THE ASSESSMENT OF HEPATOTOXIC EFFECT OF BIOACTIVE COMPOUNDS DERIVED FROM *CITRULLUS COLOCYNTHIS* ORGANIC EXTRACTS

SUBMITTED BY

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L1S20MSBC0004

In the Partial Fulfilment for the Degree of MS Biochemistry

DEPARTMENT OF BASIC AND APPLIED CHEMISTRY
FACULTY OF SCIENCES AND TECHNOLOGY
UNIVERSITY OF CENTRAL PUNJAB

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Dedication

I dedicate my thesis to my parents, siblings, supervisor, research fellows, and friends for supporting me with love and helpfulness.

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All praises, thanks to Almighty GOD, the Omnipotent, the most Beneficent and the most Merciful for guiding to conceptualize, develop, and complete this research. Indeed, without His help and will, nothing is accomplished.

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DECLARATION

I,_Anis Shahzad khan, S/O Rafiq Masih, a student of "MS in Biochemistry" at "Faculty of Science and Technology, University of Central Punjab, hereby declare that this thesis titled **"The Assessment of Hepatotoxic effect of bioactive compounds derived from *Citrullus colocynthis* Organic extracts"** is my own research work and has not been submitted, published, or printed elsewhere in Pakistan or abroad. Additionally, I will not use this thesis for obtaining any degree other than the one stated above.

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Name of Student: Anis Shahzad khan
Signature of Student: Anis Khan
Registration Number: L1S20MSBC0004
Date: 02-08-2022

PLAGIARISM UNDERTAKING

I solemnly declare that the research work presented in this thesis titled, **"The Assessment of Hepatotoxic effect of bioactive compounds derived from *Citrullus colocynthis* Organic extracts"** is solely my research work, and that the entire thesis has been completed by me, with no significant contribution from any other person or institution. Any small contribution, wherever taken, has been duly acknowledged. I understand the zero-tolerance policy of the HEC and University of Central Punjab towards plagiarism. Therefore, I as an author of the above titled thesis declare that no portion of my thesis has been plagiarized and that every material used from other sources has been properly acknowledged, cited, and referenced. I undertake that if I found guilty of any formal plagiarism in the above titled thesis, even after the award of Ms. Degree, the University reserves the right to revoke my degree, and that HEC and the University have the right to publish my name on the HEC/University website for submitting a plagiarized thesis.

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L1S20MSBC0004

CERTIFICATE OF RESEARCH COMPLETION

It is certified that this thesis titled, **"The Assessment of Hepatotoxic Effect of Bioactive Compounds Derived from *Citrullus Colocynthis* Organic Extracts"**, submitted by Anis Shahzad Khan, Registration No. L1S20MSBC0004, for MS degree at "Faculty of Sciences and Technology", University of Central Punjab, is an original research work and contains satisfactory material to be eligible for evaluation by the Examiner for the award of the above stated degree.

Supervisor's Name:

Dr Muhammad Afzal

Associate Professor,

Faculty of Sciences and Technology,

Signature

CERTIFICATE OF EXAMINERS

It is certified that the research work contained in this thesis titled ""**The Assessment of Hepatotoxic effect of bioactive compounds derived from *Citrullus colocynthis* Organic extracts**" is adequate for the award of "MS in Basic and Applied Chemistry.

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ABSTRACT

The use of traditional medicines is increasing day by day. As, the herbal products are more preferable than the synthetic medicines. The current study was designed to evaluate potential of bioactive compounds through *in-vivo* and *in-vitro* methodology; in the aforesaid study16 rats were randomly divided into four groups (3 experimental and 1 controls) to check the cytotoxicity of *C. colocynthis.* In the experimental groups a single daily dose of Methanolic, n-hexane and chloroform extract of *C. colocynthis* (300, 600, 900, 1200 mg/kg) was administered orally. Normal saline was administered in control group. After 15 days, the rats were sacrificed under deep anaesthesia and liver histopathology was done. The results indicated the minimal morphological changes of liver cells even by maximum dose of 1200mg/kg. However, the biochemical parameters like ALP, AST, and ALT can confirm that the toxicity effects are dose dependent. *C. colocynthis* have Phytoconstituents (Gallic Acid, Vanillic Acid, Feralic Acid, Cinamic acid, Ether, 3-butenyl pentyl, oxime-, methoxy-phenyl-_ Ethyl 4 chlorobutanimidoate) which were confirmed by HPLC and GC-MS. These phytochemicals have remarkable anti-oxidant, anti-diabetic, thrombolytic and haemolytic activities.

Keywords:

C. colocynthis, Hypoglycaemic agent, Hepatotoxicity, Cytotoxicity, Phytochemicals

CHAPTER ONE: INTRODUCTION

Traditional medicinal herbs have continued to play a significant part in Chinese and other indigenous medical systems across the world. Conventional medicine relates to any historically and ethnically different medical therapy that differs from modern medicine and has been handed down orally through many cultures. Conventional medicine has long been a standby in the fight against sickness and the quest of a healthier life for humankind. Despite the fact that describing the vast variety of characteristics and aspects of traditional medicine is difficult, the WHO is Diseases and Related Health Problems (WHO, 2002) explicitly indicates that a working definition is required.

Natural remedies are defined as a wide range of health procedures, perceptions, consciousness, and explanations involving plant, animal, or nutritional medicines, spiritual forms of treatment, alternative therapies, and safe from disease (Ak, 2019). Alternative treatments, often known as conventional medicine, are still practised different countries, 71% of the population in Chile, and 40 percent of the total number of the population in Colombia, to name a few 18 ttraditional healers played major part in the lives of Africa and they have the potential to be important components of an integrated health-care system. Over 21000 plant species are considered and be used as therapeutic plants, according with WHO Traditional medicine are used to cure a variation has been well recorded throughout history. For hundreds of years, plants have been utilized as treatments. Wild animals have been shown to eat specific plants on their own to treat a variety of diseases; according to research, all countries use medicinal herbs widely and effectively. Herbal medicine was a very well and documented practise throughout Asia. As a result, this region produces the majority of widely recognised medicinal plants, especially from Chinese and Indian. In Europe and North America, herbal medicine is gaining popularity, particularly for correcting the imbalances induced by contemporary diets and lifestyles (Ak, 2019)

For ages, medicinal properties have been employed as the basis of therapy in traditional communities all throughout the world. It will remain important as a primary health care strategy for around 85 percent of the world's population, as well as a drug development resource, accounting for 80 percent of all synthesised medications. Local people have been using medicinal plants as a method of treatment for thousands of years all over the planet. It will continue to be important as a general healthcare approach for around 85 percent of the country's population, as well as a foundation for pharmaceutics, with synthesis accounting for over 80% of all antidepressants. Many centuries ago, Medicinal plants were used as a means of treatment among traditional communities all over the world. It would remain important as a general healthcare strategy for roughly 85 % of the worldwide people, as well as a source of medicinal chemistry, accounting for about 80% of all contemporary pharmaceutics (Fitzgerald et al., 2019) .The medicinal plant research conducted in Africa pale into insignificance in contrast to that conducted in China and India. In reality, no African country has ever been in the top ten countries for the number of publications in this field. On the African continent, just one of the first 25 institutions is located. China is represented by thirteen of the top twenty-five countries (including the first seven), three from Brazil, two from South Korea, and one each from Saudi Arabia, Pakistan, Iran, Mexico, Cameroon, France, and Malaysia (Salmerón-Manzano *et al*., 2020).

According to United Nations, herbal medicines are used by 80 percent of people across the globe for some aspect of their basic health care. According to the WHO, about 21,000 plant strategies have the ability for use as medicinal plants. Active pharmaceutical substances, as well as non-pharmacopoeia and synthesized medicines, can all be found in medicinal plants. Aside from that, those species have had a significant influence on the development of human cultures all over the world. Furthermore, several plants have been identified as necessary source of nutrients, and their medical capabilities have been advocated as a result. These plants include ginger, green tea, walnuts, aloe, pepper, and turmeric, to name a few. Herbs are used in textile dyeing, pesticide

application, cuisine, fragrance, and tea, among so many other things, in addition to their medicinal use. To prevent ants, flies, mice, and fleas away from homes and businesses, various medical plants/herbs are utilized in various countries. Traditional medicine practitioners provide very effectual ingredients again for treating mild to moderate ailments such as diarrhoea, constipation, high blood pressure, low sperm count, dysentery, and weak male genital erection, piles, covered tongue, menstrual disorders, bronchial asthma, leucorrhoea, and fevers, as well as piles, coated tongue, menstrual disorders, bronchial asthma, leucorrhoea, and fevers (National Health Portal of India, n.d.). Medicinal plants are needed not only as a major supply of pharmaceuticals, or even as Phytoconstituents building blocks for novel drug development. Traditional medicine's that use curative medicines was indeed not spontaneous, but it is influenced by taxonomic relationships in a variety of ways. The association among taxonomic diversity and the amount of medicinally beneficial species has been investigated using a variety of statistical methods (Phumthum et al., 2019).

An indigenous medicinal medication known as the Africa flower has been used in China for decades to treating HIV-related losing symptoms, while the Africa flower has been used in Africa for decades to treat withering symptoms diseases such as HIV. Plant and natural sources (including such fungi and marine microbes) had contributed significant contributions to today's commercial pharmaceutical formulations, as have their analogues. Over 100 natural product-based medications are in clinical trials, and 11 percent of the WHO's 252 essential medicines are manufactured solely of natural ingredients of plants. (Sissi Wachtel-Galor & Iris F.F. Benzie, 2011). Plants, herbs, and ethno botanicals have been utilized for illness prevention and treatment from the beginning of time, and they are still used today all over the world.

Plants and natural sources are vital to modern medicine, and they play an important role in commercial pharmaceutical formulations. Plants are responsible for around a quarter of all medications used across the world. Herbs are still frequently utilized in medical therapy rather

than medications. Herbal medicine would be a recommended treatment alternative for some people. Others simply use herbs as a complement to prescription drugs(S. Wachtel-Galor & I. F. F. Benzie, 2011).Before medical therapy with internal preparations, diet, and behavioral restrictions, Ayurveda tries to diagnose their ailments. All plants, whether they come from the roots, flowers, fruits, seeds, or bark of plants or herbs, must be properly researched before becoming part of the Ayurvedic pharmacopoeia. Spices grow best in hot, humid areas. Spices have a different active principle than woody or herbaceous plants, which provides them distinct characteristics. Within the organism, these active principles perform distinct functions. Spices contain photochemical, which are secondary compounds which defend plants from insects, animals, fungus, diseases, and parasites (Kumar et al., 2017). Herbal extracts with therapeutic value are valuable repositories of structural and chemical variety. Surprisingly, over than 120 different photochemical from various plants have the potential to be life-saving medicines. Only 6% of the total plant species were screened chemically and pharmacologically to produce these chemicals. With the use of Ayurvedic expertise that has reached its peak of achievement thanks to verified research and better methods, there appears to be an essential need for us to use advanced research methodology to validate basic principles and pharmaceuticals used in the Ayurvedic system of medicine. As a result, continual study technique advancements are vital for the advancement of Ayurvedic medicine(Chauhan *et al*., 2015).

Fruits include bioactive chemical components including such glycosides, flavonoids, alkaloids, fatty acids. The antioxidants, cytotoxicity, anti-diabetic, anti-insecticides, antibiotic, and anti-inflammation properties of C. colocynthis fruit have all been widely studied. The nutritional benefits of the plant were also proven, owing to its high protein and important mineral content, as well as the high quality of its edible seed oil {Hussain et al., 2014}. Most ornamental and therapeutic plants include naturally produced chemicals such as flavonoids, tannins, cyanogenic glycosides, polyphenols, saponins, lignins, and lignans, as well as vitamin C, vitamin E, and

carotene, that are used as major food constituents by both humans and animals. Antioxidant ingredients (hydrolysable tannins, phenolic acid, and flavonoids) of plant materials have massive pharmacological and biological activity for the protection of cardiovascular disease, cancers, anti-carcinogenic, and anti-mutagenic effects (Hussain et al., 2011).The presence of others of medicinal plants is attributed to the presence of many complex chemical components of various compositions, which are referred to as medical plant qualities. secondary metabolite is a term that refers to a substance that seems to have it (Helmi et al., 2016).

C. colocynthis has antibacterial, antifungal, and ant-plasmodial properties. included glycosides, flavonoids, tannin, and sterols, according to the photochemical analysis. Other substances include glycosides, flavonoids, tannin, sterols, and others. The insoluble carbohydrates, total soluble sugar, and amino acids were determined using quantitative and qualitative analysis methods. The antibacterial, antifungal, and anti-palsmodial actions of extract metabolites have been determined based on the characterisation of colocynthis oil (Wadood, 2013).

The Certain foods provide a high concentration of essential amino acids. As a nutritional supplement, essential and non-essential amino acids, which are the functional units of protein required on a daily basis for human health, can be taken. It's possible that this is the first time an amino acid profile has been utilized to describe *C. colocynthis.* When paired with several other parameters being used assess the quality of *C. colocynthis,* the amino acid content revealed could offer biologists with valuable information. Cucurbitaceous plants were found as trypsin inhibitors in a simulation software. in addition to antibacterial peptides Thus according research observations, the presence or absence of a crucial Arg/Lys residue at the putativeP1 position can be used to differentiate these common cysteine-rich peptides based on the functional features. Despite their sequence similarity and broad classification as inhibitor cysteine knots, these peptides appeared to have a range of undiscovered bioactive substances (Shahin-Kaleybar et al 2020*)*

Hepatotoxicity implies chemical-driven liver damage

The liver is an important organ for processing and eliminating poisons, but it is also (Shahin-Kaleybar et al., 2020) susceptible to their toxicity. When consumed in significant doses or even when Certain pharmacological substances, when used within therapeutic guidelines, can harm the organ. Hepatotoxicity can also be caused by other chemical agents employed in laboratories and industry, as well as natural chemical and herbal therapies. Hepatotoxins are poisons that cause the liver to malfunction. Drug-induced liver injury is responsible for 5% of total of all hospitalizations and 50% of all acute liver failures. Nausea, vomiting, abdominal pain, loss of appetite, and diarrhea are all symptoms of hepatotoxicity. unexpected If you're fatigued or weak, and you have jaundice (a yellowing of the skin and eyes, there's something wrong(eyes), or have unusual oedema or weight. gain. Chemicals frequently induce liver harm that only emerges as abnormal liver enzyme tests (Paniagua, A. C., & Amariles, P. 2017). Traditional Chinese medicine (TCM) has recognised 65 regularly used herbs, herbal medications, and herbal supplements, as well as 111 other herbs or herbal blends, as being causative for liver disease, with different degrees of causation evidence that are rarely convincing (Teschke & Eickhoff, 2015).

Phytochemical analysis

The phytochemical study of plants is very significant commercially because pharmaceutical companies are very interested in producing novel medications to cure various diseases. The key phytochemical qualities identified in the indigenous medicinal plants of Mardan by our study are predicted to be highly effective in the treatment of numerous ailments in this region (Wadood, 2013). *C. colocynthis,* a Cucurbitaceous family member, was tested for phytochemicals to see if it has any therapeutic effects to prove the existence of diverse element compositions, a qualitative investigation was conducted. Alkaloids, carbohydrates, and flavonoids were detected in the plant, root, flowers (male and female), and fruit after a photochemical investigation (pulp, hull, seed). This specimen is devoid of tannins, gums, and mucilage's

Antioxidant Effects

C. colocynthis methanolic fruit extract has been shown to be a powerful antioxidant. Gallic acid, phenolic component, has good free radical scavenging activity since it was present. At a concentration of 2,500 mg/ml the fruit extract demonstrated the maximum antioxidants and free radical scavenging activity. Cucurbitacin is also a highly effective antioxidant. Free radicals such as hydroxyl, superoxide anions, and superoxide anion can indeed be removed with this method. It can also completely prevent lipid peroxidation and oxidation. Phytochemical screening was used to discover the natural compounds found in *C. colocynthis preparation*. As a result, it is an excellent antioxidant. *C. colocynthis oil* has been shown to increase antioxidant enzyme activity and protect the liver from harm. According to an in-vitro investigation, *C. colocynthis can* protect the body from free radical damage. C. colocynthis has a variety of biochemical that make it an effective antioxidant.(Li et al., 2022)

Antimicrobial activity

C. colocynthis extract demonstrated considerable antibacterial activity in chloroform and acetone extracts. Using the agar disc diffusion technique, researchers investigated the antibacterial activity of *C. colocynthis (*Cucurbitaceous), a natural plant that can be used to treat a number of ailments. *Pseudomonas aeruginosa* is resistant to the crude acetone extract, with zones of inhibition measuring 14.0 mm, according to the findings. Against *Staphylococcus aureus*, the chloroform leaf extract had little antibacterial action. For *Escherichia coli*, the chloroform extract had a minimum inhibitory concentration of 4.0mm.(Gowri et al., 2009)

Antidiabetic effect of plant *C. colocynthis*

In the United States, diabetes has risen and became one of the top causes of illness and mortality. Phytotherapy has traditionally been treated diabetes and is widely acknowledged as an effective method. *C. colocynthis is* a type of citrus fruit that has anti-diabetic properties. On the other hand, a hydro-ethanolic extract of colocynthis pulpy meat with seeds has yet to be demonstrated anti-

diabetic. The potential for -glucosidase inhibition may be linked to a hydro-ethanolic extract of *colocynthis* pulpy flesh with seeds **(**Gowri et al., 2009**).**

1.1 OBJECTIVES

- The quantitative analysis of bioactive compounds from Plant Extracts by HPLC and GC-MS.
- To determine the in vitro Biological Activities of organic extracts of *C. colocynthis*
- To determine the in vivo hepatoprotective potential effect of bioactive compound from different organic extracts.

Chapter Two: Literature Review

C. colocynthis was non-hardy, spreading perennial herbaceous vine native to subtropical Asia and Africa that has spread throughout Africa and the Mediterranean region. It's indeed related to watermelon. The abrasive and angular stems, rough, deep 3–7 lobed leaves, and single pale yellow flowers define this species. Each plant can produce 15–30 spherical (7–10 cm) fruits with tiny (6 mm) smooth brown seeds streaked with yellow. The therapeutic and oil-producing properties of *C. colocynthis* have a long history. Its tiny seeds have been unearthed in Pharaoh-era Egyptian tombs. Libyan and Middle Eastern cultural heritage Around 4000 BC, archaeologists uncovered a number of Middle Ages archaeological sites (Dane et al., 2007).

The bitter fruits of the colocynth, often known as bitter apples or bitter melon, were used to make a fatal poison. Fruits were widely used in folk medicine, especially to treat stomach disorders. The pulp is a potent hydragogue, cathartic, and laxative due to the presence of glucosides such as *C. colocynthis*. The cultivation seems to have become a source of prosperity on the island of Cyprus since the 14th century. When the seeds are mashed, they produce a coarse bread for desert Bedouins. The seeds contain a substantial amount of oil (17–19% by weight), which was historically used to light candles. In recent years, there has been a lot of interest in producing novel oilseed crops.(Dane et al., 2007).

The Bioactivity-guided extractions or fractionation aid in the isolation of active Phytoconstituents from plants, the passage of certain physicochemical tests, and the provision of a variety of clinical evidences for disease prevention. Many metabolites, or compounds found in traditional plants, are regarded non-essential for the producer's survival organisms. *(Rasool et al., 2020)* .The majority of plant metabolites contain therapeutic properties, and plant-based extractions and fractions have been employed to create a wide range of medicines. *C. colocynthis* fruit extracts and fractionations depending on bioactive components could offer a wide range of medicinal

effects (*Parveen et al., 2020).* In this study, we made AgNPs from Azadirachta indica and C. colocynthis, both of which are well enough for their therapeutic characteristics and biological activity. Azadirachta indica is a Meliaceae plant that has long been utilized in traditional medicine. It really is an antibiotic, antifungal, and antimicrobial plant because its leaves contain quercetin, -sitosterol, and nimbidin, and its seed contains azadirachtin.*(Rasool et al., 2020).*The seeds, fruits, base, and stem of C. colocynthis were shown to have analgesic and anti-inflammatory properties in mice and rats without inducing acute toxicity. Fresh fruit extract had the significant influence, preceded by seeds. The application of fruit extract to rats with androgen-induced alopecia resulted in hair growth. When compared to standard finasteride, the drug was also more effective at causing follicles to enter the antigenic phase..*(Rahimi et al., 2012).*

The Cucurbitaceae family's C. colocynthis as well as being the Old Testament's wild gourd. Ancient Greek and Roman physicians were well aware of its highly bitter taste and strong purgative qualities. Bitter apple, bitter cucumber, colocynth, and bitter gourd are some common English synonyms. It can also be a well-known medicinal herb in traditional Iranian medicine (TIM) that would be utilized for a variety of medical purposes, either alone or in conjunction with other herbs. It can be found primarily in Asia and Africa. The fruit pulp is the most therapeutic part of the plant, so it's best to keep it to a minimum before consuming it, as the strength of the pulp decreases over time and is completely gone after two years. The pulp retains its efficacy for around 4 years after it was removed from the fruit. (Rahimi *et al., 2012)*.

In today's environment, when the Western medical system has completely disappeared, it's important to remember that has reached its peak of achievement thanks to verified research and better methods, there appears to be an urgent need for us to advanced research methodology to validate basic principles and pharmaceuticals use in the Ayurvedic system of medicine. As a

result, continual study technique advancements are vital for the advancement of Ayurvedic medicine.*(Chauhan et al., 2015).*

A melancholic purgative, *C. colocynthis* leaf has been used to treating melancholia and epilepsy. Its leaf can be used to relieve inflammation and bleeding on the skin. The root can be used to treat scorpion and snail bites. *C. colocynthis seeds* and fruits have long been used in Iran as an anti-diabetic. Among Iranian herbal remedies merchants*, C. colocynthis has* been a well diabetes treatment. Herbal medicine merchants claim that this plant can also be used to treat fever. As a result, palms are cleansed with a decoction made from its fruit.(Rahim*i et al., 2012)*.

2.2. Pharmacological Activities

2.2.1 Intrinsic Toxicity in Animals

Preclinical animal research is currently being undertaken as part of pharmaceutical development. All of these experiments are based on the premise that giving a chemical to a group of animals at large doses might disclose intrinsic adverse effects (of a treatment) that would be unusual in people receiving much lower amounts. These tests are used to identify chemicals that may cause liver damage as well as to rule out compounds that pose a serious danger. Previously, several anti-TB DILI animal models based on similar ideas were reported. Male Wister rats suffer liver difficulties after 21 days of isoniazid therapy at a dosage of 100 mg/kg, and the isoniazid metabolite hydrazine plays a key role in liver abnormalities. A combination of isoniazid (50 mg/kg) and rifampicin (100 mg/kg) led to severe liver failure in mice. Further examination has become underway (Ramappa & Aithal, 2013).

According to the researchers, this model supports the concept that mitochondrial redox changes play a role in apoptotic liver cell injury in anti-TB drug hepatotoxicity. In milligrams per kilogram, the doses utilized in this animal were approximately ten times those used in humans. In contrast to clinical DILI, the mouse demonstrated histological changes associated with hepatic steatosis. Pretreatment with phorone resulted in significant glutathione depletion, which was the only way to produce hepatocyte necrosis, which is a frequent feature of DILI exhaustion in individuals. The majority of idiosyncratic DILI are induced by the pharmacological effect of a drug. 30 Idiosyncratic responses to medicine was referred to as idiosyncrasy. Anti-TB drug hepatotoxicity is similar, and it is thought to be regulated by host features. As a result, animal experiments are unable to effectively recreate such situations. Surprisingly, the phenotypes of

anti-TB DILI in people must not strongly match those of these animal models (Ramappa & Aithal, 2013).

Ant tuberculosis drugs can harm the liver, causing substantial sickness and, in exceptional instances, death. This form of toxicity may have an impact on some person's tuberculosis clinical outcome. This study looks at how and when oxidative stress and, more broadly, abnormalities in redox homeostasis, as well as mitochondrial function, play a role in anti-tuberculosis drug-induced hepatotoxicity. Aside from genetic challenges, old age, starvation, drunkenness, chronic hepatitis C, and chronic hepatitis B have all been identified as risk factors for arsenic infection, Human immunodeficiency virus (HIV, and pre-existing liver sickness. Important considerations include oxidative stress and anti-oxidant medications. These comorbid disorders treat mitochondria malfunction, and several cancer medicines are linked to mitochondrial dysfunction. The shared pathogenic mechanism for liver harm may be at work as a result of the malady interaction. Aside from affects behavior hepatotoxic medications, our ability to forecast, mitigate, or cure hepatotoxicity still seems to be limited. To better understand the etiology of ant tuberculosis drug-induced hepatotoxicity, more integrated research, covering fundamental bases but also clinical research on new therapies such as antioxidants and beyond, is required. The utility of Pharmacogenetics in the therapeutic treatment of substance hepatotoxicity is indeed expected to be researched further (Dane et al., 2007).

The liver is a crucial organ that is especially prone to the harmful effects of oxidative stress. Reactive oxygen species (ROS) are produced by hepatocyte mitochondria, microsomal, and peroxisomes, which impact signalling pathways such as transcription factor proliferator-activated binding site alpha (PPAR), which regulates fatty acid oxidation, and mitogen-activated protein kinase (MAPK) and connected anxiety kinases, which regulate proapoptosis. Furthermore, oxidative stress in Kuepfer cells can result in the generation of cytokines such tumour necrosis

factor alpha, which aid in tissue inflammation and cell death. Peroxidation caused by oxidative stress can stimulate collagen production in growth factors (Yew, Chang, & Chan, 2018).

The etiology of liver injury is thought to involve a convoluted connection between oxidative stress (nitrosative stress) and immune responses. With the advancement of genome sequencing technology, new concepts concerning the processes of drug-induced hepatotoxicity have emerged, emphasising generic indirect effects after substance upstream effects, along with complicated interactions between environmental and genetic factors. It implies that looking at genes in the context of HLA system variability, immunological response, and oxidative stress is significant beyond medication distribution and metabolic activity. In recent years, the pharmacogenomics knowledge base has grown*(Yew et al., 2018).*

DILI makes it harder to assess the safety of new pharmaceuticals, putting patients' pharmaceutical and healthcare research at risk. To investigate human DILI processes, a number of human liver cell-based in vitro models connected with toxic genomics techniques have been established as an alternative to animal testing. This research investigates the efficiency of in vitro human liver systems in omics-based drug-induced hepatotoxicity investigations. We also discuss existing and future DILI research contributions, as well as present and future bioinformatics tools for evaluating toxic genomic data generated by these models. Human pluripotent stem cells, which carry donor-specific genetic information, offer a lot of promise in toxicity studies reactions that really are different for each individual.

The defined as a result platform, which would have been co-cultured with other liver-derived non-parenchymal cells in a microfluidic device, enables us to assess immune-mediated drug hypersensitivity while also speeding up individual drug toxicity studies. Using flexible microfluidic technology, a more advanced organ-on-a-chip methodology might be developed, seeking to further narrow the gap between in vitro and in vivo environments. Conventional

transcriptome analysis of these cell cultures, combined with supernatural causality approaches, can help researchers understand better DILI causes. Statistical methods are used in these strategies to elucidate regulatory interactions in sections of these activities.

The use of more detailed human liver models in combination with causality-inferring bioinformatics techniques will pave the framework for the growth of new analysis techniques of a powerful methodology for determining the cause of disease comprehensively assessing DILI mechanisms in a variety of circumstances.(Yew et al., 2018).

Idiosyncratic hepatotoxicity seems to be a complex process that involves both concurrent and sequential events that define the pathway's orientation, the extent of liver injury, and also the end result. Recent breakthroughs in Pharmacogenetics have cleared the path for more precise algorithms that consider medication, host, and other factors. and environmental risk variables, allowing for more accurate risk–benefit ratio calculations and better drug personalization. Human tissue and specimens must be used in future investigations on the pathophysiology of hepatotoxicity whenever appropriate. so that innovative concepts can be quickly converted into therapeutic applications*(*Ramappa & Aithal, 2013*).*

2.2.2. Drug transformation, detoxification and elimination

Reactive metabolite production has been associated to a number of clinical consequences, including idiosyncratic DILI. Electrophiles can always be found in a wide variety of inhibitory compounds. They react with nucleophilic compounds on cellular proteins such as lysine and cysteine after they have been detoxified. Covalently altered cellulose proteins can be restored or eliminated. Adducts of drugs and their metabolites disturb key cellular functions, causing harm to the target organ if these mechanisms fail. Covalent protein binding, accompanied by the generation of reactive metabolites, has the potential to produce immune-mediated disease

damage. Individuals who produce a high number of reactive metabolites may have a high number of activity enzymes involves the conversion of pharmaceuticals to reactive metabolites in the body. All of those are cytochrome P450 enzymes in phase I that are involved in oxidizing, reductions, or degradation. The creation and accumulation of reactive metabolites, rather than the parent medicine, appears to be the cause of hepatotoxicity(Ramappa & Aithal, 2013).

Blood tests are taken (hematological and biochemical) Blood was collected from the interplanetary sinus and analyzed at Cairo University's Faculty of Agriculture Research Park (FARP) using a BC-2800Vet Auto Hematology Analyzer (Mindray, China) for the mentioned hematological parameters: white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), and hematocrit (HCT). A totally automated dry chemistry analyzer was used to measure lactic dehydrogenase (LDH), alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST), protein content (TP), albumin (ALB), creatinine (CREA), glucose (GLU), calcium (Ca), phosphorus (P), total cholesterol (CHO), and triglycerides (TG)(Eissa et al., 2019).

2.2.3. Histopathological examination

In the T1 and T2 groups, as well as their respective controls, small histological variations were detected in selected organs (liver, kidney, and pancreas), as well as the testes and ovaries. The treated groups' liver tissue slices revealed a normal liver morphology with basophilic isolated necrotic areas and spontaneous localized and multifocal angiectasis (arrowheads), which could occur as the animals mature. There are isolated zones of coagulation necrosis in male groups that received 50% and 100% GM soybeans, respectively. The treated groups (T1 and T2) exhibited normal histological structures in the kidneys, with symptoms of local inflammation and hemolysis, as well as amyloid bodies, as compared with untreated controls. Histological examinations were performed on pancreatic tissue slices. In the groups with 50 percent GM

soybean meal, indicated normal clinical and pathological structure with normal beta cell shape and distribution, as well as beta cells, whereas evidence of hemolysis and amyloidosis, as well as neutrophil infiltration and non-malignant pancreatic fibrosis, were identified in the 100 percent soybean meal groups.*(Eissa et al., 2019).*

This study looked into the biochemical and histological aspects of 5-FU, cisplatin, and oxaliplatin-induced liver injury in rats. Peliotic hepatic was detected in the livers of oxalipatin-treated rats (group D). Furthermore, when viewed under a microscope, the sinusoids were discovered to exhibit uneven focal dilatations. The cavernous lumen of the lesion was thickly packed with blood. The tissue lining the dilated sinusoids had no necrotic cells, indicating that it was in good health. In an oxaliplatin-treated cancer patient, lesions defined by damaged centrilobular zones in non-tumor bearing liver cells. There were bulk defects as well as substantial sinusoidal dilatation. Oxaliplatin-induced liver lesions were morphologically comparable to veno-occlusive disease produced by high-dose chemotherapy. Regenerative effects were clearly slowed in the oxaliplatin-treated group (group D), indicating lesser hepatocyte proliferation, as previously shown in oxaliplatin-treated groups Wistar rats.(Bano & Najam, 2019a). Based on the linked discussions of the mechanisms involved in adverse drug reactions, it appears that taking both medications at the very same time contributes to detrimental effects on the liver profile(Bano & Najam, 2019b).

2.2.4 Acute Toxicity

Clinical Evaluation of Acute Toxicity in rats treated 100 and 200 mg/kg GTN, no evidence of toxicity or death were identified or noted. Nevertheless, in 300 mg/kg GTN-treated rats, ocular release (after one hour of treatment), reduced immobility (without the need for a response to stimulation), and convulsions (after multiple hours of treatment) were observed; however, all organisms recovered after four hours of therapy, and no casualties were observed. Only couple

of the 400 mg/kg GTN-treated rats died inside of four hours of the court proceeding due to aftershocks and respiratory problems, while another five evolved ocular secretions (one hour after therapy), tremors (two hours after therapy), and deficient movement (without responding to stimuli), which were recovered four hours later. GTN was given at a dosage of 500 mg/kg to all rats in the GTN-treated group. After demonstrating several poisoning signs, including tremors, trouble breathing, Straub tail, opisthotonos, and distributed seizures, the group perished after four days of treatment. As a consequence, rats given 100, 200, and 300 mg/kg GTN died at a rate of 0%, but rats given 400 and 500 mg/kg GTN perished at a rate of 29% and 100%, correspondingly (Kaid et al., 2019).

According with study, all haematological parameters in rats administered 100, 200, 300, 400, and 500 mg/kg GTN did not distinguish substantially from those in the control group. The serum creatinine, urea, albumin, globulin, and immunoglobulin levels of the 100, 200, 300, 400, and 500 mg/kg GTN-treated rats did not differ significantly from those of the normal control group. Total bilirubin, alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase levels of the 100, 200, 300, 400, and 500 mg/kg GTN-treated *(Kaid et al., 2019*).

Evaluation of histopathological Microscopic examination of histological sections from the liver, kidneys, heart, lungs, spleen, and nervous system of rats administered 100, 200, 300, 400, and 500 mg/kg of GTN revealed normal structures in all organs, with no apparent changes in histological or cellular formations. Hepatocytes, sinusoids, and central veins in the liver had the same cellular structure as the control group. Cardiac muscle cells and connective tissue in the heart have normal cell structure. The bronchiole, alveoli, alveolar duct, and blood veins in the lung all had normal cellular integrity. There have been no anomalies in the spleens or brains of rats following GTN medication as compared to animals in the normal control group. The following is the situation: Toxicity has been seen in a subacute clinical context. There were very few fatalities in the 42 mg/kg GTN-treated group after 14 days of therapy Body Weight Changes.

The GTN-treated group and the normal control group had no statistically significant differences in body weight. The Absolute Weight of Selected Organs The absolute weights of the GTN-treated group's selected organs were within normal limits, and there was no significant difference between the absolute weights of the GTN-treated group's selected organs and the normal control group's absolute weights. Rats' Food and Water Requirements According to haematological and biochemical research, there were no significant differences in food and drink intake between the GTN-treated group and the normal control group(*Kaid et al., 2019).*

2.2.5. Histopathological Evaluation

A histological study was performed to confirm the chemical findings and rule out any morphological irregularities. In all GTN-treated and the control groups for sub-acute toxicity, light microscopic analysis of the rats' essential organs revealed no gross pathological alterations in the liver, kidney, heart, lungs, spleen, or brain (Kaid *et al.,* 2019). Sem micrographs of the livers and kidneys of the ordinary and GTN-treated subjects revealed normal morphological architecture. Compared with control, GTN-treated mice's livers had normal cellular architecture and binucleation, as well as no abnormalities. Injury, necrosis, congestion, fatty acid buildup, and other factors could be evident. In the area, there were no hemorrhagic patches on the skin. The sinusoids of the liver or a major vein Hepatocytes organized in cords could be seen clearly. In the case of subacute oral poisoning, a cross-section of the liver revealed no lyses in blood cells or infiltration of neutrophils, lymphocytes, or macrophages. In the case of the kidneys, there were no morphological alterations in the GTN-treated group. As in the control groups, the glomerular architecture appeared appropriate.

Liver and kidneys are important organs that play a role in xenobiotic and metabolite synthesis, detoxifying, preservation, and elimination, as well as being susceptible to external toxins. However, the liver is a complicated structure made up of a range of cell types that perform various

functions. A variety of causes can induce cell damage. Enzymes can be found in the cytosol. (AST), (ALT), and (ALP) are released into the circulation when the hepatic cell membrane is destroyed; nevertheless, both ALT and AST are internal enzymatic, and their existence in the circulation signals cellular injury (*Kaid et al., 2019).* As a consequence, the identification in plasma might be used to evaluate any biological damage, especially now that organic damage detection standards have indeed been defined. Except for AST and ALT, the serum enzymes that have been proved to be the most efficient and sensitive markers of hepatocellular damage, there is no single molecular marker that can be utilized to diagnose liver injury. AST is present in a number of organs, including the heart and muscles, and its production is not limited to acute liver failure. ALT, unlike AST, is mostly present in the liver. Many tissues, such as the liver, bone, and muscle, contain ALP. It is measured because it is found in the kidney, gut, and placenta. Despite the fact that significant increases in serum ALP have been associated to liver and bone dysfunctions, and even the fact that ALP is rapidly elevated due to bile flow obstruction or other forms of expansion lesions, the relevance of ALP differs depending on the tissue. Additionally, the presence of genetic condition could point to biliary tract malfunction or liver dysfunction as the cause of the elevated ALP levels.

According to reported data from acute and subacute trials, the levels of ALT and ALP in rats treated with different concentration of GTN were not statistically different from that in the normal control group. Toxins are metabolized and excreted mostly through the liver. It's a popular xenobiotic target, especially since even seemingly safe drugs can occasionally cause serious liver-related side effects, which are the largest cause of drug recalls. Because there are medicines that induce liver damage can reduce virtually any hepatobiliary ailment, distinguishing between adverse drug reactions and liver disease can be challenging. Despite the fact that it's practically impossible to tell the difference between drug-induced and non-drug-induced histology, and disease-induced liver damage, the findings of all rats' liver histological sections in the acute and

studies revealed normal architectures without any lesion, despite the fact that drug-induced damage encompasses virtually all types of known acute and chronic liver disease, the findings of all rats' liver histological sections in the acute and studies revealed normal architectures without any lesion sections in the acute and studies revealed normal architectures without any lesions(Kaid et al., 2019).

The liver of rat is a dark-brown organ located behind the diaphragm, primarily on the right hand side. The centre lobe is the largest while the right lateral lobe, the left lateral lobe and the central lobe are the smallest. The right lateral lobe is a tiny caudal lobe are followed by the right lateral lobe, the left lateral lobe, and the right lateral lobe, and a small caudal lobe. The typical hepatic duct is formed by the confluence of the hepatic ducts from each lobe. It then travels to the duodenum, where it opens at the papilla, about 25mm below the pylorus(Quaresma et al., 2007). There are some animals required anesthetics reinforcement near the end of surgery, which was accomplished by inhaling a small amount of ether through a mask. Because this anesthetic produces respiratory depression, only tiny doses should be administered while the animal's respiratory status is stable. Heart rates were also monitored.

To prevent hypothermia, a 40-watt bulb light was utilized to keep the animals warm throughout the early post-operative period until they were fully recovered from an aesthesia. It's worth noting that having an assistant remove the hepatic lobes during surgery is crucial for a proper biliary network exposition as well as suitable sectioning as a retractor, the cotton buds were soaked in saline solution were utilized, and they were successful in exposing the lobes while causing little tissue damage. The dissection of the right hepatic lobe of liver, which was adjacent to the portal vein, was another source of worry. Even the tiniest misstep could result in the patient's death. In one case, a duct dysfunction caused hypovolemic shock and hemo peritoneum. and the animal's death as a result of uncontrollable bleeding. The right hepatic lobe needs to be treated with

caution. As with any other tissue, rapid motions must be avoided because it is a small, delicate structure(Quaresma et al., 2007).

2.2.6 Phytochemical analysis

The researchers wanted to evaluate how effective different extracts from *C. colocynthis seeds* preparations were at scavenging DPPH free radicals. To investigate the antioxidant activity of this plant, polyphenols and flavonoids in plant seeds extracts were produced using standard protocols, including a crude extract and an aqueous extract (E1), a hydromethanolic extract (HM), , an ethyl acetate extract (EA), a defatted aqueous extract (E2) and an n-butanol extract (n-B). None of the extracts contained any alkaloid, quinone, antraquinone, or reducing sugar. Although terpenoids were abundant in E1 and n-B, catechic tannins were only found once a week in HMaFlavonoids were found in abundance in E1, HM, and EA. Coumarins have been discovered to be efficacious in E2, EA, and n-B. When calculated as gallic acid equivalents, polyphenols in EA, HM, and E1 were 329, 1002, and 150 mg per 100 g plant matter, respectively.

In terms of the catechin equivalent, flavonoids of per 100 g plant matter in EA, HM, and E1 were 620, 241, and 94 mg, respectively. Similar results were achieved in n-B and E1, with lower results in E2. Quercetin compound was discovered via thin layer chromatography in the EA and HM extracts, myricetin and gallic acid were found. The ant oxidative impact of these extracts was reduced by 88.8% with EA, 74.5 percent with HM, and 66.2 percent with E1, with IC50s of 350, 580, and 500 g/mL, respectively, in a 1,1-diphenyl-2-picrylhydrazyl assay. The concentration of ascorbic acid is 1.1 g/mL, while that of vitamin C is 1.1 g/mL. The presence of flavonoids, which are responsible for antioxidant activity, in C. colocynthis extracts, as well as other biological activities of this plant, is demonstrated by these qualitative and quantitative analytical data.(Chekroun et al., 2015)

2.2.7. Antioxidant Effects

Medicinal herbs are frequently used in traditional medication established all over the world. They are a priceless, wonderful, and time-honored sources of cures to a variation of ailments. The researchers intended to see if *C. colocynthis* seeds have antiulcer genic or antioxidant properties. The goal of this research was to show how important seed is as a source of bioactive chemicals when eaten in a traditional way. Many phytoconstituents with pharmacological activities were discovered during phytochemicals study of methanol extract seed extract. A spectrophotometric approach using 1, l-diphenyl-2-picryl hydrazyl and a free radical scavenging approach with Hydrogen Peroxide were used to test the antioxidant properties of the extracted. When all these compounds procedures were combined at 300 g mL -1, this methanol extracts seed extract showed maximum percentage of inhibition of 79.4 and 72.4 point margin, respectively, indicating that the leaf extracts has great antioxidant capability(Birem *et al*., 2017).

2.2.8 Antimicrobial activity of plant

Herbal plants have long been exploited in medicine to cure communicable diseases. In this study, the antimicrobial activity of *C. colocynthis* is explored that *C. colocynthis* ' antibacterial activity in various solvents is comparable to that of commercial antibiotics and antifungals such as imipenem and miconazole to some extent. Salmonella typhi, the pathogen that causes typhoid fever, was more sensitive to ethyl acetate extracts and methanolic extracts compared to the other extracts. Only two extracts (Methanolic and ethyl acetate) decrease Shigella flexneri growth, whereas the rest have no impact. Gram-positive bacteria like Staphylococcus aureus have a wide growth inhibition zone against methanolic extract while Escherichia coli has the biggest inhibition zone against ethyl acetate extract. With the exception of Aqueous Extract (AE), which had no effect on any bacteria or fungi, all of the subjective extracts influenced all of the bacterial and fungal strains investigated in this study in some way.

Those findings are same to which was discovered that *C. colocynthis* fruit has a significant effect on *Candida spp., E. coli,* and *Pseudomonas aeruginosa*. Flavonoids, steroids, alkaloids, and terpenoids are all major elements of C. colocynthis *(Neelam, 2020).*

2.2.9 Antidiabetic activity of *C. colocynthis*

Diabetes mellitus is one of the most frequent endocrine illnesses worldwide, according to current studies. *C. colocynthis* is a traditional herb that is used to cure diabetes. It has a well-known hypoglycemic effect, which is backed up by modern phytotherapy. Gastrointestinal and urinary tract abnormalities are among the adverse effects. This review summarizes the findings of many blood glucose lowering studies that have been completed to date. Plant materials used in extract preparation included roots, fruits, seeds, rinds, and leaves. The extracts were ethanolic, methanolic, or aqueous in nature, with daily doses ranging from 10 to 500 mg/kg body weight. In all of these published articles, C. colocynthis is mentioned as a possible ant glycemic medicinal plant.(Shi et al., 2014).

CHAPTER THREE: MATERIALS AND METHODS

3.1. Collection of plant

C. colocynthis was purchased from local market of Lahore, Pakistan.

3.2. Preparation of plant extract

Liquid nitrogen was used to wash and dry the leaves, which were then pulverized with a pestle and mortar. An electric grinder was used to make a fine powder. The leaves powder methanolic extract was made by dissolving 100 g of powder in 400 ml of 100% methanol and shaking it for 72 hours at 37°C on an orbital shaker. After the plant materials settled, the supernatant solution containing plant compounds was collected and filtered. The crude methanolic extract was then produced by rotational evaporation of the isolated and purified supernatant. (Perveen and colleagues, 2020) We put this crude extract in a hot air oven in a petri dish at 50 degrees Fahrenheit for 5 days to improve it. With the same procedure we prepare the extract of (CTC) in chloroform, n-Butanol and n-Hexane.

3.2.1 Identification of bioactive constituents by the GC-MS analysis

A GC-MS was used to analyze the ethanolic extract of the plant seeds. This experiment performed using a Clarus 580 chromatography equipment with a capillary column (5 percent phenyl, 95 percent methypolysiloxane) (30.0 MX 250 m) and a mass spectrometer (Polaris Q) (EI 70 eV). Helium at 1 mL/min was used as the carrier gas. The injection volume was 1L and the split was 1/75. Temperatures for injection and monitoring were set at 250 and 280°C, respectively. The temperature of the mixture determining the temperatures of column were designed to rise at a rate of 11°C/min from 50°C to 200°C, then 6°C/min from 200°C to 240°C. The spectra of the major

unknown compounds were compared to the spectrum of the known component in the NIST library ***(Bourhia et al., 2020).***

3.3. Preparation of sample for hepatotoxicity

3.3.1 Experimental Animals:

Swiss albino Rats were taken of approximately of the same age group having weight of 110g and were purchased from National Institute of Health Sciences Islamabad Pakistan. These rats were maintained in Animal House of University of Central Punjab, Lahore, Pakistan. They were kept in the cages, had free access to food and water, and maintained under temperature-controlled environment (23±2 °C) with the 12 hours of light –dark cycle.

3.3.2 Experimental Protocol:

Total 16 animals were divided into one control and three treated groups with Methanol extract, Chloroform extract and N-hexane extracts.

Group I: Consider as control group was administered 1ml/kg normal saline per oral for 15 days.

Group II: 300mg/kg,600mg/kg ,900mg/kg and 1200/kg Methanol extract was given to rats by gavage for 15 days (once a day).

Group III: 300mg/kg,600mg/kg ,900mg/kg and 1200/kg Chloroform extract was administrated to rats for 15 days (once a day).

Group IV: 300mg/kg, 600mg/kg, 900mg/kg and 1200/kg n-Hexane extract was administrated to rats for 15 days (once a day).

3.3.3. Selection and preparation of dose for the pharmacological screening:

The different extracts of the plant (Methanol, Chloroform and n-Hexane) was suspended in normal saline to prepare four doses of 300mg/kg, 600mg/kg, 900mg/kg, and 1200 mg/kg according to animal body weight. According to this formula.

$$\text{Dose} = \frac{Rat\ weight}{1000} \times Dose$$

The doses were made for 15 days' usage.

3.3.4. Acute oral toxicity study:

The testing on rats were conducted using OECD-423 criteria. The dosage was chosen from a list of four options: 300, 600, 900, and 1200 mg/kg. To assess mortality and behavioural reactions, the rats were keep under direct monitoring for 48 hours, then once daily for the next fourteen days. Putting together an experiment A total of 16 rats were acquired and divided into four groups of similar size. There were four rats in each group. As a normal control group, Group I was given a daily oral dose of 100 ml/kg of saline for 15 days. The toxicant control dose was given to Group II, whereas the daily oral dose was given to Group IV. of300mg/kg,600mg/kg ,900mg/kg and 1200/kg n-Hexane extract.(Vakiloddin et al., 2015)

3.3.5. Protocol:

Total 16 rats were collected and divided into 4 of equal groups. There were four rats in each group. A normal control group which was Group I was given a daily oral dose of 100 ml/kg of saline for 15 days. Group II was administered daily by the multiple doses of 300, 600, 900, and 1200 mg/kg Methanol extract for 15 days as a toxicant control group. Group III was administered a daily oral dose of C. colocynthis fruit chloroform extract (MECCF). (MECCF). The 300mg/kg,600mg/kg ,900mg/kg and 1200/kg doses were given for 15days. The Group IV was a recipient of daily oral dose of300mg/kg,600mg/kg ,900mg/kg and 1200/kg n-Hexane extract. On day fifteenth, the rats of group II, III, and IV were anesthetized using Chloroform and blood samples were drawn from respective groups of rats and collected in sterile tube for

the clotting. After sometime, the sterile tubes were subjected to centrifugation for 15 min at 3000 rpm and serum were separated. The serum obtained were subjected to several biochemical assays to check the level of enzymes dysfunction. On the other hand, after last blood sample withdrawn all groups of rats were operated and their livers were isolated for the histopathological studies from all groups of rats respectively.(Vakiloddin et al., 2015)

3.3.7. Biochemical parameters

The various groups of animals were arranged (according to standard methods) for different bio components such as SGOT, sserum glutamate pyruvate transaminase(SGPT), alkaline phosphatase (ALP) and Total protein(TP).

3.3.8. Histopathological studies

After treatment, all animals were slaughtered, and tiny parts of each liver were preserved for 48 hours in a 10% formalin solution. The livers were then processed to use the paraffin embedding procedure. After that, the liver was sectioned (cutting up to 5m thickness).

Following that, the liver slices were stained with eosin and hematoxylin. Finally, the stained liver slices were studied histopathological below a microscope.

3.4. Biological Activities

3.4.1. Free radical scavenging activity

The sample's anti-oxidant strength was determined using the DPPH free radical test. 3 mg of DPPH was dissolved in 1 mL of methanol to make the DPPH stock solution. At 490 nm, the absorbance was read using a Biochemistry ELISA plate reader. For this, 20 liters of crude sample, 50, & 70% precipitated samples were taken and 200 µL of DPPH solution was added in ELISA plate wells in triplicates. Then wrapped the plate in aluminum foil to avoid any light exposure. First absorbance at recorded at 60 min interval. In the same way, absorbance was noted after 120 and 180 minute at 490nm.

The antioxidant activity was calculated using the formula:

$$\textbf{\% radical scavenging activity} = \frac{\boldsymbol{A\ control - Asample}}{\boldsymbol{Acontrol}} \times \mathbf{100}$$

IC_{50} value was determined graphically from the non-linear regression analysis,(Chekroun et al., 2015) and by the inverse of the value of IC50 which means antiradical activity (ARA) value. ARA = 1/IC

3.4.2. Thrombolytic Activity

100 mg Serum were completely removed after clot formation (aspirated out without disturbing the clot formed). The clot mass was calculated by multiplying the mass of the fibrin tube by the weight of the tube alone. Each clot-containing alpine tube was appropriately labelled, and 100 l of plant extract was added to each tube. 100 l of SK and 100 l of distilled water were separately introduced to the control tubes numbered as a positive and negative non-thrombolytic control, respectively. After that, all of the tubes were incubated for 90 minutes at the 37°C and lysis of clot was detected. After incubating, the serum recovered was withdrawn and the tubes were weighed again to determine the weight difference after the clot breakup. The percentage of clot lysis was calculated from the difference in weight acquired in between clot lysis. The test was carried out 10 times in total (Mannan and colleagues, 2011). Formula was used to

Q compute the percent clot lysis.

$$\textbf{Clot lysis \%} = \frac{\boldsymbol{weight\ of\ lysis}}{\boldsymbol{weight\ of\ clot}} \times \mathbf{100}$$

3.4.3. Anti-diabetic activity

Starch Iodine Assay is another name of this activity. The inhibition of α amylase has observed in the *in vitro* analysis. Firstly, the stock solution (3mg/1ml) was prepared and then dilution of different concentration formed such as 50%, 100%, 150%, 200%, 250% and 300%. For

example, 250µl of extract and 750µl DMSO were mixed together to form 250% dilution. Secondly, sample (145µl) was taken from each dilution and α-amylase (10µl) in the wells of ELISA plate. Incubated the plate for 10 minutes at the temperature of 37°C. Added 40µl starch solution in the wells and incubate for an hour. After the incubation, the 40µl of 1% iodine solution added in the extracts of plate. Metformin had taken as standard value and their different concentration had formed. Absorbance of diluted fraction of sample was measured at the 630nm.

3.5.4. Haemolytic activity

Blood (3ml) of O^+ blood group was taken from healthy person and put in the EDTA tube immediately so that the blood can be prevented from clotting. Now put the blood into the falcon tube and centrifuged the blood at 15000rpm for 5 minutes. The supernatant was discarded and washed the blood with NaCl three times. Put NaCl (2.5ml) into the falcon tube from their walls and placed the tube for 5 minutes. Then removed the supernatant and process was repeated for two times more. After washing, add PBS (phosphate buffer saline) into the tube to make the total volume of 20ml.

Prepared the stock solution of each extract by the addition of 3mg extract into 1ml of DMSO. Then dilutions of 50%, 100%, 150%, 200%, 250%, and 300% were made from the stock solutions. For example, 50% of dilution formed by the addition of extract (50µl) into DMSO (950µl) solution. The other dilutions were also formed by this method. Marked the Eppendorf's with the fraction names and their dilution concentration.

Put extract (100µl) and PBS (180µl) blood into the marked Eppendorf's and homogenized them at 37°C in the shaking incubator for 35 minutes. Then cooled them instantly on the ice for 5 minutes. Again, centrifuged the Eppendorf's at 1500rpm for 5 minutes. Supernatant of 100µl was added and add chilled PBS (900 µl). PBS was taken as negative control and 0.1% of Triton

X-100 as the standard. Addition of 200µl of each dilution into the wells of ELISA plate and control and standard were also added. The absorbance was checked at 630nm wavelength.

Chapter Four: Results

Citrullus colonsythis is a traditional medicinal fruit having valuable medicinal properties such as Hepatotoxicityty, antidiabetic, antioxidant, thrombolytic and hemolytic activity. The study was conducted on plant alcoholic extract and their biological properties. Various analysis was done quantitatively which were represented with tables and graphs along with their mean and standard deviation.

4.1Hepatotoxicity of rat's liver:

In hepatotoxic rats, the effect of MECCE on biochemical parameters. SGOT, SGPT, ALP, and Total Protein levels were acquired as a result of the LFTs levels collected during this investigation. Hepatotoxicity is not present in the group I supervise. Hepatotoxicity was seen in rats in groups 2 (methanolic extract), 3 (Chloroform extract), and 4 (and n-hexane extract). In comparison to a healthy control, increased blood levels of biochemical such SGOT, SGPT, and ALP, as well as total protein, were observed. Resultant data of SGOT, ALP and SGPT serum level are shown in table 4 and graph.

4.2 Statistical analysis

Table 4.2b

The comparison between the group of ALT, AST, ALP and TP and within the groups ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
ALT	Between Groups	5062.7	3	1687.6	10.40	.001
	Within Groups	1947.3	12	162.3		
	Total	7009.9	15			
AST	Between Groups	11051.7	3	3683.9	6.30	.008
	Within Groups	7015.8	12	584.6		
	Total	18067.4	15			
ALP	Between Groups	12615.5	3	4205.2	7.17	.005
	Within Groups	7037.5	12	586.5		
	Total	19653.0	15			
TP	Between Groups	2.13	3	.709	3.78	.040
	Within Groups	2.25	12	.188		
	Total	4.38	15			

The ratio in ALT between the groups was determined using a one-way analysis of variance (ANOVA). The results revealed a significant variance 1 and 4 (control) ($p = 0.004$), 2 and 4 (control) ($p = 0.008$), and 3 and 4 (control) ($p = 0.002$). The distinction 1 and 2, group 1 and 3, and group 2 and 3 appears to just be minor, according to statistics.

When compared with the control group, the ALT levels were found to be considerably higher.

Table 4.2c Comparison of AST between the group

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.
AST	G 1	G 2	13.3	17.1	.864
		G 3	17.0	17.1	.755
		G 4	69.0*	17.1	.008
	G 2	G 3	3.8	17.1	.996
		G 4	55.8*	17.1	.030
	G 3	G 4	52.0*	17.1	.044

The one-way analysis of variance (ANOVA) was analysed to find the difference of AST among the groups. The results have shown that a significant difference exists between group 1 and group 4 (control) $p = 0.008$, group 2 and 4 (control) $p = 0.03$ and group 3 and 4 (control) $p = 0.044$. However, data has suggested that the difference between group 1 and 2, group 1 and 3, group 2 and 3 is insignificant.

Results have shown that the AST levels are significantly higher as compared to the control group.

Table 4.2d Comparison of ALP between the group

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.
ALP	G 1	G 2	12.3	17.1	.889
		G 3	18.5	17.1	.707
		G 4	73.3*	17.1	.005
	G 2	G 3	6.3	17.1	.983
		G 4	61.0*	17.1	.018
	G 3	G 4	54.8*	17.1	.034

The one-way analysis of variance (ANOVA) was calculated to find the difference of ALP among groups. The results have shown that a clear difference exists between group 1 and group 4 (control) $p = 0.005$, group 2 and 4 (control) $p = 0.018$ and group 3 and 4 (control) $p = 0.034$. However, data has suggested that the difference between group 1 and 2, group 1 and 3, group 2 and 3 is insignificant.

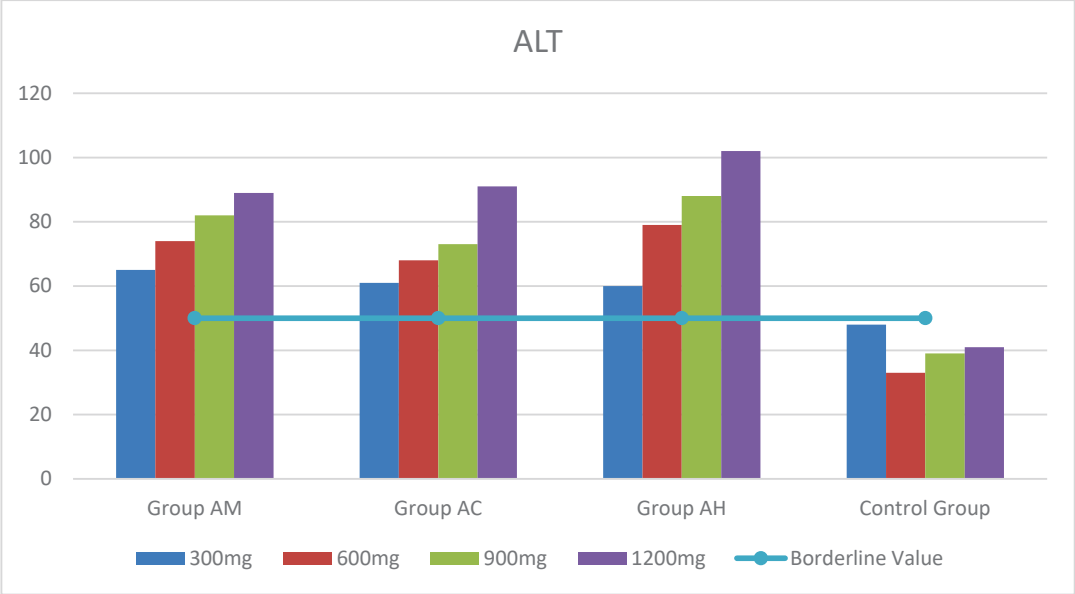
Results have shown that the ALP levels are significantly higher as compared to the control group.

Table 4.2f Comparison of TP between the group

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.
TP	G 1	G 2	.35	.31	.671
		G 3	.30	.31	.763
		G 4	1.00*	.31	.030
	G 2	G 3	-.05	.31	.998
		G 4	.65	.31	.201
	G 3	G 4	.70	.31	.156

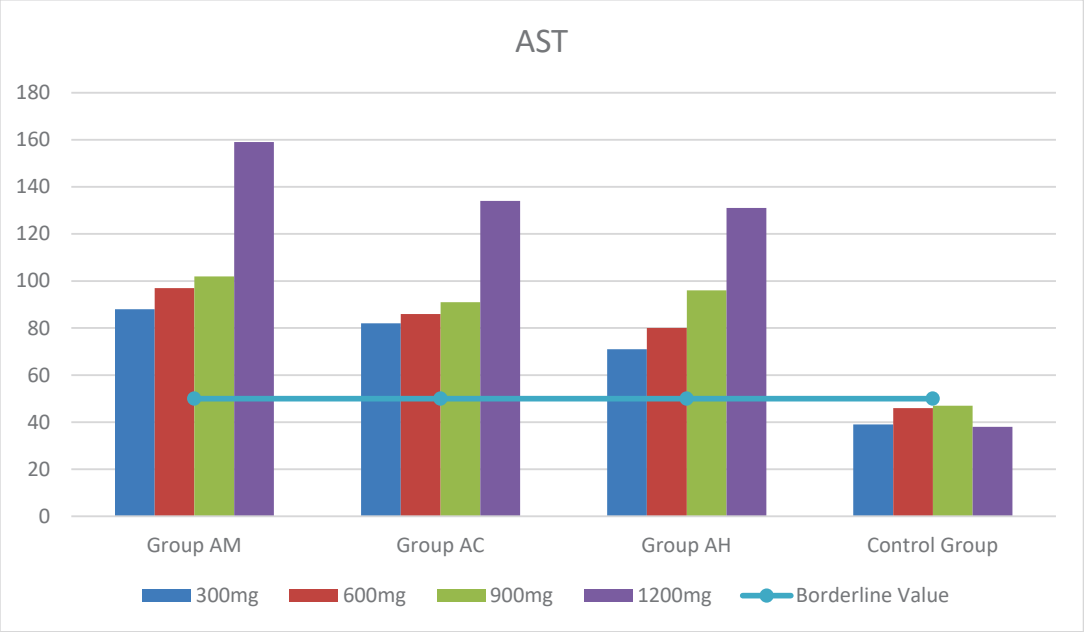
A one-way analysis of variance (ANOVA) was calculated to find the difference of ALP among the groups. The results have shown that a significant difference exists between group 1 and group 4 (control) $p = 0.03$. However, data has suggested that the difference between group 1 and 2, group 1 and 3, group 2 and 3, group 2 and 4, group 3 and 4 is insignificant.

Results have shown that the clinical difference between the total protein levels among the groups is insignificant however, statistically significant difference exists between the TP levels of group 1 and group 4 (control).



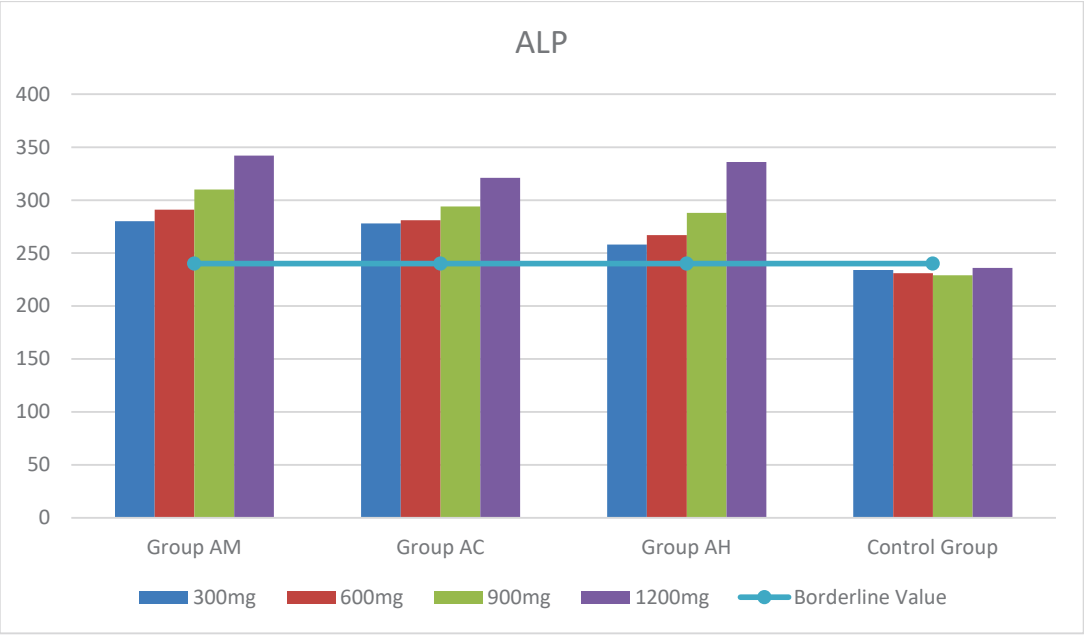
Graph 4.2g **Comparison of ALT between the group by graph**

Graph 4.2f Results have shown that ALT levels are elevated in group AM, AC and AH above upper borderline. Contrary to this, the ALT levels are normal in the control group. Data also suggests that ALT levels are higher with 1200mg dose in all groups as compared to other doses.



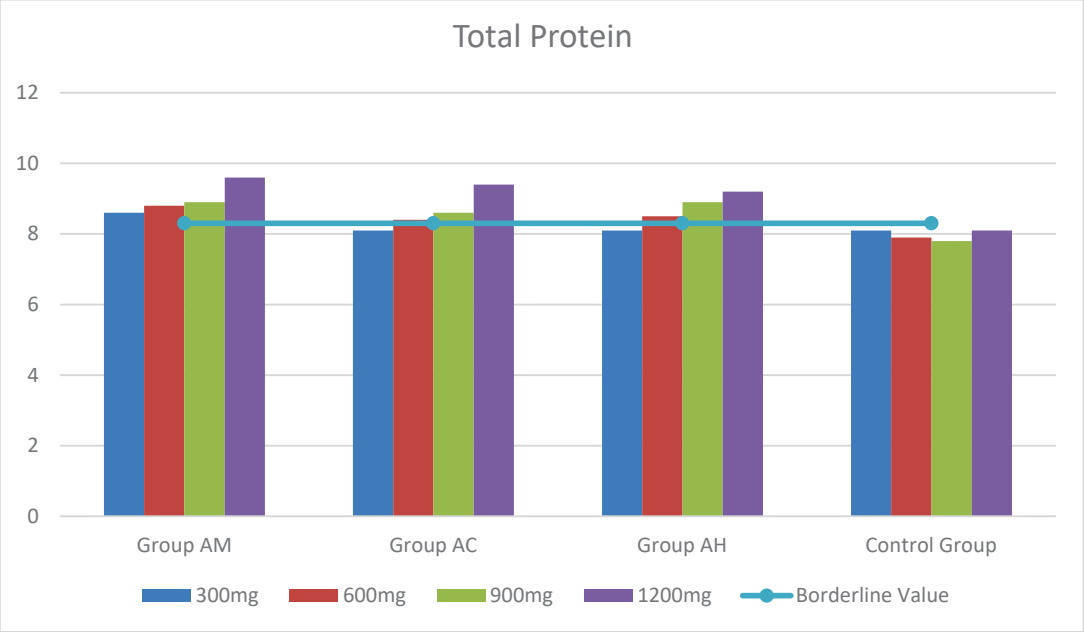
Graph 4.2h **The comparison of AST between the group by graph**

Graph 4.2I Results have shown that AST levels are elevated in group AM, AC and AH above upper borderline. On the other hand, the AST levels are normal in the control group. Data also suggests that AST levels are higher with 1200mg dose in all groups as compared to other doses.



Graph 4.2J Comparison of ALP between the group by graph

Results have shown that ALP levels are elevated in group AM, AC and AH above upper borderline. On the other hand, the ALP levels are normal in the control group. Data also suggests that ALP levels are higher with 1200mg dose in all groups as compared to other doses.



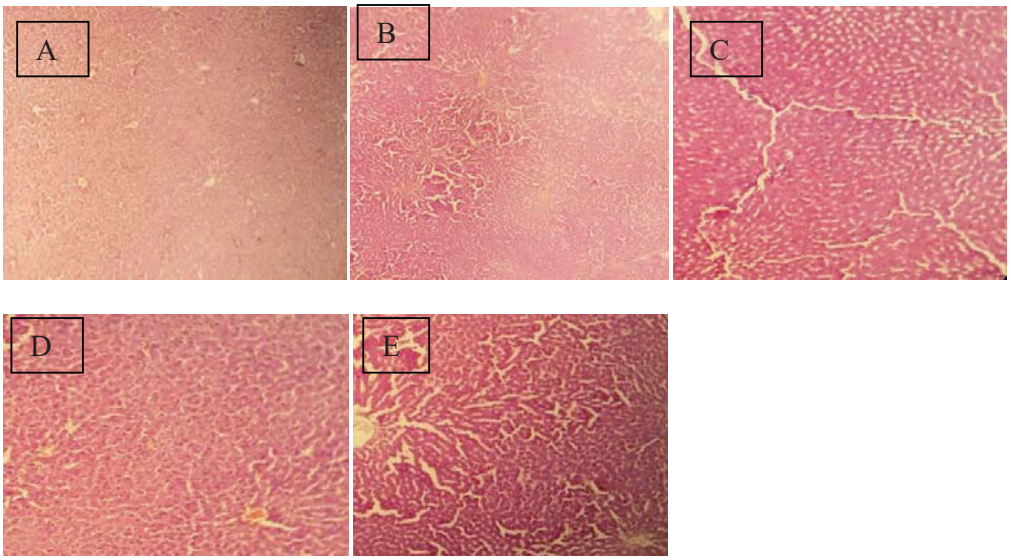
Graph 4.2k **Comparison of TP between the group by graph**

Results have shown that TP levels are elevated in group AM, AC and AH above upper borderline. On the other hand, the TP levels are normal in the control group. Data also suggests that TP levels are higher with 1200mg dose in all groups as compared to other doses.

4.3 Caused hepatotoxicity histopathology investigations of the liver

The histological assessment of CTC extract toxicity in all groups was investigated and represented in figures. Section A of rat liver treated with vehicle control group revealed intact architecture in the liver parenchyma, which is the usual look. Hepatocytes that have a little ballooning. Central vein was complete. There's no fibrosis. The toxicant was applied to section B of the liver. The 300mg/kg methanolic extract group revealed somewhat effaced architecture. Hepatocytes are somewhat inflated. There is no ECM build-up. Overall, the liver tissue appears to be in good condition. The architecture of the rat liver in reference drug treated groups (600mg/kg) was intact, with just few granular alterations. Fatty droplets were observed in the sinusoids, no ballooning , Overall tissue seems healthy 90-95%. Whereas Section D of liver treated with (900mg/kg) dose group showed mild vacuolar tubules in focal Slightly ballooning, accumulation of ECM was observed, nuclear Variation, Vacuolar degeneration, are present. Section E of liver treated with (1200mg/kg) dose group showed Hydropic Degeneration, vacuolar Degeneration and fatty droplets were observed, Slight accumulation of ECM Overall health is 87%).

Fig 4.3a

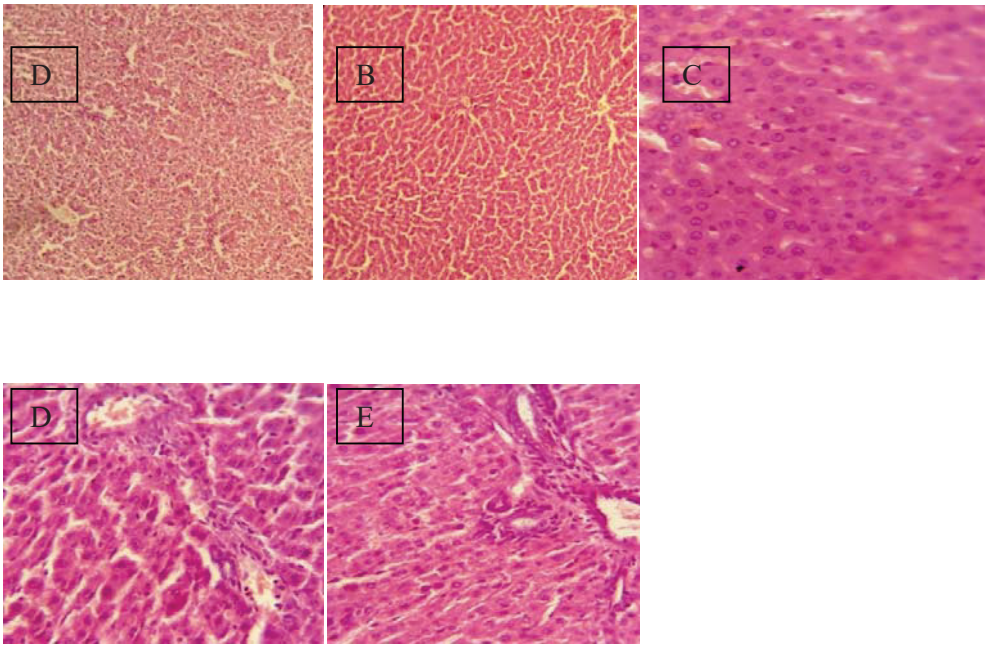


A (Normal hepatocytes with slight ballooning. Intact central vein. No fibrosis.) B (Slightly ballooning of hepatocytes. No accumulation of ECM Overall liver tissue seems healthy) C (Fatty droplets were observed in the sinusoids, no ballooning, Overall tissue seems healthy 90-95%.) D(Slightly ballooning, accumulation of ECM was observed, nuclear Variation , Vacuolar degeneration, are present) E(Hydropic Degeneration, vacuolar Degeneration and fatty droplets were observed, Slight accumulation of ECM Overall health is 87%) .

Fig 4.3b the section A of rat's liver were treated with vehicle control group and it showed liver parenchyma with intact architecture, which is normal appearance Normal hepatocytes with Normal hepatocytes, No fibrosis, No necrosis. Section B of liver in treated with toxicant Chloroform extract dose is given 300mg/kg group showed No ballooning 100%. No fibrosis or accumulation of immune cells. Section C of rat liver in test dose treated groups (600mg/kg) No fibrosis, no necrosis, Nuclear Variation is present, no ballooning, Overall tissue seems healthy 90-95%. Whereas Section D of liver treated with (900mg/kg) dose group showed Hepatocyte ballooning, very slight portal activation with some sort of hepatocyte damage. Overall health 90%. Section E of liver treated with (1200mg/kg) dose

group showed Hepatocyte ballooning, nuclear variation, vacuolar degeneration hydropic degeneration present, Overall health 86%.).

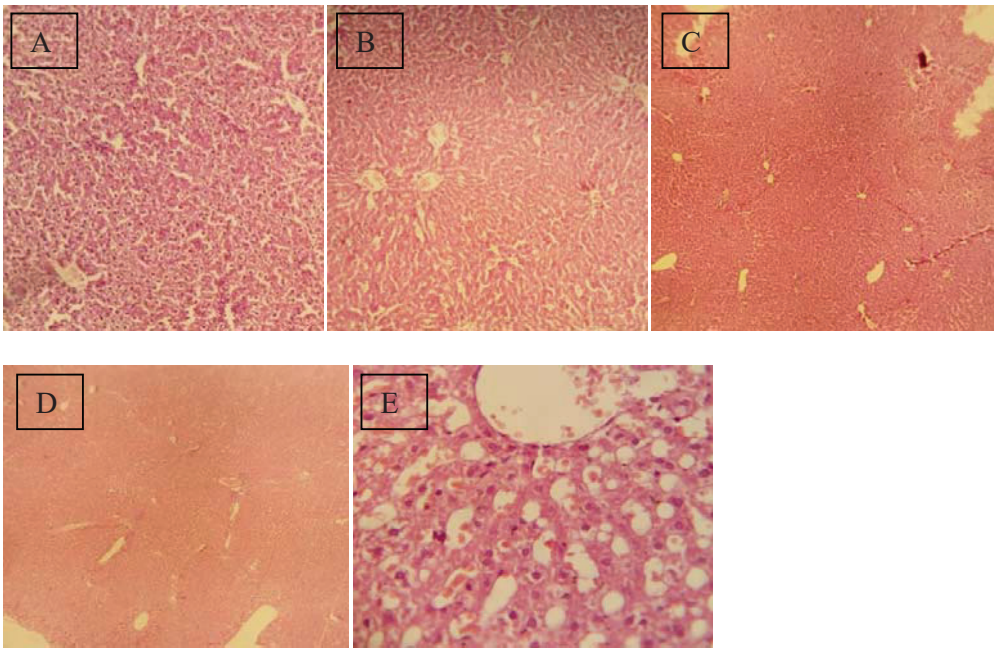
Fig 4.3b



A (Normal hepatocytes, No fibrosis, No necrosis) B (No ballooning 100%. No fibrosis or accumulation of immune cells. Hydropic Degeneration and nuclear variation are present.) C (No fibrosis, no necrosis, Nuclear Variation is present) D (Hepatocyte ballooning, very slight portal activation with some sort of hepatocyte damage. Overall health 90%.) E (Hepatocyte ballooning, nuclear variation, vacuolar degeneration hydropic degeneration present, Overall health 86%.)

Fig 4.3c Section A of rat liver treated with vehicle control group showed Normal. No ballooning No fibrosis & Necrosis tissue is 100 % healthy. Section B of liver in treated with toxicant n-hexane extract dose is given 300mg/kg group showed Normal tissue. No ballooning No fibrosis or accumulation of immune cells. Section C of liver in test dose treated groups with the concentration of (600mg/kg) and it showed slight fibrosis with no

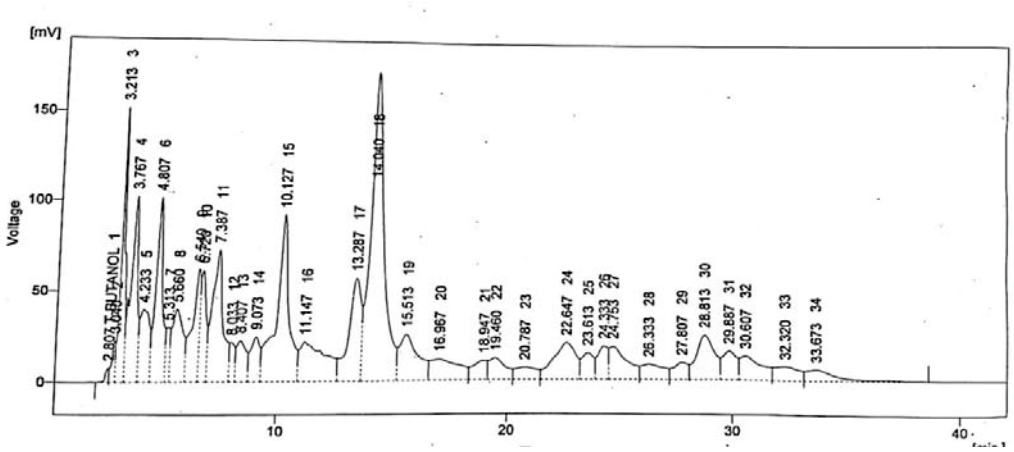
ballooning, nuclear degeneration, Tissue health is 90%. Whereas Section D of liver treated with (900mg/kg) dose group Slight portal activity, hydropic degeneration. Tissue health is 85-90%. Section E of liver treated with (1200mg/kg) dose group showed Ballooning of the hepatocytes, slight infiltration of macrophages nuclear variation, vacuolar degeneration hydropic degeneration present. Tissue health is 85 %.



A (Normal. No ballooning No fibrosis & Necrosis tissue is 100 % healthy.) B (Normal tissue. No ballooning No fibrosis or accumulation of immune cells.) C (Slight fibrosis with no ballooning, nuclear degeneration, Tissue health is 90%.) D (Slight portal activity, hydropic degeneration. Tissue health is 85-90%) E (Ballooning of the hepatocytes, slight infiltration of macrophages nuclear variation, vacuolar degeneration, hydropic degeneration present. Tissue health is 85 %)

HPLC Results:

The HPLC result different compounds in figure 4.3d show different peaks



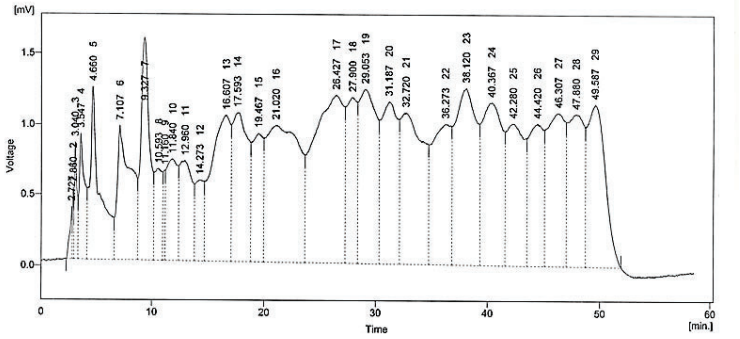
Quantitative representative of different bioactive compounds

Table 4.3 list of compounds finds through HPLC

Sr no	Compound	Retention time	%Area	Conc (PPM)
1	Quercitin	3.040	0.6	12.2127
2	Gallic Acid	4.807	5.0	74.8542
3	Vanillic Acid	13.287	4.7	122.6160
4	Chlorogenic Acid	15.513	3.4	110.7420
5	Syringic acid	16.967	2.4	25.2823
6	Feralic Acid	22.647	3.4	101.0458
7	Cinamic acid	24.753	2.4	35.4156
8	Sinapic Acid	26.333	1.4	1.5497

HPLC chromatogram of *n*-butanol

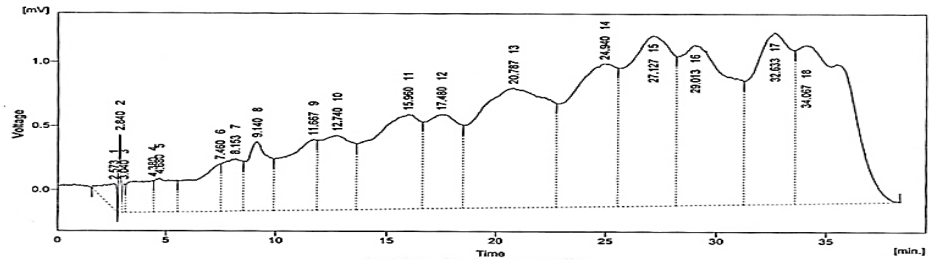
HPLC analysis of *n*-butanol extract of *Citrullus colocynthis* revealed the presence of seven phenolic compounds after evaluating the retention times and UV spectra i.e. quercetin, gallic acid, caffeic acid, benzoic acid, syringic acid, *p*-coumeric acid and sinapic acid (Figure 5). Highest concentration in ppm was observed for benzoic acid (3.13283PPM) followed by syringic acid (2.985425 PPM).



Compound name	Retention time	Area [%]	Concentration in PPM
Quercetin	2.860	0.1	0.186295
Gallic acid	4.660	2.9	2.727072
Caffeic acid	12.960	2.1	2.53538
Benzoic acid	14.273	1.1	3.13283
Syringic acid	16.607	4.5	2.985425
p-coumeric acid	17.593	3.8	1.309217
Sinapic acid	26.427	8.4	2.887105

HPLC chromatogram of chloroform fraction

HPLC analysis of chloroform extract of *Citrullus colocynthis* revealed the presence of six phenolic compounds after evaluating the retention times and UV spectra i.e. quercetin, gallic acid, caffeic acid, Chlorogenic, *p*-coumeric acid and cinnamic acid (Figure 7). Highest concentration in ppm was observed for chlorogenic acid (8.973978 PPM) followed by cinnamic acid (5.77745 PPM).



HPLC chromatogram of chloroform extract of Citrullus colocynthis

Compound name	Retention time	Area [%]	Concentration in PPM
Quercetin	2.840	0.2	0.206647
Gallic acid	4.380	1.2	0.673956
Caffeic acid	12.740	3.7	2.71216
Chlorogenic acid	15.960	7.2	8.973978
p-coumeric acid	17.480	4.9	1.010308
Cinnamic acid	24.940	10.3	5.77745

GC-MS Analysis

Table 4.3.6 list of compounds that is finds through GCMS

Compound	Pub Chem Id	Retention time	Retention Area
Ether, 3-butenyl pentyl	536090	5.047	93.6
oxime-, methoxy-phenyl-_	9602988	5.620	5.78
Betaine	247	6.277	0.91
Ethyl 4 chlorobutanimidoate	18474	7.417	1.82
Cyclotrisiloxane, hexamethyl	10914	8.604	1.26
.2-Methoxy-4-vinylphenol	332	9.745	5.27
2,4-Di-tert-butylphenol	7311	13.471	0.88
Silane, trimethyl (4-methyl-3-pen	586141	15.589	1.14
Triisopropyl phosphite	8304	16.354	1.68
cis-13-Octadecenoic acid, methyl	5312441	23.211	3.16
Methyl stearate	8201	23.511	9.00
.2-Butanone, 4-phenyl	17355	23.762	1.40
9,12-Octadecadienoic acid, methyl	8203	24.442	1.26
Cyclohexane, 1,3,5-tripheny	119930	28.138	1.03

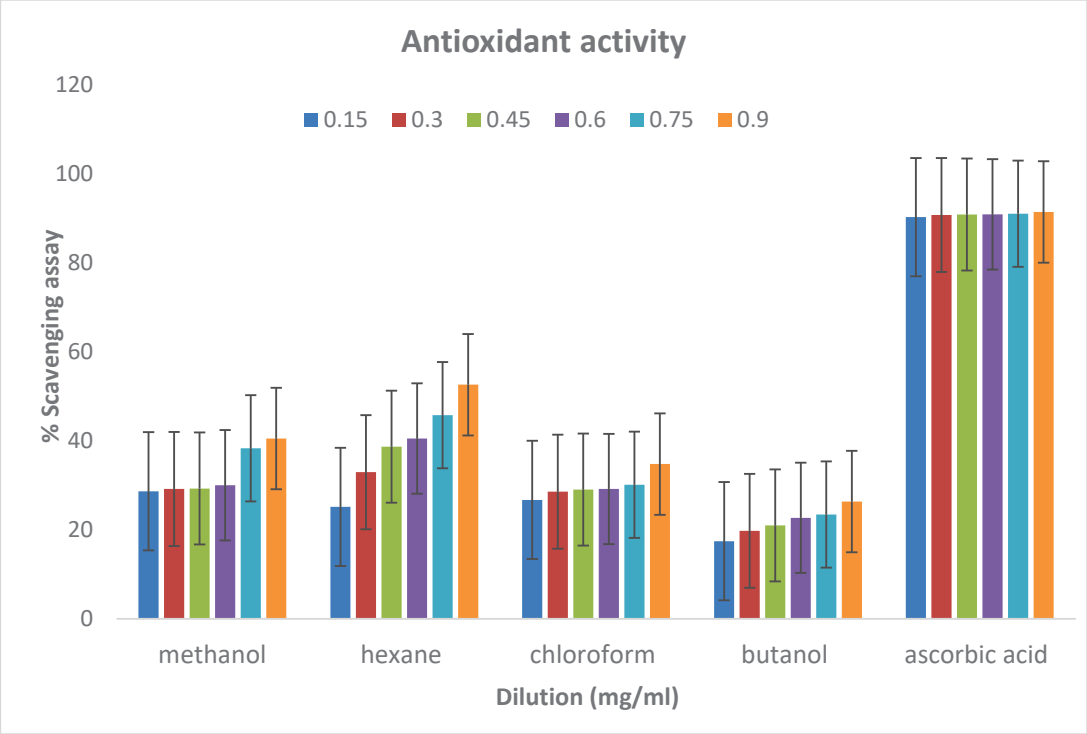
6-Chloropiperonyl alcohol	10322	40.044	4.25

Biological activities

Table 4.4.1 Anti-oxidant Activity

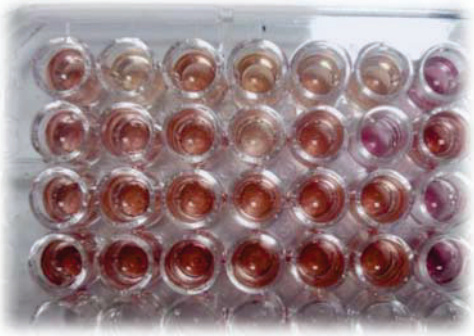
Sr. no	Extracts names	Concentrations (mg/ml)	% Anti-oxidant activity
1	Methanol	0.15	28.59±0.45
		0.3	29.38±0.43
		0.45	29.36±0.39
		0.6	30.57±0.49
		0.75	38.41±0.45
		0.9	40.57±0.30
2	Hexane	0.15	25.66±0.45
		0.3	32.39±0.49
		0.45	38.34±0.31
		0.6	40.51±0.49
		0.75	45.59±0.51
		0.9	52.54±0.49
3	Chloroform	0.15	26.67±0.45
		0.3	28.57±0.40
		0.45	29.61±0.49
		0.6	29.39±0.52
		0.75	30.44±0.39
		0.9	34.59±0.42
4	Butanol	0.15	17.48±0.49
		0.3	19.59±0.51
		0.45	21.33±0.56
		0.6	22.61±0.44
		0.75	23.75±0.27
		0.9	26.45±0.49
5	Ascorbic acid (standard)	0.15	90.28±0.28
		0.3	90.58±0.50
		0.45	90.57±0.28

		0.6	90.59±0.35
		0.75	91.63±0.51
		0.9	91.50±0.35



Graph 4.1 Graphical Presentation

DPPH radical scavenging activity is commonly used method for screening of antioxidant potential of any extract containing phytochemicals. The results of antioxidant assay are shown in Table-V. 0.9 mg/ml of *n*-hexane extract produced high DPPH scavenging activity among all the experimental plant extracts with highest DPPH scavenging activity (52 %) followed by 0.9 mg/ml of Methanol extract (40 %), Chloroform extract (34 %), *n*-butanol extract (26 %) whereas least DPPH scavenging activity was observed in 0.9 mg/ml .Similarly minimum concentration 0.15 mg/ml of Methanol extract produced high DPPH scavenging activity (28%) followed by 0.15 mg/ml Chloroform extract (26 %), *n*-hexane extract (25 %), *n*-butanol extract (17 %), Thus it was observed that by increasing extract concentration from 0.15 mg/ml to 0.9 mg/ml, % DPPH scavenging activity will be increased.

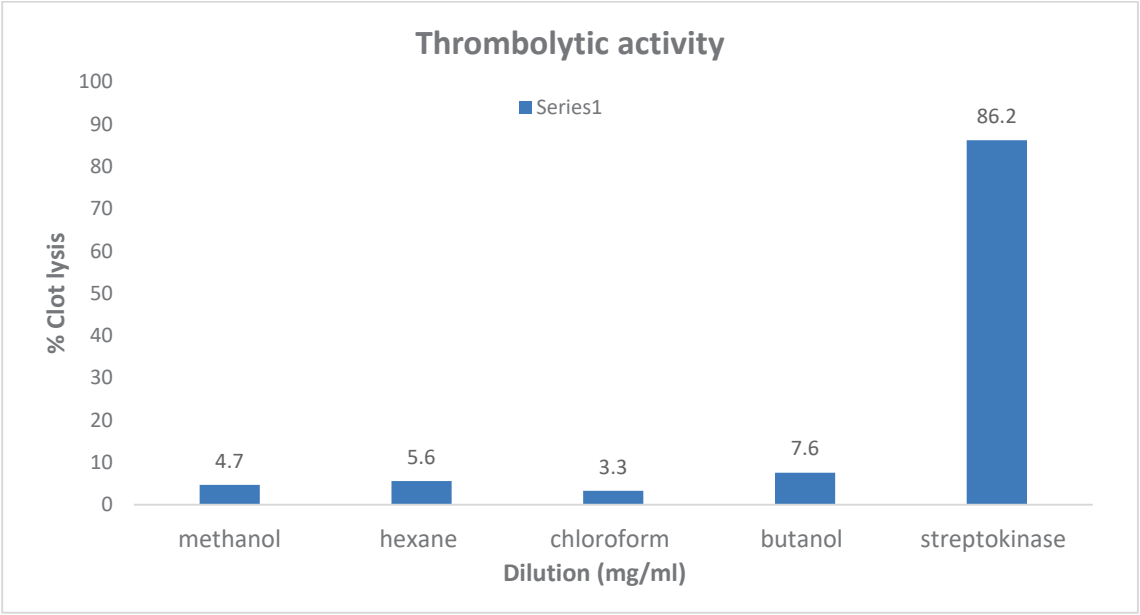


ELISA Plate of Antioxidant activity

Thrombolytic activity

Table 4.4.2 *Thrombolytic Activity*

Sr. no.	Extracts names	Thrombolytic activity (%)
1	Methanol	4.5±0.41
2	Hexane	5.5±0.40
3	Chloroform	3.4±0.37
4	Butanol	7.5±0.40
5	Streptokinase	86.4±0.43



Graph 4.2 Graphical presentation of thrombolytic activity

The thrombolytic activity of the *C. colocynthis* was observed against human red blood cells. The positive control was SK (streptokinase), which showed 86.4±0.43clot lysis and when treated with negative control (distilled water) they showed minor clot lysis (4.7%). Significant percentage of clot lysis was observed when clot lysiswas observed when clot incubated with *C. colocynthis* extract samples (Methanol, Hexane, Chloroform and Butanol) The Methanol sample was showed 4.5±0.41clot lysis, Hexane5.5±0.40 clot lysis was observed of Chloroform sample, 3.4 ± 0.37, &7.5 ± 0.40clot lysis was observed with butanol separately.

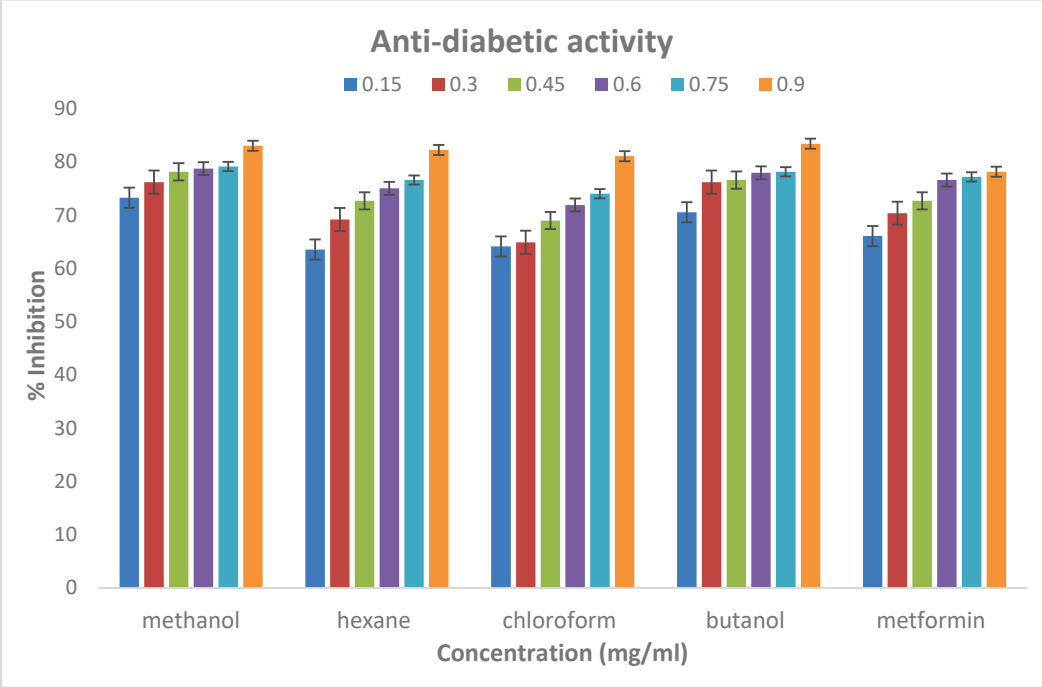
.

Thrombolytic activity of extracts

Anti-diabetic activity

Table 4.4.4

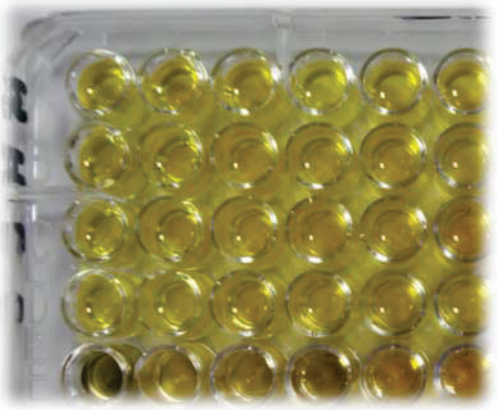
Sr. no	Extracts names	Concentrations (mg/ml)	% anti-diabetic activity
1	Methanol	0.15	73.38±0.24
		0.3	76.40±0.42
		0.45	78.58±0.40
		0.6	78.50±0.33
		0.75	79.58±0.42
		0.9	83.34±0.47
2	Hexane	0.15	63.57±0.38
		0.3	69.55±0.34
		0.45	72.64±0.37
		0.6	75.45±0.47
		0.75	76.61±0.23
		0.9	82.64±0.36
3	Chloroform	0.15	64.99±0.99
		0.3	64.60±0.50
		0.45	69..19±0.16
		0.6	71.71±0.18
		0.75	74.53±0.47
		0.9	81.54±0.45
4	Butanol	0.15	70.54±0.45
		0.3	76.45±0.24
		0.45	76.82±0.20
		0.6	77.44±0.45
		0.75	78.60±0.41
		0.9	83.64±0.27
5	Metformin (standard)	0.15	66.13±0.10
		0.3	70.45±0.49
		0.45	72.27±0.37
		0.6	76.77±0.30
		0.75	77.23±0.06
		0.9	78.67±0.44



Graph 4.3 Graphical presentation of ant-diabetic activity

Antidiabetic activity of unripen *C. colocynthis* was determined by analyzing α-amylase inhibition at different concentration, metformin (standard) was showed minimum % of inhibition at minimum concentration. Decreasing concentration of metformin decreased the % of α-amylase inhibition. Metformin showed maximum inhibition % at 0.9 mg/mL (78.67±0.44), then at minimum at 0.15 mg/mL (66.13±0.10). Extract samples (Methanol, hexane, chloroform, and butanol) were showed variable % of inhibition as compared to the standard, because the inhibition % was

increasing with decreasing extract concentration. The Inhibition % of α-amylase of Butanol sample was observed 83.64±0.27at 0.9 mg/mL is the maximum Inhibition % of α-amylase followed by Methanol sample was observed 83.34±0.47 at 0.9 mg/mL, Hexane 82.64±0.36 at 0.9 mg/mL and Chloroform81.54±0.45at 0.9 mg/mL. Amylase inhibition percentage of 0.15 mg/mL sample was measured as methanol73.38±0.24 at 0.15mg/mL, Butanol70.54±0.45, Chloroform64.99±0.99 at 0.15 mg/mL and Hexane 63.57±0.38 at 0.15 mg/mL respectively.

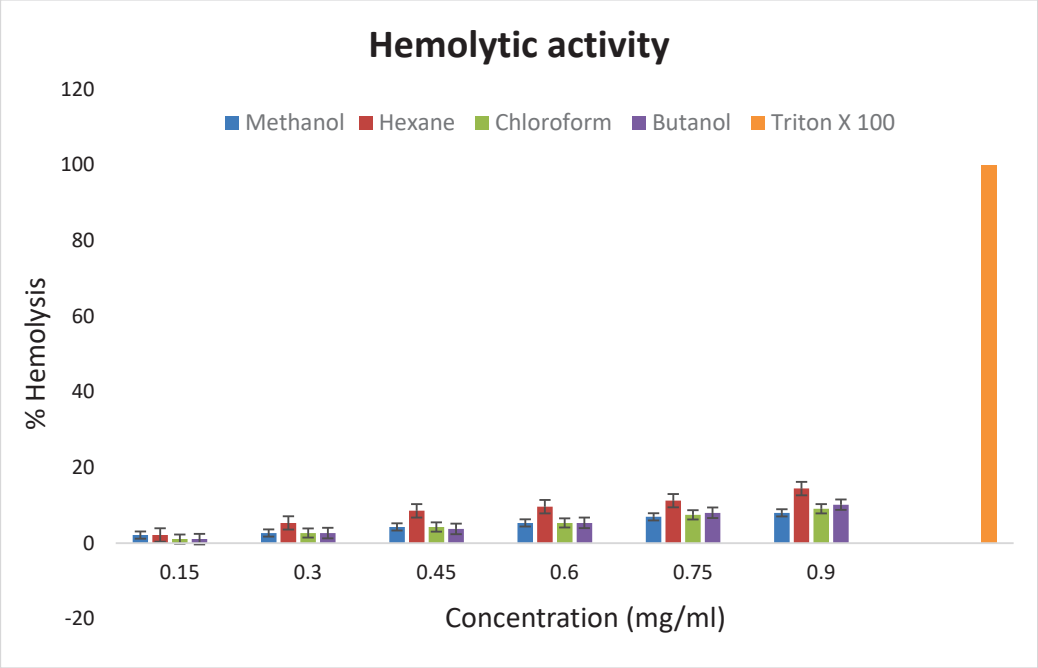


Anti-diabetic activity

Haemolytic activity:

Table 4.4.6

Sr. no	Extracts names	Concentrations (mg/ml)	% anti-diabetic activity
1	Methanol	0.15	2.50±0.43
		0.3	2.30±0.33
		0.45	4.48±0.33
		0.6	5.45±0.44
		0.75	6.51±0.47
		0.9	8.43±0.38
2	Hexane	0.15	2.45±0.40
		0.3	5.42±0.45
		0.45	8.43±0.27
		0.6	9.63±0.23
		0.75	11.50±0.41
		0.9	14.66±0.22
3	Chloroform	0.15	1.49±0.40
		0.3	2.67±0.33
		0.45	4.32±0.06
		0.6	5.19±0.16
		0.75	7.68±0.22
		0.9	9.53±0.40
4	Butanol	0.15	1.53±0.30
		0.3	2.50±0.40
		0.45	3.32±0.37
		0.6	5.45 ± 0.49
		0.75	8.37 ± 0.38
		0.9	10.26 ± 0.40
4	Triton X-100	0.1%	100.19 ± 0.15



Graph 4.4 Graphical presentation

C. colocynthis having little or no toxic effect on human health. Different samples (Methanol, Hexane, Chloroform and Butanol) of *C. colocynthis* showed variable percentage of cell lysis by comparing with Triton X- 100 (positive control). The hemolysis percentage of extract sample, and % extract samples were observed as 14.66±0.22 of hexane at 0.9 mg/mL, 10.26±0.40 of Butanol extract, 9.53±0.40 of chloroform and 8.43±0.38 Methanol at 0.9 mg/mL respectively. The results showed less cytotoxic effect observed as1.49±0.40 at 0.15 mg/mL chloroform, Butanol 1.53±0.30, Hexane 2.45±0.40 and Methanol 2.50±0.43 extract sample of *C. colocynthis* as compared to the other samples concentration. The hemolysis percent of positive control (Triton X-100) was 100.19±0.15. As hemolysis percentage shown by the extract samples is less than 15%. *C. colocynthis* did not show marked hemolytic activity.

CHAPTER 5: DISCUSSION

Medicinal plants are recommended worldwide due to their high potential and high nutritional values. Thus, plants have therapeutic potential and are recommended for their therapeutic properties (Rasool Hassan, 2012). Traditional medicine uses a variety of medicinal plants for treat diabetes mellitus. *C. colocynthis* has been identified one of the most widely utilized plants in ethnobotanical investigations. Patients with diabetes are advised to drink an infusions made from the fruits of the cucurbitaceous plant. Certain antidiabetic plants also include polysaccharides, gums, and glycans in addition to alkaloids and polyphenols. The phytochemical screening of different extracts of *C. colocynthis* seeds were performed in order to scrutinize the presence or absence of chemical compounds such as tannins, flavonoids, alkaloids and terpenoids, total content of polyphenols and flavonoids in such extracts and to assess their antioxidant activity on the DPPH free radical scavenging and many other biological activities. (Najafian & Babji, 2012).

The focus of this research are the fruits portion of *C. colocynthis* and analyzed that whether the plant have hepatoprotective action against methanolic, n-hexane, and chloroform extract as hepato-toxins in Swiss albino rats, in order to refute its claims in folklore medicine for liver issues. The extent of liver damage is determined by histological examination and the level of several biochemical markers in circulate. However, in experimental animals and human, overdosing on plant extract has been found to be hepatotoxic and nephrotoxic. Hydropic degeneration, vacuolar degeneration, nuclear degeneration, and no ballooning are all symptoms of a possibly fatal overdose of plant extract as evidenced by the aforementioned results. There are a variety of enzymes present in the cytosol that were released into the bloodstream when the plasma membrane of the liver cells is disturbed. The research indicates the effects of too much paracetamol on the liver were demonstrated in this study by increased levels of

biochemical markers such as AST, ALT, and ALP. This seems that liver cells perform a wide variety of metabolic functions and it includes a significant number of enzymes. The cytoplasm and mitochondria had higher amounts of SGPT and SGOT respectively. With liver injury, hepatocyte transport potential is disrupted, resulting in bile flow. Such enzymes leak across the plasma membrane, leading to higher serum levels. Another research, though, found that paracetamol induces cytotoxicity in mice.

The levels of AST, ALT, ALP, and total protein all increased significantly after taking paracetamol. The administration dose of 200 mg/kg of *C. colocynthis* methanolic extract was successfully decreased the pathological effects of paracetamol toxicity. Similarly, there are other serum parameter treatment of 200 mg/kg be of methanolic extract of *C. colocynthis* also promotes the body weight in albino rats (Dar *et al*., 2012). The histopathological alterations in the liver samples were compared to the normal control group. The results show that the cell membrane stabilisation, hepatic cell regeneration, and normalisation of serum parameters are the mechanisms by which the 90 percent ethanolic extract of *C. colocynthis* (200 mg/kg b) provides *in-vivo* hepatoprotective activity against paracetamol. In comparison, when we gave 1200mg/kg extract (methanolic, n-hexane, and chloroform) to rats in an experimental group, they showed liver injury, which we evaluate from histopathological results indicating hydropic degeneration.

There was evidence of vacuolar degeneration and lipid droplets. We achieved a result from 900mg/kg extract (methanolic, n-hexane, and chloroform) dosage. Slight ballooning, ECM accumulation, nuclear variation, and results in histopathology for 600mg/kg extract dose demonstrated intact architecture, with granular modifications in a few locations. There was fatty droplet inside the sinusoids, but no ballooning. When we gave 300mg/kg extract (methanolic, n-hexane, and chloroform) to groups rats, they did not show that much impairment to liver cells.

There is no ballooning and no fatty droplets, Figures 1,2,3 demonstrate no nuclear degeneration (A section). The DPPH radical scavenging activity is a widely used method for determining the antioxidant capacity of any phytochemical extract. Among all the experimental plant extracts with the highest DPPH scavenging activity, the antioxidant assay on conc 0.9 mg/ml of n-hexane extract demonstrated significant DPPH scavenging activity.DPPH scavenging activity (52 %) and Similarly minimum concentration 0.15 mg/ml of Methanol extract produced high DPPH scavenging activity (28%) followed by 0.15 mg/ml Chloroform extract (26 %), *n*-hexane extract (25 %), *n*-butanol extract (17 %), Thus it was observed that by increasing extract concentration from 0.15 mg/ml - 0.9 mg/ml, % DPPH scavenging activity will be increased. The indication of many organic compounds or secondary metabolites are actually responsible for the antioxidant activity.

In this study, methanolic fruit extract of *C. colocynthis* was screened and this medical plant showed highest anti-diabetic activity. Thus, this plant is using worldwide for the treatment of diabetic mellitus. The highest antioxidant and free radical scavenging ability of the fruit extract was observed at a concentration of 2500mg/ml (Kumar et al., 2008).

Thrombolytic effect with the Butanol sample, clot lysis was found at 7.50.40, while with chloroform test, clot lysis was observed at 3.40.37. Furthermore, 250, 500, and 1000 g/mL A. fragrantissima (87.91.0, 97.95.1, and 112.51.1 s, respectively), 500 g/mL and 1000 g/mL

C. colocynthis (65.11.0 and 106.40.4 s, respectively), and 1000 g/mL A. herba-alba (157.03.0 s) had the highest PTT values. Using thrombosis tests, the anticoagulant impact of methanol extracts *C. colocynthis* was assessed and results indicate that the methanolic extract showed highest thrombolytic activity. These findings suggest that these plants could be used to treat arterial and venous thrombosis. (Alabdallat & Bin Dukhyil, 2021). As a consequence, a sample of anti-diabetic Butanol was found. At 0.9 mg/mL, the highest -amylase inhibition percent was reported to be 83.640.27, followed by methanol sample at 83.340.47 mg/mL. The 0.15 mg/mL sample percentage Amylase inhibition were estimated using hemo percent extract samples, which were determined to be 14.660.22 of hexane at 0.9 mg/mL and 2.50.0.43 of methanolic at 0.15 mg/mL, respectively. Plant materials used in extract production included roots, fruits, seeds, rinds, and leaves. The extracts were ethanolic, methanolic, or aqueous in nature, with daily doses varies from 10 to 500 mg/kg body weight (Shi *et al.,* 2014).

CHAPTER 6: CONCLUSION

From the afforest studies it is recommended medicinal C. colocynthis is a potent plant that contains good profile of biological active compound that is confirmed through GCMS and HPLC Analysis. The biological active compound shows strong bioactivity i.e. anti-diabetic activity, anti-oxidant activity, haemolysis and thrombolytic activity. Cytotoxicity of ***C. colocynthis*** extracts from the above discussion it is concluded that extracts of *C. colocynthis* (1200 mg/kg) exhibited no significant toxicity in rat's liver. So recommended that this plant can be use, as medicinal plant for the treatment of different disease. Further investigation is required to study the mechanism that is involved in causing toxicity in rats and human as well.

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