INVESTIGATION FOR ESTROGENIC ACTIVITY OF CRINUM LATIFOLIUM IN FEMALE ALBINO RATS

A Thesis Submitted
in Partial Fulfillment of the Requirements for the Degree of

MASTER OF PHARMACY

in

Pharmacology

by

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LUCKNOW

July, 2024

DECLARATION

I hereby certify that the work, titled "**Investigation for Estrogenic Activity of *CRINUM LATIFOLIUM*in female Albino rats,"** submitted by **Omer Alam**for award of degree of "Master of pharmacy" with specialisation in Pharmacology, is true and correct. includes legitimate research. I conducted in our labs, library, and computer center under the Guidance of **Qumre Alam**, Associate Professor. and Co-guidance of, **Anjali Singh** (Department of Pharmacy)**)** the department's head of pharmacology, at Innovative College of Pharmacy, Greater Noida.

Additionally, I declare that no other degree or fellowship has ever been awarded on the basis of the current work. The specifics given in this summation are to best of my insight.

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ABSTRACT

In the present study, the estrogenic activities of the ethanolic extract derived from the fruits of Crinum latifolium were investigated. This plant, belonging to the amaryllis family (Amaryllidaceae), is a herbaceous perennial that grows from an underground bulb. It features stout flowering stems reaching approximately 2 meters in height, with long, linear, and ligulate leaves. The plant produces white flowers arranged in an umbel formation.Crinumlatifolium is native to various parts of Asia, spanning from India and Sri Lanka through much of mainland Southeast Asia to southern China, specifically in the regions of Guangxi, Guizhou, and Yunnan. It has also reportedly naturalized in the West Indies and the ChagosArchipelago.To assess the estrogenic activity of the Crinum latifolium fruit extract, a standardized method with some modifications was employed. The study was conducted using bilaterally ovariectomised immature albino rats. The parameters used to evaluate estrogenic activity included vaginal cornification, uterine wet weight, vaginal opening, uterine glycogen content, and uterine histopathology. These measurements were compared against the known estrogenic activity produced by diethylstilbestrol (DES), which served as a reference compound.

The results of the study revealed several notable effects of the Crinum latifolium extract. An increase in uterine wet weight among the treated rats was observed. Additionally, there was a dose-related rise in vaginal opening, indicating a response to the extract's estrogenic properties. Specifically, the extract induced a 0.292-fold increase in uterine wet weight compared to the control group.Histopathological examination of the uterine tissue showed that Crinum latifolium extract caused proliferative changes in the uterine endometrium. These changes were characterized by an increased height of the luminal epithelium, accompanied by a loosening of the stromal tissue and an increased number of glands. These histological observations further support the estrogenic activity of the plant extract.This study provides valuable insights into the potential estrogenic properties of Crinum latifolium fruit extract, suggesting its possible applications in areas related to hormonal health or as a natural source of estrogenic compounds. Further research may be warranted to explore the specific compounds responsible for these effects and to investigate potential therapeutic applications.

Key words: Estrogenic activity; vaginal cornification; uterine wet weight; uterine glycogen content etc.

CHAPTER 1

INTRODUCTION

Since the dawn of civilization, humanity has turned to the natural world for healing. Plants, with their diverse chemical profiles, have offered a rich source of potential medicines for millennia. This journey, spanning ancient traditions to cutting-edge research, continues to unveil the remarkable potential of plant-based drugs, offering promising avenues for treatment and prevention of various ailments.

This introduction delves into the captivating world of plant-based drugs, exploring their historical significance, diverse applications, and exciting discoveries. We will embark on a journey through time, witnessing the evolution of their use from ancient practices to modern scientific advancements.

A Legacy of Healing: A Glimpse into the Historical Significance of Plant-Based Drugs

Our ancestors, through trial and error, observed the therapeutic effects of various plants. Traditional knowledge systems like Ayurveda in India, Unani in the Middle East, and Traditional Chinese Medicine all incorporated various plants into their therapeutic approaches. These practices formed the foundation for future exploration and laid the groundwork for the development of modern medicines.

Latest Discoveries in Plant-Based Drugs

Modern scientific advancements about plant-based drugs. Here are some of the discoveries in this field:

- Identification and isolation of bioactive compounds: Advanced techniques in natural product chemistry allow scientists to isolate and characterize the specific molecules within plants responsible for their therapeutic effects. This knowledge is crucial for developing standardized herbal extracts and potentially designing novel drugs based on these natural compounds.
- Genome sequencing and targeted breeding: Advances in plant genomics enable researchers to identify and understand the genes responsible for the showing of specific medicamental properties of herb. This knowledge can be utilized to breeding programs for cultivating plants with enhanced levels of these beneficial compounds.

- Synergy and interaction of plant constituents: Recognizing the complex interplay between various components within a plant is crucial. Modern research is exploring the synergistic effects of different plant constituents, leading to a more holistic understanding of their therapeutic potential.

1.2 Weighing the Advantages and Challenges of Plant-Based Drugs Plant-based drugs offer various advantages, including:

- Natural source: Many individuals view natural remedies as safer and less invasive than synthetic drugs.
- Fewer side effects: Plant-based drugs are often perceived to have milder side effects compared to synthetic medications.
- Cultural significance: Traditional medicine practices incorporating plant-based drugs hold significant cultural and historical value in various communities.

However, it's crucial to acknowledge the challenges and limitations associated with plant-based drugs:

- Variability in composition: The chemical composition of plant-based remedies can change according to topography area and growing procedures,This variability can pose challenges in ensuring consistent quality and efficacy.
- Dosage considerations: Determining the appropriate dosage for plant-based remedies can be complex due to the aforementioned variability in their composition.
- Potential interactions: Plant-based drugs can react on other medicament .

1.3 Phytoestrogens - Nature's Mimicry of Female Hormones

The intricate dance of hormones shows un-substituatal importance in maintaining robustness , especially for women. Among these hormones, estrogens stand out, regulating various physiological processes, including the menstrual cycle, bone health, and cardiovascular function. However, in recent times, a fascinating class of compounds called phytoestrogens has captured scientific interest. These plant-derived molecules, found in numerous dietary sources, possess structural and functional similarities to human estrogens.

This introductory chapter delves into the captivating realm of phytoestrogens, exploring their diverse types, potential health benefits, and ongoing research endeavors.

1.4 History of Phytoestrogens

The story of phytoestrogens has its roots in the late 19th century. In 1896, researchers observed that sheep grazing on red clover developed reproductive issues, leading to the discovery of the first identified phytoestrogen, coumestrol. This pivotal finding sparked further investigations, revealing the presence of various phytoestrogens in numerous plants consumed by humans. Over the past century, research has intensified, unraveling the diverse types of phytoestrogens and their potential impact on human health.

1.5 Types of Phytoestrogens

The world of phytoestrogens encompasses a diverse array of compounds, classified into three main categories:

1. Isoflavones: These are the most extensively studied and abundant type of phytoestrogen, primarily found in soybeans, legumes, and nuts. Examples include genistein, daidzein, and glycitein.
2. Lignans: These phytoestrogens are present in whole grains, flaxseeds, and fruits like vegetables. Examples include secoisolariciresinol and enterodiol.
3. Coumestans: These less common phytoestrogens have been proven in a few plants like clover, alfalfa sprouts, and kudzu root.

Each type of phytoestrogen possesses unique structural characteristics and varying degrees of estrogenic activity, influencing its potential effects on the body. Isoflavones, for instance, exhibit the strongest estrogenic activity among the three categories.

1.6 Understanding the Mechanism of Action of Phytoestrogens[22]

Phytoestrogens, due to their structural resemblance to estradiol (the primary human estrogen), can interact with the body's hormonal system in two distinct ways:

- Estrogen agonist: In some tissues, bound to estrogenic receiving domins and copies the effects of estradiol, potentially influencing various physiological processes.
- Estrogen antagonist: In other tissues, they can compete with endogenous estradiol for binding to receptor sites, potentially blocking or reducing its effects.

The complex interplay between phytoestrogens, their specific types, and the body's tissues determines their overall impact on human health.

1.7 Health Benefits of Phytoestrogens

- The ability of phytoestrogens to interact with the estrogenic system has led to growing interest in their potential health benefits, particularly:
- Menopausal symptoms: Research suggests that phytoestrogens may offer some

relief from common signs , like Vasomotor symptom and pubic depletion.

- Bone health: It is found out phytoestrogens functions to protectingosteocytes's health and reducing chances of osteoporosis, particularly postmenopausal . However, the mechanisms behind this potential benefit require further investigation.
- Cardiovascular health: Some research suggests that phytoestrogens does enhances cardiovascular health due to lowering blood lipid conc.. However, definitive conclusions are yet to be drawn.
- Cancer prevention: While the evidence is not conclusive, some studies suggest that phytoestrogens may have potential benefits in reducing the chances of illness such as cancers .

  However, further research is needed to confirm these findings and understand the underlying mechanisms.

It is crucial to emphasize that the potential health benefits of phytoestrogens mentioned above are still under investigation.

1.8 Challenges and Considerations in Phytoestrogen Research

While the potential benefits of phytoestrogens are enticing, several challenges remain in fully comprehending their impact on human health:

- Variability in composition: The amount and type of phytoestrogens in plants can vary depending on factors like species, cultivation practices, and processing methods[1]. This variability poses challenges in conducting rigorous research and ensuring consistent results.
- Individual differences: The way individuals absorb and metabolize phytoestrogens can vary significantly, impacting their overall effects. This necessitates personalized approaches to utilizing phytoestrogens for potential health benefits.

CHAPTER -2

PLANT INTRODUCTION

Crinum latifolium, commonly known as the milk and wine lily, is a perennial herb in the Amaryllidaceae family. This plant is notable for its healing functions .

Fig No. 1 :*Crinum Latifolium*

2.1 Common names

1. English: Milk and Wine Lily
2. Chinese: 西..南文殊兰(x.ī. n.á.nw.é.n .s.hūlán)

3. Hindi: सुधष न (Sudarsana)
4. Tamil: ஷ சாJகி(Vishamungil)
5. Marathi: गदांबीखंड(Gadambikanda)
6. Malayalam: ജോവപൊലലി(Jovannapolatali)
7. Kannada: 9ಷ 2ಾofi8 (Visha Mungali)
8. Telugu: £eąъ˘o£ట´&gరమc (Krishnavenkateswaramu)
9. Bengali: চWর7R7 (Chokhranga)

Fig No. 2 :Leaves of *Crinum Latifolium*

2.1TAXONOMY CLASSIFICATION

Table No.1 : Taxonomy Classification

Kingdom	Plantae
Phylum	Angiosperms
Class	Monocot
Order	Asparagales
Family	Amaryllidaceae
Subfamily	Amaryllidoideae
Genus	Crinum
Species	C.latifolium

MACROSCOPIC CHARACTERISTICS

Robust herbaceous perennial Crinum latifolium with large tunicate bulbs, rosette-like, stoloniferous clusters, and ancient leaf sheaths. It originates from a subterranean bulb. The edge leaves are smooth, simple, fleshy, and linear-lanceolate throughout.coriaceous, measuring between 45 and 100 cm in length.

The broad, enormous leaves resemble a snake's hood. Beautiful white blooms with a hint of scarlet are present. Flowers have thick, lengthy stems that measure between two and three millimeters in length. The fruit has a short pedicel and is spherical, about two -three cm in radius. It contains nine-ten seeds. whitish lobes that occasionally have purple undertones.

The tip is acuminate and brief.The linear anthers are 1.2–1.8 cm long with filaments shorter than the perianth, and they are composed of six stamens.

Few ovules and three inferior carpels oval-shaped capsule, 1-3 cm long. Flowers are in

bloom throughout May and June.
Although insects and birds are the primary pollinators of most lilies, C. latifolium does not need insect pollination to thrive.

Applications of Crinum Latifolium

Crinum latifolium, also known as the Spider or River Latifolium, is a captivating angiosperms belongingAmaryllidaceae family.Enthralled both horticulturalists and researchers with its captivating beauty, diverse applications, and intriguing biological properties. This comprehensive introduction delves into the world of Crinum latifolium, exploring its botanical characteristics, historical significance, diverse applications, ongoing research, and potential future prospects.

A Detailed Look at Crinum Latifolium

A Majestic Presence: Crinum latifolium boasts an impressive stature, typically reaching heights between 1-2 meters (3.3-6.6 ft). Its captivating beauty lies in its:

- Elegant foliage: The plant features long, strap-like leaves that can grow up to 1 meter (3.3 ft) in length and 5-10 cm (2-4 in) in width. These leaves emerge from the base, forming a lush, vibrant green clump.
- Dazzling blooms: Crinum latifolium produces breathtaking, trumpet-shaped flowers typically ranging from white to pale pink or lavender in color. The blooms, borne on a tall stalk, boast six petals and long, delicate stamens that extend outwards, resembling the legs of a spider, hence the name "Spider Lily."
- Fragrant allure: The flowers release a sweet, intoxicating fragrance, especially in the evenings, adding to the plant's captivating character.

2.2 Adaptability and Resilience

Crinum latifolium exhibits remarkable adaptability, thriving in various environments. It can tolerate:

- Diverse light conditions: The plant can adapt to full sun, partial shade, and even shade-tolerant conditions.
- Varying moisture levels: While it prefers consistently moist soil, it demonstrates some drought tolerance, making it suitable for various climatic regions.
- Multiple soil types: Crinum latifolium adapts well to different soil types, including well-drained loam, sandy loam, and even slightly clay-rich soils.

These characteristics contribute to the plant's widespread distribution and popularity in various landscaping applications.

2.3 History of Crinum Latifolium

Crinum latifolium transcends its captivating presence to hold cultural significance in various regions:

- Traditional medicine: In some cultures, Crinum latifolium is applied in herbal medicament practices for decadess. various components of the plant, such as bulbs, roots etc, have been employed to address various ailments, although scientific validation of these uses is crucial.
- Symbolism and folklore: The plant holds symbolic value in various cultures. In some regions, it symbolizes purity, rebirth, and new beginnings, while in others, it is associated with mourning and remembrance.

Understanding the cultural significance of Crinum latifolium underscores its multifaceted nature and deep-rooted connection to human history and traditions

2.4 Applications of Crinum latifolium

Crinum latifolium transcends its captivating beauty to offer a multitude of applications:

- Ornamental plant: The plant's elegant foliage and stunning blooms make it a popular choice for landscaping. Its adaptability allows for diverse applications in gardens, borders, and even containers.
- Cut flower: The long-lasting blooms of Crinum latifolium are valued in the floristry industry, adding a touch of elegance and fragrance to floral arrangements.
- Potential medicinal applications: While extensive scientific research is necessary,

Crinum latifolium is being explored because of its possible use addressing many ailments, including:

- Infectious Illness : Some studies suggest potential antimicrobial and antifungal properties.
- Wound healing: Certain studies indicate potential wound healing properties.
- Cancer: Preliminary research suggests potential anti-cancer properties, although further investigation is crucial.

It is important to emphasize that these applications are based on ongoing research, and the therapeutic potential of Crinum latifolium requires rigorous scientific evaluation and clinical trials to ensure safety and efficacy.

2.5 Ongoing Research on Crinum latifolium

The captivating properties and potential applications of Crinum latifolium have fueled ongoing research in various areas:

- Phytochemical analysis: Researchers are actively investigating the presence and characterization of bioactive compounds within the plant, aiming to understand their potential health benefits.
- Biological activity exploration: Scientific studies are exploring the potential against microbes , against fungus, wound healing, properties of Crinum latifolium.
- Cultivation optimization: Research efforts are underway to optimize cultivation practices for Crinum latifolium, aiming to improve yield and enhance the production of desired bioactive compounds.

Crinum latifolium, also known as the Spider or River Latifoloium, is a captivating angiosperms belonging to the Amaryllidaceae family. Enthralled both horticulturalists and researchers with its captivating beauty, diverse applications, and intriguing biological properties.One of the ongoing research areas on Crinum latifolium is the investigation of its constituents, which are the chemical compounds that make up the plant. These constituents contributes to the various properties and potential applications

2.6 Constituents in Crinum latifolium

2.6.1 Alkaloids

One of the primary classes of compounds identified in Crinum latifolium is alkaloids. These nitrogen-containing compounds are known for their potent pharmacological activities.
Among the alkaloids isolated from this plant, lycorine stands out due to its notable anticancer, antiviral, and anti-inflammatory properties. Lycorine has shown efficacy in stops the development of mutated cells, making it a focal point of cancer research. Another significant alkaloid, crinamine, exhibits cytotoxic activities against cancer cells, contributing further to the plant's anticancer potential. Additionally, powelline, another alkaloid present in Crinum latifolium, adds to the plant's pharmacological arsenal with its bioactive properties.

2.6.2 Flavonoids

Flavonoids are another crucial group of compounds found in Crinum latifolium. These polyphenolic compounds are renowned for their antioxidant properties, nulliefy harmful unpaired electron in the cell. Notable flavonoids in Crinum latifolium include quercetin

and kaempferol. Quercetin is a powerful antioxidant that also proves action against inflammation and anticancer. It has been studied extensively for its ability to modulate cell signaling pathways, which is crucial in cancer treatment and prevention. Kaempferol, similarly, has been recognized for its antioxidant, anti-inflammatory, and anticancer effects, therefore enhancing the possible applicability of Crinum latifolium.

2.6.3 Saponins

Saponins are glycosides that have been identified in Crinum latifolium and are known for their wide range of biological activities[55]. These compounds possess significant anticancer, anti-inflammatory, and immune-boosting properties. The presence of saponins in Crinum latifolium contributes to its effectiveness in traditional medicine, particularly in enhancing immune function and combating inflammation and cancer.

2.6.4 Tannins

Tannins are carbolic acid structure with strongaction against oxidant ,infalmation, and microbes effects. The tannins in Crinum latifolium help protect cells from oxidative damage and reduce inflammation, supporting the plant's use in treating various inflammatory conditions and infections. Their antimicrobial properties also make them valuable in preventing and treating infections, adding to the medicinal value of the plant.

2.10.4 Polysaccharides

Polysaccharides in Crinum latifolium are known for their immunomodulatory and anticancer properties. These long-chain carbohydrates can modulate the Immunobiology improve restaurants to fall ill. The Immunobiology effects of these polysaccharides are particularly important in the context of cancer therapy, where boosting the immune response can help in targeting and eliminating cancer cells.

REVIEW OF RESEARCH ACTIVITIES CARRIED ON CRINUM LATIFOLIUM

Anticancer Properties

Studies have shown that Crinum latifolium extracts have significant antiproliferative effects on various cancer cell lines. For example, Crinumlatifolium indicates to inhibit TGF-β-induced proliferation in prostate stromal cells (WPMY-1), suggesting potential use in treating conditions like benign prostatic hyperplasia and prostate cancer (BioMed Central). Additionally, aqueous extracts of Crinum latifolium demonstrated inhibitory effects on the proliferation of Adenocarcinoma and enlargement of prostate, highlighting its possible role in managing prostate disorders (MDPI).

Phytochemical Analysis

Research has isolated several bioactive compounds from Crinum latifolium, including alkaloids such as lycorine and 6α-hydroxybuphanidrine. These compounds have been

studied for their biological activities, including their role in inhibiting cell proliferation and modulating immune responses (BioMed Central) (MDPI).

Traditional Medicine Uses

Historically, Crinum latifolium in Vietnamese medicament for cure of tumors, rheumatism, and infections. Its continued use in these practices underscores its importance and potential as a source of natural therapeutic agents (MDPI)

CHAPTER 3

LITERATURE SURVEY

1. Jalal M.,etal., 2023: Mohammad Jalal's study examines the environmentally friendly synthesis of gold nanoparticles (AuNPs) using extract from Crinum latifolium and assesses the biological activities of these nanoparticles. In order to produce stable and evenly distributed AuNPs, the biosynthesis process makes use of the plant extract's inherent reducing and stabilizing agents. Several methods are employed to confirm the size, shape, and stability of the synthesized nanoparticles. The study looks into the AuNPs' anticandidal qualities and finds that they have a lot of action against Candida species. Furthermore, the AuNPs have strong antibiofilm activity, which prevents biofilms—which are protective layers made by microbial communities—from forming and remaining intact. Additionally, by blocking virulence factors that increase the pathogenicity of microbes, the nanoparticles demonstrate encouraging antivirulenceactivity.According to the research, AuNPs mediated by Crinum latifolium have the potential to be powerful weapons against microbial infections and to be applied in the creation of novel antimicrobial treatments.s.

2. Boussekine, et al., (2022): The current study aims to target and assess impact about selenium supplementation on diabetic nephropathy, which is caused by reactive species produced by hyperglycemia. Tissue biochemical parameters are used in the assay and statistical study of blood biochemical parameters. By controlling plasma insulin, blood glucose, G6PDH, urea, creatinine, calcium, and phosphorus, we conclude that mineral selenium supplementation protects cells from reactive species caused by alloxane.

3. Arica C.D., etal., (2022): This paper explains about the effects of curcumin, an antioxidant phytochemical, on diabetic nephropathy treatment and care. The MannWhitney U test and the Kruskal-Wallis test were used to identify differences in glomerular damage and GH scores between the groups. Curcumin treatment reduced it in experimental diabetic rats in this study, and the results were superior to those of other antidiabetic medications like Diamicron and metformin.

4. Aakruti K., et al., 2022: The goal of this paper is to standardize and form rat models of type 1 and type 2 diabetic nephropathy that are comparable to human clinical symptoms. This study aimed to improve a T2DM rat model, which causes diabetic nephropathy, and to distinguish it from a T1DM model by evaluating various parameters such as metabolic, antioxidant, and renal damage meters.

5. Kishore D.V., et al., 2022: The anti-arthritic properties of the complete extract of plant Crinum latifolium are examined in this study by Prof. Dr. D.V. Kishore e in Wistar albino rats. The purpose of the study is to determine whether the plant extract can slow the progression and symptoms of arthritis in an experimental rat model. After causing arthritis in the Wistar albino rats, different dosages of Crinum latifolium extract were administered. The study gauges a

number of factors in the treated rats, including paw swelling, joint inflammation, and histopathological alterations. The plant extract's anti-inflammatory and anti-arthritic qualities are demonstrated by the results, which show a significant reduction in arthritis symptoms. The results point to Crinum latifolium as a possible natural treatment for arthritis, which opens up new avenues for investigation and the creation of novel therapeutic compounds derived from this plant.

6. Kishore D.V., et al., 2022: The study examines the antidepressant properties of an extract from Crinum latifolium in laboratory animals. Using animal models of depression, the study aims to assess the plant extract's possible antidepressant benefits. The experimental animals were administered varying dosages of Crinum latifolium extract, and their behavior was observed by established tests like the tail suspension test and the forced swim test, which are frequently employed to evaluate antidepressant efficacy. According to the findings, the extract considerably lowers depressive-like behaviors in the treated rats in a way that is similar to that of conventional antidepressant medications. These results point to the potential of Crinum latifolium extract as a natural medicinal agent for the treatment of depression by suggesting that it demonstrates noteworthy antidepressant characteristics. It is advised that more research be done to clarify the underlying mechanisms and investigate its therapeutic uses.

7. Nijhawan P., et al., 2022: The study "Exploring the effect of Crinum latifolia in obesity: possible role of oxidative, angiogenic, and inflammatory pathways" The purpose of the study is to ascertain whether the plant extract can alter these pathways in order to lessen obesity and the problems that come with it. After administering Crinum latifolia extract to experimental models of obesity, levels of inflammation, angiogenesis, and oxidative stress were evaluated using a variety of indicators. The findings show that in the treated models, the extract dramatically lowers oxidative stress indicators, suppresses aberrant angiogenesis, and lowers inflammatory cytokines. These results imply that by focusing on important biological pathways connected to obesity, Crinum latifolia has a multifarious role in its management. The results of the study demonstrate the potential of Crinum latifolia as a natural therapeutic agent for obesity, indicating the need for more investigation to fully comprehend its mechanisms and create efficacious remedies.

8. Kumar N., et al., 2022: Neeraj Kumar study from 2022 focuses on assessing a hydroalcoholic extract from Crinum latifolium's in-vitro antioxidant properties utilizing a variety of techniques. The purpose of the study is to ascertain whether the extract has the ability to scavenge free radicals and stop oxidative damage. Several in-vitro tests, including reducing power assays, DPPH radical scavenging, and ABTS radical cation decolorization, were used to evaluate the extract's antioxidant capabilities. According to the findings, the Crinum latifolium extract has considerable antioxidant activity that is on par with conventional antioxidants in all evaluated methodologies. These results imply that Crinum latifolium hydroalcoholic extract is a rich source of natural antioxidants that may help shield against illnesses linked to oxidative stress.

9. Tian H., et al., 2021: The study "Antimicrobial crinane-type alkaloids from the bulbs of Crinum latifolium" by Hong Tian et al. (2021) describes the extraction and characterization of three novel alkaloids of the crinane type from Crinum latifolium bulbs. The purpose of the study is to

investigate the plant's chemical components and assess their antibacterial qualities. Spectroscopic methods like NMR and MS were used to characterize the structure of the isolated alkaloids. The investigation evaluated these substances' antibacterial efficacy against a range of bacterial and fungal species. The findings show that the recently discovered alkaloids have strong antibacterial activity, indicating their potential as naturally occurring antimicrobial agents. The results open the door for more investigation into the possible therapeutic uses of bioactive compounds in the fight against microbial illnesses and emphasize the significance of Crinum latifolium as a source of these chemicals.

10. Mohammed A., et al., 2021: The aim of this study was to see if treating rats with streptozotocin-induced diabetes and non-diabetic rats with metformin would affect adenine induced chronic kidney disease (CKD). Several physiological and novel biochemical biomarkers, including albumin/creatinine ratio, uric acid, and N-acetyl-beta-d-glucosaminidase, were altered in plasma, urine, and kidney homogenates of adenine-treated rats. Metformin may be a useful drug for halting the progression of CKD in diabetic and non-diabetic rats, according to the physiological data, histopathology, and renal variables results. We used metformin at a dose of 200 mg/kg/day in this study, which is known to be safe for rats. One drawback of this study is that it only used one dose of metformin.

11. Guo L, et al., 2021: This study examines how adropin protects rats from diabetic nephropathy caused by streptozotocin. The processes by which adropin reduces kidney damage brought on by diabetes are the main focus of the research. According to the study, adropin dramatically lowers oxidative stress and inflammatory markers in the kidneys of diabetic rats. The protective effects are facilitated by the suppression of the NF-κB signaling pathway, an essential mechanism in the regulation of oxidative stress and inflammation. The findings imply that adropin protects against diabetic nephropathy by lowering oxidative damage and renal inflammation. This work emphasizes the value of targeting the NF-κB pathway to reduce difficulties related to diabetes and underlines the potential of adropin as a therapeutic agent for controlling diabetic kidney disease.

12. Chen M., et al., 2018: Crinum latifolium has cytotoxic, antimicrobial, antioxidant, and anti-inflammatory activities.examines the many biological actions of Crinum latifolium, including as its anti-inflammatory, cytotoxic, antibacterial, and antioxidant capabilities. The goal of the study is to assess the plant extract's medicinal potential in a thorough manner. A range of assays were employed to evaluate each of the four activities: radical scavenging assays for antioxidant activity, cancer cell line cytotoxicity, bacterial and fungal strains for antimicrobial activity, and the inhibition of pro-inflammatory mediators for anti-inflammatory effects. The findings show that Crinum latifolium has great antioxidant qualities, powerful antibacterial activity, considerable anti-inflammatory benefits, and significant cytotoxic effects on cancer cells. These results point to Crinum latifolium as a promising natural source for the development of therapeutic compounds with a variety of health advantages, laying the groundwork for more research into the plant's potential medical uses.

13. Liu J., et al., 2018: Estrogenic activity of cylindrospermopsin and anatoxin-a and their oxidative products.Jishan Liu et al.'s (2018) study looks on the estrogenic properties of anatoxin-a and

cylindrospermopsin, as well as the oxidative products that result from these substances. The purpose of the study is to find out if these cyanotoxins and their derivatives can mimic or disrupt estrogenic activity, which could have an effect on endocrine functioning. The study assesses these drugs' capacity to activate estrogen receptors and elicit estrogenic responses using in vitro assays, including the E-SCREEN assay and estrogen receptor binding assays. The findings demonstrate the strong estrogenic activity of cylindrospermopsin, anatoxin-a, and associated oxidative byproducts. These results raise questions about the cyanotoxins' capacity to modify hormones in the environment, raising the possibility that exposure to these substances could be harmful to both human and animal health.

14. Ashrafuzzaman MD., et al., 2016: Antidiarrheal Activity of Three Medicinal Plants in Swiss Albino Mice.In traditional medicine, various portions of Bruguiera cylindrica (BC), Crinum latifolium (CL), and Allamanda neriifolia (AN) are used to treat diarrhea. Thus, the purpose of this study was to assess and examine any potential antidiarrheal effects of crude extracts from the roots, barks, and stems of AL, CL, and BC in Swiss albino mice.

15. Akram, et al., 2016: Finding out if artificial antioxidant therapy affects diabetic nephropathy and how it relates to changes in serum oxidative markers is the aim of this research. Enzymaticscavengers such as catalase, glutathione peroxidase, superoxide dismutase, and lipid peroxidase were examined, as well as the total amount of thiol molecules and antioxidant capacity. In diabetic rats, Tempol can alter oxidative stress indicators and possibly nephropathy. This study's weakness was the lack of comparison and measurement of GFR among several rat groups.

16. Dangi N., et al., 2015: In the current work, diabetic nephropathy in STZ-induced diabetic rats was examined , determine the impact of an Aloe vera leaf extract. Aloe vera's powerful antihyperglycemic, antioxidant, and improvement of renal function parameters may be the cause of its strong impact on diabetic nephropathy.

17. Shelia, et al., 2015: This study's goal was to assess the impact of oxidative stress in a rat experimental model of streptozotocin-induced diabetic nephropathy. The combination ofhyperglycemia and uninephrectomy led to increased renal impairment, proving that the model for the study of diabetic nephropathy. This study supports that diabetic rats exhibit lipid peroxidation and vigorous antioxidant defence system usage.

18. Mohamed A., et al., 2014: In this study, carvedilol prevents STZ-induced early diabetic nephropathy in rats. Its anti-inflammatory, antioxidant, and podocyte injury-relieving properties may contribute to this protective effect. Nephroprotective efficacy as demonstrated by a drop in blood creatinine, kidney index, and renal levels of malondialdehyde as compared to diabetic rats who were not receiving treatment.

19. Gang Ko., et al., 2008: We looked at the impact and molecular mechanism of Crinum Latifolium, a PPARgamma agonist, on the development of diabetic nephropathy in type 2 diabetic rats. Western blot, EMSA, immunocytochemical staining, and RT-PCR were used to identify inflammatory markers such as NF-kB, MCP-1, and pro-fibrotic cytokines. According to

these findings, pioglitazone not only improves lipid profiles, glycaemic management, and insulin resistance. but in type two diabetic rats, decresaes kidney injury via an anti-inflammatory mechanism. The study was limited by the fact that glucose levels varied between experimental groups.

CHAPTER -4

AIM&OBJECTIVE

AIM

The study aims about "Investigation for Estrogenic Activity of *CRINUM LATIFOLIUM* in female Albino rats,"

OBJECTIVE

1. Evaluation of the acute toxic effects of the leave of of*Crinum Latifolium.*

2.Comparision of estrogenicityof ethanolic extract of leave of *Crinum Latifolium*with diethylstilbestrol in immature ovariectomized albino rats by following parameters.

a) Vaginal cornification.
b) Uterine wet weight.
c) Uterine glycogen content.
d) Vaginal opening.
e) Uterine histology.

CHAPTER -5

PLAN OF WORK

Survey of Literature

↓

Adequate Availability of *CRINUM LATIFOLIUM*

↓

Preparation of ethanolic extract

↓

Adjustment of Animals in appropriate environment

↓

Experimental Design: Estrogenic Activity

↓

Physical observation of possible side effects

↓

Observation of time vaginal opening of female animals for estrogenic activity

↓

Histopathological examination for estrogenic activity

↓

Submission of thesis

CHAPTER

6METHODOLOY

6.1 PRELIMINARY PHYTOCHEMICAL ANALYSIS [3]

Using established procedures, the alcoholic extract of *Crinum Latifolium*was qualitatively examined for several phytoconstituents such as glycosomes, alkaloids, tannic acid, triterpene, Plant stanols, and bioflavonoids.

I) PLANT STANOLS (LIEBERMAN- BURCHARD'S TEST)

Chloroform, three droplets of ethanoic anhydride, and two droplets of strong H2SO4 were combined to treat the extract. The existence of phytosterols is indicated by the appearance of purple colour that changes to blue or green.[3]

II) ALKALOIDS

Mayer's test: After dissolving the alcohol extract inside 2N HCL, 1-2 droplets of recently made Mayer's reagent is alsoput in the solution. There are alkaloids present when a white precipitate appears.[33]

Dragendroff's test: After dissolving the alcoholic extract in 2N hydrochloric acid, 1-2 droplets of recently made Dragendroff'sreagent put in the solution.The presence of alkaloid is indicated by emergence of brick red ppt.

III) FLAVONOIDS (SHINODA'S TEST)

One gram Mg powder and one milliliter of strong HCL were used to treat extract of alcohol. The emergence of an orange hue suggests existence of flavonoids.[43]

IV) TANNINS

Plumbous acetate solution was used to treat the alcoholic extracted leaves . The existence of tannins is indicated by the formation of white precipitate.

V) GLYCOSIDES (BORNTRAGER'S TEST)

Chloroform was used to extract the hydrolyzate after the alcoholic extracted leaves was dehydrated about150-250 minson water bath using diluted HCL[88]. An equivalent amount of diluted ammonia was added to the chloroform layer. The existence of glycosides is indicated by the glowing of pink color.

VI) SAPONINS (FOAM TEST)

A vigorous shake of distilled water was applied to the alcoholic extract. Saponin is present when stable foam starts to form.

VII) PHENOLIC COMPOUNDS[66] (FERRIC CHLORIDE TEST)

Ferric chloride solution that had been diluted was used to treat the alcoholic extract.
There are phenolic compounds present because of the blue color that appears.

6.2 ACUTE TOXICITY STUDY

To evaluate the drug's safety and acute pharmacological effects, an initial pharmacological investigation was carried out.

Using the "up and down" method, female or male albino rats were used for the acute toxicity investigation.

PROCEDURE: OECD GUIDELINE

Limit test:

1. Dose one animal at 2000 mg/kg.

2. If the animal passes away, perform the primary test to determine the LD50.

3. In order to test five animals in total, do four more animals in succession if the animal survives[2].
4. End the limit test and proceed with the main test if three animals pass away at any time during the sequential dosing.

5. The median lethal dose > two thousand milligrams per kilogram if 3 minimum rat survive.

6. The median lethal dose < milligrams per kilogram if three or more animals die.

7. Stop administering the medication and keep an eye on all the animals to see if any more die during the same observation period if an animal unexpectedly passes away toward the end of the study and there are still survivors.

This procedure outlines a limit test for determining whether the LD50 (median lethal dose) is above or below 2000 mg/kg. It provides guidelines for when to continue dosing, when to stop and conduct a main test, and how to interpret the results based on animal survival.

MAIN TEST:

Doses for individual animals are given sequentially, typically every 48 hours. Delaying treatment until one is certain the previously dosed animal will survive is advised before administering the subsequent dose to the animal. The1st rat is doped lesser than the most accurate LD50 preparatory value. 2nd rat is given a larger dosage if it survives.[3]

If the animal dies , the 2nd animal is given a smaller doped. 3.2 dose progression factor is employed. Doses from the following list would be chosen using the default progression factor: 1.75, 5.5, 17.5, 55, 175, 550, and 2000.

Throughout the first 30 minutes following dosage and on occasion throughout the first 24 hours, each animal is observed separately. With minimal changes, the data were tallied in accordance with Irwin's table.

6.3 DRUG EXTRACT'S IMPACT ON BIOCHEMICAL PARAMETERS:

Male or female albino rats were used in a study assessing biochemical changes in serum and urine. Two groups of four albino rats, weighing between 150 and 200 grams apiece, were formed.

Group I (control) : For 14 days, received an oral dose of 10 milliliters per kilogram every day.

Group II :received treatment with an oral dose of 400 mg/kg of alcohol-based Crinum Latifolium extract every day for 14 days.
Animals were given cardiac punctures on the fourteenth day of the protocol in order to draw blood. After 30 minutes of blood coagulation at 37°C, the serum was separated by centrifuging the mixture for 10 minutes at 15,000 rpm. [69]Next, using standard protocols, . Serum TC, serum High-density lipoprotein, Serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase.

The animals' urine was sent creatinine content tested at the end of the dosage period.
At the conclusion of the dosage period, the animals' urine was also collected and its creatinine content examined.

6.4 EVALUATING ESTROGENETIC ACTIVITY

With only minor modifications, the standardized method of assessing the estrogenicity of the alcoholicastion extract in adult albino rats underwent bilateral ovariectomies. The parameters of assessment, which included uterine glycogeniocontentopion, vag1inal cornification, qwvaginal opening, and uterous histology, were compared with the established estrogenic activity of diethylbestrol (DES).

Requirements :

1. Adult females albino rats weighing 1150-2100 grams.
2. Diethylstilbestrol (DES) (0.2 mg/ml suspension).
3. Anthrone Reagent (0.15% w/v in 95% v/v sulfuric acid).

6.4.1 METHOD OF (OOPHORECTOMY)

Anesthesia was performed by 87 miligram ketamine/kilogram of body weight and 13 mg xylazine/kg i.p. About halfway between the base of the tail and the middle of the back (the hump), a small midline incision at dorsal side was performed . The peritoneal cavity was used to identify the female genital tract, which includes the uterus and ovary.

The ovary was extracted by means of a muscle incision, with caution exercised to prevent any small pieces of the ovary from becoming detached and re-implanted.[9] Each uterine horn's distal end, or the area close to the ovary, was tied off with a 3-0 silk suture. The uterine horn and its fallopian tube junction, along with any surrounding fat and blood vessels, were cut with sharp scissors in one cut on each sides, and the horn was then removed and returned to the abdominal cavity. Two layers of closure were used on the abdomen: a 2-0 silk suture was used on the skin, and a 3-0 chromic catgut was used on the peritoneum and muscle.[56]

The rats that had their ovariectomies were kept in cages made of polypropylene and given unlimited access to water and standard laboratory food.

FIG NO .3 **SUTURING**

FIG NO .4 OVARIECTOMY

Rats that had their ovariectomies were given a 15-day period to heal. Every day after the surgery, vaginal smears were obtained to detect any lingering estrogenic activity. Rats that had undergone ovariectomies were separated into five groups, each with four rats, after 15 days.

6.4.2 SELECTION OF DOSES

Three dose levels were selected for the evaluation of estrogenic activity: a lower dosage that was 50 percent of the 10th dose , a higher dosage that was double the 10th dosage , and a mid dosage that was roughly 1/10th of the max dosing administered during ACT.

Group 1: Was given distilled water orally every day for seven days at a dose of 10 milliliters per kilogram.

Group 2: Given a dosing of2 Milligrams per kilogram of diethylstilbestrol orally every day for seven days.

Group 3 : Give a regular oral dosing of 100 Milligrams per kilogram of alcohol-based extract of Crinum Latifolium for seven days.

Group 4: Underwent a seven-day oral treatment of 200 mg/kg of alcohol-based Crinum Latifolium extract.

Group 5: Underwent a seven-day oral treatment of 400 mg/kg of alcohol-based Crinum Latifolium extract.

Every day, the vaginal opening and cornification were checked. Following the eighth day, or 24 hours after the last treatment, all rats underwent a hysterectomy (uterine removal) using the "Waynforth"73 technique, receiving 13 mg of xylazine and 87 mg of ketamine per kilogram of body weight.

6.4.3 Uterine wet weight

After carefully removing any adherent connective tissue, harvested uteri were immediately weighed using an electronic balance. An rise in the wet weight indicates estrogenic activity.

6.4.4 Histology of the uterus

Each group's 3 removed uteri were preserved by Bouin's fluid and prepared for tissue analysis. Under a microscope, slides stained with hematoxylin and eosin were inspected for alterations in cellular organization, as well as for increases in uterine diameter, endometrial thickness, and endometrial epithelium height. 5, 71
An increase in the aforementioned parameter indicates estrogenic activity.

6.4.5 Uterine Glycogen Content

For the estimation of glycogen, the final three uteri from each group were utilized. The anthrone method was used to estimate the amount of glycogen.

On ice, 5 milliliters of 30% potassium hydroxide were used to homogenize the removed uteri. After 15 minutes of boiling in a water bath, the homogenate was allowed to cool before 0.05 milliliter of sodium sulfate solnwas used together and mixed. After adding 3.5 ml of 96 percent volume/volume Ethanoll, the mixture placed on ice about five mins.

After centrifuging the contents for ten minutes at 2000 round per minutes , the liquid floating on the surface was removed out. After that, 3 ml of absolute alcohol was added, and the mixture was recentrifuged for 10 minutes at 2000 rpm. Again the supernatent was decanted out.

Three milliliters (ml) of distilled water were added to the resulting sediment. In different test tubes, two milliliter of the diluted sediment and Two milliliter of distilled H20 were added. After cooling the test tubes in an ice bath, 4 ml of anthrone reagent was added to each test tube, dropping it in and thoroughly mixing it in. After that, the test tubes were placed in a bath of boiling water for ten minutes. After the test tubes were cooled, a spectrophotometer was employed to measure the absorbance at 650 nm.

Test uterus specimen's glycogen content (µg/mg of uterine tissue) was determined by following equation:

$$\frac{\text{density} \times 1/\text{Slope}^* \times \text{Dilution factor}}{\text{Uterine weight (mg)}}$$

*where Slope is the result of matching different known glycogen concentrations to optical density to construct a standard graph.

One indicator of estrogenic effects is an increase in the amount of glycogen in the uterus.

6.5 Preparation of standard graph for glycogen estimation

Glycogen was measured using the anthrone method, and graded concentrations of the glycogen solution—that is, 5-100 µg/ml—were prepared in distilled water. A spectrophotometer was used to measure absorbance at 650 nm in relation to a blank..

Table No: 2 Concentration of glycogen and its absorbance

Serial Number	Concentration (mcg/ml)	Absorbance
1	0	0
2	5	0.0432
3	10	0.095
4	20	0.1923
5	40	0.402
6	60	0.7218
7	80	0.76
8	100	1.009

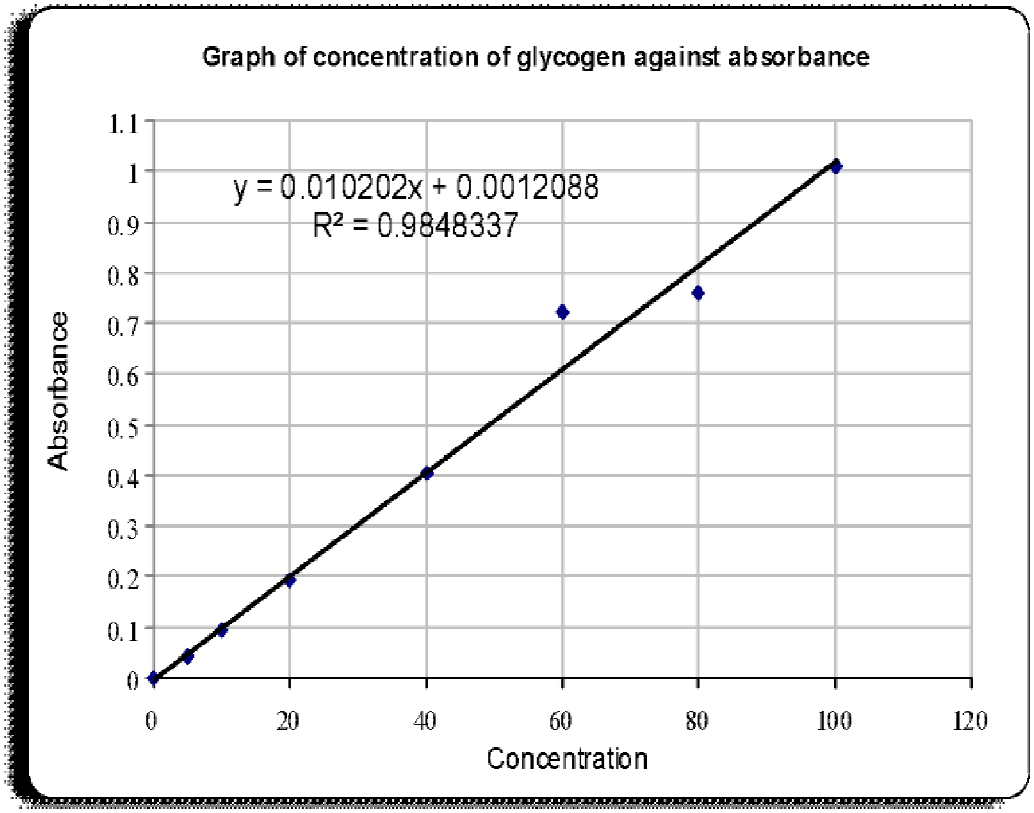

Fig. 5: Graph of Concentration of Glycogen against Absorbance

Calib Curve: Linear

Expression: Abs = A + B Conc.

Factor : A = 0.0012

B = 0.0102

Coefficient: 0.9848337

Vaginal cytology

The vaginal smear method was used for this. Using an eye dropper, a little droplets of saline were introduced into the vagina,to obtain a vaginal smear. Two or three times, the saline was withdrawn and ejected into the vagina. The eye dropper's contents were spread out onto a glass slide, and the smear was immediately fixed for 5–6 minutes with one percent weight/volume aqueous methyla1ene blueqw. Any remaining stain was then washed off with distilled water, and the smear was counterstained for 2–3 minutes with 2% w/v eosin. Ultimately, extra stain was removed using distilled water and allowed to air dry. The percentage vaginal cornification was calculated using the relative proportion of each cell type, leukocytes, nucleated epithelial cells, and cornified epithelial cells, in either case. Presence or absence of leukocytes, nucleated epithelial cells and

cornified epithelial cells, and the relative proportion of each cell type was used to quantify the percentage vaginal cornification.

Vaginal opening

Every day, the vaginal opening was watched and recorded. An increase in vaginal opening is a sign of increased estrogen production.

Statistical Analysis

The Crinum Latifolium alcoholic extract treated group and the control group's biochemical parameters were compared using a paired student t test. The % of quterine wet weight and uterine glycogen content that varied among treatment groupson were examined using one-way analysis of variance and Dunnet's "t" test.

CHAPTER

7RESULT

1.1 PRELIMINARY PHYTOCHEMICAL ANALYSIS

Flavonoids, phytosterols,tannic acid, and phenols are observed in the alcoholic extract of Crinum latifolium, according to a preliminary phytochemical analysis .

1.1.1 ACUTE TOXICITY STUDY

Even when max dosage of 12000 mg/kg was administered, no deaths were reported. The amount of some autonomic responses, including piloerection, scrotal enlargement, sedation, elevated muscular action and ynskeletal muscle contraction, staggering posture, and purgation, was dose-dependently increased .

1.1.2EFFECT OF DRUG EXTRACT ON BIOCHEMICAL PARAMETERS

A fourteen days treatment regimen was introduced to provide information on possible health risks related recurrent over a restricted period . Rats given Crinum latifolium treatment had slightly higher serum levels of total protein, serum globulin, SGPT, cholesterol, and serum creatinine as matched to to the controled group, according to bio-chemical studies of their serum. These changes weren't statistically of significance , though. A minor drop in LDH, albumin, alkaline phosphatase, and HDL. Once more, these alterations were not weren't statistically of significance. There was a noted significant drop in serum triglycerides ($t=3.71$, $df=6$, at $p<0.05$) and blood glucose ($t=4.88$, $df=6$, at $p<0.01$) when compared to the control group.

The rate of creatinine excretion on a daily basis increased slightly, according to urine creatinine estimation.

The rate of creatinine excretion on a daily basis increased slightly, according to urine creatinine estimation. However it was statistically insignificant .

1.2 Evaluation of estrogenicity

The alcoholic extract of Crinum latifolium was tested for its estrogenic activity in bilaterally ovariectomized albino rats. The parameters used for this evaluation were percentage vaginal cornification, percentage vaginal opening, uterine wet weight, uterine glycogen content, and uterine histology.

A) Uterine wet weight

When compared to the control group, the alcoholic extract of Crinum latifolium gave a rise uterine wet weight with respect to dose and statistically evident manner ($F=180.80$; df =4, 15 at $p<0.010$).

On doses of 100, 200, and 400 mg/kg, respectively, there was a 0.004, 0.06, and.128 fold increase in uterine wet weight in comparison to the control. However, a 0.004-fold increase in uterine wet weight [$F=180.80$; df =4, 15 at $p<0.05$] does not show statistical significance. In contrast, DES at a dose of 2 mg/kg increased uterine wet weight by a statistically significant 1.27 -fold when compared to the controled group [$F=180.80$; df =4, 15 at $p<0.01$].

B) Uterine Histology

For both the 200 mg/kg and 400 mg/kg doses, Crinumlatifolium caused proliferating changes in the uterine endometrium, as shown by increased height of luminal epithelium, with loose stroma and increased number of glands . However, no proliferative alterations were seen at 100 mg/kg (Fig. 18). DES, on the other hand, displayed proliferative changes, as shown by an increase in gland number, loose stroma, and epithelium height . In the control rats, uterine endometrium was disintegrated.

C) Uterine glycogen content

There was observed to be a dosage -related ,statisticaally relevant increase in uterine glycogen after Crinum latifolium extract was administered. A statistically insignificant [F=72.02; df=4, 15, at p>0.05] 0.292 fold increase was observed at a dose of 100 mg/kg. In contrast, the 200 and 400 mg/kg doses demonstrated statistically significant increases in comparison to the control [F=72.02; df= 14, 15, at p< 0.01] of 0.3622 and 0.6119 folds, respectively.
indicates that there was a statistically evident - 2.01 fold enhancement in diethylstilbestrol extract when compared to the controled group (F=72.02; df=4, 15, at p< 0.011).
.

D) Vaginal cytology

During the treatment period, there was no vaginal cornification observed in the vaginal smear of the ovariectomized control group (Fig. 11). In contrast, starting on day four, the alcoholic extract of Crinum latifolium at doses of 1200 mg/kg & 0400 mg/kg increased the proportion about vaginal cornification. However, on 11100 milligrams per kilogram there was very little cornification (Tab. 8 & Fig. 13). It was observed that the % of vaginal cornification at 1400 milligrams per kilogram was comparable to diethylstilbestrol.

E) Vaginal Opening

Compared to the control, which stayed closed, the extract demonstrated a dosage -related rise in vaginal opening starting on fourth cycle. Vaginal opening was significantly demonstrated by Crinum latifoliumdosage of 2001 mg/kg and 1400 mg/kg. However, the vaginal opening was negligible at 1100 mg/kg. A percentage rise in vaginal openness was also observed with DES.

Table No. 3: Phytochemical analysis of the extract of *Crinum latifolium*,.

Sr. No	*Crinum latifolium,*	Tests conducted	Observation	Conclusion
1. Test for Alkaloids	Alcoholic extract	a)Dragendroff's test b) Mayer's test	Brick red ppt. whitish ppt.	Positive
2. Test for saponins	Alcoholic extract	Foam test	No stable foam was seen	Negative
3. Test for Phytosterols	Alcoholic extract	Lieberman-Burchard's test	Purple color was produced	Negative
4. Test for Tannins	Alcoholic extract	Lead acetate test	White ppt. seen	Positive
5. Test for Phenols	Alcoholic extract	Ferric chloride test	Blue colour seen	Positive
6. Test for Flavonoids	Alcoholic extract	Shinoda's test	Orange color was produced	Positive
7. Test for Glycosides	Alcoholic extract	Borntrager's test	No pink color seen	Negative

Table 4: Acute Toxicity

A dose dependent increase in the magnitude of above mentioned parameters wereobserved.

DRUG	PARAMETERS OBSERVED								
	Aware-ness	**Motor Activity**	**Posture**	**CNS Excita-tion**	**Muscle Tone**	**Reflexes**	**Autonomic Profiles**	**Miscella-neous**	**Death**
Crinum latifolium, extract	Sedation	Spontaneous motor activity was increased	Staggering posture	seen	Skeletal muscle contraction	Decreased	Piloerection and increase in scrotum size	Purgation	Even at 2000 mg/kg dose, no death was observed.

Table.5: Effect of alcoholic extract of Crinum latifolium, (at the dose of 400 mg/kg p. o for 14 days) on biochemicalparameters. Values are mean ±S.E.M of 4 animals in each group. Data were analysed by unpaired‘t’ test.

Group	Cholesterol	HDL	SGOT	SGPT	Creatinine	Protein	Albumin	Globulin	ALP	TG	LDH	Glucose	Urine creatinine
Control	56.75 ± 7.98	34 ± 5.52	218 ± 5.58	65.5 ± 6.76	0.73 ± 0.048	5.68 ± 0.34	2.8 ± 0.071	2.88 ± 0.33	4.25 ± 0.48	51 ± 4.81	158.25 ± 5.53	103.77 ± 1.63	4.69 ± 0.22

Crinum latifolium extract	60.5 ± 8.34	24.75 ± 2.75	218.5 ± 1.66	70.5 ± 15.25	0.78 ± 0.06	6.1 ± 0.35	2.73 ± 0.09	3.38 ± 0.43	2.75 ± 0.75	*32.5 ± 1.32	139.5 ± 16.63	** 93.58 ± 1.31	6.95 ± 0.91

*p<0.05 compared to control group

** p<0.01 compared to control group

Note: Changes in other biochemical parameters were statistically insignificant.

Table.6: Effect of alcoholic extract of *CRINUM LATIFOLIUM*on uterine wet weight, uterine glycogen content and uterine histology in bilaterally ovariectomized albino rats.

Group	Treatment (route)	Dose (mg/kg)	Uterine wet weight (mg)	Uterine glycogen content (µg/mg of uterine tissue)	Uterine histology
1	Control distilled water (p.o)	10	97.50± 0.4601	0.3056± 0.004	The uterine endometrium was disintegrated.
2	Standard DES (p.o)	2	221.8± 0.6700 [x]	0.9201± 0.005 [a]	Height of luminal epithelium was increased and number of glands increased.
3	*CRINUM LATIFOLIUM* extract (p.o)	100	97.93± 0.3881 [ns]	0.3950± 0.0117 [ns]	
4		200	104.53± 0.1315 [xx]	0.4163± 0.018 [b]	
5		400	110.23± 0.111 [xx]	0.4926± 0.020 [a]	
ANOVA			F=180.80 D.F:(4, 15) p<0.001 b/w grps.	F=72.02 D.F:(4, 15) p<0.001 b/w grps.	

[x]p<0.01 compared to control group
[xx]p<0.05
D.F: 15

[a]p<0.01 compared to control group
[b]p<0.05 compared to control group
D.F: 15

Values are mean ± S.E.M of 4 animals in each group. Data were analysed by one-way ANOVA followed by Dunnet's 't' test.

Table 7: Effect of alcoholic extract of *CRINUM LATIFOLIUM*on vaginal cornification inbilaterally ovariectomized albino rats.

Group	Treatment (route)	Dose (mg/kg)	VAGINAL CORNIFICATION (%)							
			Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
1	Control, distilled water (p.o)	10	0	0	0	0	0.5±0.28	0	0	0.5±0.5
2	Standard, DES (p.o)	2	0	0	0	86.5± 0.28	88.5± 0.28	93± 0.40	97.5± 0.28	98.5± 0.81
3	*CRINUM LATIFOLIUM* extract (p.o)	100	0	0	0	2.5± 0.28	3.5± 0.28	4± 0.40	5. 5± 0.28	6.25± 2.52
4		200	0	0	0	52.25± 0.85	67± 0.40	76.5± 0.65	86.5± 0.64	97.1± 0.70
5		400	0	0	0	65± 0.28	74.5± 0.28	83± 0.40	94.5± 0.28	98.5± 0.28

Table 8: Effect of alcoholic extract of *CRINUM LATIFOLIUM*on vaginal opening inbilaterally ovariectomized albino rats.

Group	Treatment (route)	Dose (mg/kg)	VAGINAL OPENING (%)							
			Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
1	Control Distilled water (p.o)	10	0	0	0	0	0	0	0	0
2	Standard, DES (p.o)	2	0	0	0	60	100	90	80	70
3	*CRINUM LATIFOLIUM*	100	0	0	0	5	5	8	9	5
4		200	0	0	0	55	70	75	80	85

5	extract (p.o)	400	0	0	5	50	70	76	79	84

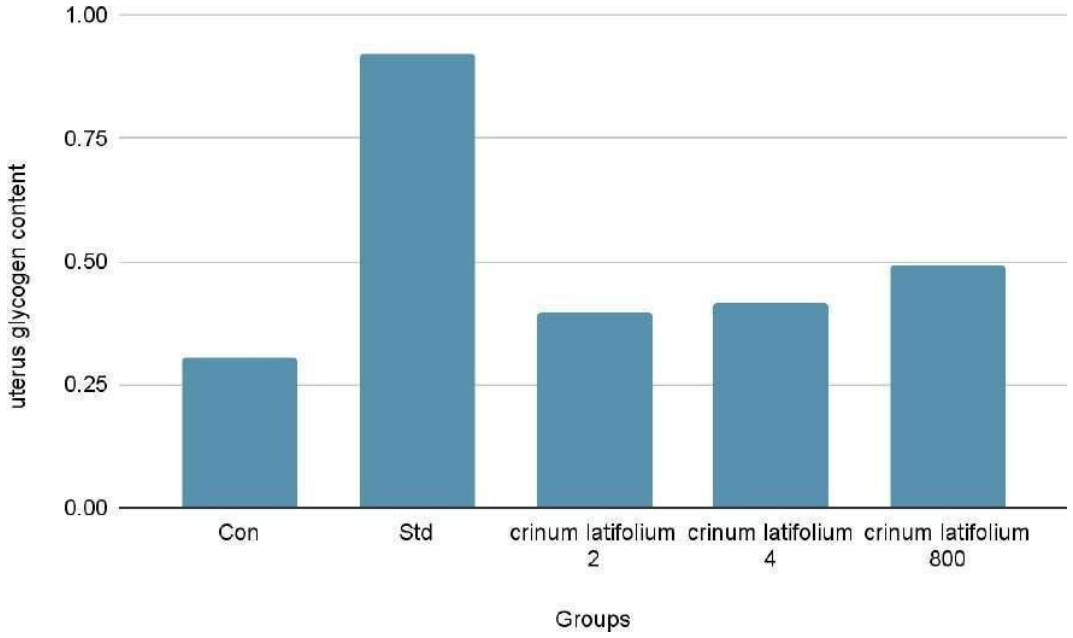

Fig. 6: Impact of CRINUM LATIFOLIUM alcoholic extract on uterine wet weight

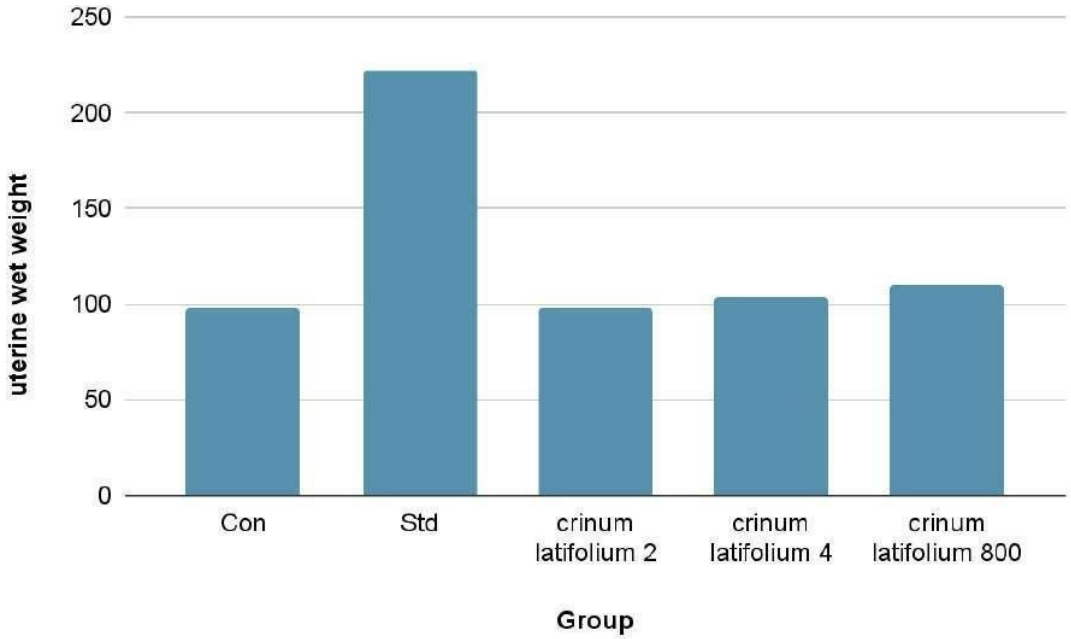

Fig. 7 Impact of CRINUM LATIFOLIUM alcoholic extract on utreous glycogen content.

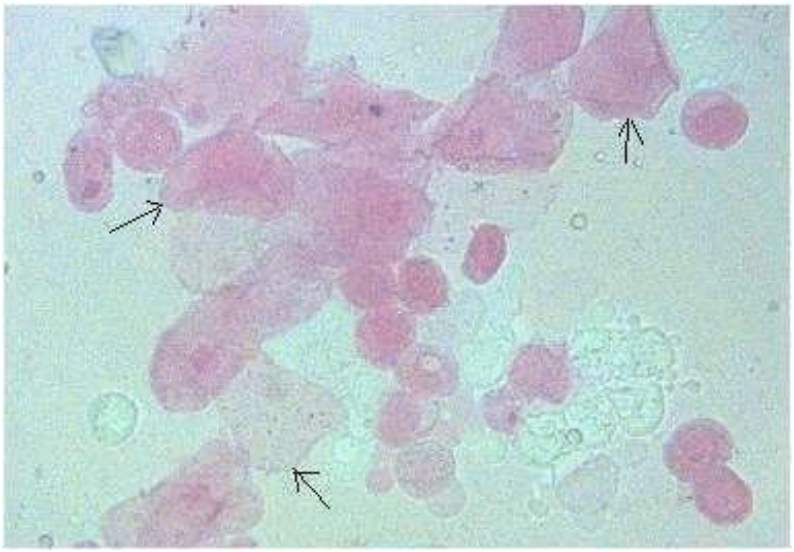

Fig.8:Photomicrograph (x100) of a vaginal smear of a rat treated withdiethylstilbestrol (2 mg/kg, p.o.), stained with methylene blue and eosin; onlycornified epithelial cells (i.e., in estrous stage).

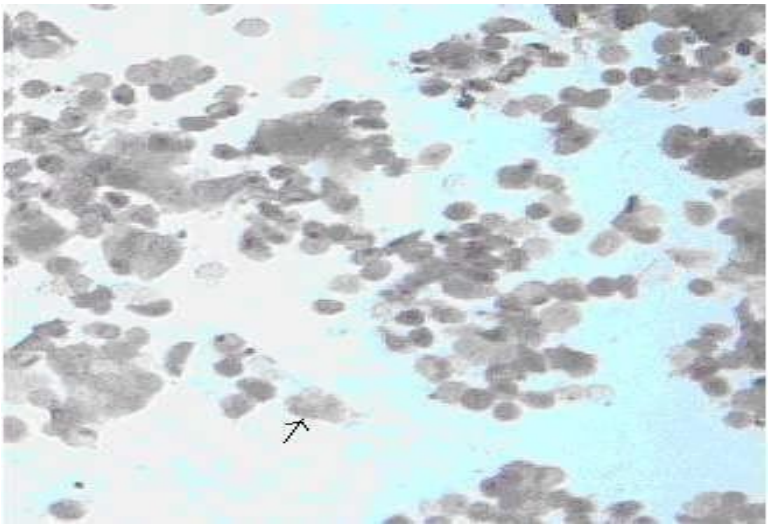

Fig. 9: Photomicrograph (x100) of vaginal smear stained with methylene blue andeosin from rats treated with CRINUM LATIFOLIUM extract (100 mg/kg, p.o.),revealing a small number of cornified epithelial cells (i.e., between estrous anddiestrous stage).

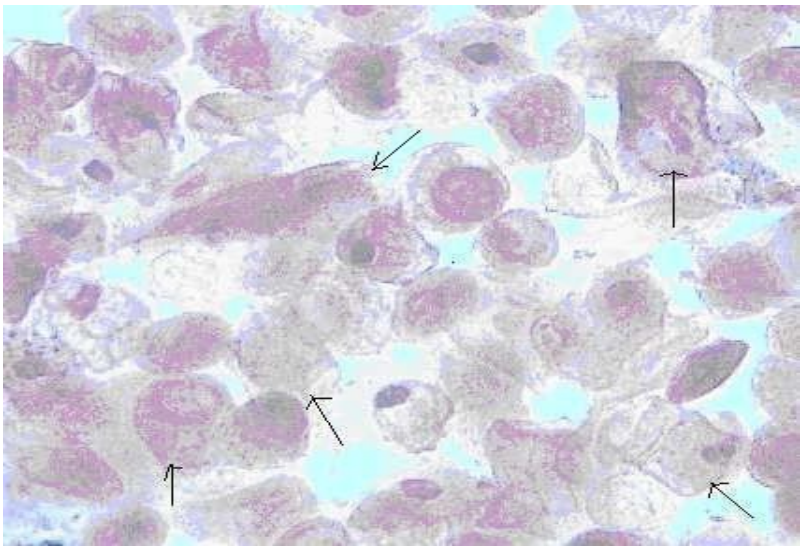

Fig.10: A photomicrograph (x100) of a vaginal smear of a rat treated with CRINUMLATIFOLIUM extract (200 mg/kg, p.o.) that only displays cornified epithelial cells (i.e.,in the estrous stage)

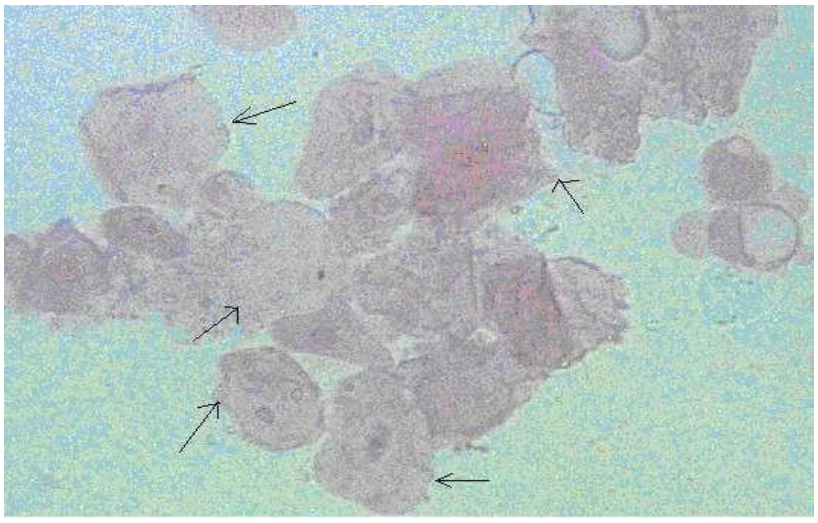

Fig. 11: A photomicrograph (x100) of a vaginal smear of a rat treated with 400 mg/kg ofCRINUM LATIFOLIUM extract (p.o.) that only displays cornified epithelial cells (i.e.,in the estrous stage).

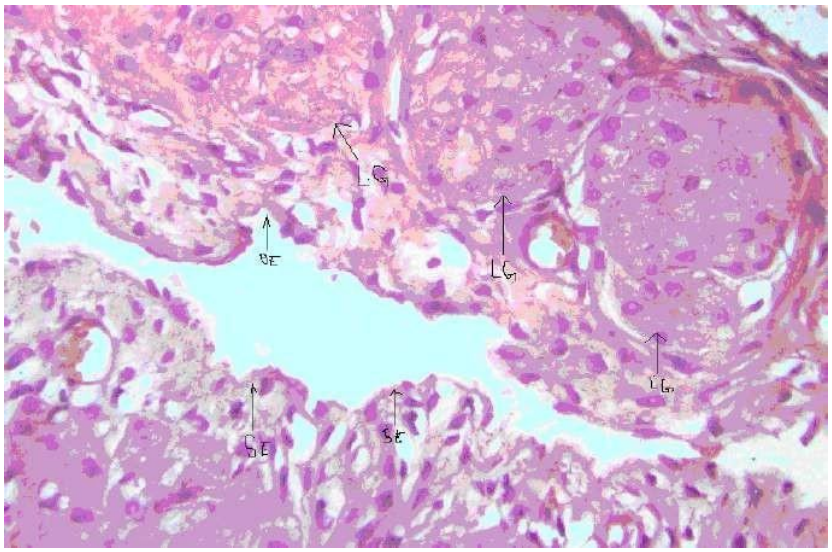

Fig. 12: A photomicrograph (x100) of the transverse section of the control rat's uterusstained with hematoxylin and eosin, demonstrating the endometrium'sdisintegration.

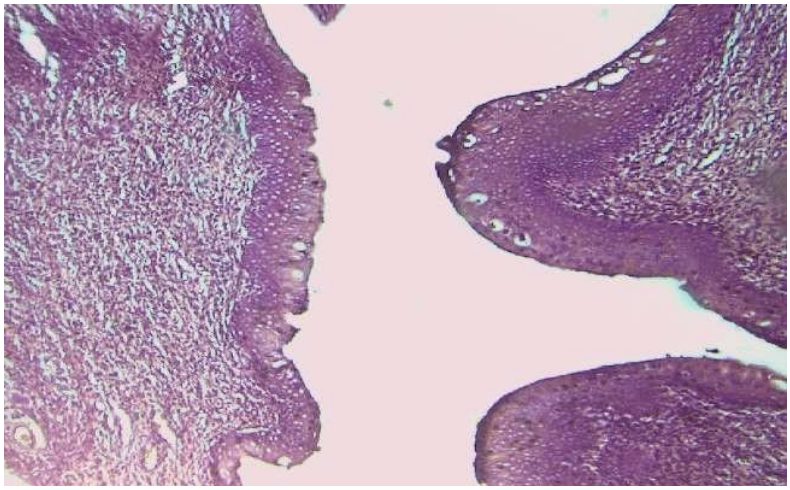

Fig. 13: A photomicrograph (x100) of the transverse section of the uterus of a rattreated with diethylstilbestrol (2 mg/kg, p.o.) stained with hematoxylin and eosindemonstrates the proliferative stage, which is characterized by a stimulatedendometrium with loose stroma and glands.

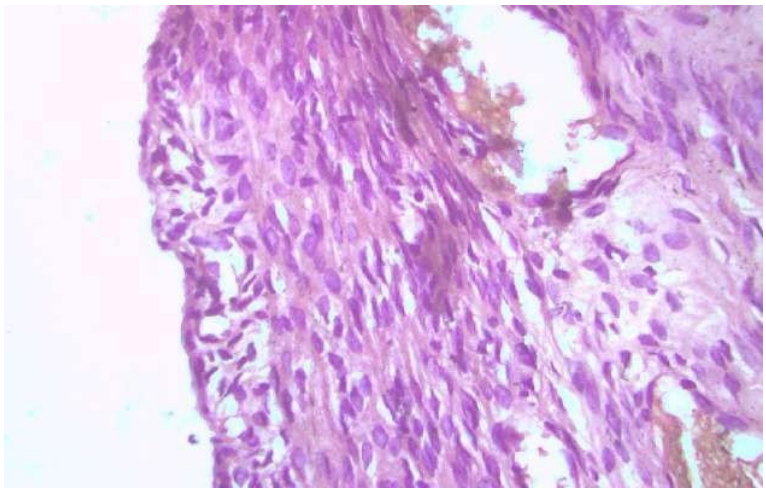

Fig. 14: A photomicrograph (x100) of the transverse section of the uterus of a rattreated with CRINUM LATIFOLIUM extract (100 mg/kg, p.o.) demonstrating theabsence of a proliferative stage, or unstimulated endometrium with no loose stroma.

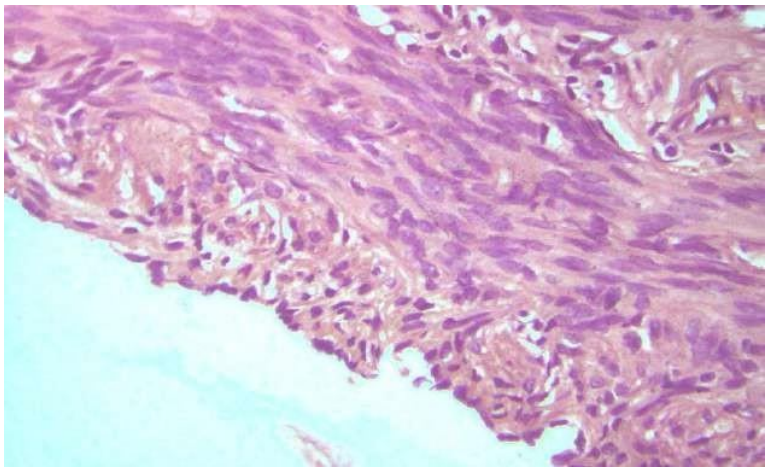

Fig. 15: A photomicrograph (x100) of the transverse section of the uterus of a rattreated with CRINUM LATIFOLIUM extract (200 mg/kg, p.o.) that shows theproliferative stage (i.e., stimulated endometrium with loose stroma)

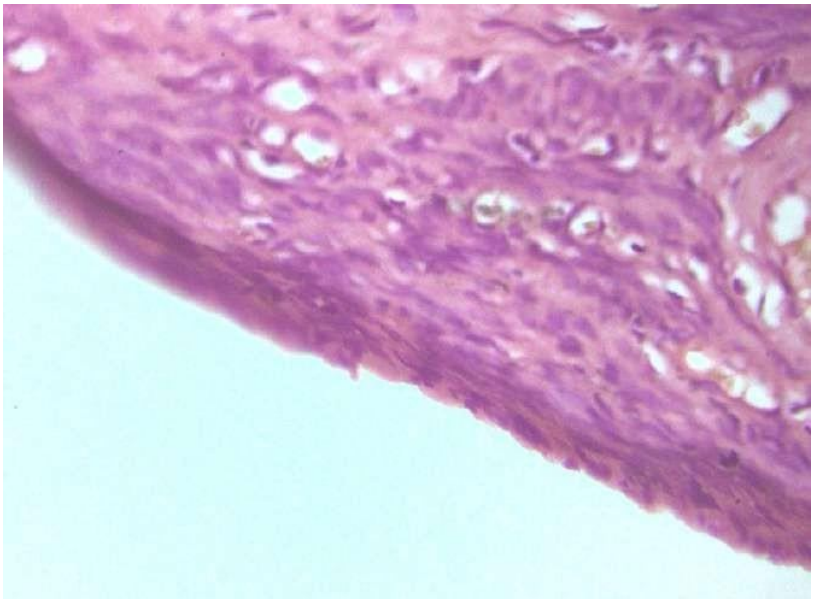

Figure 16: A photomicrograph (x100) of the transverse section of the uterus of a rat treated with 400 mg/kg of CRINUM LATIFOLIUM extract (p.o.) demonstrating the

Fig.17: Vaginal Opening in the albino rat

CHAPTER- 8

DISCUSSION

By assessing percentages of vaginal cornification and opening, uterine wetweight, uterineglycogencontent, and uterine h istology, the currentstudy aimed to assess the estrogenic activity of an alcoholic extract derived from Crinum Latifolium leaves in bilaterally ovariectomized albino rats. Diethylstilbestrol was used as a reference medication.

An examination of the literature on this plant revealed that it contains phenolic chemicals, phytosterols, and flavonoids.Alcohol was selected as the extraction solvent due to its solubility in phenolic compounds, phytosterols, and flavonoids. The estrogenic qualities of phenolic compounds and flavonoids are widely recognized. Crinum Latifolium is a plant that also contains solasodine, a substance with a steroid skeleton and phenolic A rings that have a strong affinity for the estrogen receptor.

A preliminary phytochemical (i.e., qualitative) analysis of the alcoholicextract of Crinum Latifolium revealed the presence of flavonoids, phytosterols, and phenolic compounds. This suggests that alcohol was used to extract the medication's main active ingredients. Up to a maximum dosage of 4000 mg/kg, no toxic symptoms or fatalities were observed in male or female albino rats participating in an oral acute toxicity investigation. The extract is therefore considered to be "practically nontoxic."

However, a number of pharmacological activities, such as drowsiness, reduced skeletal muscle activation, altered posture, piloerection, scrotal enlargement, and purgation, showed a dosage -based rise in strength when the medication was used. A 14-day investigation meant to evaluate potential health risks that can result from recurrent exposure over a brief period oftime did not any toxic effects on heart, kidney and liver.

There was a drop in serum HDL, albumin, and ALP and an increase in serum cholesterol, SGPT, creatinine, globulin, SGOT, and total protein, but statistically not significant. There was a statistically significant drop in triglycerides and blood sugar. For diabetic individuals, a drop in blood sugar and triglycerides can be considered a positive outcome.Many physiologic and biochemical changes occur in the uterus and the female reproductive system as a result of ovarian hormones like estrogen.

The administration of estrogenic substances to ovariectomized rats causes uterotrophic effects, vaginal cornification, opening of the vagina, increase in uterine glycogen content, and proliferative changes in the uterine endometrium.
It has been noted that the administration of1alcoholic extract at various doses resulted in a statistically significant rise in the weight of the uterus as well as an increase in the percentage of cornification and vaginal opening.As the dosage was increased, the uterine wet weight increased gradually and successively.

Histological analysis of the uterus of rats given extract treatment revealed an influence of estrogen, as seen by an increase in the number of glands and the height of the luminal epithelium with loose stroma.The amount of uterine glycogen rose in response to alcoholic extract administration in a dose-dependent manner. A one-fold rise in uterine glycogen content was observed at 400 mg/kg, which was in line with previous findings of "Prakash and Mathur 1979".

It has been demonstrated that estrogens improve the hexose transport into the rat uterus, which raises the uterine synthesis of glycogen. It is still unknown, though, exactly what the uterine glycogen in rats does. The increase in uterine glycogen content in ovariectomized rats may be due to the estrogenic activity of the alcoholic extract of Crinum Latifolium. The 400 mg/kg dose of Crinum Latifolium extract was found to have an effect that was roughly equivalent to half of the 2 mg/kg dose of DES. It was discovered that the 400 mg/kg dose of Crinum Latifolium extract had an approximately same impact to half of the 2 mg/kg dose of DES. Therefore, it may be concluded that the potency of the Solanum xanthocapum extract is 1/300th that of the normal medicine DES.

Moreover, it is possible that the extract's flavonoids, phytosterol, and phenolic components are what give it its estrogenic action.given that the estrogenic action of flavonoids and phenolic chemicals is well established.Previous pharmacological investigations on this plant using animal models revealed antifungal, antinociceptive, antiasthamatic, hypoglycemic, and protective properties against sexual debility, gonorrhea, and a possible antifertility effect due to the drug extract's estrogenic activity.

Finding precisely the substance that causes its estrogenic action and researching its effectiveness in treating menopausal illnesses including osteoporosis and hot flashes may lead to the development of an alternative non-hormonal treatment for a variety of postmenopausal ailments.

CHAPTER 9

CONCLUSION

The study found that in bilaterally ovariectomized albino rats, the alcoholic extract of Crinum Latifolium leaves demonstrates strong estrogenic activity. A thorough set of observations and analyses carried out throughout the study bolster this conclusion.

Since alcohol is a solvent that can effectively extract flavonoids, phytosterols, and phenolic compounds from plant material, the investigation started with a careful selection of the extraction method. Previous research showing these compounds' presence and known estrogenic qualities in Crinum Latifolium influenced this choice. The plant also contains a substance called solasodine, which has a phenolic A ring structure and a steroid skeleton and exhibits a strong affinity for estrogen receptors.

The presence of flavonoids, phytosterols, and phenolic compounds in the alcoholic extract was validated by a preliminary phytochemical analysis, indicating the effectiveness of the extraction method in isolating the active ingredients of the plant. Acute toxicity tests were also used in the study to determine the extract's safety profile. The results showed that the extract is "practically nontoxic" in albino rats of both sexes up to a max 4000 mg/kg.

Primary goal of the study was to evaluate the extract's estrogenic activity using uterine histology, uterine glycogen content, vaginal cornification and opening percentages, and uterine wet weight. All of these measures showed dose-dependent increases, which made the results very convincing.

Uterine weight, vaginal cornification percentages, and vaginal opening all increased statistically significantly when the alcoholic extract was administered at different doses. In particular, the uterine wet weight increased gradually and successively as the dosage was increased. These effects are similar to what is usually seen when estrogenic drugs are given to rats that have had their ovaries removed; they counteract the atrophy of the uterus and reproductive tract that is typically caused by low estrogen levels after ovariectomy.

Additional proof of the estrogenic influence was provided by histological examination of the uteri from rats treated with extract. Upon examination, the uterus exhibited characteristic signs of estrogen activity, including an increase in the number of glands and the height of the luminal epithelium, along with loose stroma.

Additionally, uterine glycogen content increased in a dose-dependent manner following the administration of the extract, according to the study. There was a one-fold increase in uterine glycogen at 400 mg/kg. This result is consistent with earlier studies by Prakash and Mathur (1979), who found that estrogen treatment in ovariectomized rats increased the amount of uterine glycogen by three to four times. Although the precise purpose of rat uterine glycogen is still unknown, its elevation is in line with established estrogenic effects on hexose transport and uterine glycogen synthesis.

One particularly significant finding was that the reference estrogenic medication used in the study, diethylstilbestrol (DES), had an effect roughly half that of a 400 mg/kg dose of Crinum Latifolium

extract. Based on this comparison, an approximate estimate of the extract's potency of roughly 1/300th that of DES can be made.

The study also discussed additional possible advantages of the extract from Crinum Latifolium. Repeated exposure did not have any harmful effects on the liver, kidney, or heart, according to a 14-day investigation. Serum biochemical parameter changes were noted, however the majority were not statistically significant. Nonetheless, the study did find statistically significant drops in blood sugar and triglyceride levels, which may be especially helpful for those with diabetes.

These results confirm the estrogenic activity of Crinum Latifolium and corroborate and supplement earlier pharmacological studies of this plant. Previous research has shown that it has hypoglycemic, antifungal, antinociceptive, antiasthmatic, and protective qualities against gonorrhea and sexual debility. The current study contributes to this body of knowledge by offering an explanation for the plant's estrogenic activity, which may account for some of these effects.

This study's conclusion provides a number of directions for further investigation. Finding the precise substances causing the estrogenic action may help create novel plant-based remedies for a range of ailments. The potential use in treating menopausal symptoms like osteoporosis and hot flashes is especially intriguing. For those women who are unable or unwilling to use conventional hormone replacement therapy, the development of non-hormonal alternative treatments for postmenopausal disorders may offer important alternatives.

This study clarifies the safety profile, offers compelling evidence of the estrogenic activity of Crinum Latifolium extract, and highlights potential therapeutic uses. Future research can build on the thorough approach of this study, which looked at several aspects of estrogenic activity and compared the extract's potency to a known estrogenic compound. In the ongoing quest for secure and efficacious substitutes for traditional hormone treatments, Crinum Latifolium shows promise and merits additional investigation in preclinical and clinical contexts.

CHAPTER-10

SUMMARY

The milk and wine lily, or *Crinum latifolium*, is a perennial herb belonging to the Amaryllidaceae family. This plant is well-known for its therapeutic qualities and is frequently utilized in Asian traditional medicine.

It is mentioned in the literature to have antioxidant, anti-inflammatory, and anti-cancer effects. In the current investigation, phytochemical, pharmacological, and subacute toxicity analyses are conducted using an ethanolic extract of *Crinum Latifolium*leaves. Following an initial phytochemical screening, flavonoids, triterpenoids, phenolic compounds, phytosterol, and tannins were identified in the Crinum Latifolium extracts.

The ethanolic extract of *Crinum Latifolium*fruits was found to be safe in an acute toxicity study carried out in accordance with OECD guidelines, with a maximum dose of 2000 mg/kg body weights in rats.

Rats treated with *Crinum Latifolium*had slightly higher serum levels of total protein, serum globulin, SGPT, cholesterol, and serum creatinine, according to a biochemical analysis of their serum, than the control group. However, statistical significance was not demonstrated by these modifications. The levels of albumin, HDL, alkaline phosphatase, LDH, and other substances slightly increased. Again, there was no statistical significance to these changes. there was a statistically significant drop in serum triglycerides and blood glucose when compared to the control group.

A wide variety of steroidal and non-steroidal substances have estrogenic properties. 17-β-estradiol, an 18-carbon steroid, is the strongest estrogen found in humans naturally. Many treatments for postmenopausal osteoporosis, fertility management, and menstrual cycle regulation involve the use of estrogens and some of their derivatives.
Numerous plants naturally contain nonsteroidal compounds with estrogenic activity, such as steroidal derivatives, isoflavones, and flavones. Eating these plants may have estrogenic effects, according to a study.

In albino rats that had undergone bilateral ovariectomies, the ethanolic extract of Crinum Latifolium was evaluated for its estrogenic activity using the following parameters: percentage vaginal cornification, percentage vaginal opening, uterine wet weight, uterine glycogen content, and uterine histology. The findings demonstrated the estrogenic activity of the extracts at doses of 200 and 400 mg/kg body weight by showing significant ($P<0.05$ & $P<0.01$) results for a number of parameters, including uterine histology, uterine glycogen content, uterine wet weight, and % vaginal cornification. However, the dosage of 100 mg/kg of Crinum Latifolium was shown to be statistically insignificant in the parameters listed above.

Ethanol extracts of *Crinum Latifolium*fruits were subjected to subacute toxicity tests, which demonstrated that the fruits exhibited no toxic effects even at the highest dose of 2000 mg/kg.

CHAPTER-11

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CERTIFICATE

Certified that **Omer ALAM** (**enrollment** no.220227098050333) has carried out the research work presented in this thesis entitled **"Investigation for Estrogenic Activity of *CRINUM LATIFOLIUM* in female Albino rats"**for the award of **Master of Pharmacy** from Dr. APJ Abdul Kalam Technical University, Lucknow under my supervision. The thesis embodies results of original work, and studies are carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this University or any other university/Institution.

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