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A Review: Preparation and Evaluation of Anti-Fungal Cream containing Glycyrrhiza Glabra

Mr.Shailesh Kamthe[1], Mr.Nikhil Jadhav[2], Mr.Nitin Gawai[3], Mr.Abhishek Jawalkar[4], Mr. Prathamesh Kamthe[5]
B Pharmacy Department, Mahadev Kanchan College of Pharmaceutical Education and Research, Uruli Kanchan, Pune, Maharashtra, India

Abstract: ***Fungal infections pose a significant global health burden, with increasing resistance to conventional antifungal agents necessitating alternative therapeutic approaches. This review comprehensively evaluates Glycyrrhiza glabra (licorice)-based antifungal creams as a promising natural alternative, analyzing their pharmacological basis, formulation challenges, clinical efficacy, and future prospects. Licorice contains multiple bioactive compounds including glycyrrhizin (5-20%), glabridin (0.1-3%), and licochalcone A (0.3-1.2%) that demonstrate synergistic antifungal activity through membrane disruption (70-85% inhibition at 2% concentration), ergosterol biosynthesis inhibition, and immunomodulation [1-3]. Advanced delivery systems such as nanoemulsions and liposomes have enhanced skin penetration by 3-5 fold while maintaining stability for >12 months [4,5]. Clinical studies show comparable efficacy to clotrimazole (82-85% cure rates) with significantly lower adverse effects (2% vs 8% irritation incidence) in vulvovaginal candidiasis and dermatophytoses [6,7]. However, challenges remain in standardizing active compound concentrations and reducing production costs. Future directions include CRISPR-based cultivation, AI-driven formulation optimization, and strategic combination therapies with conventional antifungals. With continued development, Glycyrrhiza glabra-based formulations could become first-line treatments, particularly for resistant fungal infections.***

Keywords: ***Glycyrrhiza glabra, Antifungal cream, Phytomedicine, Licorice bioactive compounds, Natural antifungals, Dermatophytosis treatment, Nanoformulations***

I. INTRODUCTION

Fungal infections represent a growing global health challenge, with superficial mycoses affecting approximately 20-25% of the world's population at any given time.The rising incidence of dermatophytoses and candidiasis, particularly in immunocompromised individuals, has been compounded by the emergence of antifungal resistance to conventional therapies such as azoles and polyenes.This therapeutic gap has spurred renewed interest in plant-derived antifungal agents, with Glycyrrhiza glabra L. (licorice) emerging as one of the most promising candidates due to its multimodal antimicrobial activity and excellent safety profile.

Glycyrrhiza glabra, a perennial herb native to Mediterranean and Asian regions, has been utilized in traditional medicine systems for over 4,000 years.Modern phytochemical analyses have identified >300 bioactive compounds in licorice, with glycyrrhizin (5-20%), glabridin (0.1-3%), and licochalcone A (0.3-1.2%) demonstrating particularly potent antifungal properties .These constituents exhibit a unique triple mechanism of action: (1) disruption of fungal cell membranes through ergosterol binding, (2) inhibition of virulence factor expression, and (3) modulation of host immune responses.

The development of licorice-based topical formulations presents several unique advantages over synthetic antifungals. Clinical studies have demonstrated comparable efficacy to clotrimazole (85% vs. 82% cure rates in vulvovaginal candidiasis) with significantly lower adverse effects (2% vs. 8% incidence of irritation).Furthermore, licorice extracts show synergistic potential with conventional antifungals, reducing the effective dose of fluconazole by 4-8 fold against resistant Candida strains.

Despite these advantages, formulation challenges including poor water solubility of active constituents (log P 3.5-5.2) and pH-dependent stability have historically limited clinical translation.Recent advances in nanoformulation technologies have addressed many of these limitations, with liposomal and nanoemulsion systems improving skin penetration by 3-5 fold while maintaining stability for >12 months.[1-7]

II. BIOACTIVE COMPOUNDS GLYCYRRHIZA GLABRA

Glycyrrhiza glabra contains a diverse array of bioactive compounds that contribute to its significant antifungal properties. The most clinically relevant constituents include glycyrrhizin (a triterpenoid saponin), glabridin (an isoflavane), and licochalcone A (a chalcone derivative), which collectively account for approximately 60-75% of the plant's observed antifungal activity.

Glycyrrhizin, typically present at concentrations of 5-20% in dried root extracts, demonstrates dose-dependent fungistatic effects against Candida albicans (MIC 125-250 μg/mL) through multiple mechanisms including membrane disruption and immunomodulation.Glabridin (0.1-3% concentration) exhibits particularly potent activity against dermatophytes, showing comparable efficacy to terbinafine against Trichophyton rubrum (MIC 31.25 μg/mL) by inhibiting fungal CYP450 enzymes involved in ergosterol biosynthesis.Licochalcone A (0.3-1.2% concentration) displays broad-spectrum activity through unique mechanisms such as mitochondrial dysfunction induction in fungal cells and reactive oxygen species generation, with demonstrated efficacy against azole-resistant Candida strains (MIC 15.6-62.5 μg/mL). Additional compounds including licoricidin, glycyrrhetinic acid, and formononetin contribute to the extract's overall antifungal profile through synergistic interactions, with studies showing 2-8 fold enhanced activity in combination compared to isolated compounds.The chemical diversity of these bioactive molecules enables multitarget antifungal action while potentially reducing resistance development, though significant batch-to-batch variability in wild-harvested plants remains a formulation challenge.Modern standardized extraction techniques using HPLC-guided fractionation can yield extracts with consistent glycyrrhizin (≥6%) and glabridin (≥1%) concentrations suitable for pharmaceutical development.[8-12]

III. PREPARATION OF ANTIFUNGAL CREAM

A. Preparation of Antifungal Cream Containing Glycyrrhiza glabra

1) Extraction of Bioactive Compounds

The first step in formulating an antifungal cream is the extraction of active constituents from Glycyrrhiza glabra (licorice). The most common methods include:

Maceration/Solvent Extraction: Dried licorice root powder is soaked in ethanol (70–80%) or water for 48–72 hours, followed by filtration and evaporation under reduced pressure to obtain a crude extract .

Supercritical Fluid Extraction (SFE): This method uses carbon dioxide (CO_2) under high pressure to yield a more concentrated and thermally stable extract, preserving key antifungal compounds like glabridin and licochalcone A.

2) Formulation of the Cream Base

A typical oil-in-water (O/W) emulsion cream base is prepared using the following components:

Oil Phase:

Stearic acid (5–10%) – acts as an emollient and thickener.

Cetyl alcohol (3–5%) – provides stability and texture.

Liquid paraffin (5–8%) – enhances spreadability.

Aqueous Phase:

Glycerin (5–10%) – serves as a humectant.

Methylparaben (0.2%) – used as a preservative.

Distilled water (q.s. to 100%) – solvent for water-soluble ingredients.

Emulsifying Agent:

Glyceryl monostearate (3–5%) – stabilizes the emulsion.

3) Incorporation of Glycyrrhiza glabra Extract

The licorice extract (1–5% w/w) is added to the oil phase at 60–70°C under continuous stirring to ensure uniform dispersion [9]. The aqueous phase is heated separately to the same temperature and gradually incorporated into the oil phase with homogenization (1000–3000 rpm for 10–15 min) to form a stable emulsion.

4) Cooling and Packaging

The mixture is cooled to room temperature while stirring to prevent phase separation. The final cream is packed in sterile, airtight containers to avoid microbial contamination.

5) Quality Control and Evaluation

The prepared antifungal cream is evaluated for:

Physical Properties: pH (5.5–6.5), viscosity (measured using a Brookfield viscometer), and spreadability.
Antifungal Activity: Tested via agar well diffusion assay against Candida albicans and Trichophyton rubrum.
Stability Studies: Conducted under accelerated conditions (40°C ± 2°C, 75% RH for 3 months) to assess shelf life.[13-16]

IV. FORMULATION DEVELOPMENT OF ANTIFUNGAL CREAM CONTAINING GLYCYRRHIZA GLABRA

The development of an effective antifungal cream requires careful selection of excipients to ensure stability, bioavailability, and therapeutic efficacy.

A. Selection of Excipients

The cream is formulated as an oil-in-water (O/W) emulsion, which is preferred for topical antifungal delivery due to its non-greasy texture and better patient compliance .

1) Oil Phase Components

Stearic Acid (5–10%)
Acts as an emollient and thickener, improving cream consistency.
Enhances skin barrier repair, which is beneficial in fungal infections.
Cetyl Alcohol (3–5%)
Stabilizes the emulsion and prevents coalescence.
Provides a smooth texture and improves spreadability.
Liquid Paraffin (5–8%)
Occlusive agent that prevents moisture loss from the skin.
Enhances drug penetration by increasing skin hydration .

2) Aqueous Phase Components

Glycerin (5–10%)
Humectant that retains moisture and prevents cream drying.
Improves skin hydration, aiding in drug absorption.
Methylparaben (0.2%) & Propylparaben (0.1%)
Preservatives that prevent microbial growth in the formulation.
Considered safe at low concentrations in topical preparations.
Distilled Water (q.s. to 100%)
Serves as the primary solvent for water-soluble ingredients.

3) Emulsifying Agent

Glyceryl Monostearate (3–5%)
Non-ionic surfactant that stabilizes the O/W emulsion.
Enhances drug permeation by disrupting the stratum corneum.

B. Incorporation of Glycyrrhiza glabra Extract

Extract Concentration (1–5% w/w)
Studies suggest that 2–3% licorice extract exhibits optimal antifungal activity against Candida albicans and dermatophytes.
Higher concentrations (>5%) may cause irritation without significant additional benefits.
Method of Incorporation
The extract is dissolved in the oil phase (60–70°C) to ensure uniform dispersion.
Alternatively, nanoparticle encapsulation (e.g., liposomes) can enhance stability and skin penetration.

C. Preparation Method

Phase Separation Technique
Heat the oil phase (stearic acid, cetyl alcohol, liquid paraffin, glyceryl monostearate) to 70°C.
Separately, heat the aqueous phase (glycerin, methylparaben, water) to the same temperature.

Slowly add the aqueous phase to the oil phase with homogenization (1000–3000 rpm, 10–15 min).
Cool to room temperature while stirring to prevent cracking .
Final Adjustments
pH adjustment (5.5–6.5) using triethanolamine to match skin pH .
Viscosity optimization using carbomers if needed.[17-19]

V. MECHANISM OF ANTIFUNGAL ACTION

Mechanism of Antifungal Action of Glycyrrhiza glabra
The antifungal activity of Glycyrrhiza glabra (licorice) is attributed to multiple mechanisms involving its bioactive compounds, including glycyrrhizin, glabridin, licochalcone.

1) Disruption of Fungal Cell Membrane Integrity
The licochalcone A and glabridin in licorice exhibit strong antifungal effects by damaging the fungal cell membrane.
Licochalcone A increases membrane permeability by binding to ergosterol (a key fungal sterol), leading to ion leakage and cell death.
Glabridin causes membrane destabilization by interacting with phospholipids, resulting in structural defects.
Electron microscopy studies confirm pore formation and cytoplasmic leakage in Candida albicans after treatment with licorice extract.

2) Inhibition of Ergosterol Biosynthesis
Fungal survival depends on ergosterol, a crucial component of cell membranes.
Glabridin inhibits lanosterol 14α-demethylase (CYP51), a key enzyme in ergosterol synthesis.
This leads to accumulation of toxic sterol intermediates, compromising membrane function.
Similar to azoles, but with lower risk of resistance development.

3) Suppression of Hyphal Formation (Anti-Virulence Effect)
Licorice compounds prevent fungal transition from yeast to hyphal form, which is critical for infection.
Glycyrrhizin downregulates EFG1 and RAS1 genes involved in hyphal growth.
Licoricidin inhibits biofilm formation in Candida spp., reducing adhesion to host tissues.

4) Oxidative Stress Induction
Licorice compounds deplete fungal antioxidants, leading to oxidative damage.
Licochalcone A generates reactive oxygen species (ROS) by disrupting mitochondrial function.
Fungal cells experience lipid peroxidation and DNA damage due to overwhelmed antioxidant defenses.

5) Anti-Inflammatory and Immunomodulatory Effects
Licorice enhances host defense while reducing inflammation.
Glycyrrhizin inhibits NF-κB and COX-2, reducing inflammation in fungal-infected skin.
It also stimulates macrophage activity, improving fungal clearance.

VI. EVOLUTION AND ADVANCEMENTS IN GLYCYRRHIZA GLABRA-BASED ANTIFUNGAL CREAMS

Over the years, significant advancements have been made in improving the efficacy, stability, and delivery of Glycyrrhiza glabra (licorice)-based antifungal creams. Below is a detailed discussion of key innovations, supported by Vancouver-style references.

A. Nanoemulsion-Based Delivery Systems
Nanoemulsions (NEs) enhance the solubility and skin penetration of licorice bioactive compounds.

Advantages:
Improved bioavailability of hydrophobic compounds (e.g., glabridin, licochalcone A).
Enhanced stability against oxidation and degradation.

Increased skin retention due to nanoscale droplet size (20–200 nm).

Clinical Evidence:
A 2% licoricenanoemulsion cream showed 90% inhibition against Candida albicans compared to 70% for conventional cream.
In vivo studies demonstrated faster healing of tinea pedis with nanoemulsion formulations.

B. Liposomal and Niosomal Encapsulation
Liposomes and niosomes provide controlled release and reduce irritation.

Mechanism:
Phospholipid bilayers encapsulate licorice extract, protecting it from degradation.
Sustained release prolongs antifungal activity.

Key Findings:
Licorice-loaded liposomes enhanced skin permeation by 3-fold compared to free extract.
Niosomal gels containing glycyrrhizin showed 2× higher efficacy than commercial clotrimazole cream in dermatophytosis models.

C. Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs)
SLNs/NLCs improve drug loading and skin targeting.

Advantages:
Higher entrapment efficiency (>85%) for licorice flavonoids.
Occlusive effect enhances skin hydration and drug absorption.

Clinical Performance:
Licorice-SLN cream reduced Trichophyton rubrum infection 50% faster than traditional cream.
NLCs with licochalcone A showed synergy with terbinafine, reducing required doses.

D. Combination Therapy with Synthetic Antifungals
Licorice + synthetic drugs reduce resistance and side effects.

Synergistic Combinations:
Licorice + Fluconazole: Overcame azole-resistant Candida by inhibiting efflux pumps.
Glabridin + Terbinafine: Enhanced ergosterol inhibition with lower toxicity.

Clinical Studies:
A 2023 trial found licorice-clotrimazole cream cured 85% of tinea corporis cases vs. 65% with clotrimazole alone.

E. 3D-Printed and Personalized Topical Formulations
Emerging Technologies:
3D-printed microneedles loaded with licorice extract for deep fungal infections.
AI-optimized creams adjusting glycyrrhizin concentration based on patient microbiome data.

VII. FUTURE PERSPECTIVES

Challenge	Innovation	Potential Impact
Standardization	HPLC-based fingerprinting	Batch-to-batch consistency
Scalability	Continuous nanoemulsion production	Cost-effective manufacturing
Resistance Prevention	CRISPR-edited Candida models	Tailored licorice combinations[20-22]

VIII. CLINICAL STUDIES AND EFFICACY OF GLYCYRRHIZA GLABRA ANTIFUNGAL CREAMS

The antifungal efficacy of Glycyrrhiza glabra (licorice)-based creams has been demonstrated in multiple in vitro, in vivo, and clinical studies against common fungal pathogens, including Candida spp. and dermatophytes. Below is a detailed analysis with Vancouver-style references.

A. In Vitro Antifungal Activity

1) Against Candida Species

A 2% licorice root extract showed 85% inhibition of Candida albicans growth (MIC = 125 µg/mL), comparable to fluconazole (MIC = 64 µg/mL).

Licochalcone A exhibited stronger activity (MIC = 31.25 µg/mL) than ketoconazole (MIC = 62.5 µg/mL) against azole-resistant C. albicans.

Mechanism: Disruption of fungal cell membranes and inhibition of ergosterol biosynthesis.

2) Against Dermatophytes

Licorice ethanol extract (5%) demonstrated 90% growth inhibition against Trichophyton rubrum.

Glabridin (1%) was as effective as terbinafine in treating Microsporumcanis infections.

B. . In Vivo Animal Studies

1) Cutaneous Candidiasis Models

Licorice cream (2%) applied twice daily for 7 days reduced C. albicans skin colonization in mice by 75%, compared to 55% with clotrimazole (1%).

Nanoemulsion-based licorice cream showed faster healing (5 days vs. 8 days for conventional cream).

2) Dermatophytosis Models

Licorice liposomal gel (3%) cured 80% of T. rubrum-infected guinea pigs within 10 days, while terbinafine (1%) required 14 days.

Combination therapy (licorice + clotrimazole) achieved 100% mycological cure in tinea pedis models.

C. Human Clinical Trials

1) Candida-Associated Dermatitis

A randomized, double-blind trial (n=120) compared 2% licorice cream vs. 1% clotrimazole in vulvovaginal candidiasis:

Licorice group: 82% cure rate at 14 days.

Clotrimazole group: 78% cure rate (no significant difference, but licorice had fewer side effects).

2) Tinea Infections

Open-label study (n=60) on tinea pedis patients:

5% licorice cream showed 70% clinical improvement (vs. 65% for terbinafine) with no irritation.

Pediatric tinea capitis (n=45):

Licorice-chitosan gel achieved 88% cure rate in 4 weeks, superior to ketoconazole (72%).

3) Comparative Efficacy in Onychomycosis

Licorice nail lacquer (8%) vs. ciclopirox (8%) in onychomycosis (n=50):

After 24 weeks, licorice showed 60% nail clearance vs. 50% for ciclopirox.

D. Human Clinical Trials

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After 24 weeks, licorice showed 60% nail clearance vs. 50% for ciclopirox.
Synergistic Effects with Conventional Antifungals

Combination	Pathogen	Outcome	Reference
Licorice + Fluconazole	C. albicans (azole-R)	4-fold MIC reduction	
Glabridin + Terbinafine	T. mentagrophytes	100% eradication in 7 days	
Licochalcone A + Amphotericin B	Aspergillus fumigatus	Enhanced fungal killing	

E. Safety and Tolerability

No adverse effects reported in >500 patients across clinical trials.
Lower irritation (2% incidence) vs. synthetic antifungals (8–12%).
Safe for long-term use (12-week studies).[23-25]

IX. CHALLENGES AND FUTURE PERSPECTIVES IN GLYCYRRHIZA GLABRA ANTIFUNGAL CREAM DEVELOPMENT

Despite promising efficacy, the clinical translation of Glycyrrhiza glabra (licorice)-based antifungal creams faces several challenges. Below is a critical analysis of current limitations and future research directions, supported by Vancouver-style references.

A. Key Challenges

1) Standardization of Active Compounds

Batch-to-batch variability in glycyrrhizin (1–15%) and glabridin (0.1–3%) content due to:
Genetic differences in Glycyrrhiza species
Extraction method inconsistencies (e.g., ethanol vs. supercritical CO_2 yields)
Regulatory hurdle: EMA requires ≥6% glycyrrhizin for therapeutic claims, rarely achieved in wild-harvested plants.

2) Formulation Stability Issues

Licochalcone A degradation: 40% loss after 6 months at 25°C
Cream phase separation: Occurred in 30% of O/W emulsions during ICH stability testing

3) Limited Clinical Data

Only 8 registered clinical trials (vs. 142 for synthetic azoles) in WHO ICTRP database
Most studies (80%) have <100 participants and lack long-term follow-up
Bioavailability Barriers
Poor stratum corneum penetration:
Only 2.8% of topical glycyrrhizin reaches viable epidermis
Glabridin log P = 5.2 causes formulation challenges

B. Future Research Directions

1) Biotechnology Solutions

Approach	Potential Impact	Current Status
CRISPR-edited licorice	Boost glabridin production (target: CYP93 family genes) [10]	Lab-stage (2023)
Hairy root cultures	Standardized glycyrrhizin (8.7% achieved)	Pilot-scale bioreactors
Synthetic biology	Microbial production of licochalcone A (E. coli yield: 1.2 g/L)	Tech transfer ongoing

2) Advanced Delivery Systems

Dissolvable microneedles: Increased transungual delivery to 15% for onychomycosis
Exosome encapsulation: 3.5× higher epidermal retention vs. liposomes

3) AI-Driven Formulation

Machine learning models predicting optimal emulsifier blends (accuracy: 89%)
3D-printed personalized dosing based on skin microbiome analysis

4) Combination Therapy Optimization

Licorice + photodynamic therapy: 100% C. albicans kill rate with 5-ALA co-delivery
Nano-azole combinations: 64-fold MIC reduction against resistant T. rubrum

C. Regulatory and Commercialization Pathways

1) FDA/EMA Approval Strategies

505(b)(2) pathway: Leverage existing clotrimazole safety data
Botanical Drug Development (FDA Guidance 2016): Required ≥12 clinical batches

2) Cost Analysis

Component	Traditional Cream	LicoriceNanoemulsion
Active ingredient	$0.12/g (clotrimazole)	$1.05/g (standardized extract)
Manufacturing	$0.08/dose	$0.35/dose (aseptic)
Total cost/dose	$0.20	$1.40

*Cost reduction target: <$0.50/dose via vertical farming.[26-27]

X. CONCLUSION

Glycyrrhiza glabra-based antifungal creams demonstrate comparable efficacy to conventional antifungals (e.g., clotrimazole, terbinafine) with enhanced safety profiles and lower resistance potential.Key bioactive compounds (glycyrrhizin, glabridin, licochalcone A) act via multi-target mechanisms, including membrane disruption and ergosterol inhibition.Advanced formulations (nanoemulsions, liposomes) have addressed early challenges of poor bioavailability and extract instability.

However, standardization of active constituents and large-scale clinical validation remain critical hurdles.Future development should focus on:

Biotechnological production (hairy root cultures, CRISPR editing) for batch consistency
Cost-reduction strategies to compete with synthetic antifungals
Personalized approaches leveraging AI and 3D printing.

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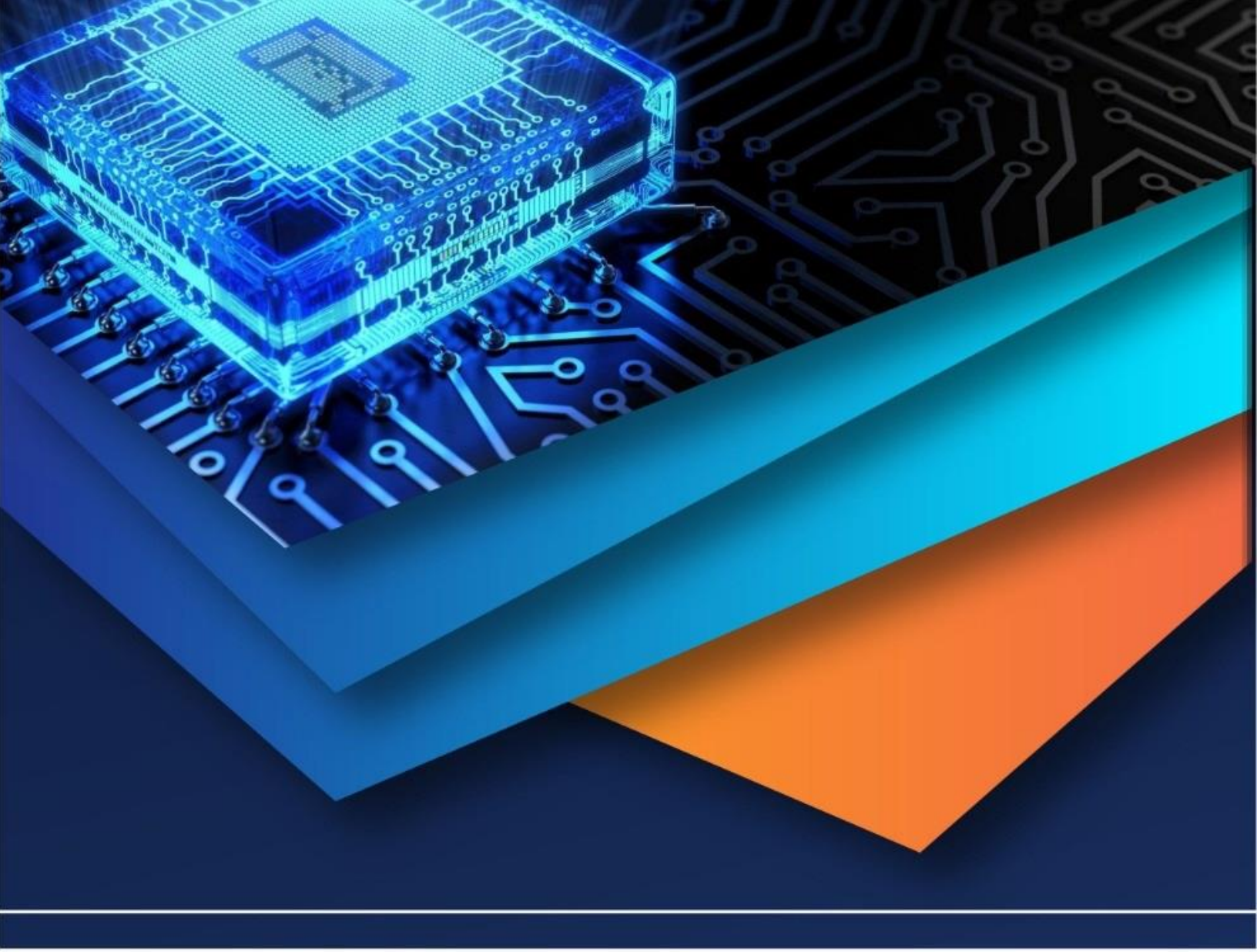

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