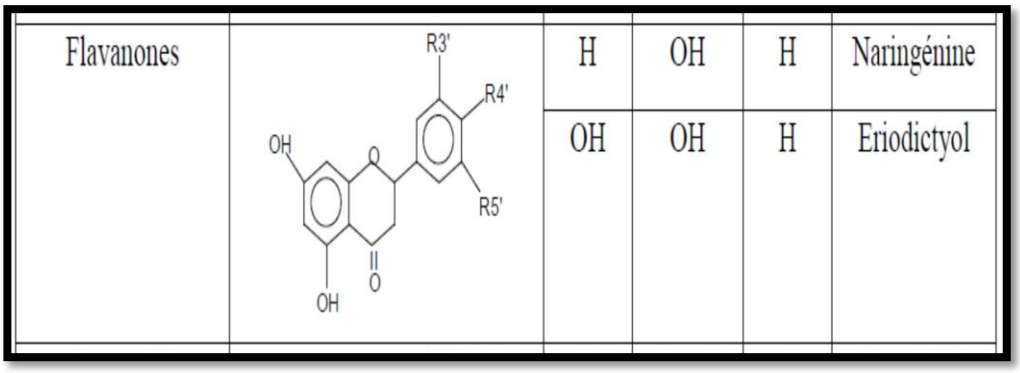


R5	R7	R4'	
OH	OH	OH	Genisteine
H	O-Glu	OH	Daidezine

Figure 18: Basic structure of Isoflavones and its main type (Narayana et al., 2001).

III-1-2-2-Flavanones

Flavanones are widely distributed in about 42 higher plant families they can be found in all plant parts, above and below ground, from vegetative part to generative organs: stem, branches, bark, flowers, leaves, roots (Khan et al., 2014). The main directions of the pharmacological activity of flavanones are: radical scavenging, anti-inflammatory, anticancer, cardiovascular, and antiviral effects (figure 19) (Małgorzata Brodowska, 2017).



H	OH	H	Naringénine
OH	OH	H	Eriodictyol

Figure 19: Basic structure of Flavanones and its main type (Narayana et al., 2001).

III-1-2-3-Flavonols

Flavonols are a category of flavonoids family, comprised of double-bond between C2-C3, and carbonyl C4 they share a similar structure with flavones, except that there is an extra hydroxyl group substituted at C-3 they are commonly found in fruits and vegetables, such as berries, grapes, tomatoes, onions (figure 20) (Shan Ku et al., 2020).

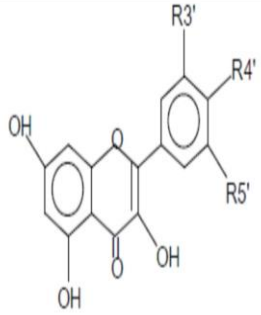
Flavonols		H	OH	H	Kaempférol
		OH	OH	H	Quercétine
		OH	OH	OH	Myrecétine

Figure 20: Basic structure of Flavonols and its main type (Narayana et al., 2001).

III-1-2-4-Flavones

Flavones define one of the largest subgroups. Their natural distribution is demonstrated for almost all plant tissues. Various flavone aglyca and their O- or C-glycosides have been described in the literature. The diverse functions of flavones in plants as well as their various roles in the interaction with other organisms offer many potential applications, not only in plant breeding but also in ecology, agriculture and human nutrition and pharmacology (figure 21) (Martens and Mithöfer, 2005).

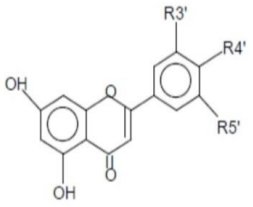
Flavones		H	OH	H	Apigénine
		OH	OH	H	Lutéoline
		OH	OCH3	H	Diosmétine

Figure 21: Basic structure of Flavones and its main type (Narayana et al., 2001).

III-1-2-5-Anthocyanins

Anthocyanins (Greek anthos: flower and kyaneos: dark blue) are coloured water-soluble pigments representing one of the major subclasses of compounds. They rarely exist in nature as free aglycons, instead, they attach to one or more sugar moieties. Anthocyanins are found within different plant organs: flowers, leaves, fruits, roots, tubers and grains. They appear in different attractive colors depending on their structure, pH, and other factors (Nassour et al., 2020).

The basic chemical structure is flavylium cation (2-phenyl-1-benzopyrilium), which links hydroxyl (-OH) and/or methoxyl (-OCH_3) groups, and one or more sugars.

The sugar-free molecule is called anthocyanidins**.** In addition, anthocyanins have dietary importance for human health and reported encouraging results in the treatment of different syndromes like cancer and other cardiac diseases (figure 22) (Tazzini, 2014; Pervaiz et al., 2017).

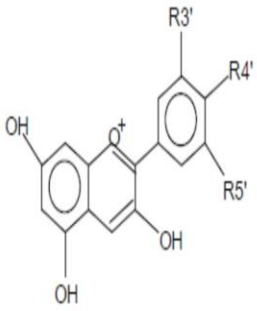
Anthocyanidines					
		H	OH	H	Pelargonidine
		OH	OH	H	Cyanidine
		OH	OH	OH	Delphénidine

Figure 22: Basic structure of Anthocyanins and its main type (Narayana et al., 2001).

III-2-Biological properties of polyphenols

Polyphenols represent one of the most well-known secondary plant metabolites characterized by a large and diverse array of unique bioactive properties, which makes them highly appreciated for their beneficial effects on both plants and humans (Cvitanović et al., 2018).

The biological properties of polyphenols include anticancer, antioxidant and anti-inflammatory effects. Polyphenols possess anti-microbial and anti-cariogenic properties and is an important source as anti-infective agents against antibiotic-resistant human pathogens. As antioxidants, polyphenols are the most abundant in man diet. Dietary intake requirement is 1 g/d that can be achieved by consuming a wide array of plant foods. Cereals, vegetables, dry legumes and chocolate contribute to the intake of polyphenols (Abdel-shafy and Mansour, 2017).

III-2-Oxidative stress

Oxidative stress is defined as an imbalance between production of free radicals and reactive metabolites, so-called oxidants or reactive oxygen species (ROS), and their elimination by protective mechanisms, referred to as antioxidants. This imbalance leads to damage of important biomolecules and cells, with potential impact on the whole organism. Oxidative stress can damage lipids, proteins, carbohydrates and DNA in cells and tissues (figure 23) (Vladimir et al., 2014).

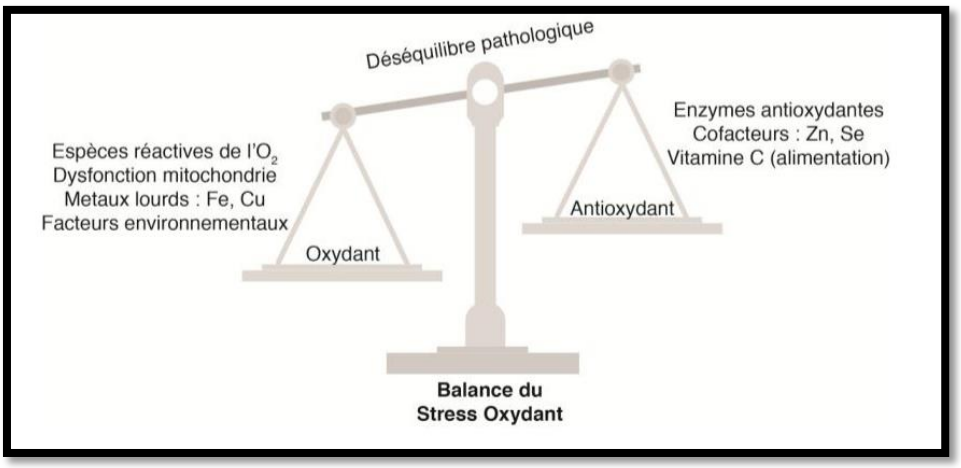


Figure 23: Antioxidant / Oxidant Imbalance (Ndong, 2019).

Free radicals are the products of normal cellular metabolism. A free radical can be defined as an atom or molecule containing one or more unpaired electrons in valency shell or outer orbit and is capable of independent existence. The odd number of electrons of a free radical makes it unstable, short lived and highly reactive. Because of their high reactivity, they can abstract electrons from other compounds to attain stability. Thus, the attacked molecule loses its electron and becomes a free radical itself, beginning a chain reaction cascade which finally damages the living cell (Phaniendra et al., 2014).

Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. They are found in many foods, including fruits and vegetables. Multiple types of antioxidants, such as glutathione, vitamin C, vitamin A, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxides. Traditional herbal medicines, dietary foods were the main source of antioxidant for ancient peoples that protected them from the damage caused by free radicals (Yadav et al., 2016).

Antioxidants may protect cells from the damage caused by unstable molecules known as free radicals. Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause. Free radical damage may lead to cancer (Hamid et al., 2010).

Antioxidants are grouped into two namely;

- ***Natural Antioxidants:***

They are the chain breaking antioxidants which react with lipid radicals and convert them into more stable products; they can be further divided into two categories, i.e., enzymatic antioxidants and nonenzymatic antioxidants.

- Enzymatic antioxidants are uniquely produced in the human body include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)
- Nonenzymatic Antioxidants they are a class of the antioxidants which are not found in the body naturally but are required to be supplemented for the proper metabolism include the following:
  - ✓ Antioxidants minerals: These are co factor of antioxidants enzymes. Their absence will definitely affect metabolism of many macromolecules such as carbohydrates. Examples include : selenium, copper, iron, zinc and manganese.
  - ✓ Antioxidants vitamins: It is needed for most body metabolic functions. They include- vitamin C, vitamin E, vitamin B.
  - ✓ Phytochemicals: These are phenolic compounds that are neither vitamins nor minerals. These include: Flavonoids (Kshipra Misra et al., 2014).

➢ ***Synthetic Antioxidants :***

These are phenolic compounds that perform the function of capturing free radicals and stopping the chain reactions, the compounds include:

- ➢ Butylated hydroxyl anisole (BHA)
- ➢ Butylated hydroxyrotoluene (BHT)
- ➢ Propyl gallate (PG) and metal chelating agent (EDTA)
- ➢ Tertiary butyl hydroquinone (TBHQ)
- ➢ Nordihydro guaretic acid (NDGA) (Hamid et al., 2010)

III-2-1-Polyphenols as antioxidants

Phenolic compounds are able to act as antioxidants which can neutralize free radicals by donating an electron or a hydrogen atom (Lee, 2009). They are classified according to their mode of action: free radical eliminators, metal ion chelators, oxygen scavengers in closed systems (figure 24) (Chew et al., 2009).

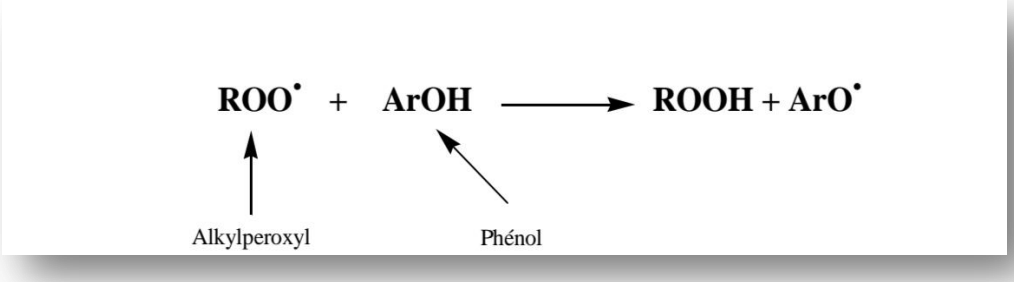


Figure 24: Mechanism of action of polyphenols: donation hydrogen (Di Meo et al., 2013)

III-2-1-1-Polyphenols scavenge free radicals

Polyphenols scavenge free radicals by H-atom transfer and may thus decrease noxious effects due to oxidative stress. Free radical scavenging by polyphenols has been widely theoretically studied from the thermodynamic point of view whereas the kinetic point of view has been much less addressed (Di Meo et al., 2013).

Polyphenols are known to cure certain disease conditions by their ability to scavenge the active free radical's species. A number of methods have been used to determine the effectiveness of the scavenging activity of a particular plant or food. DPPH was claimed to be the most widely and efficient method for the measurement of scavenging activities of polyphenols due to it is simplicity, accuracy inscavenging activities (figure 25) **(**Yunusa et al., 2015).

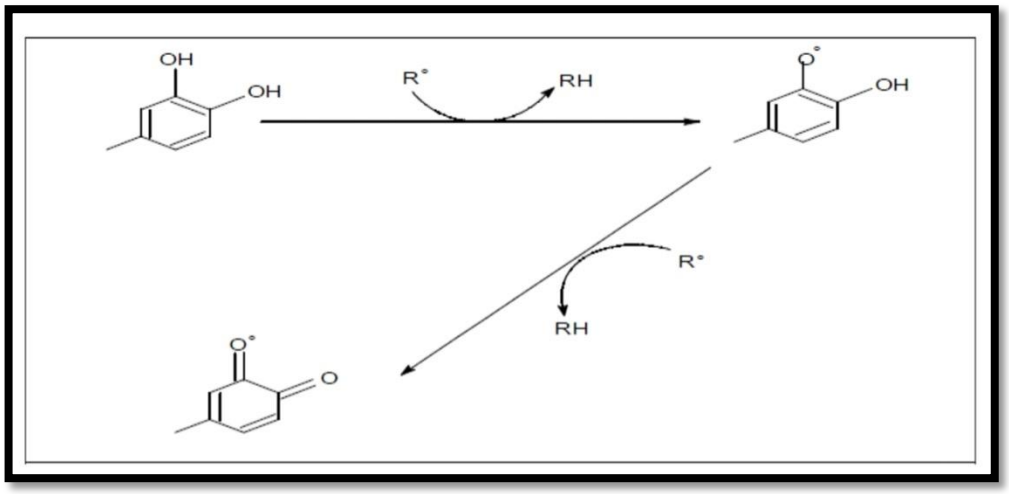


Figure 25: Trapping of reactive oxygen-derived species (R •) byflavonoids and the formation of a stable structure (Tiqwari, 2001)

III-2-1-2-Metal Chelation of Polyphenols

Iron and copper are biologically important metals involved innumerous phenomena of transport (oxygen) and electron transfer. In general, highly complexed with transport or storage and enzymes they could exist in "free" (weakly complexed) form in trace amounts and then participate in "oxidative stress", by production of toxic oxygen radicals (Achat, 2014).

Several studies have shown that the reducing properties of polyphenols and their ability to form stable complexes with transition metals, especially iron and copper, can modulate various biologically important processes involving the redox state of the metal ion. The

interactions of copper and iron ions with polyphenols, and more particularly with flavonoids, are often proposed as one of the antioxidant action mechanisms of these natural products (Achat, 2014).

Flavonoids exert anti-oxidant activity through interactions with the reduced form of transition metals, primarily Fe(II), Fe(III) and Cu(I), which participate in reactions generating free radicals. Flavonoids may sequester metal ions by chelating and preventing metal-mediated generation of free radicals and, accordingly, may protect the potential biological targets from oxidative stress (figure 26) (Malešev and Kunti, 2007).

Flavonoids have several potential sites of metal complexation:

- 5-hydroxyflavones can chelate metal ions thanks to the 5- grouphydroxy-4-oxo to give a six-center chelate.
- The 3-hydroxyflavones form with the metal ions a complex with five centers thanks to the 3-hydroxy-4-oxo group.
- 3'4'-dihydroxyflavones chelate metal ions through their ortho-dihydroxybenzene (catechol) group present on ring B (Heim et al., 2002; Pietta, 2000).

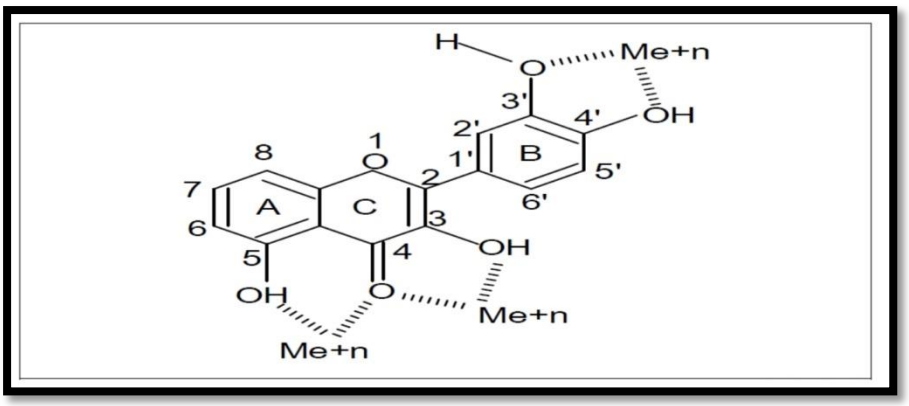


Figure 26: Main sites involved in the chelation of metal ions (Me^{+n}) (Tiqwari, 2001)

VI- *Ephedra alata* subsp. *Alenda*

Ephedra alata, commonly known in Algeria as" alenda", belongs to the *Ephedraceae* family containing more than 60 species of nonflowering seed plants, with light green densely branched dioecious small and perennial stiff shrub about 50–100 cm tall. This species is mainly distributed in arid environments, often near shifting sand dunes of Iran, Algeria, Iraq, Chad, Egypt, Palestine, Lebanon, Jordan, Saudi Arabia, Morocco, Syria, Libya, Mauritania, Mali, Somalia, and Tunisia, where it grows wildly on the gravely rocky, sandy, and clay soil. The decoction of *E. alata* stem is used in folk medicine as a potential stimulant, to treat different disorders (e.g., kidney, bronchi, circular system, digestive system disorders), to relieve asthma

attack, and as antifungal. The plant stems are usually chewed to treat bacterial and fungal infections (Soua et al., 2020; Jaradat et al., 2021). The *in vitro* and *in vivo* pharmacological studies on the crude extracts, fractions and few isolated compounds of *Ephedra* species showed anti-inflammatory, anticancer, antibacterial, antioxidant, hepatoprotective, anti-obesity, antiviral and diuretic activities (Sioud et al., 2021).

- ***Systematic position***

Branch Spermaphytes
Sub-branch Gymnospermes
Class Gnetopsida
Order Ephedrales
Family Ephedracrae
Genus *Ephedra*
Species *alata*

Materials and Methods

I- Materials and methods

I-1- Plant material

The plant *Ephedra alata* subsp. *Alenda* was bought from an herbalist, we used the aerial part. The plant material was dried in the dark and protected from moisture and at room temperature, the plant is crushed and stored carefully after drying.

Figure 27 : Ephedra alata

I-1-1-Extraction

The crude hydro-methanolic extract is prepared by cold maceration of 100 g of plant material in methanol-water (70:30 V/V) at room temperature for 48 hours. This operation is repeated three times, with renewal of the solvent every 48 hours.

After filtration, the filtrates are combined and then concentrated under vacuum at 45 ° C. using a rotary evaporator. The dry residue obtained is weighed and then stored in a dark flask for subsequent studies.

I-2- Experimental animals

The twenty-four male rats of *Wistar Albino* rats (212-293g) used for this study were obtained from the animal facility of the Department of Animal Biology, Faculty of Natural Sciences and life, University of the Brothers Mentouri Constantine. They were kept under natural light/dark cycle (12 hours light and dark) at 27°C and had access to standard rodent pellet diet and water ad libitum. They were left to acclimatize in the laboratory for 2 weeks before the commencement of the study.

Figure 28 : Experimental animals

I-2-1- Gentamicin-induced hepatotoxicity

To perform the hepatotoxicity model, we used gentamicin dissolved in 0.9% sodium chloride solution and administered by intra-peritoneal injection with a dose of 80 mg/kg (a volume of 2 ml / kg), once daily for 10 days.

For the controls (group I and II), an equivalent volume of 0.9% sodium chloride was administered intra-peritoneally.

I-2-2-Treatment of animals

All the rats were divided into four groups of 6 rats each:

Group I (T): Control group received distilled water daily by gavage and 1 hour later, physiological water intra-peritoneally for a period of 10 days.

Group II (EXT): Received each day by gavage 100 mg/kg of the extract and physiological water intra-peritoneally (from the 4th day).

Group III (GM): Gentamicin: received intra-peritoneally, 80 mg/kg of gentamicin for a period of 10 days.

Group VI (GM + EXT): Gentamicin + Plant Extract; received 100 mg/kg of the extract daily by gavage and 1 hour later, intra-peritoneally 80 mg/kg of gentamicin (from the 4th day).

After the treatment, the animals were anesthetized with chloroform after 16 h of fasting and are sacrificed (by decapitation). At the time of sacrifice, blood was collected for biochemical

analyzes. The recovered blood is immediately centrifuged at 4000 rpm for 10 minutes at 10 ° C centrifuge, for analysis of biochemical parameters.

After the dissection the liver was carefully removed, rinsed with physiological water, and then weighed. Part of the liver was recovered in formalin vials (10%) for histological study. The homogenates of the organs were prepared for the determination of the parameters of tissue oxidative stress (malondialdehyde, reduced glutathione and glutathione peroxidase).

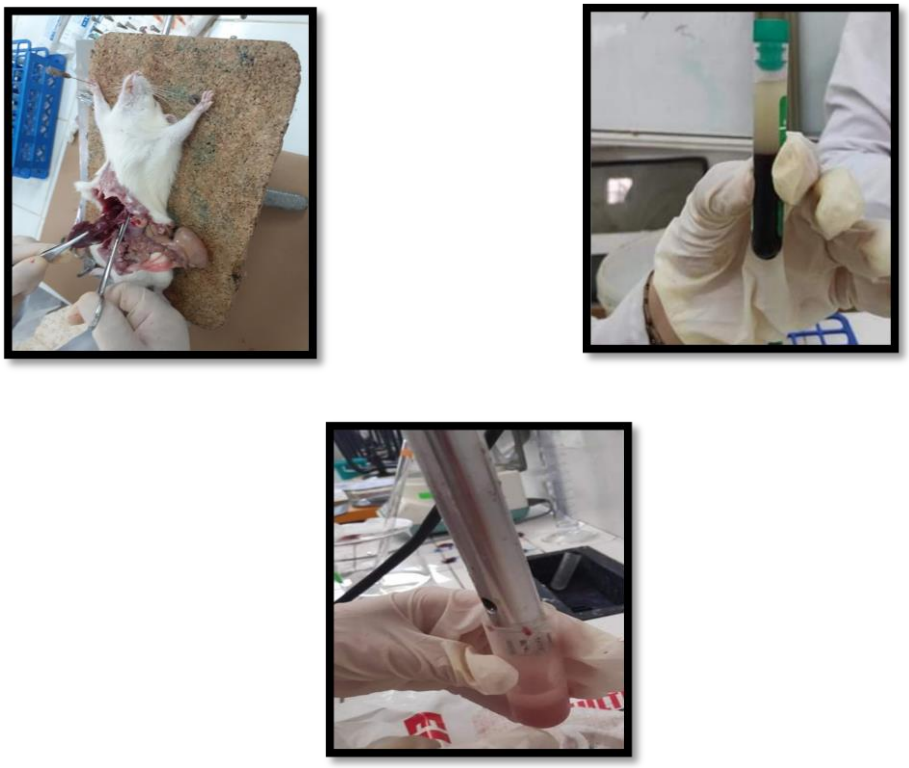


Figure 29: A: Sacrifice of animals; B: blood was collected for biochemical analyzes. C: Homogenate of liver

I-2-3- Preparation of serum and liver function test

The enzymatic activities of AST, ALT, and ALP, biomarkers of liver function are determined by colorimetry using commercial kits (SPINREACT, SPAIN).

Alanine transaminase (ALT) is used by your body to metabolize protein. If the liver is damaged or not functioning properly, ALT can be released into the blood. This causes ALT levels to increase. A higher than normal result on this test can be a sign of liver damage.

Aspartate aminotransferase (AST) is an enzyme found in several parts of your body, including the heart, liver, and muscles. Since AST levels aren't as specific for liver damage as ALT, it's usually measured together with ALT to check for liver problems.

When the liver is damaged, AST can be released into the bloodstream. A high result on an AST test might indicate a problem with the liver or muscles.

Alkaline phosphatase (ALP) is an enzyme found in your bones, bile ducts, and liver. An ALP test is typically ordered in combination with several other tests.

High levels of ALP may indicate liver inflammation, blockage of the bile ducts, or a bone disease

I-2-4-Determination of oxidative stress parameters

I-2-4-1-Estimation of Lipid peroxidation

Lipid peroxidation in the liver was evaluated by measuring malondialdehyde (MDA) according to the method of Uchiyama and Mihara, 1978. MDA is one of the end products of the decomposition of polyunsaturated fatty acids (PUFAs) as a result of lipid peroxidation and reacts with thiobarbituric acid (TBA) at 100°C temperature and in acidic environment (pH 2-3). A molecule of MDA is condensed with two molecules of thiobarbituric (TBA) to form a colored complex in pink (reading at 532 nm).

For the determination of MDA, 20% of the liver was added to a KCl solution (1.15%) then grinding using a Dounce homogenizer (Kontes, Glass companyan ISO-9001 steered firm, New Jersey USA). 0.5 mL of the homogenate; 0.5 mL of 20% phosphoric acid and 1 ml of 0.67% thiobarbituric acid (TBA) are added. The mixture is heated at 100 ° C. for 45 minutes, cooled and then added with 4 mL of *n*-butanol. After centrifugation for 15 minutes at 3000 rpm, the absorbance is determined on the supernatant using a spectrophotometer at 532 nm.

I-2-4-2-Estimation of glutathione (GSH)

The determination of GSH is based on the colorimetric method of Ellman, 1959. The glutathione assay is based on the oxidation reaction of GSH with acid 5,5'-dithiobis-2 nitro benzoic acid (DTNB) freeing the thionitrobenzoic acid (TNB) which absorbs at 412 nm.

I-2-4-3- Evaluation of Glutathione peroxidase (GPx) activity

GPx activity was measured by the method described by Flohe and Gunzler, 1984. Briefly, reaction mixture containing 0.4mL GSH (0.1 mM), 0.2mL TBS solution (Tris 50mM, NaCl

150mM PH 7.4) and 0.2 mL of tissue homogenate. After 5 min incubation at 25 °C, 0.2 mL of H_2O_2 (1.3mM) was added in the mixture. The content was incubated at 37°C for 10 min. the reaction was stopped by 1ml of 1% trichloroacetic acid (TCA) and centrifuged. Absorbance was recorded at 412 nm, and GPx activity was expressed as units/mg protein.

I-3- Histopathological studies of liver

Immediately after sacrifice of rats, liver samples were removed and fixed in 10% formalin. The tissues were kept in the fixative for 12 h, dehydrated with serial ethanol cycles (70% to absolute), and then embedded in paraffin. The paraffin-embedded tissue was cut into 5 µm sections. Tissue sections were stained with hematoxylin and eosin and observed under light microscopy.

I-4-Statistical evaluation

The results were expressed as means and standard deviations. Statistical evaluation is performed using Student's t test. The value found by the calculation of t can affirm that the populations are different with a risk of error p such that:

ns: $p > 0.05$ = the difference is not significant

*: $0.05 > p > 0.01$ = the difference is significant

**: $0.05 > p > 0.001$ = the difference is highly significant

***: $p < 0.001$ = the difference is very highly significant

Results and Discussion

I- Results

I-1-Effect of Gentamicin and extract of *Ephedra alata* on liver marker enzymes

Gentamicin caused significant increases in serum AST, ALT, and ALP levels (*p<0.001; p<0.01*) Compared to the control group indicated cell liver damage. While 100mg/kg of extract plant administration caused a significant reduction (P< 0.01) in these parameters compared to GM treated group (Fig.23, 24 and 25). So, the crud extract of *Ephedra alata* (100mg/kg) protects the liver against toxicity caused by GM.

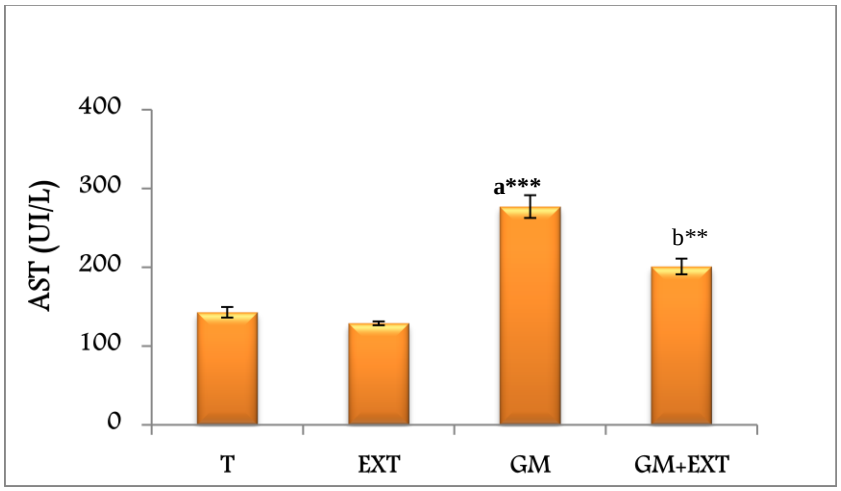


Figure 30: Effects of extract of Ephedra alata and GM in serum AST
***P<0.01; ***P<0.001, a:compared to control group. b: compared to GM group; T: the untreated rats; GM: Gentamicin group; EXT: extract at the dose 100mg/kg; GM+EXT:gentamicin+extract (100mg/kg)*

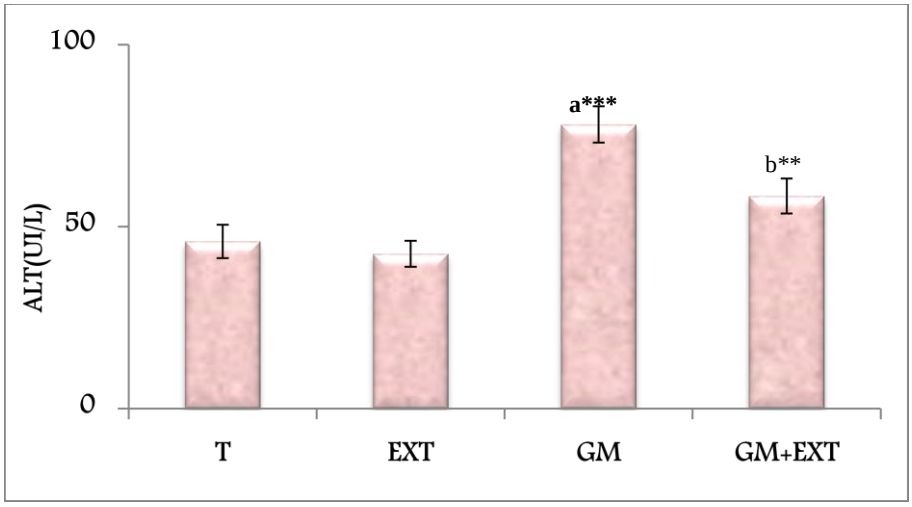


Figure 31: Effects of extract of Ephedra alata and GM in serum ALT
***P<0.01; ***P<0.001, a:compared to control group. b: compared to GM group; T: the untreated rats; GM: Gentamicin group; EXT:: extract at the dose 100mg/kg; GM+EXT:gentamicin+extract (100mg/kg)*

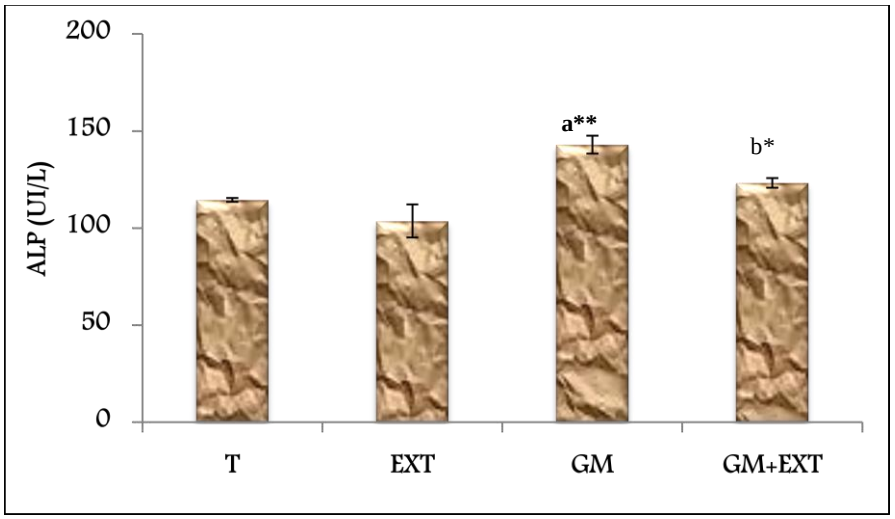


Figure 32: Effects of extract of Ephedra alata and GM in serum ALP
**P<0.05; **P<0.01, a:compared to control group. b: compared to GM group; T: the untreated rats; GM: Gentamicin group; EXT: extract at the dose 100mg/kg; GM+EXT:gentamicin+extract (100mg/kg)*

I-2-- Effects of Gentamicin and extract of *Ephedra alata* on hepatic TBARS, GSH and GPx in rat

Oxidative stress induced by GM caused significant (*P< 0.001*) alterations in hepatic antioxidant defense system as GSH, GPx, and MDA levels compared to controls (Fig. 26,27,28). A significant change was observed in MDA level in rats treated with gentamicin+extract (100mg/kg) (*p<0.05*). Extract treatment increased the level of reduced glutathione (GSH) and normalized the value of MDA compared to the GM group. The activity of the antioxidant system is maintained at normal levels in rats pretreated with the extract.

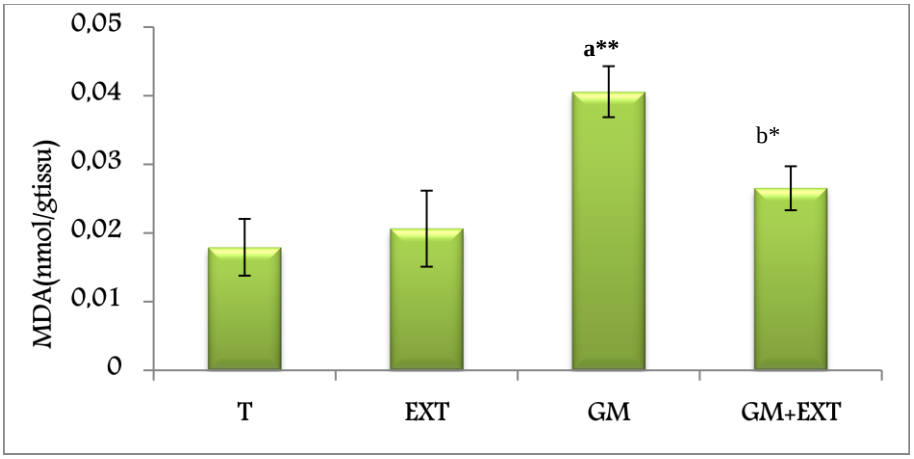


Figure 33: Effects of extract of Ephedra alata and GM in MDA level

**P<0.05; **P<0.01, a:compared to control group. b: compared to GM group; T: the untreated rats; GM: Gentamicin group; EXT: extract at the dose 100mg/kg; GM+EXT:gentamicin+extract (100mg/kg)*

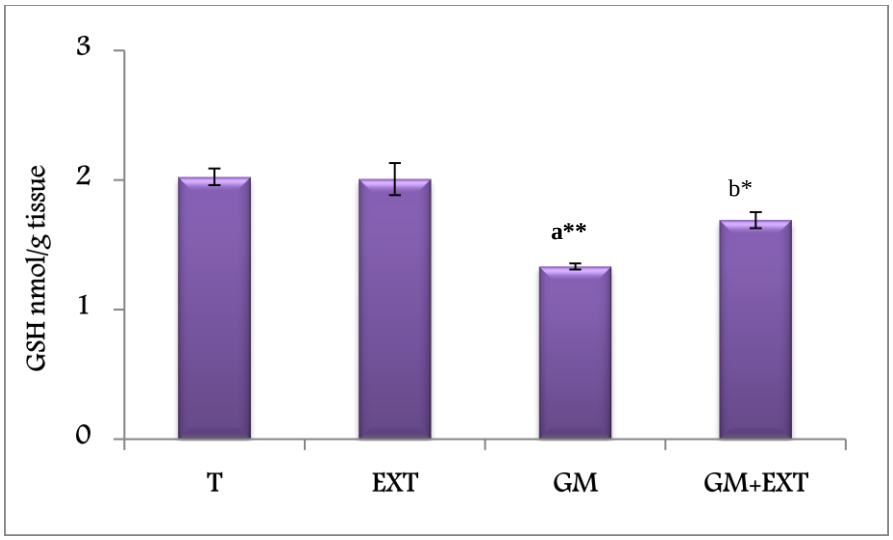


Figure 34: Effects of extract of Ephedra alata and GM in GSH level

**P<0.05; **P<0.01, a:compared to control group. b: compared to GM group; T: the untreated rats; GM: Gentamicin group; EXT: extract at the dose 100mg/kg; GM+EXT:gentamicin+extract (100mg/kg)*

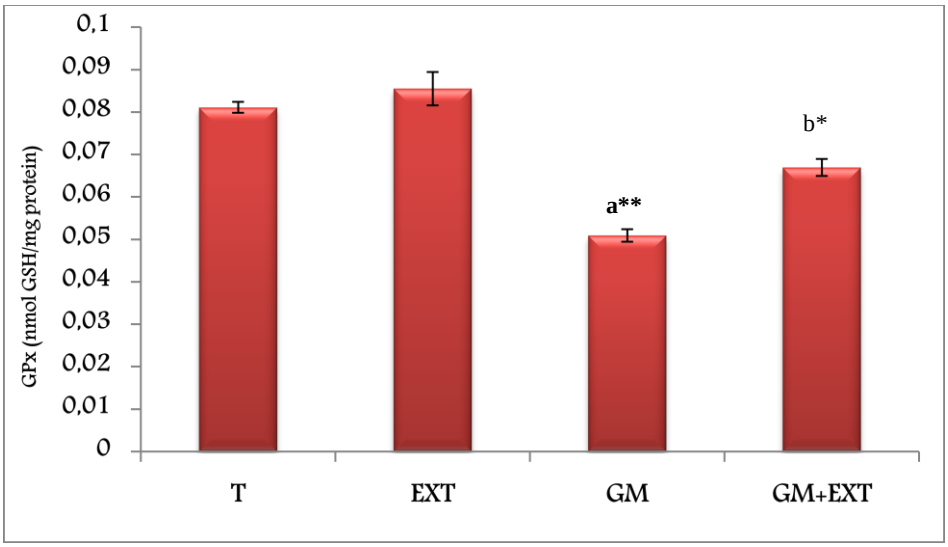


Figure 35: Effects of extract of Ephedra alata and GM in GPx avtivity
**P<0.05; **P<0.01, a:compared to control group. b: compared to GM group; T: the untreated rats; GM: Gentamicin group; EXT: extract at the dose 100mg/kg; GM+EXT:gentamicin+extract (100mg/kg)*

I-3-Effect of extract of *Ephedra alata* on Gentamicin induced histopathological alteration in hepatic tissue

Histologically, by light microscopic examination, the liver cells appeared with normal architecture in control group. The hepatocytes appeared pentagonal and contained large nuclei. The hepatocytes appeared as cell strands radiating from a central vein with intervening blood sinusoids.

In gentamicin treated group, the hepatocytes appeared irregularly arranged with disorganization of hepatic architecture, and appeared large with light and foamy cytoplasm filled with numerous vacuole-like spaces. The central vein appeared dilated and congested. Also, there were focal degenerative and necrotic changes along with inflammatory cell infiltration.

Liver treated with crude extract of *Ephedra alata* and GM showed decrease in the sever histopathological changes caused by gentamicin.

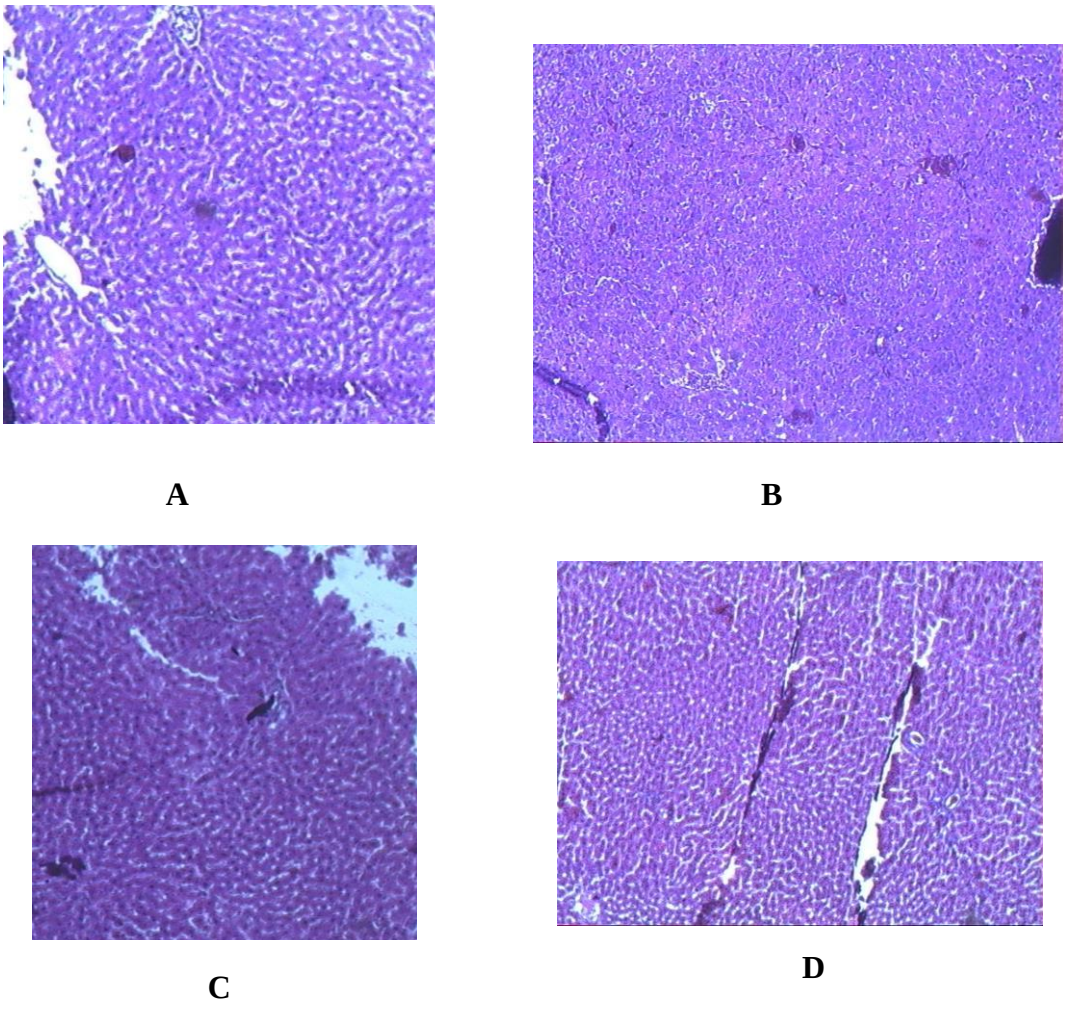


Figure 36: Histopathology showing the alterations induced with gentamicin and preventive effects crude extract of Ephedra alata in hepatic tissues of rats. *A: Control group, Hepatic section showing the normal architecture of hepatocytes;B: GM group, Gentamicin (80 mg/kg b.wt) induced injuries in liver; C:EXT group; D:GM+EXT group, reduced injuries of liver (×100).*

II-Discussion

Aminoglycosides are a family of broad-spectrum antibiotics that exhibit bactericidal action and interrupt bacterial protein synthesis by binding to the ribosome. This inhibits the formation of initiation complex with mRNA and thus inducing a misreading of the genetic code, which give rise to defective proteins that leads to cell death. Clinical features of aminoglycoside antibiotics are an excellent antibacterial profile against Gram-negative life-threatening infections, low cost, synergism with the wall-affecting antibiotics high bactericidal efficacy and low level of resistance. Mechanism of gentamicin toxicity is based on the formation of cytotoxic metabolite. The suppressive effect of gentamicin on the non-enzymatic and enzymatic antioxidants results in an excess production of reactive oxygen species which not only deleteriously affects membrane lipids but also deteriorates proteins and nucleic acids (Noorani et al., 2011; Galaly et al., 2014; Azab et al., 2016).

In order to protect the liver from the gentamicin, several studies showed that herbs can alter the toxicological effect of gentamicin. The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently uses herbal medicine for some aspect of primary health care due to their safety and efficacity (GALALY et al., 2014; Azab et al., 2016; Mishra and Shrivastava, 2018).

In Algeria, *E. alata* is used against influenza, whooping cough and general weakness in herbal tea and by inhalation as well as in the form of nasal drops against colds (Atef Chouikh, 2020), it is one of the oldest drugs, having been used by the Chinese for at least 5000 years. The *in vitro* and *in vivo* pharmacological studies on the crude extracts, fractions and few isolated compounds of *Ephedra* species showed anti-inflammatory, anticancer, antibacterial, antioxidant, hepatoprotective, antiviral and diuretic activities. The chemical compounds isolated from *Ephedra* species include alkaloids, amino acids and derivatives, volatiles, tannins, polysaccharides and phenolic compounds (Sioud et al.,2021).

Based on these informations, we have set the following objectives:

- Study of gentamicin induced hepatotoxicity in *Wistar Albino* rat's type.
- Study of the hepatoprotective and antioxidant effect of crude extract of *Ephedra alata* after 10 days of treatment in conjunction with gentamicin by measure the activity of AST, ALT, ALP in serum, and the antioxidant defense system MDA, GSH and GPx in the liver.

In our study it was found that rats treated by gentamicin at a daily dose of 80 mg/kg for 10 days induced hepatotoxicity characterized by highly signification elevation (*p<0.001; p<0.01*) of AST, ALT, ALP in the serum these markers are associated with liver injury. We found that our results are consistent with those of (Noorani et al.,2011; Azab et al.,2016), who have found that the activities of AST, ALT, ALP in serum, are indicative for hepatic function, their increase is correlated with the hepatic injury and considered the most sensitive markers of liver injury (Galaly et al.,2014); have also recorded similar results he found that administration of rats with 80 mg/kg of gentamicin for more than a week produced a significant elevation of AST, and ALT activities, reflect the damage of hepatocytes and liver injury. Alam and Galal, 2013 reported that intraperitoneal administration of gentamicin (100 mg/kg) for 10 successive days produced a remarkable hepatotoxicity in rats, characterized by significant increase in the serum ALT, and ALP with significant hypoproteinaemia when compared with control group. Sivachandran and Hariharan, 2012, illustrated that gentamicin administered at a dosage of (80 mg/kg) once daily for seven days produced significant elevation of serum ALT with significant hypoproteinaemia and hypoalbuminemia due to damaging of the hepatic cells.

AST is an enzyme found primarily in liver cells in the form of two isoenzymes, one located in the cytoplasm and the other in the mitochondria, the presence of these enzymes outside the cell represents damage to the cell hepatic (Ferrandi et al., 2018).

It is well known fact that some traditional herbs act as remedy, which can alter the toxicological effect of gentamicin. In this study, we found that oral and daily administration of the crude extract of *Ephedra alata* at a daily dose of 100 mg/kg for 10 days significantly decreased (P< 0.01) plasma activity of AST, ALT and ALP compared to GM treated group. These results suggest that the plant extract may have protected the liver from damage caused by gentamicin. These data are in concurrence with those reported by (Gong et al., 2015), who obtained results showed an increase in the serum level of AST.ALT, these effects could be a consequence of the disturbing of several antioxidant system functioning.

Certain drugs may induce oxidative stress by forming drug-derived radicals that can not only deplete the antioxidant defenses but can also react directly with biomolecules.

In gentamicin-treated rats, there was a significant increase in oxidative stress suggesting the liver damage. It induces an increase in the oxidative stress and production of free radicals and supresses the antioxidant defense system in liver (Priuska and Schacht, 1995; Noorani et al., 2011).

Our results are consistent with these studies, there was a highly significant increase of MDA in GM treated rats (*P< 0.001*) and decrease in GSH level and GPx activity compared to control group, these data are in concurrence with those reported by Yazar et al., 2006, was shown that gentamicin caused decreased in GPx activity and GSH level and increases in MDA levels, which is related to the relationship between gentamicin and antioxidant enzymes, oxygen free radicals involved in aminoglycoside hepatotoxicity and singlet oxygen inactivate GPx and GSH activities.

MDA value, measure of oxidant-antioxidant balance, could provide complementary information about the homeostasis of the animal than coventional metabolic parameters alone (Castillo et al., 2006). The exacerbated increase of lipid peroxidation by gentamicin impairs membrane lipids and causes hepatocytes necrosis and damage. The suppressive effect of gentamicin on the nonenzymatic and enzymatic antioxidants results in an excess production of reactive oxygen species which not only deleteriously affects membrane lipids but also deteriorates proteins and nucleic acids. This in turn leads to liver toxicity, dysfunction and damage (Galaly et al., 2014).

Many experimental studies have shown the beneficial effect of administration of plant extract, used in traditional medicine, in prevention drug hepatotoxicity and oxidant/antioxidant balance.The treatment with *Ephedra alata* reversed oxidative damage in the kidneys and liver, and prevented acute kidney injury and histopathological liver damage (Ghedira and Chekir-Ghedira, 2020). Our results are consistent with these studies, it was found that rats treated with the gentamicin 80 mg/kg and the plant (*Ephedra alata*) extract 100 mg/kg (*p<0.05*) shown a significant change compared to GM group, we noticed that the extract treatment increased the level of GSH and GPx activity and normalized the value of MDA compared to the GM group. These results run in parallel with (Sioud et al., 2021), the treatment with *E alata* decreased the MDA levels and enhanced the levels of antioxidants enzymes; it reversed oxidative damage in the liver.

The previous results of the present study were confirmed by histopathological examination of the liver in gentamicin treated rats. The hepatocytes appeared irregularly arranged with disorganization of hepatic architecture; the central vein appeared dilated and congested. Also, there were focal degenerative along with inflammatory cell infiltration. Similar observations were reported by Azab Elsayed et al., 2016 who found that administration of rats with 100 mg/kg body weight gentamicin for 3 weeks induced liver histological alterations including hydropic degeneration of hepatocytes, inflammatory cell infiltration and congestion of portal vein. Oluwadare et al., 2021 reported that the liver of rats treated with gentamicin showed

focal coagulative necrosis and severe hydropic degeneration. This is due to extensive production of ROS which damage the cells of liver. These results may indicate degenerative changes and hypofunction of liver as well as hepatic cell necrosis which increase the releasing of these enzymes in the blood stream (Azab Elsayed et al., 2016).

In our study, the appearance of inflammatory cells in the hepatic tissue, due to gentamicin administration, may suggest that gentamicin could interact with proteins and enzymes of the hepatic interstitial tissue interfering with the antioxidant defense mechanism and leading to reactive oxygen species generation which in turn may imitate an inflammatory response. Biochemical and Histopathological changes induced by gentamicin were reduced to a moderate extent in crude extract of *Ephedra alata* treated rats. Treatment with *Ephedra alata* back the cellular arrangement around the central vein and reduced necrosis. Crude extract of *Ephedra alata* protects the liver from gentamicin-induced damage, which may be due to its antioxidant property.

Conclusion

Conclusion

Using Gentamicin antibiotic at a dose of 80 mg / kg for 10 days had significant toxic effects on the biochemical parameters of blood, and the functional performance of hepatic tissues. On the other hand, the crude extract of *Ephedra alata* subsp. *Alenda* showed potent anti-oxidative, and protective activities against the toxicity that caused by this type of antibiotic, as well as crude extract of *Alenda* exhibited an ameliorative efficiency to the biological functions of the liver, therefore, this study recommends to use it as a natural remedy.

The protective effect of *Ephedra alata (Alenda)* extract on gentamicin induced hepatoxicity

Abstract

The most vulnerable tissue against damages and side effects of medications is the liver tissue. Among the medications, most frequently used ones are antibiotics with among which toxicity with aminoglycosides, especially gentamicin has special importance. Studies have shown that gentamicin exerts its hepatotoxicity property through creating free radicals. The present study was conducted to evaluate the protective effect of crude hydro-methanolic extract of *Ephedra alata* subsp. *Alenda* on gentamicin-induced hepatotoxicity in rats.

The effect of crude hydro-methanolic extract of *Ephedra alata* at a dose of 100 mg/kg was studied on gentamicin induced hepatic damage (80mg/kg i.p. once daily for 10 days) in *Wistar Albino* rats. Serum transaminases, lipid peroxidation (MDA), reduced glutathione (GSH), and glutathione peroxidase (GP_X) were estimated to access liver damage. A histological study was determined.

The activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and MDA were significantly increased; a significantly decrease in GSH level and GPx activity in rats exposed to gentamicin. Moreover, administration of gentamicin resulted in damage to liver structures. Administration of hydro-methanolic extract of *Ephedra alata* before gentamicin exposure prevented severe alterations of biochemical parameters and disruptions of liver.

In conclusion, this study obviously demonstrated that pretreatment with crude hydro-methanolic extract of *Ephedra alata* significantly attenuated the physiological and histopathological alterations induced by gentamicin. Also, the present study identifies new areas of research for development of better therapeutic agents for liver, and other organs dysfunctions and diseases.

Key words: Liver; Gentamicin; Hepatotoxicity; *Ephedra alata*

L'effet protecteur de l'extrait *Ephedra d'alata (Alenda)* sur l'hépatotoxicité induite par la gentamicine

Résumé

Le tissu le plus vulnérable aux dommages et aux effets secondaires des médicaments est le tissu hépatique. Parmi les médicaments, les plus fréquemment utilisés sont les antibiotiques avec lesquels la toxicité avec les aminosides, en particulier la gentamicine a une importance particulière. Des études ont montré que la gentamicine exerce sa propriété d'hépatotoxicité en créant des radicaux libres. La présente étude a été menée pour évaluer l'effet protecteur de l'extrait brut hydro-méthanolique d'*Ephedra alata* subsp. *Alenda* sur l'hépatotoxicité induite par la gentamicine chez le rat.

L'effet de l'extrait brut d'*Ephedra alata* à une dose de 100 mg/kg a été étudié sur les dommages hépatiques induits par la gentamicine (80 mg/kg i.p. une fois par jour pendant 10 jours) chez des rats *Wistar Albinos*. Les transaminases sériques, la peroxydation lipidique (MDA), le glutathion réduit (GSH) et la glutathion peroxydase (GP_X) ont été estimées pour accéder aux dommages au foie. Une étude histologique a été déterminée.

Les activités de l'alanine aminotransférase sérique (ALT), de l'aspartate aminotransférase (AST), de la phosphatase alcaline (ALP) et du MDA ont été significativement augmentées ; une diminution significative du taux de GSH et de l'activité GPx chez les rats exposés à la gentamicine. De plus, l'administration de gentamicine a entraîné des dommages aux structures hépatiques. L'administration d'extrait brut d'*Ephedra alata* avant l'exposition à la gentamicine a empêché de graves altérations des paramètres biochimiques et des perturbations du foie.

En conclusion, cette étude a bien évidemment démontré qu'un prétraitement avec un extrait brut d'*Ephedra alata* atténue significativement les altérations physiologiques et histopathologiques induites par la gentamicine. En outre, la présente étude identifie de nouveaux domaines de recherche pour le développement de meilleurs agents thérapeutiques pour le foie et d'autres dysfonctionnements et maladies des organes.

Mots clés : Foie ; Gentamicine ; Hépatotoxicité ; *Ephedra alata*

التأثير الوقائي لمستخلص *Ephedra alata* ***(Alenda)*** على السمية الكبدية المستحثة بواسطة الجنتاميسين

ملخص

يعتبر الكبد من أكثر الأنسجة الأكثر عرضة للأضرار الناتجة عن سمية الأدوية. من بين هذه الأدوية الأكثر استخداما هي المضادات الحيوية ،من بينها الأمينوغليكوزيدات ، وخاصة جنتاميسين ، حيث يعمل هذا الأخير على علاج التعفنات الناتجة عن التهابات البكتيرية. وقد أظهرت الدراسات أن جنتاميسين يتسبب في اختلالات كبدية من خلال تخليق الجذور الحرة و كذلك إحداث تشوهات نسيجية على مستوى الكبد. أجريت هذه الدراسة لتقييم التأثير الوقائي للمستخلص الخام لنبات العلندة *Ephedra alata* اتجاه السمية الكبدية المحرضة بجنتاميسين على جرذان ذكور من سلالة *Albino Wistar*.

تمت معاملة الجرذان بالمستخلص الخام بجرعة (100 mg/kg) عن طريق الفم, في حين تم حقن جنتاميسين (80mg/kg) تحت السفاق يوميا لمدة عشرة أيام. في نهاية التجربة تم تشريح الحيوانات و أخذ ، الكبد لتقدير مؤشرات الجهد التأكسدي: MDA، GSH، GPx . كما تم تقدير مختلف المؤشرات البيوكيميائية: ALT , AST و ALP, إضافة إلى الدراسة النسيجية للكبد.

تشير النتائج المتحصل عليها عند المجموعة المعاملة بالجنتاميسين إلى ارتفاع معنوي في مستوى كل من الأنزيمات الكبدية و الأكسدة الفوقية للدهون, في حين سجلنا انخفاض معنوي في مستوى GSH و نشاط Gpx. إضافة إلى إحداث أضرار على مستوى النسيج الكبدي. بالمقابل لدلك أدت المعاملة المسبقة بالمستخلص النباتي إلى الحد من التأثيرات السامة الناتجة عن جنتاميسين.

في الختام، أظهرت هذه الدراسة بوضوح أن المعالجة المسبقة بمستخلص النباتي قد حدّ بشكل كبير من التغيرات الفسيولوجية والنسيجية الناجمة عن جنتاميسين. كما تمكن هذه الدراسة من فتح مجالات جديدة للبحث لتطوير مركبات فعّالة لحماية الكبد.

كلمات مفتاحية: كبد ، جونتاميسين ، تسمم كبدي، *Ephedra alata*

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TITLE: **The protective effect of *Ephedra alata (Alenda)* extract on gentamicin induced hepatotoxicity**

NATURE OF THE DIPLOMA: master's degree in animal biology
Option: Toxicology

Abstract:

The most vulnerable tissue against damages and side effects of medications is the liver tissue. Among the medications, most frequently used ones are antibiotics with among which toxicity with aminoglycosides, especially gentamicin has special importance. Studies have shown that gentamicin exerts its hepatotoxicity property through creating free radicals. The present study was conducted to evaluate the protective effect of crude hydro-methanolic extract of *Ephedra alata* subsp. *Alenda* on gentamicin-induced hepatotoxicity in rats.

The effect of crude hydro-methanolic extract of *Ephedra alata* at a dose of 100 mg/kg was studied on gentamicin induced hepatic damage (80mg/kg i.p. once daily for 10 days) in *Wistar Albino* rats. Serum transaminases, lipid peroxidation (MDA), reduced glutathione (GSH), and glutathione peroxidase (GP_X) were estimated to access liver damage. A histological study was determined.

The activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and MDA were significantly increased; a significantly decrease in GSH level and GPx activity in rats exposed to gentamicin. Moreover, administration of gentamicin resulted in damage to liver structures. Administration of hydro-methanolic extract of *Ephedra alata* before gentamicin exposure prevented severe alterations of biochemical parameters and disruptions of liver.

In conclusion, this study obviously demonstrated that pretreatment with crude hydro-methanolic extract of *Ephedra alata* significantly attenuated the physiological and histopathological alterations induced by gentamicin. Also, the present study identifies new areas of research for development of better therapeutic agents for liver, and other organs dysfunctions and diseases.