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Development and Evaluation of Novel Polyherbal Formulation for Rheumatoid Arthritis

Shrikrushna Unhale[1], Saloni Khandelwal[2], Quazibilal[3], Proff. Pavanfolane[4]
[1, 2, 3, 4]*Anuradha College of Pharmacy, Chikhali, Dist- Buldana (M.S) India 443201*

Abstract: ***Rheumatoid arthritis (RA) is an autoimmune disease particularly affecting elderly people which leads to massive bone destruction with consequent inflammation, pain, and debility. Allopathic medicine can provide only symptomatic relief. However, Zingiberofficinale , Turmeric, Boswellia is a plant , which has traditionally been used for treatment of RA in alternative medicines of many countries. Many of the phytochemical constituents of the rhizomes of this plant have therapeutic benefits including amelioration of RA. This review attempts to list those phytochemical constituents with their reported mechanisms of action. It is concluded that these phytochemicals can form the basis of discovery of new drugs, which not only can provide symptomatic relief but also may provide total relief from RA by stopping RA-induced bone destruction. As the development of RA is a complex process, further research should be continued towards elucidating the molecular details leading to RA and drugs that can stop or reverse these processes by phytoconstituents of ginger, turmeric, boswellia***
Keywords: ginger, essential oil, gingerol, arthritis, mice, inflammation, rats, turmeric, curcumins, boswellia

I. INTRODUCTION

Rheumatoid arthritis is an autoimmune disease characterized by chronic inflammation due to synovial hyperplasia which further progresses with massive irreversible bone destruction .Other symptoms include stiffness and loss of physical movement and systemic features including cardiovascular, pulmonary, physiological, and skeletal disorders .Recent epidemiological study shows that about 1% of people all over the world are now affected with rheumatoid arthritis, which exerts significant impact on the quality of life .In all populations, it is more prevalent among women rather than men. Generally, RA is developed (almost in 80% of cases) from the mid of the fourth decade in life to the last of the fifth .Medications and lifestyle changes are considered as treatment for RA. Current treatment provides nonsteroidal anti-inflammatory drugs (NSAIDs), for example, salicylic acid and steroids (typically cortisone injection) .Though these drugs ease the pain, they are incapable of repairing damaged tissues. Although a broad range of drugs are prescribed for managing the pain and slowing the progression of RA, no drug is known to cure the disease completely . Moreover stomach ulcer is an adverse effect observed in RA patients, regularly taking NSAIDS, and adrenal suppression by steroids . These undesirable side effects frequently force the patients to look for complementary and alternative medicine (CAM) .A recent survey indicates that people suffering from chronic pain in RA and those dissatisfied with allopathic treatment are more prone to seek alternative medicine, where 60–90% of arthritic patients use CAM .Therefore, it is highly desirable to find out a potential alternative to eradicate the drawbacks of present allopathic treatment. Natural products from plants have played a remarkable role to cure and avert different diseases from ancient times . A study conducted by World Health Organization (WHO) has reported that about 80% of world's population relies on traditional medicine . In USA, nearly 121 drugs are prescribed today, where 90 of them come from the natural sources particularly from plants in a direct or indirect manner . Herbal remedies can form an alternative source to relieve symptoms in patients having RA as well as to address the drawbacks associated with present treatment methods with allopathic drugs. Among all investigated plants , it is scientifically palpable that Zingiberofficinale Roscoe (Zingiberaceae) turmeric and bowswlli has a pivotal role to lessen the unbearable pain and inflammation associated with RA .

A. Phytochemical Constituents.

Table : I - ginger oil.

Groups	Examples
Phenolic compounds	Shogaols , Parsdols and Gingerols.
Sesquiterpens	Bisapolene , Zingiberene , Zingiberol.
Vitamins	Thiamine , Riboflavin , Niacin , Vitamin – A , C , E .
Others	Galanolactone , Zingerone.

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Table: II – Turmeric Oil.

Ingredients	Persentage
Volatile oil	5 %
Resin	5.1%
Starch	3.5%
Curcumin	3-5%
Carbohydrates	69.4%

Table: III – BOSWELLIA ACID

Ingredients	Persentage
Volatile oil	8 – 9 %
Gum	20 – 30 %
Resin	55 %

II. METHOD OF EXTRACTION AND ISOLATION

A. Gingerols

1) Dry ginger is crushed to a coarse powder and extracted with 95% ethanol from alcoholic extract
2) The solvent is evaporated by distillation to obtained thick pasty mass
3) The thick pasty mass is suspended in water
4) The ginger resin precipitates in water which is removed by filtration and be residue obtained is dried under vaccum
5) The suspended oleo resin is extracted with solvent ether and the ether extracted is evaporated to dryness at low temperature to yield total ginger

B. Curcumins

1) Turmeric powder is extracted with alcohol in soxhlet extractor .
2) The alcoholic extract is concentrated under reduced pressure and dried.
3) In another procedure turmeric powder is first extracted with hexane followed by acetone
4) The acetone extract is concentrated and dried to yield curcumin
5) The most efficient way of isolated curcumin is, to extract with hot ethanol ,concentrate the filterate ,and through the concentrate into superior grade kerosene when solid mass seprate
6) The mass is stripped of kerosene with petroleum ether and recrystalised from ethanol
7) The final product obtained is recrystalised from hot ethanol to yield orange red needles

C. Boswellic acid

1) The oleo gum resin of B. serreta is first defatted with petroleum ether 62 -80 degree clc
2) The dried marc of oleo gum resins is further exhaustively extracted with methanol .
3) The methanollic extracted is concentrated and then treated with 10% KOH to produce acid fraction and neutral fraction .
4) The acid fraction of methalloic extracted is subjected to column chromatography fractionation over silica gels using increasing amount of ethyl acetate and hexane to effort first acetyl beta boswellic acid and then acetyl 11 keto beta boswellic acid .

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III. EXPERIMENTAL PROCEDURE

A. *Animal Study*

Animal studies were performed in accordance with institutional guidelines using approved protocols. Using the identical protocol previously described for assessment of other polyherbaldrug.female Lewis rats (Harlan, Indianapolis, IN) were administered a single intraperitoneal (i.p.) injection of vehicle (normal saline) or peptidoglycan-polysaccharide polymers (25 µgrhamnose/g body weight) isolated from the sonicated cell wall of Group A *Streptococcus pyogenes* (Lee Laboratories, Grayson, GA).

At the indicated times, control and streptococcal cell wall (SCW)-treated animals received an intraperitoneal injection of botanical sample or vehicle (0.5-1 µL/g DMSO) .

Intraperitoneal treatments with the polyherbal drug extract or vehicle (1 ul/g DMSO) were administered as in our previous studies beginning 4 days prior to SCW administration and continuing daily until the beginning of the chronic phase (day 14), when treatment frequency was decreased to five days per week.

Polyherbal drug was dosed at 28 mg/kg/d in order to: 1) facilitate comparison with the *in vivo* potency of isolated polyherbal drug which were dosed at 25 mg/kg/d and to 2) approximate the polyherbal drug dose received by rats treated with the crude herbal drug extract in prior experiments .

In separate experiments, in order to compare the anti-inflammatory effects of polyherbal drug to those of estrogen, rats were treated beginning 4 days prior to SCW injection with 17-β estradiol (E_2, Sigma, St. Louis, MO) using pharmacologic doses with demonstrated anti-arthritic efficacy in other rat arthritis models that also normalize bone parameters and prevent uterine atrophy in ovariectomized rats (200 µg/kg or 600 µg/kg subcutaneously five times a week, as indicated, vs. vehicle alone [1 µl/g sesame oil]).

Joint inflammation was determined in a blinded fashion by daily assessment of arthritic index (AI) in each distal limb using standard criteria (0 = normal; 1 = slight erythema and edema; 2 = increased edema with loss of landmarks; 3 = marked edema; 4 = marked edema with ankylosis on flexion).

Bone mineral density (BMD) of the total femur was determined using a Piximus densitometer (GE Lunar, Madison, WI) at end of experiment (days 28-30) as previously described.

To monitor for possible toxic effects of treatments in normal or SCW-treated animals, daily weights were recorded, and serum creatinine and alanine aminotransferase (ALT) levels in blood samples obtained 28 days after injection of SCW (or vehicle) were determined using a Hemagen Diagnostics Endocheck Plus Chemistry Analyzer to monitor for possible renal- or hepatotoxicity, respectively .

Circulating white blood cell counts and hematocrit in whole blood were assayed using a Hemavet 880 analyzer (CDC Technologies, Oxford, CT), with manual determination of differential WBC counts

B. *Test*

1) *Gingerole Oil*: The extract or gingerol is dissolved in alcohol . The spots are applied over silica gel-G plate and eluted in solvent system ether-n hexane (7:3). The dried TLC plate is sprayed with 1 percent vanillin-sulphuric acid and the plate is heated at 110^0 C for 10 minutes .spots due to gingerols occur at Rf value value 02.
2) *Turmeric Oil:* 1 mg of curcumin is dissolved in 1 ml methanol . The spots are applied on silica gel –G plate and the plate eluted in the solvent system chloroform –ethanol-glacial acetic acid (94:5:1). The eluted plate is dried and visualized under 366 nm light .curcumin exhibits a bright yellow fluorescents spot at Rf value 0.79 the other spot appearing at Rf values 0.60 and 0.43 correspond to desmethoxycurcumin and bisdesmethoxycurcumin
3) *Boswellic Acid:* 1mg of boswellic acid is dissolved in 1 ml of methanol. the silica gel G plates spotted with the sample are eluted with chloroform –methanol (95:5).acetyl beta boswellic acid and beta boswellic acid correspond to the Rf values 0.49 and 0.45 respectively

IV. OBSERVATION

A. *In Vivo Effect Of Polyherbal Drug On Joint Inflammation*

A polyherbal drug dosing scheme (28 mg/kg/d beginning 4 days prior to SCW injection) similar to that previously used for the crude extract . Polyherbal drug fraction was employed to explore whether the excess joint protection previously documented with the crude extract in SCW-induced arthritis couldbeattributable to bioactivity of its polyherbal drug content . Acute joint swelling in SCW-injected rats was unaltered by polyherbal drug treatment .

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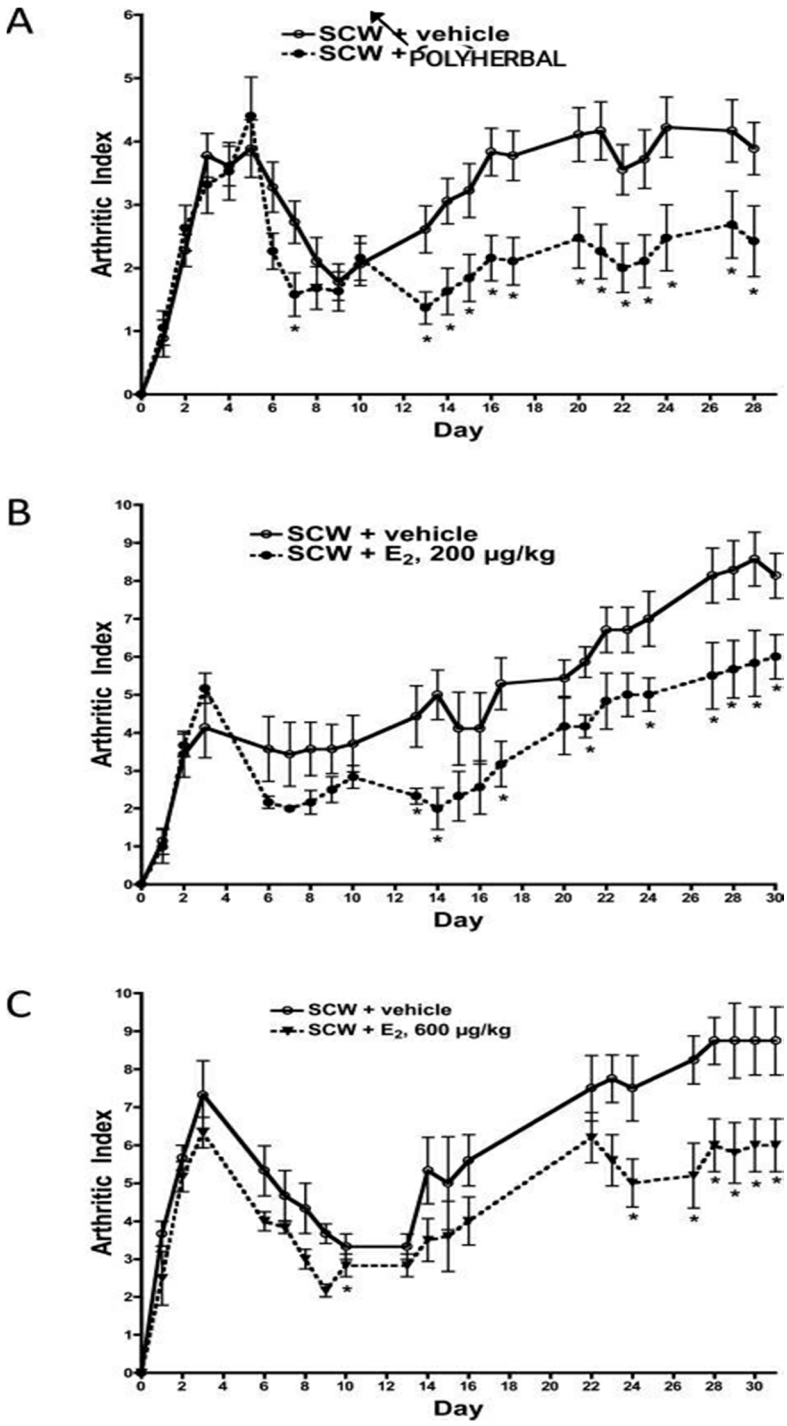

Fig 1: Effects of polyherbal drug or E_2 on joint inflammation. Female Lewis rats were injected on day 0 with SCW (or vehicle) to induce arthritis with daily polyherbal drug , E_2, or vehicle treatments starting 4 days prior to SCW injection as described in Methods and Materials. Joint swelling in limbs of SCW-injected rats was assessed at times indicated and expressed as mean arthritic index (AI, mean ± SEM)) (scale 0-4/limb for total possible score of 16) with statistical significance assessed by ANOVA with Mann Whitney analysis. *$p < 0.05$, treated vs. vehicle control. A. Polyherbal drug (28 mg/kg, n = 19) or vehicle alone (DMSO, 1 µl/g, n = 18) were dosed ip daily 5-7 times a week. B. E_2 (200 µg/kg, n = 9) or vehicle alone (sesame seed oil, µl/g, n = 9) were dosed sc daily 5 times a week. C. E_2 (600 µg/kg, n = 9) or vehicle alone (sesame seed oil, µl/g, n = 9) were dosed sc daily 5 times a week.

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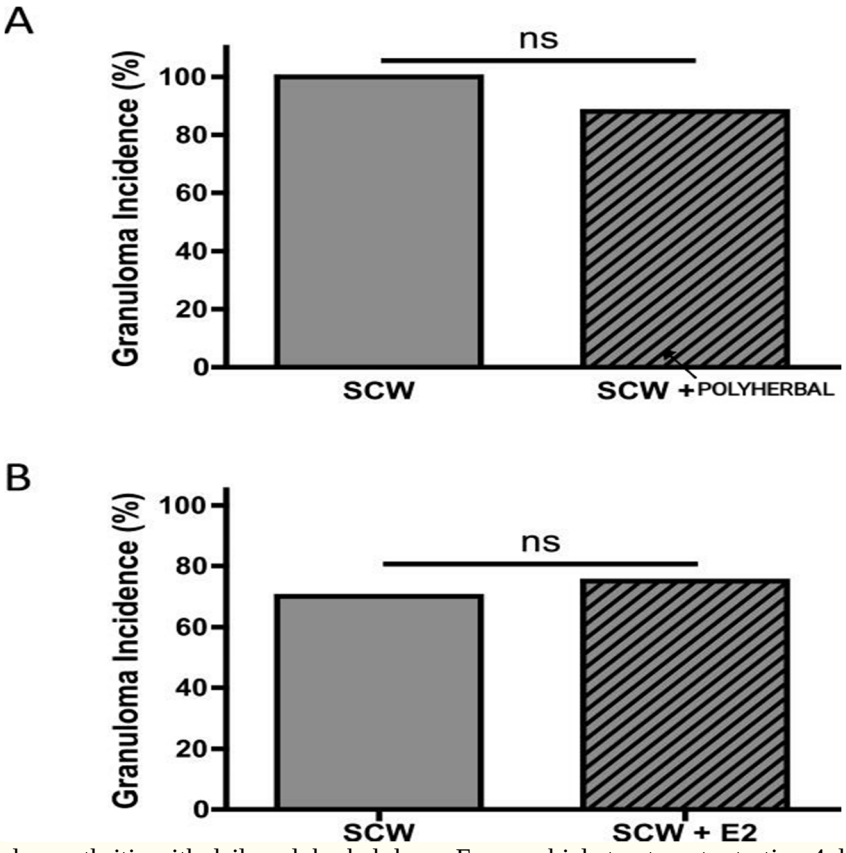
B. In Vivo Effect Of Polyherbal Drug On Hepatic Granuloma Formation

In previous experiments, the crude polyherbal drug extract decreased the incidence of granuloma formation at site of SCW deposition in the liver by 76% while polyherbal drug only extracts were without effect , suggesting that blockade of granuloma formation by the crude extract might be attributable to it's the polyherbal drug content. However, treatment of SCW-injected animals with polyherbal drug in isolation did not alter the granulomatous response; the incidence of granuloma formation in the livers of SCW-injected rats was unchanged in polyherbal drug vs. vehicle-treated animals.

Fig. 2Effects of polyherbal drug or E_2 on incidence of hepatic granuloma formation. Female Lewis rats were injected on day 0 with



SCW (or vehicle) to induce arthritis with daily polyherbal drug , E_2, or vehicle treatments starting 4 days prior to SCW injection as described in Methods and Materials. The incidence of granuloma formation on day 28-30 was assessed histologically as described in SCW-injected animals. Statistical significance was determined by Fisher's exact test. NS = non-significant. A. Polyherbal drug(28 mg/kg, n = 8) or vehicle alone (DMSO, 1 µl/g, n = 8) were dosed ip daily 5-7 times a week. B. E_2 (200 µg/kg or 600 µg/kg, n = 12) or vehicle alone (sesame seed oil, µl/g, n = 10) were dosed sc daily 5 times a week. The E_2 doses tested did not alter granuloma incidence when analyzed separately (data not shown) or in combination, as demonstrated here.

C. Tolerability of Polyherbal Drug

Mortality, which only occurred in SCW-injected animals, was not altered by polyherbal drug(vs. vehicle) treatment in control or SCW-injected rats (Table). GEO treatment did not alter body weight, which tends to decrease in SCW-treated rats, in control or SCW-injected rats (Table) . Circulating leukocyte counts, which are elevated in SCW-injected rats, were unchanged by polyherbal drug treatment in control or SCW-injected rats (Table) . Neutrophil, monocyte and lymphocyte counts were similarly unaltered by polyherbal drug treatment in control or SCW-injected animals (Table). Blood hematocrit values, which decrease in SCW-injected animals, were unchanged by GEO treatment in SCW-injected or control animals (Table). Hepatic function, which remained normal in SCW-injected animals as assessed by ALT, was unaltered by polyherbal drug treatment in control or SCW-injected animals (Table) . Creatinine levels, which were unchanged by polyherbal drug treatment in control animals, were slightly but statistically increased in SCW-injected animals treated with polyherbaldrug,a change that is of questionable physiologic significance given its small magnitude (Table).

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Toxicity Monitorig	Control (vehicle)	Polyherbal drug	SCW	SCW + Polyherbal
Mortality (number died/total)	0% (0/9)	0%(0/5)	4% (1/23)	7% (2/29)
Body Weight (g)	159.7 ± 4.6	158.6 ± 6.8	146.7 ± 3.5	143.3± 2.5[a]
ALT (U/L)	17.6 ± 3.0	13.9 ± 3.3	13.4 ± 1.6	13.1 ± 2.5
Creatinine (mg/dL)	0.24 ± 0.02	0.26 ± 0.05	0.28 ± 0.02	0.34 ± 0.02[a]
WBC (K/µl)	9.1 ± 1.3	10.2 ± 1.9	18.04 ± 1.5[b]	18.2 ± 2.0[b]
Neutrophils (K/µl)	1.6 ± 0.5	2.9 ± 1.1	8.0 ± 1.2[b]	7.2 ± 1.0[b]
Lymphocytes (K/µl)	6.5 ± 1.0	6.9 ± 0.9	9.1 ± 0.7	10.1 ± 1.2
Monocytes (K/µl)	0.24 ± 0.03	0.35 ± 0.07	0.90 ± 0.09[a]	1.21 ± 0.21[c]
Hematocrit (%)	44.9 ± 1.3	39.9 ± 4.0	7 34. ± l.2[b]	34.3 ± 1.2[b]

Values are expressed as mean ± SEM with statistical significance of differences between all groups determined by ANOVA with post-hoc testing or Fisher's exact test, as appropriate.
[a]p< 0.05 vs. vehicle[b]p<0.01 vs. vehicle[c]p<0.001. vs. vehicle

V. CONCLUSION

In conclusion, various Poly herbal constituents of ginger, trmeric, boswellli have potential therapeutic roles in RA symptoms and even possibly RA itself. It is expected that further elucidation of the molecular mechanisms behind the action of these Polyherbl drugs not only can lead to discovery of new drugs for symptomatic relief of RA conditions like inflammation and pain, but also may make it possible to stop further progress or even reverse the damage caused by RA.

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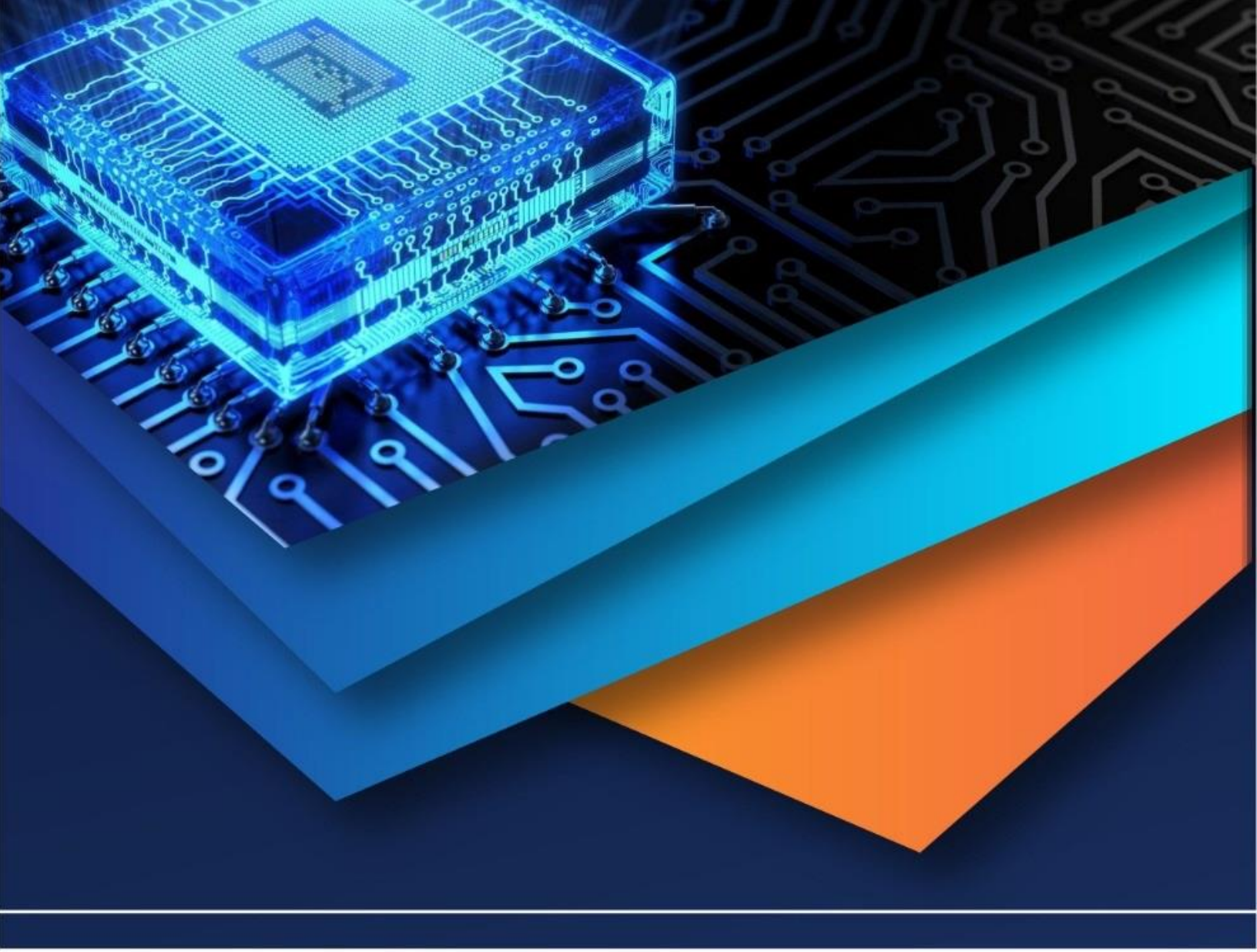

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