Thesis Title

"Hepatorenal Pharmacovigilance study of marketed herbal preparation of ***Carica papaya*** leaves in experimental rat."

[A thesis report submitted to the department of pharmacy, Daffodil International University in the partial fulfillment of the requirements for the degree of master of pharmacy.]

Submitted To

Department of Pharmacy,

Faculty of Allied Health Sciences,

Daffodil International University

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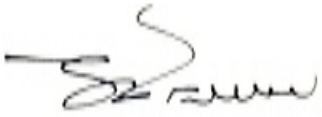
APPROVAL

This thesis paper, "Hepatorenal Pharmacovigilance study of marketed herbal preparation of *Carica papaya* leaves in experimental rat", submitted to the Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, has been accepted as satisfactory for the partial fulfillment of the requirements for the degree of Master of Pharmacy and approved as to its style and contents.

BOARD OF EXAMINERS



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DECLARATION

I, Md. Shadman Muttake Al - Rafid, hereby declare that, this thesis is done by me under the guidance of Md. A.K. Azad, Assistant Professor, Department of Pharmacy, Daffodil International University, in partial fulfillment of the requirements for degree of Masters of Pharmacy. The results embodied in this project have not been submitted to any other university or institute for the award of any degree.

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"Acknowledgement"

I might want to communicate my profound applause to the All-powerful Allah who has given me the capacity to finish my undertaking work and the chance to concentrate in this subject.

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Finally, I would like to express my gratitude towards my parents and other family members for their kind cooperation and encouragement which helped me in completion of this project.

Dedication

To

My Parents and Supervisor.

Abstract

Herbal medications are natural remedies that have been used for hundreds of years because of their better patient tolerance & beneficial effects on health, however, safety issues still exist. Present study was conducted with the aim to find out the pharmacovigilance effects of the herbal preparation over two primary organs (liver & kidney) in experimental rat model. As sample, Carica papaya leaf extract and supplied marketed herbal drug & Peracetamol as toxicity inducing agent were used. Few parameters (CRP, ALT, and creatinine) and morphological alterations were observed after carrying out this treatment for 21 days. It was found that marketed herbal preparation have shown harmful effect over liver. CRP levels have been found to be elevated (0.60±0.01 mg/L), as well as ALT level (88±2.5 iU/L). However, the serum creatinine was within the normal range (0.41±0.02 mg/dl), concluding that there were no adverse effects observed on the kidneys. A morphological investigation shows that the liver and kidney have not undergone any detrimental or abnormal alterations. However, further research is required to fully comprehend how these medications affect our major organs.

Key words: Herbal, Nutraceuticals, Papaya Leaf, Pharmacovigilance, CRP, ALT, Creatinine, Liver, Kidney

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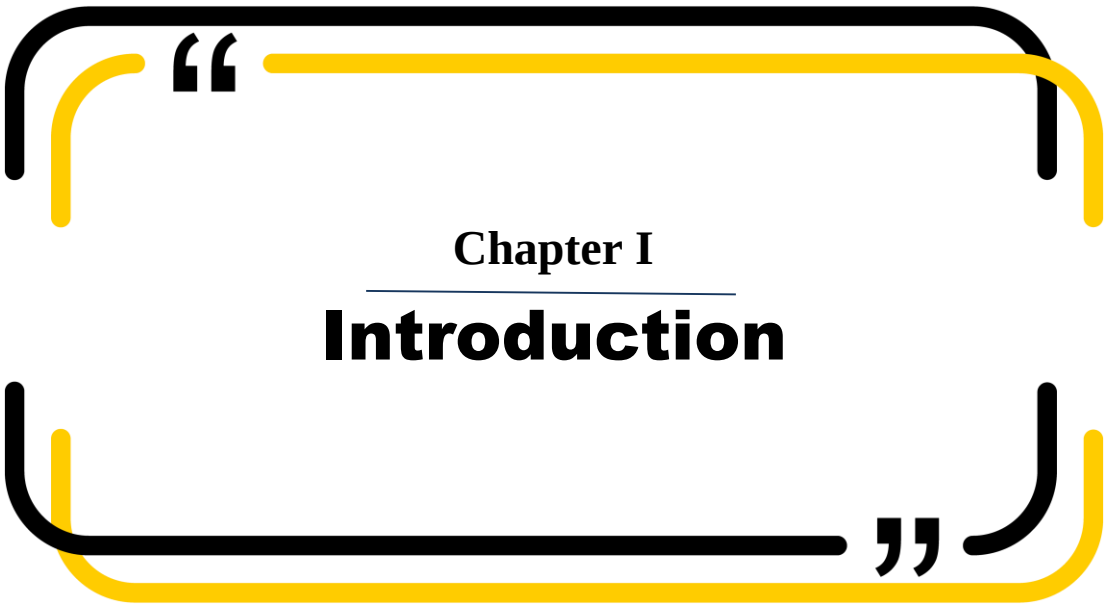

Chapter I

Introduction

1. Introduction

About 60% of people on the planet utilize traditional medicines. These are utilized for basic healthcare not just in rural parts of impoverished nations, but also in industrialized nations where the use of contemporary medications is more prevalent.[1] Herbal medicines are exclusively made from medicinal plants, as opposed to conventional medicines, which also contain minerals and organic substances. The Indian subcontinent's health care system includes a significant amount of plant-based medicine, which has been passed down through generations. Since most Indian medical practitioners create and administer their own prescriptions, thorough documentation and research are needed. The usage of herbal medicines is slowly expanding in the western world as well, with 40% of the population reporting using herbs to address medical conditions in the previous year.[2] Due to the rising prevalence of adverse medication responses and the financial load of the contemporary medical system, there is an exponential rise in public, academic, and governmental interest in traditional remedies. [3][4]

Nutraceuticals introduce a brand-new classification that blurs the line between pharmaceuticals and food. A nutraceutical is defined as "a food or component of a food that delivers health advantages in addition to its nutritional content." Given their shown clinical usefulness, active compounds, whether they are derived from plants (phytocomplexes) or animals, and provided in an appropriate pharmaceutical form, can produce a highly promising toolbox used to prevent and/or assist the therapy of specific pathologic illnesses. The importance of nutrition and lifestyle in promoting and maintaining good health, in addition to assisting in the possible prevention of illness development, is well acknowledged. Pathological problems can in reality be caused by improper eating practices and lifestyle choices. One particularly good illustration is the pandemic threat posed by the metabolic syndrome. This syndrome is characterized by a series of cardio metabolic risk factors, such as hypertension, dyslipidemia, insulin resistance, and obesity. The key to effective proactive medicine is prevention. In this approach, efforts are directed toward prevention and, as a result, to reduce the risk associated with some lifestyle-related diseases while also lowering the cost to the National Health Systems required to ensure the appropriate therapeutic approach based on pharmaceuticals. Utilizing nutraceuticals to avoid health issues is a proactive, backwards-looking strategy. Since they combine the therapeutic qualities of natural active chemicals with the nutritional and healthful benefits of food extracts, they may be utilized

successfully by integrating them in the daily diet in a range that veers toward "beyond the diet, before medicines." [5]

1.1. Nutraceuticals and Modern medication

Nutrigenetics is a young field that is rapidly growing and riding the wave of "personalized medicine," presenting prospects in the creation of nutraceutical products. In this study, the topic of nutrigenetics and metabolic disorders was limited to a few select genetic polymorphisms. Due to their roles in a number of chronic human illnesses, other crucial topics relating to inflammation and oxidative stress require special consideration in nutrigenetic research. The function of oxidative stress in apoptotic pathways in heart disease was explored in a recent review[6], which amply demonstrated the necessity for antioxidant supplementation as a preventative medicine. To relate particular mutations in the oxidant/antioxidant systems to lower plasma antioxidant capacity and an increased risk of heart disease, nutrigenetic investigations are still required. This allows for the development of customized nutraceuticals (antioxidants) that target certain metabolic sites. Other recent intriguing studies suggesting possible locations for nutraceutical intervention include those on the ABCG5 polymorphism73, uncoupling protein 2 genotype,[8], and perilipin gene variation[7,8]. For instance, the ABCG5 genotype can be used to identify those who respond more strongly to dietary cholesterol. A diet high in nutrients that prevent these people from absorbing cholesterol starting early in life, including plant sterols, may reduce the prevalence of CHD in this nation. Nutrigenetics will become a science that is fundamental to the practice of nutrition and dietetics as more study is done on individual variances linked to metabolism, dietary requirements, and genetic profile. [9]

It is acknowledged that using traditional medicine can teach us about prospective new medications. According to estimates from the World Health Organization, 80 percent of people worldwide currently utilize herbal medicine for some part of primary healthcare. The idea of a nutraceutical has gained acceptance across the world. Benefits from food that go above and beyond those due to basic nutrients are what give rise to the labels "functional foods" and "nutraceuticals." Nutraceuticals, which offer health advantages and serve as an alternative to contemporary medicine, have attracted increasing attention in recent years. Major components of nutraceuticals include nutrients, herbs, and dietary supplements, making them essential for preserving health, fighting off various illness conditions, and enhancing quality of life. [10]

Consumers today are very conscious of their dietary choices, health, and way of life. The standard of living has increased as a result of economic growth and globalization. In addition to progress, a significant problem has emerged in the shape of lifestyle illnesses. Junk food consumption has multiplied, which has resulted in a variety of disorders linked to nutritional inadequacies. The use of nutritional supplements can be quite effective in reducing them. The phrases "nutrition" and "pharmaceutical," two general terms, are the origin of the word "nutraceutical." These goods might be anything from isolated minerals, nutritional supplements, and particular diets to designer meals made through genetic engineering and herbal remedies. The definition of nutraceuticals is "alternative beneficial products produced completely or partially from food that preserve optimal health and combat nutritionally caused disorders, consequently increasing the quality of life." [10, 11]

Importance

From the perspective of the consumer, functional foods and nutraceuticals provide a number of advantages.

- ✓ Improve the nutritional value of our diet.
- ✓ Please make us live longer.
- ✓ Please assist us in preventing certain medical issues.
- ✓ Seen as more "natural" and less likely to have negative side effects than conventional medication
- ✓ May provide food for groups with unique dietary requirements, such as nutrient-dense meals for the elderly [12].
- ✓ It can be provided with the goal of restoring, correcting, or changing physiological functioning in people. It is used for the prevention, treatment, or cure of a condition or illness.
- ✓ Nutraceuticals not only support a healthy diet but also help with illness prevention and/or treatment.
- ✓ Nutraceuticals are advertised for use as a regular food, as the only component of a meal, or as a diet supplement. [13]

1.2. Herbal medicine & Challenges

The use of various sections of therapeutic plants is connected to herbal medicine or phytomedicines. Herbal medicine has a long history of use outside of traditional medicine. It has

been gaining popularity in recent years as a result of improvements in clinical research, analysis, and quality control. According to the World Health Organization (WHO), phytomedicine, also known as herbal medicine, is the culmination of all knowledge, skill, and practices that are based on the theories and experiences that are unique to various cultures, whether or not they can be explained, and are used to maintain health as well as to prevent, diagnose, improve, or treat physical and mental illness. [14]

Herbal medicine and herbal therapy are nothing new to humanity; they have been used for thousands of years and are still in use now. Because of its many advantages, both industrialized and developing nations are currently concentrating on treatments based on herbal medicine. India is regarded as the "land of herbs," and its ancient medical system, known as Ayurveda, was similarly founded on herbs and medicinal plants. India is a well-known source of herbal plants with a history of therapeutic usage. Botanical or phytomedicine are other names for herbal medicine. According to a World Health Organization study report, 80% of the world's population relies on traditional herbal medicine for their main healthcare needs. Alternative medicine is really becoming more and more popular in industrialized nations because of its effectiveness, safety, and lack of negative side effects. Herbal medicines have a long history of use and are better tolerated by patients when used. The limitations and difficulties in producing herbal medicines are covered in this review article. [15]

Historical Perspective Over the years, humans have utilized natural products derived from natural sources, such as plants, as food and medicines, particularly plant parts or the entire plant to treat and prevent disease. [16] Although it is very difficult to pinpoint the precise moment when people began utilizing plants as medicine, there is some ancient literature and other evidence that points to its emergence. The earliest known written record of the use of medicinal herbs for medication manufacture was discovered on a Sumerian clay slab from Nagpur that is thought to be about 5000 years old. It included 12 drug production methods and references to more than 250 different plants, some of which included alkaloids including poppies, henbane, and mandrake. [17] The Vedas, the holy writings of India, recommend using plants to heal illnesses since they are widely distributed there. 365 medications (dried sections of medicinal plants) are covered in the Chinese book "Pen T'Sao," which was authored by Emperor Shen Nung around 2500 BC and is based on the usage of roots and grasses. Many of these drugs are still in use today.[18,19]

The father of medicine, Hippocrates, focused much of his work on human anatomy and physiology and produced more than 60 works on medicine. [20] In addition, he proposed the humoral hypothesis, which states that the four humors—blood, phlegm, yellow bile, and black bile—that make up the human body play a major role in both healthy and diseased bodily functioning. [21] The most famous quote attributed to Hippocrates is, "Let your food be your medicine and let medicine be your food." "The body's incapacity to assimilate its surroundings is what causes illness," [22] Early in the 19th century is considered to be the turning point for the usage and use of medicinal plants. The investigation, confirmation, and separation of alkaloids from poppies and other plants in 1806. The discovery of glycosides, meanwhile, signaled the start of scientific pharmacy. Other active components from medicinal plants were also found with the improvement and advancement of the chemical processes. [23]

1.3. Present Scenario

The global market for herbal or phytomedicines is still growing quickly. In many national health-care contexts, many individuals are now turning to herbal medications for the treatment of a variety of health issues. Both in industrialized and emerging nations, public interest in natural medicines has increased over the past few decades. [24] In poor nations like Africa, up to 90% of the population still relies on traditional doctors and herbal remedies for their basic care, as does up to 70% of the population in India. Around 40% of all healthcare services in China are provided through traditional medicine, and more than 90% of general hospitals there include traditional medicine departments. [25,26,27] Currently, herbal remedies are used to treat both acute and chronic illnesses, as well as a wide range of diseases and conditions, including inflammation, depression, prostate issues, cardiovascular disease, and prostate difficulties, to mention a few. The Africa flower, a traditional herbal remedy, has long been used in Africa to alleviate the wasting symptoms brought on by HIV. [28, 29] The new medicine compound is thought to include 90% of natural components. Many efficient pharmaceuticals have been produced by nature, including doxorubicin, dactinomycin, and bleomycin (anticancer), vinblastine, irinotecan, topotecan, etoposide, and paclitaxel (antimalarial), mefloquine chloroquine, amodiaquine artemisinin, dihydroartemisinin, artemether, and arteether (antimalarial), met (anti-HIV drugs). [30, 31] With approximately 1.5 million users of the traditional medical system, India has roughly 25,000 efficient plant-based remedies. In India, there are 7800 production facilities for pharmaceuticals, and they use roughly 2000 tonnes of herbs annually. [32] The information at hand indicates that

there is a sizable demand for traditional medicine. With a growth rate of 14% annually, the Indian herbal industry is close to 50 billion rupees. Exports of herbal products are estimated to be worth 1 billion rupees. The worldwide herbal market is expected to rise from its present level of $ 62 billion to $ 5 trillion in 2050 as a result of the rising demand for therapeutic plants. India and China are the countries that create more than 70% of the world's variety. In addition to the EU, USA, Canada, Australia, Singapore, and Japan, other important herbal export markets include Brazil, Argentina, Mexico, China, and Indonesia. [33]

1.4. Challenges Associated

Herbal medications are released into the market without being subject to any required safety or toxicological testing on their effects. Many of these nations also lack efficient apparatus to control production procedures and herbal medicine quality requirements. The regulatory status, evaluation of safety and efficacy, quality control, safety monitoring, and a lack of or incomplete understanding about traditional, complementary, and alternative therapies are challenges that are frequently faced and shared by many nations. [34, 35]

1.4.1. Challenges Associated to the Regulatory Status of Herbal Medicaments

A dietary supplement is defined as a product that is consumed, is meant to complement the diet, and contains a "dietary element." These items' nutritional constituents may comprise various vitamins, minerals, herbs, or other botanicals that the body needs. [36] If a herb was sold before 1994, no further toxicity tests are typically required under the DSHEA. The FDA is responsible for carrying the burden of demonstrating that a herbal therapeutic product or "dietary component" is hazardous or unfit for use. The fact that regulatory information on herbal medications is frequently not exchanged between regulatory bodies and safety monitoring or pharmacovigilance centers is an extra significant difficulty in many countries. [37]

1.4.2. Challenges in the Evaluation of Safety and Efficacy

No one can contradict the fact that the requirements as well as the research protocols, standards, and methods needed for the evaluation of the safety and efficacy of herbal medicines are much more complex than those required for conventional or orthodox pharmaceuticals. A single herbal medicine or medicinal plant may contain more than hundreds of natural constituents, and a mixed herbal medicinal product may contain several times the number of one. In such an analysis of single active constituents may practically be impossible especially where an herbal product is a mixture of two or more herbs. [38]

1.4.3. The challenges in maintaining the quality of herbal medicines

The safety and efficacy of herbal medicines are largely based on the quality of the raw ingredients used in their preparation. Source or raw material quality is influenced by environmental variables, excellent farming practices, and good collecting techniques for medicinal plants, such as plant selection and culture, in addition to intrinsic (genetic) aspects. It is challenging to conduct quality controls on the raw materials used to create herbal medicines because of a variety of elements working together. [39] Correct identification of medicinal plant species, specific storage, and unique cleaning processes for diverse materials are significant criteria for the quality control of beginning materials, according to good manufacturing practice (GMP). The main difficulties are in the quality control of completed herbal medicines, particularly herbal mixtures. [38] As a result, compared to other medicines, the overall specifications and procedures for quality control of final herbal products continue to be far more complicated. The WHO continues to support the implementation of quality assurance and control measures, such as National Quality Specifications and Standards for Herbal Materials, GMP, labeling, and licensing systems for production, to assure the safety and efficacy of herbal medicines.

1.4.4. The challenges in monitoring the safety of herbal drugs

Issues about the rising usage of herbal or natural medications or goods in industrialized nations have come up in recent years. Furthermore, there is a greater awareness of the need to monitor safety and an understanding of both the potential risks and benefits associated with the use of herbal medicines as a result of the dependence of many people living in developing countries on plants as their primary source of medicines, as well as the lax regulation of herbal medicines in the majority of countries and the occurrence of high-profile safety concerns. [40] The use of the incorrect plant species, adulteration of herbal products, contamination, overdosing, misuse of herbal medications by consumers or healthcare professionals, and the combination of herbal medications with other medications are just a few of the causes of adverse effects resulting from the consumption of herbal medicines. Most makers of herbal medicines are not well informed about the value of taxonomic botany and documentation, and this presents specific obstacles during the identification and collecting of medicinal plants used in herbal treatments. It is vital to use the most widely used binomial names for medicinal plants in order to avoid the confusion caused by the common names. An active narcotic derivative is present in Artemisia absinthium L.,

which has at least 11 common names. Therefore, good cooperation between botanists, phytochemists, pharmacologists, and other key stakeholders is required for the proper monitoring of herbal medicine. [15]

1.5. Pharmacovigilance (PV)

Pharmacovigilance is the investigation of the efficacy of commercially available medications as they are used clinically in often sizable populations. The effectiveness of the drug, whether it is used on healthy or ill individuals, the pharmaceutical quality of the drug, the nature and severity of any side effects and the extent to which these can be treated, the threat posed by the disease that is being treated with the drug, and the rest of the complex of issues that constitute holistic patient care must all be taken into account before safety can be considered. [40]

1.5.1. Pharmacovigilance in drug regulation

The basis for a national ethos of drug safety and for the public's faith in medicines is sound drug regulatory framework. Clinical trials, the safety of complementary and alternative medicines, vaccines, and biological medicines, as well as developing channels of communication between all parties with an interest in drug safety and ensuring that they are open and able to function effectively, particularly during times of crisis, are among the issues that drug regulatory authorities must deal with.

To guarantee that authorities are adequately informed on safety concerns in daily practice that may be relevant to future regulatory action, pharmacovigilance programs need to have strong connections with regulators. Regulators are aware of the crucial and specialized role that pharmacovigilance plays in maintaining the continuous safety of pharmaceuticals. To fulfill their goals, pharmacovigilance programs need to have enough funding.[41]

1.5.2. PV of Herbal and Traditional Medicines

Concerns about the safety of traditional and herbal medications are raised by their use. There is a common misunderstanding that "natural" equates to "safe." There is a widespread misconception that a medicine's efficacy and safety are guaranteed by long-term usage, which is based on tradition. Traditional and herbal treatments have been shown to be tainted or adulterated with allopathic drugs, chemicals including corticosteroids and non-steroidal anti-inflammatory drugs, and heavy metals. Many traditional medicines are produced for use worldwide, transcending the traditional and cultural contexts for which they were first developed. Patients' risks are significantly increased by self-medication. When traditional and herbal treatments are taken in

combination with other drugs there is the risk of major adverse drug interactions. Herbal medications should be included into a regulated framework, much as other items meant for human use (medicines, nutritional supplements, and foods). These goods should be controlled by criteria of safety, quality and efficacy that are equal to those needed for other pharmaceutical products. The growing gray area between foods and medicines, into which an increasing number of herbal items fall, makes it difficult to do this. The legal status of herbal items varies greatly from nation to nation. Few nations now have procedures in place to govern traditional healers, and less than 70 countries currently control herbal medicines. The distribution of these items internationally and the differences in regulation across nations are extremely important. For instance, a herbal product could only be available with a prescription and from a licensed pharmacy in one nation, but it might be available through a health food store, by mail order, or even online, as has been customary. Due to all of these factors, it is essential and unavoidable to include herbal and traditional medicines in national pharmacovigilance programs. A shared duty for their informed and secure usage is shared by healthcare professionals, including conventional healthcare practitioners, regulators, producers, and the general public. The World Health Organization has published recommendations for assessment of the safety, effectiveness and quality of herbal medicines. The development of new, organized methods for assessing the security of pharmaceuticals produced from plants is ongoing. Several national pharmacovigilance organizations are now keeping an eye on the security of conventional medications. Consumers, traditional healthcare doctors, traditional and herbal medicine suppliers, and other specialists must work together and support this for it to be successful. Research in this area needs to be prioritized, as well as consumer and provider education. [41]

1.5.3. Importance of PV for herbal medicines

As the potential for herbal goods and herbal medications increases globally, one of the most important responsibilities in pharmacovigilance systems is to consider the value of herbal treatments. Nowadays, medication development is centered on identifying novel active substances or combinations, but expenses are also rising, making herbal medicines an alluring, risk-free, and less expensive substitute for synthetic treatments. Like any medications, herbal ones carry some risk, and several studies point to possible combinations and unpleasant responses. Statistics available indicate that several herbal drugs, used in conventional medicine for centuries, may have harmful effects such as cancer, hepatotoxicity, cardiotoxicity, and others. For drugs intended to be

used continuously for more than three months or intermittently for more than six months, long-term rodent carcinogenicity tests, reproductive and developmental toxicity studies, and an analysis of the effects on drug-metabolizing enzymes should at the very least be included in the safety assessment process. [42] Any medical technique or therapy must always adhere to the core premise of safe and effective medicine. Due to the widespread usage of herbal medicine across the world, ensuring their safety becomes crucial and a top priority. Pharmacovigillance is essential for researching the safety of herbal medications. The research and practices involved in the identification, evaluation, comprehension, and prevention of side effects or any other drug-related issue are referred to as pharmacovigilance (PV). Case reports of acute and chronic poisoning, adverse drug reactions, and medication errors (toxicity) Every herbal medication should undergo pharmacovigilance testing to look for drug abuse and misuse, drug-drug interactions, and food-drug interactions. Herbal medicine items are frequently used nowadays to avoid synthetic ones, but they are not necessarily "safe," especially when taken in conjunction with other medications, and can have detrimental effects on health. Every herbal drug should undergo a pharmacovigilance study to prevent these issues. [43]

1.6. Hepatotoxicity

The primary organ for preserving the body's interior environment is the liver. Currently, there is no way to make up for lost liver function. It primarily affects nutrient flow and regulates the metabolism of carbohydrates, proteins, and lipids. Drug abuse is a significant factor in liver damage. There have been over 900 substances—drugs, poisons, and herbs—reported to harm the liver. In over 75% of cases, fatal medication responses or liver transplants are the outcome. Acute-dose dependent liver damage, acute fatty infiltration, cholestatic jaundice, liver granulomas, active chronic hepatitis, liver cirrhosis, liver tumors, etc. are a few examples of drug-induced liver illnesses. Acute liver failure affects about 2000 Americans each year, and pharmaceuticals are to blame for more than half of those instances (37% of which are brought on by acetaminophen and 13% by unusual drug interactions). About 10% of all instances of acute hepatitis and 2-5% of individuals hospitalized with jaundice, respectively, are related to drugs. In 17 nations, chronic liver disease and cirrhosis cause around 2% of all fatalities, or about 40,000 people annually. This study sheds light on several medicines that create hepatotoxicity, together with their mechanism of liver damage and clinical situation, taking into account the significance of drug-induced hepatotoxicity as a significant cause of liver damage.

The liver performs an amazing variety of essential roles in the body's upkeep, operation, and homeostasis regulation. It participates in practically all metabolic pathways that lead to development, illness prevention, nutrition uptake, energy generation, and reproduction (Sharma et al., 1991). The metabolism of carbohydrates, proteins, and fats, detoxification, bile secretion, and vitamin storage are the liver's main tasks. Maintaining a healthy liver is therefore essential for one's overall health and wellbeing. Hepatotoxicity denotes liver damage brought on by chemicals. Certain medications have the potential to harm the organ when taken in excess or occasionally even when administered within therapeutic parameters. Hepatotoxicity can also be brought on by other chemical agents, including those found in industry and labs, natural compounds (such microcystins), and herbal medicines. The term "hepatotoxins" refers to substances that harm the liver. The most frequent reason for a drug's removal from the market is liver damage, which has been linked to more than 900 different medications. Subclinical liver damage brought on by chemicals frequently only emerges as abnormal liver enzyme testing. 50% of all acute liver failures and 5% of all hospital admissions are caused by drug-induced liver damage. Idiosyncratic medication responses frequently lead to liver transplantation or death in more than 75% of instances. [44, 45]

1.7. Nephrotoxicity

Acute kidney damage (AKI) may affect 60% of patients admitted in an intensive treatment unit and is a fairly frequent diagnosis in both hospital and pre-hospital settings. The rise in risk factors such old age, chronic kidney disease (CKD), and diabetes mellitus can be blamed for the increase in its occurrence in recent decades. Nephrotoxicity is the third most prevalent cause of AKD (Acute Kidney Disease), according to epidemiological research. Nephrotoxic medication usage has been found to occur up to 20% of the time in critically ill patients, making it one of the most common causes of kidney damage in recent years. [46]

Up to 60% of critically ill patients have an acute kidney damage diagnosis, and medication toxicity is its third leading cause. Any renal damage brought on by medicine, whether directly or indirectly, is referred to as nephrotoxicity. Acute renal failure, tubulopathies, and glomerulopathies are some typical clinical manifestations. Anti-inflammatory medications, antibiotics like vancomycin and aminoglycosides, and chemotherapeutic treatments like cisplatin and methotrexate are a few examples of medications that are frequently linked to the acute lowering of glomerular filtration rate. Amphotericin B, polymyxins, and tenofovir frequently cause tubulopathies, while VEGF

inhibitors, bisphosphonates, and immunotherapy frequently cause glomerulopathies. It is also typical to have more than one clinical manifestation associated with a single treatment. Knowing the risk factors and biomarkers is necessary for an early diagnosis, which is crucial for the patient's positive course of treatment and a reduction in renal exposure to the toxic chemical. The foundations for the treatment of complications that are still common and frequently preventable include general measures like correcting hydroelectrolytic disorders and hypovolemia, monitoring the serum level, avoiding combinations with the synergy of renal injury, and looking for comparable options which are less toxic. [47]

Nephrotoxicity is the malfunctioning of kidney-specific detoxification and excretion as a result of endogenous or exogenous toxins damaging or destroying renal function. Kidney toxicity from drug exposure frequently affects the body's primary homeostasis control system, making it particularly vulnerable to xenobiotics. Knowing the harmful processes underlying nephrotoxicity helps scientists create medications with therapeutic benefits and fewer side effects. Modifications in glomerular hemodynamics, tubular cell toxicity, inflammation, crystal nephropathy, rhabdomyolysis, and thrombotic microangiopathy are a few of the mechanisms for drug-induced nephrotoxicity. It has become possible to identify biomarkers for the evaluation of nephrotoxicity. For the efficient prevention of drug-induced nephrotoxicity, new biomarkers that can detect kidney damage early and more precisely must be discovered and developed. Several interesting possibilities for biomarkers have recently been validated for the assessment of nephrotoxicity, even if some of them lack specificity and sensitivity. This review includes a list of medicines that cause nephrotoxicity, a summary of the processes underlying drug-induced nephrotoxicity, as well as biomarkers that may be used to detect nephrotoxicity early on. [48]

1.8. C-reactive protein (CRP)

The plasma protein known as C-reactive protein (CRP), which has homologs in many vertebrate and invertebrate species, is involved in the body's overall response to inflammation. Its plasma content rises in inflammatory situations, which is a property that has long been used in medicine. CRP is a molecule that recognizes patterns and binds to particular chemical configurations that are frequently exposed after cell death or seen on the surfaces of pathogens. The fact that its synthesis increases quickly within hours of tissue damage or infection shows that it supports host defense and is a component of the innate immune response. The American Heart Connection and the

Centers for Disease Control have recommended that people at intermediate risk of coronary heart disease could benefit from CRP testing as a result of a recent association between mild CRP rise and future significant cardiovascular events. The majority of this study will be devoted to our present knowledge of CRP's structure, ligands, effector molecules with which it interacts, and functions that are now known to be performed by it. [49]

The pentraxin family of proteins includes the ancient, highly conserved molecule known as C-reactive protein (CRP). The liver releases CRP in response to certain inflammatory cytokines. In reaction to injury, inflammation, and infection, CRP levels rise quickly, and they fall just as quickly after the disease has passed. As a result, CRP testing is frequently used to track different inflammatory states. In a calcium-dependent way, CRP binds to injured tissue, nuclear antigens, and some pathogenic organisms. It is believed that CRP's participation in the innate immune system is connected to how it works. It triggers complement, attaches to Fc receptors, and functions as an opsonin for certain pathogens, just as immunoglobulin (Ig)G. Pro-inflammatory cytokines are produced as a result of CRP's interaction with Fc receptors, which heightens the inflammatory response. Contrary to IgG, which only detects certain antigenic epitopes, CRP uses pattern recognition to distinguish between changed self- and foreign molecules. CRP is therefore believed to function as a monitoring molecule for infections and changed selves. This early detection results in a pro-inflammatory signal, the activation of the humoural, adaptive immune system, and early defense. [50] Greater CRP concentrations are related to higher liver enzyme levels. The low-grade inflammation linked to the metabolic syndrome may be influenced by hepatic inflammation related to liver steatosis. Higher plasma CRP concentrations are linked to mild liver enzyme increases. The persistent low-grade inflammation linked to metabolic risk factors and the metabolic syndrome may be exacerbated by hepatic inflammation owing to NAFLD. [51]

1.9. Alanine transaminase (ALT)

The main screening method for identifying acute liver damage is now an assay of the blood level of the enzyme alanine aminotransferase (ALT). But what does a high value actually mean? Not what it is frequently misinterpreted to mean. It does not assess liver health. It doesn't always foretell negative outcomes (in a given person). It is not a reliable indicator of the degree of liver malfunction or damage. It is too general to be effective in detecting somewhat infrequent liver damage. Even with these significant drawbacks, ALT may be a highly helpful biomarker when

utilized appropriately. It is critical to reflect on how and why these false notions came to be widely accepted as well as how to better understand increases in ALT activity when assessing patients and study subjects. [52]

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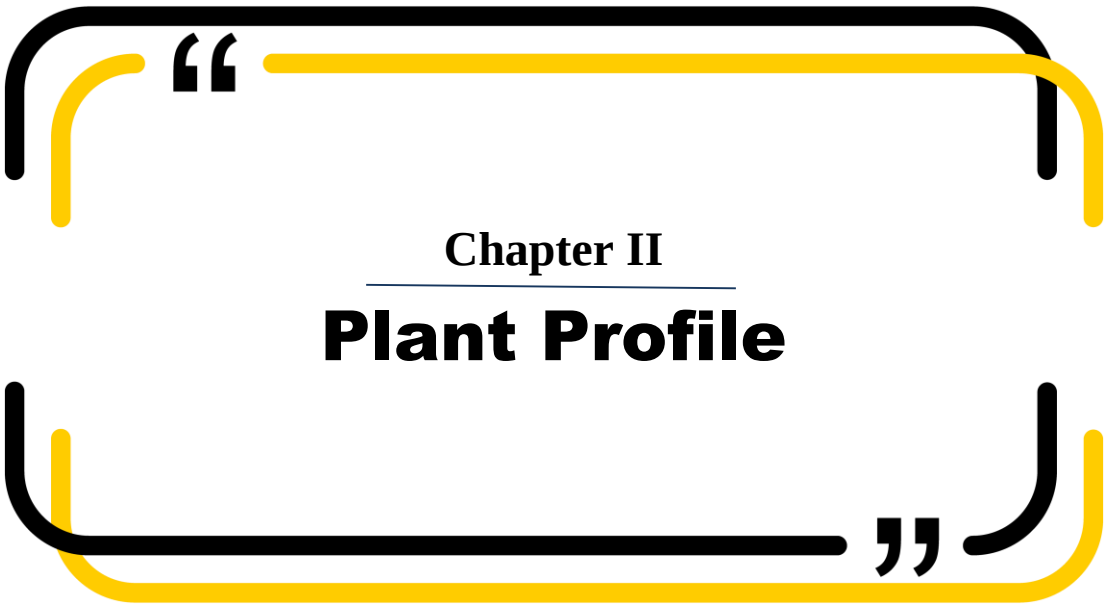
1.10. Creatinine

However, in the 1940s and 1950s, measures of serum and urine creatinine (as well as the computation of creatinine clearance) were made clinically available and acquired popularity, which has persisted to the present day. Biomarkers are often employed for diagnosis, severity categorization, result prediction, and, most critically, outcome modification. The first three can be treated with creatinine (except for outcome modification). Numerous clinical studies have demonstrated that new renal biomarkers, particularly neutrophil gelatinase-associated lipocalin and cystatin C, can diagnose acute kidney injury more quickly and accurately, have a better correlation with disease severity, and accurately predict the prognosis of patients with acute kidney injury than creatinine. More clinical research will be needed, nevertheless, to demonstrate the

novel renal indicators' actual superiority to creatinine. Such studies include those that use therapies to better the outcomes (particularly mortality) of patients with acute renal injury as well as those that link certain indicators to the positive effects of particular interventions. [54, 55]



Chapter II

Plant Profile

2. Plant Profile

2.1. Scientific classification

Kingdom	Plantae
Orde	Brassicales
Family	Caricaceae
Genus	Carica
Species	C. papaya
Binomial name	*Carica papaya*

Fig-1: *Carica papaya*

2.2. Local names

Pappaiya, Papita, Papeya.

2.3. Botanic description

Carica papaya is an evergreen, tree-like plant that grows 2–10 m tall, is often unbranched but occasionally does so owing to damage, and has white latex in every portion. Have hollow stem with a cylinder shape, 10–30 cm in diameter, with pronounced leaf scars. It has a complex rooting structure. Petiole up to 1 m long, hollow, greenish or purplish-green; lamina orbicular, 25–75 cm in diameter, palmate, deeply 7-lobed, glabrous, conspicuously veined; lobes deeply and extensively serrated; leaves spirally arranged, grouped close to top of stem. Female flowers are 3-5 cm long, with a large functional pistil, no stamens, and an ovoid-shaped ovary. Male flowers are on long, drooping panicles with 10 stamens in 2 rows, no gynoecium other than a pistillode.

Hermaphrodite flowers are larger than males and have a 5-carpellate ovary. Flowers are small, yellow, funnel-shaped, solitary or clustered in Fruits come in a variety of sizes, shapes, and colors. They have a juicy orange pulp and hollow berries. Female flowers produce oblong, spherical, or pear-shaped fruits; hermaphrodite blooms produce long, obovoid, or pyriform fruits. Numerous, tiny, spherical, black seeds with a gelatinous aril covering. Small latex vessels can be seen all over the tree, but they are particularly common in fruit that has grown to maturity but has not yet started to mature. Because of how similar the leaves are, the genus name, "carica," comes from the latin for "delicious fig."

2.4. Biology

Papayas from the Carica genus bear fruit within 5 months and survive for 4–5 years. Some flowers are bisexual, albeit typically male and female blossoms are on separate branches. Numerous insects, including hummingbird moths (Macroglossa), bigger bees (Xylocarpa, Trigona), honeybees, long-tongued sphinx moths (Sphingidae), and wind, are pollinating agents. A cultivar may quickly lose its distinctive characteristics when exposed to open (uncontrolled) pollination.

2.5. Products

The year-round availability of ripe papaya makes it a popular fruit for breakfast and dessert. Fruit salads, cool drinks, jam, jelly, marmalade, sweets, and crystallized fruit may all be made with it. Green fruit can be pickled, boiled, or used in place of applesauce. The amount of edible ripe fruit is about 60%. It contains approximately 86.6 g of water, 0.5 g of protein, 0.3 g of fat, 12.1 g of carbohydrates, 0.7 g of fiber, 0.5 g of ash, 34 mg of calcium, 11 mg of phosphorus, 1 mg of iron, 3 mg of sodium, 450 mg of vitamin A, 74 mg of vitamin C, 0.03 mg of thiamine, and 0.5 mg each of niacin and riboflavin. 200 kJ/100 g is the energy value. Sucrose (48.3%), glucose (29.8%), and fructose (21.9%) are the three main sugars. The blossoms are used to make a sweetmeat in Java. Sometimes, young leaves are consumed. Rubber or latex: Large-scale plantations of C. papaya are planted in various nations in order to harvest papain, a proteolytic enzyme found in the latex and mostly harvested from green fruit. Papain has a wide range of applications in the food, beverage, and pharmaceutical sectors, including the ability to chill-proof beer, tenderize meat, create medications for digestive disorders, and cure gangrenous wounds. Additionally, it is used to bathe hides, degum silk, and soften wool. The papain output for latex can range from 70 to 130 kg per hectare per year.

2.6. Medicine

Papaya contains the alkaloid carapine, which has heart-depressing, amoebic, and diuretic properties. For gastrointestinal issues, the fruit and juice are consumed; for wounds, a fresh leaf poultice is applied. To relieve rheumatism, the raw root mixed with sugarcane alcohol can be consumed or used topically. For chest colds, bronchitis, asthma, and coughs, a floral infusion is ingested orally. The seeds are employed as a vermifuge and an abortifacient in various nations. [56]

2.7. Pharmacology

The papaya plant, comprising the fruit, leaves, seeds, bark, latex, and other parts of it, is crucial in controlling the spread of illness. Alkaloids, glycosides, tannins, saponins, and flavonoids are some of the active ingredients in carica papaya leaf that give it its therapeutic properties. Papaya leaf juice also raises platelet counts in dengue fever patients, another benefit. Additional signs include:

- May support good blood sugar levels;
- May aid digestion;
- May have anti-inflammatory benefits;
- May support hair development;
- May support healthy skin;
- May have anticancer potential.

The main research results showed that papaya leaf extract possesses potent antibacterial, antiviral, anticancer, hypoglycemic, and anti-inflammatory effects. Clinical trials are also required to investigate the papaya leaf's potential as a medicine. [57,58]

Chapter III

Literature Review

3. Literature Review

3.1. Therapeutic application of Carica papaya leaf extract in the management of human diseases

Papaya (Carica papaya Linn.), a member of the Caricaceae family, is renowned around the world for its medicinal and dietary benefits. Since antiquity, various papaya plant components have been used for medicinal purposes. Here, we set out to examine papaya leaf's anticancer, anti-inflammatory, antidiabetic, and antiviral properties.

This review article's material on the medicinal use of Carica papaya leaf extract was compiled by consulting a number of online sources, including Scopus, Google Scholar, Web of Science, and PubMed. Up till December 2019, the terms Carica papaya, anticancer, anti-inflammatory, immunomodulatory, and phytochemicals were researched.

The papaya plant, comprising the fruit, leaves, seeds, bark, latex, and other parts of it, is crucial in controlling the spread of illness. Alkaloids, glycosides, tannins, saponins, and flavonoids are some of the active ingredients in carica papaya leaf that give it its therapeutic properties. Papaya leaf juice also raises platelet counts in dengue fever patients, another benefit.

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Ref: Singh, S. P., Kumar, S., Mathan, S. V., Tomar, M. S., Singh, R. K., Verma, P. K., ... & Acharya, A. (2020). Therapeutic application of Carica papaya leaf extract in the management of human diseases. *DARU Journal of Pharmaceutical Sciences*, *28*(2), 735-744.

3.2. Phytochemical studies on *Carica papaya* leaf juice

Carica papaya pulp, fruits, roots, bark, peel, and seeds are all recognized to have medical benefits. It has been used to treat a wide range of conditions, including thread worms, warts, corns, sinusitis, eczema, cutaneous tubercles, blood pressure, dyspepsia, constipation, amenorrhea, and general debility. According to Ayurvedic literature, papaya leaf extract has hemostatic qualities, and more modern research has shown that it can increase platelets in rat models of cyclophosphamide-induced thrombocytopenia. Pilot investigations in dengue patients showed that leaf juice had a positive effect on increasing white blood cells, platelet count, and recovery without admission to

the hospital. Liquid chromatography-mass spectrometry was used in the current work to investigate the phytochemical composition of papaya leaf extract (LCMS). Young leaves' aqueous extract was obtained, and it was analyzed using LCMS for phytochemical profiling with water and acetonitrile as the mobile phase. Alkaloids, phenolics, flavonoids, and amino acids were among the 21 ingredients discovered during LCMS analysis and an integrated library search, along with phytochemicals that are pharmacologically active. Additional research on these elements can be done to determine and isolate the most active bio constituent responsible for platelet augmentation, anticancer property, anti-acne activity, reducing menstruation discomfort, and nausea relief.

Ref Akhila, S., & Vijayalakshmi, N. G. (2015). Phytochemical studies on Carica papaya leaf juice. *International Journal of Pharmaceutical Sciences and Research*, *6*(2), 880.

Chapter IV

Purpose of the Study

4. Purpose of the Study

At least 80% of individuals now use herbal medications and supplements for some part of primary healthcare, reflecting a huge increase in their use over the previous three decades. [59] Many herbal products have been shown to be effective, but treatments using these substances have shown promise. But many of these medications are still experimental, and there is either little or no regulation of their use. Because there is insufficient knowledge regarding these agents' modes of action, potential adverse reactions, contraindications, and combinations with conventional pharmaceuticals and functional foods already on the market, it is challenging to promote their safe and responsible usage.[60] The right regulatory organizations must provide the essential measures to protect the public health by guaranteeing that all herbal drugs are secure and of sufficient quality because safety is still a key worry when using herbal treatments.

This study aims –

- To find out the safety of Herbal medication.
- To observe if there is any pharmacovigilance effect on body's two primary organs (Liver & Kidney).

Chapter V

Methodology

5. Method

5.1. Animal Model

For this study we chose Rat as our animal model, where number of rats, n=9. The mean weight of the rats were 150-165g. And the mean age of the rats were 6-7 weeks. And the gender were male. (Fig-2)

Fig-2: Animal Model (Rat)

5.2. Study Design

9 rats were divided into 3 groups. Each group contained 3 rats. Group-1 was the control group where the rats given given paracetamol (8 mg) to induce liver and kidney toxicity. Group-2 was Sample-1 group that treated with Papaya leaf extract (3 ml) and the Group-3, the Sample-2 group was given the Supplied Papaya capsule from local market (4 mg). After 21 days of successful administration of the experimental drugs and samples, the rats were slaughtered, and blood samples were taken. A sample of 2-3 ml blood was taken using the cardiac puncture technique. The serum was then taken from the blood and kept for later research after centrifuging it. Then few parameters were observed.

5.3. Parameters

After successive administration of dosage, following parameters were observed.

- For Liver function test: CRP, ALT test
- For Kidney function test: Serum-creatinine test
- Organoleptic observation - Morphological study of 2 major organs.[Liver, Kidney]

Chapter VI

Result & Discussion

6. Result

We performed 3 tests and morphological observation in order to assess the liver & Kidney damage.

6.1. For Liver

CRP is used to check for internal inflammation, while an ALT test is used to determine if there is hepatocellular damage. The results are shown in tables-1 and tables-2.

6.1.1. C-reactive protein (CRP)

A test for C-reactive protein (CRP) quantifies the amount of CRP in a sample of blood. The liver produces a protein known as CRP. When there is inflammation in the body, the liver often produces more CRP into the circulation. The normal range of CRP of a healthy rat is 0.30-0.50mg/L[61]

Group	CRP Level Mean ± SEM (mg/L)	Normal Range
Sample-1 (Leaves Extract)	<0.51±0.01	0.30-0.50 mg/L
Sample-2 (Marketed Herbal Preparation)	<0.60±0.01	
Control Paracetamol	<0.60 ±0.008	

Tables-1: C-reactive protein (CRP) Level

Discussion: The mean CRP level for the Sample-1 group is <0.51±0.01 mg/L, the mean for the Sample-2 group is <0.60±0.01 mg/L, and the mean for the Control group is <0.60 ±0.008 mg/L. This demonstrates that the given sample drug slightly elevated the CRP level similarly to the control group, suggesting the potential induction of inflammation.

6.1.2. An alanine transaminase (ALT)

The level of ALT in the blood is determined using an ALT test. ALT is released into the circulation by injured liver cells. A liver injury or illness may be indicated by high levels of ALT in the blood. Before the illness's symptoms appear, several forms of liver disease raise ALT levels. The normal range of ALT of a healthy rat is 10 to 40 IU/L. [62]

Group	ALT Level Mean ± SEM (IU/L)	Normal Range
Sample-1 (Leaves Extract)	64±2.1	10 to 40 IU/L
Sample-2 (Marketed Herbal Preparation)	88 ±2.5	
Control Paracetamol	85 ±2.3	

Tables-2: Alanine transaminase (ALT) Level

Discussion: The mean ALT level in the Sample-1 group was 64±2.1 IU/L, the Sample-2 group's mean was 88 ±2.5 IU/L, and the Control group's mean was 85±2.3 IU/L. The ALT level was markedly elevated above the normal range in both the Sample-2 group and the Control group, possibly suggesting the probability of liver damage.

6.1.3. Liver morphology

After the dissection of the rat's body, the organoleptic observation was made for the livers of each rats. There is no abnormalities or color change observed. **(Fig-3)** The mean weight of the livers of 3 groups is 2.98(±0.01) g which is normal.

6.2. For Kidney

Serum creatinine Level is observed.

6.2.1. Serum Creatinine

A creatinine test evaluates how well the kidneys are removing waste from your blood. A chemical byproduct of the energy-generating mechanisms in muscles is creatinine. The blood is filtered by healthy kidneys to remove creatinine. The normal range of Serum Creatinine of a healthy rat is 0.4–0.8 mg/dL.[63]

Group	Creatinine Level Mean ± SEM (mg/dL)	Normal Range
Sample-1 (Leaves Extract)	0.33±0.006	0.4–0.8 mg/dL
Sample-2 (Marketed Herbal Preparation)	0.41±0.02	
Control Paracetamol	0.42±0.02	

Tables-3: Creatinine Level.

Discussion: The mean Creatinine level for the Sample-1 group is 0.33±0.006 mg/dl, the mean for the Sample-2 group is 0.41±0.02 mg/dl and the mean for the Control group is 0.42±0.02 mg/dl. This explain that the both Sample-1 & Sample-2 have similar effects over kidney as like the control group. Besides the range of the serum creatinine level is normal for 3 groups indicating no possible harmful effects over kidney.

6.2.2. Kidney morphology

After the dissection of the rat's body, the organoleptic observation was made for the kidneys of each rats. There is no color change observed neither any abnormality is observed. **(Fig-4)** The mean weight of the kidneys of 3 groups is 0.59±0.02 gm g which is normal.

Fig-3: Livers

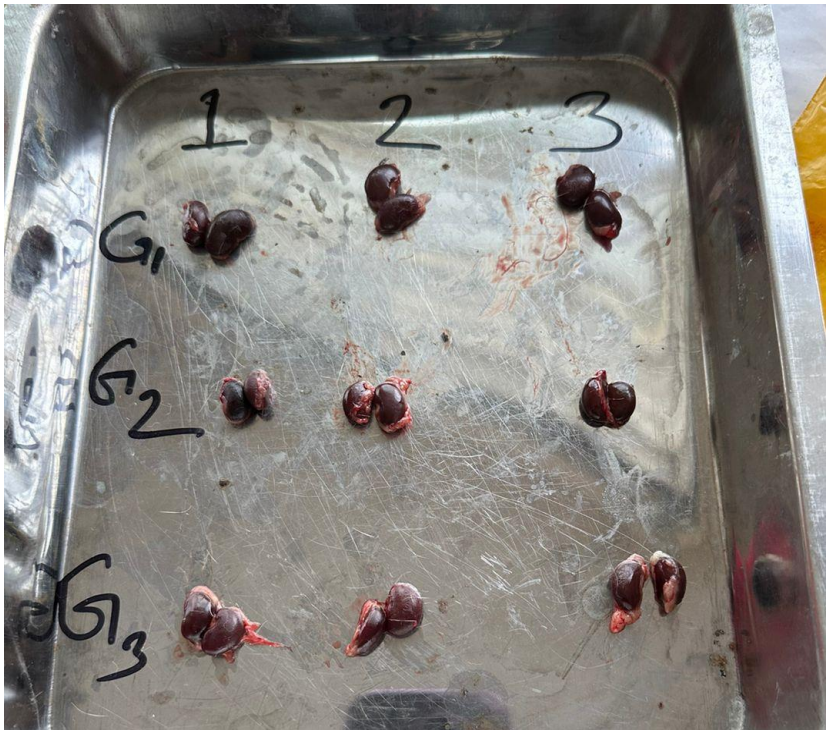

Fig-4: Kidneys

Chapter VII

Conclusion

7. Conclusion

Civilization has been using herbal medicine and herbal treatments for thousands of years, and they are still utilized today. 80% of the world's population relies on traditional herbal medicine for their primary healthcare requirements, according to WHO study report. Alternative medicine is really gaining popularity in industrialized countries due to the long history of use and higher patient tolerance of these drugs. The new category of "nutraceuticals" blurs the line between "food" and "herbal medications." A nutraceutical is defined as "a food or component of a food that delivers health advantages in addition to its nutritional content." Although this medications have positive effects on health, safety concerns remain. After the study was finished, it was discovered that these herbal and nutritional supplements have a minor impact on the liver. CRP levels have been found to be elevated (0.60±0.01 mg/L) as well as ALT levels (88±2.5 iU/L). However, as the serum creatinine is within the normal range (0.41±0.02 mg/dl), there are no adverse effects on the kidneys. A morphological investigation shows that the liver and kidney have not undergone any detrimental or abnormal alterations. However, further research is required to fully comprehend how these medications affect our major organs.

Chapter VIII

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