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Effects of Different Ethanolic Extract of *Azadirachata indica* on Blood Glucose Level in Alloxan Induced Diabetic Swiss Albino Mice

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Abstract: ***The plant, Azadirachta indica A Juss, family Meliacea is a very valuable herb in Ayurvedic medicine. The tree is found in every part of India and considered an excellent herb that believes to cure almost hundred diseases. In the present study, an attempt was made to investigate the effects of ethanolic extract of A. indica leaves and seeds on the blood glucose level in diabetic albino mice. A.indica leaves extract (250mg/kg & 500mg/kg) and A.indica seeds extract (100mg/kg & 200mg/kg) were administered single doses and repeated doses for 21 days. Single doses of the extract in diabetic albino mice produced maximal dose dependent percentage reduction after 6 hours of administration with percentage reductions of 18.97% (250 mg/kg A.indica leaves extract), 24.30% (500 mg/kg A.indica leaves extract) , 20.94% (100 mg/kg A.indica seeds extract) and 28.14% (200 mg/kg A.indica seeds extract) respectively.***

The result shows that 500 mg/kg A.indica leaves extract and 200 mg/kg A.indica seeds extract repeated doses gave the highest percentage reduction in fasting blood glucose in alloxan induced diabetic mice after 21 days as follows 39.28% and 42.01% respectively. In conclusion, it was observed that the higher dosage of A.indica leaves extract (500 mg/kg/body wt) and seeds extract (200 mg/kg/body wt) for prolonged treatment exhibited increased reduction in the blood glucose levels compared to low dosage administration.

Keywords: Alloxan, Diabetes, Azadirachta indica, Pancreas.

I. INTRODUCTION

Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and are still widely used and have considerable importance in international trade [1]. In rural areas where access to modern health facilities is limited by the level of development, plants/herbs remain the mainstay of the health care system[23]. Additionally, current research in medicinal plants is beginning to lend credence to their efficacy and potency and in most instances over and above the existing conventional and chemotherapeutic options particularly as it concerns degenerative disease complexes including diabetes mellitus.

A. indica belongs to the family Meliaceae and has a long history of use in folkmedicine as a treatment against various ailments[15]. Reported pharmacological and biological properties are also numerous[20]. The hypoglycemic actions of its leaves stem bark and seeds have been articulated in a review by Biswas et al.[2], and Ebong et al[5] indicate recently in their studies, The Neem tree is native to the Western Himalayas, growing in the warmer areas of India and Southeast Asia. It is commonly found in the forested areas of Andhra Pradesh, Tamil Nadu and Karnataka. Neem is also cultivated in other tropical areas of Asia, as well as West Africa, the Caribbean, South America, Central America, Indonesia and Australia.However, The chemical constituents contain many biologically active compounds that can be extracted from neem, including alkaloids, lavonoids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones, biologically most active compound is azadirachtin.

Moreover, *Azadirachta indica* is used for a wide range of purposes. The oil extracted from the seed (Neem oil) has insecticidal properties [9, 11]. Neem can treat many skin disorders, including scabies and lice. It is used Pharmacologically as abortifacient, analgesic, antihelminthic, antibacterial, antiyeast, antiulcer, antifertility, antifilarial, antifungal, antihyperglycemic, anti-inflammatory, antiviral, antimalarial, diuretic, antinematodal, antipyretic, antispasmodic, insecticidal, antispermatogenic, antitumor, hypercholesteremic, hypoglycaemic, immunomodulator [16,17].

The present study evaluates the hypoglycaemic effect and histopathological analysis of *Azadirachta indica* on alloxan induced diabetic albino mice with specific objectives to find the effect of single dose administration of the extract on blood glucose level as well as the effect of repeated and prolonged administration of the extract on blood glucose level in alloxan induced diabetic mice.

II. MATERIAL AND METHODS

A. *Plant Materials*

1) *Collection of plant materials:* Fresh matured leaves and seeds of *Azadirachta indica* (Neem) were harvested from Science College Campus, Patna University, Patna and was identified by Dr. S. R. Padmadeo, Professor, Department of Biochemistry, Patna University, Patna (Bihar). The leaves and seeds were washed with distilled water and dried completely under the mild sun.
2) *Preparation of Plant Materials:* 1 kg of dried leaves and seeds were crushed with electrical grinder in coarse powder and soaked separately in absolute ethanol (95%) for 48 hours. The supernatant was collected and the residue was further soaked in absolute ethanol (95%) for 24 hours. The supernatant was collected and filtered. The filtrate was subjected to Rota vapour extraction at a temperature below 60°C for 24 hours. The concentrated form of the extract was obtained and freeze-dried. The dose was finally made for oral administration after the LD_{50} estimation.

B. *Experimental Animals*

8 weeks old male Swiss albino mice (25 to 30 gms) were carried out and acclimatized till 2 weeks for laboratory condition. Animals were housed in animal house at 25°C $\pm$ 2°C with 12-12 hrs dark- light cycle. Standard food and water provided *ad libitum* throughout the experimental period. Animals care and handling were taken according to standard protocol. This project was approved by the DRC, Department of Biochemistry, Patna University, Patna.

1) *Induction of diabetes:* Alloxan (150mg/kg bw) was prepared in distilled water and administered intra-peritonial to the mice three times at the interval of 72 hrs. Diabetes was confirmed by blood sugar test, with the help of glucometer (Lever Check Pvt. Ltd.) and its chemical method. Animals have more than 250 mg/dl blood sugar levels were selected for the further study and maintained up to 4 days in diabetic condition for well establishment of diabetes.

C. *Experimental Protocol*

Mice were divided into six groups and each group had 6 mice as follows:

1) Group I: Normal Control (NC),
2) Group II: Alloxan induced Diabetic Control (DC),
3) Group III: Diabetic + *A. indica* leaves extract (250 mg/kg body weight),
4) Group IV: Diabetic + *A. indica* leaves extract (500 mg/kg body weight),
5) Group V: Diabetic + *A. indica* seeds extract (100 mg/kg body weight),
6) Group VI: Diabetic + *A. indica* seeds extract (200 mg/kg body weight)

The plant extract was orally administered daily for 21 days. Whole blood was collected from retro orbital venous puncture into sodium fluoride treated tubes for estimation of glucose and blood was collected in plane tubes for other biochemical test. Serum was separated by centrifugation at 2500 rpm at 4°C for 15 minutes.

D. *Measurement of Blood glucose*

Under aseptic conditions, blood samples were collected on the first day before inducing alloxan and later after 72 hrs from the tail vein. Blood glucose level was measured by using Accucheck glucometer.

E. *Statistical Analysis*

Comparison between control and drug treated groups were analyzed with one-way ANOVA by using Graph Pad Prism 5.04 software. The results were expressed as mean ± Standard Error of mean (S.D), N=6. P-Values<0.05 were considered to be statistically significant.

F. *Pancreatic Histopathology*

For the study and comparison of pancreatic tissue, mice were sacrificed and tissue was collected. The tissue was fixed in 10% formalin, stained in Hematoxylin and Eosin, and subsequently, examined by a microscope at 300X magnification.

G. *Toxicity Evaluation in mice*

The ethanolic extract of *A.indica* was tested for its acute and short term toxicity in albino mice. To determine short term toxicity, different doses of the *A.indica* leaves extract (250, 500,1000,2500,4500 and 5000mg/kg) and *A.indica* seeds extract (200, 400, 800,

2000, 3000 and 3600 mg/kg) were administered daily for 7 days (p.o) to different group of mice (10 mice were used for each group). After administration of extracts, the animals were observed for the first 3 hours for any toxic symptoms followed by observation at regular intervals for 24 hours up to 7 days. At the end of the study, the animals were also observed for general toxicity, morphological behaviour and mortality. The number of animals dying during this period was noted[7]. The LD_{50} of leaf and seeds extract of *A.indica* was calculated by the method of Litchfield and Wilcoxon[12].

III. RESULT

Intra-peritoneal (i.p) treatment of Swiss albino mice with alloxan monohydrate (150 mg/kg) significantly ($p<0.001$) increases the blood glucose level. The changes in the blood glucose levels before and after receiving the treatment in normal and diabetic mice are listed in Table 1 & 2. Upon administration of different ethanolic extract of *A.indica,* significant changes were recorded in blood glucose levels, both in acute as well as in chronic study groups.

During preliminary toxicity study, no adverse effect or mortality was observed in albino mice with oral administration of ethanolic *A.indica* leaves extract up to 500mg/kg b.wt and seeds extract upto 400mg/kg b.wt. Hence a high dose of 250 mg/Kg body wt. & 500 mg/Kg body wt. of *A.indica* leaves extract and 100 mg/kg body wt. & 200 mg/kg body wt. of *A.indica* seeds extract were selected as a test dose.

A. Single Dose Study

Administration of single dose of *A.indica* leaves extract (250 mg/Kg body wt & 500 mg/Kg body wt) and *A.indica* seeds extract (100 mg/kg body wt & 200 mg/kg body wt), oral, each to four study groups which are diabetic induced by alloxan, significant reduction in blood glucose levels was observed. The study period encompassed 6 hrs. *A.indica* leaves extract at 500 mg/Kg body wt and *A.indica* seeds extract at 200 mg/kg body wt produced more hypoglycaemic effects (significant decreases of 24.30% and 28.14%, respectively) compared to *A.indica* leaves extract administered at 250 mg/Kg body wt (significant decrease of 18.97%) and *A.indica* seeds extract at 100 mg/kg body wt (significant decrease of 20.94%).

B. Chronic study

During chronic study which encompassed a period of 21 days, the *A.indica* leaves extract at 500 mg/kg body wt and seeds extract at 200 mg/kg body wt, oral, produced a significant ($P<0.01$) reduction in blood glucose levels of the diabetic mice compared to normal control. *A.indica* leaves extract at the dose of 500 mg/kg body wt/day and seeds extract at 200 mg/kg body wt/day exhibited better blood glucose reduction (significant decrease of 39.28% and 42.01%, respectively) than *A.indica* leaves extract at 250 mg/kg body wt/day and seeds extract at 100 mg/kg body weight/day (decrease of 28.20% and 29.70%, respectively).

C. Effect on Oral Glucose Tolerance

The effects of different *A.indica* extract at different doses on oral glucose tolerance (OGTT) in alloxan induced diabetic mice are presented in graph 2. The results in diabetic mice showed abnormal glucose tolerance during OGTT. The blood glucose level of normal non–diabetic mice had 96.7± 26.85 mg/dl that was much lower than that of the diabetic non- treated ones, reached its peak value at 60 minutes following glucose intake (2 g/kg B.W.) and began to decrease during the next 60 minutes to reach 116.43 ± 19.84 mg/dl after 2 hours of glucose administration. In the diabetic non-treated male albino mice, blood glucose also attained its maximal level after 60 minutes of glucose administration recording 446.25 ± 71.20, after the 21 days of daily treatment. Subsequently, these values begin to decline during the next 60 minutes but in slower rate and still elevated than that of the normal ones. The prolonged treatment of diabetic mice with ethanolic extract of *Azadirachta indica* showed considerable improvement on Glucose tolerance values alleviating hyperglycaemia. When the mice were first administrated orally with glucose, mild rates of increase in the blood glucose level (BGL) were consistent for *A.indica* leaves and seeds extract at different doses during the first 60 min, after which BGL level decreased significantly in comparison with diabetic control ($p<0.01$). Evidentially, *A.indica* leaves extract at 500mg/kg/body wt/day and *A.indica* seeds extract at 200mg/kg/body wt/day showed a more beneficial effect on OGTT.

D. Effect of Different A.indica extract on Body Weight

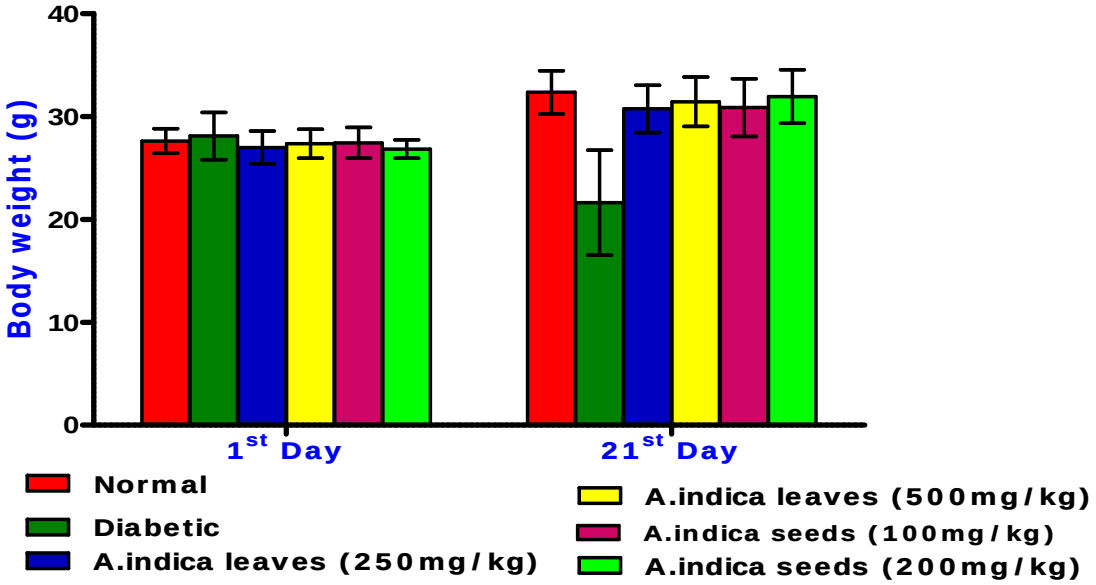
The diabetic control mice showed a remarkable decrease in body weight compared to the control mice at 21st days of treatment. In contrast, diabetic mice treated with higher dose of *A.indica* leaves extract (500mg/kg/body wt.) and *A.indica* seeds extract (200mg/kg/body wt.) showed a significant increase in body weight compared to the diabetic control group (graph-1).

E. Toxicity evaluation

The result of the acute toxicity study showed that mice administered with *A.indica* leaves extract (250mg/kg & 500mg/kg) and seeds extact (200mg/kg &400mg/kg) did not show fatality. Even at these doses there were no gross behavioural changes. But with doses of above 1000mg/kg of *A.indica* leaves extract and above 800mg/kg of *A.indica* seeds extract, fatality was observed.

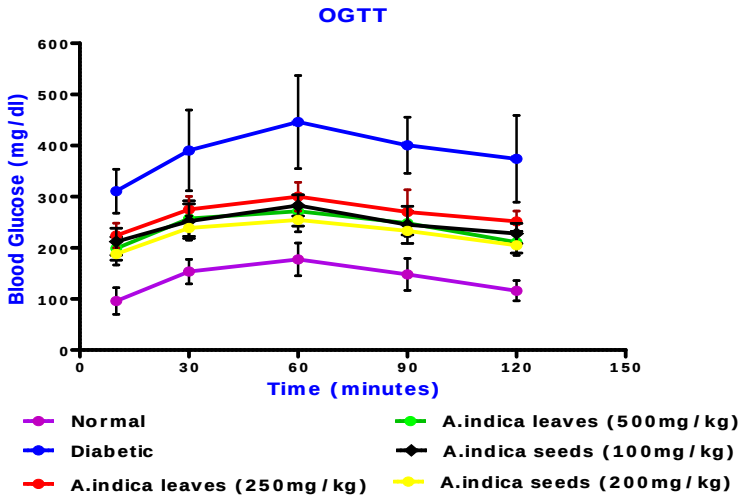
F. Histopathological analysis of Pancreas

Photomicrograph of pancreas (Figure 1) showed normal population of pancreatic islets (Pi) and serous acini (Sa) in the pancreas of normal control A. Extensive damage to islets of Langerhans and shrinkage of islets in diabetic control B and regeneration of pancreatic islets and serous acinal cells were observed in C & D when compared to the section of pancreas of the diabetic control group.



* Values expressed as Mean ± S.E.M, n=6 in each groups

Graph 1: Effect of *A.indica* extract on body weight changes in the normal and diabetic mice



Effects of different *A.indica* extract on glucose tolerance (mean±S.D.) curve of diabetic and diabetic treated mice at 21st days of treatment

Graph 2: Effect on Oral Glucose Tolerance Test

Table1: Effect of different *A.indica* extract on Blood glucose level in alloxan induced diabetic mice after single dose administration:

GROUPS	DOSES	Blood Glucose Level (mg/dl) (mean ± S.D.)					
		Pretreatment	Post treatment				
		0 Hr.	1hr.	2hr.	4hr.	6hr.	
						Mean+S.D.	%
Normal control	Distilled water	121 ± 5.73	121 ± 5.75	112.3 ± 8.31	119 ± 7.43	124 ± 9.81	+ 2.47
Diabetic control	Distilled water	427 ± 74.09***	460 ± 67.38***	423 ± 88.71***	474 ± 37.44***	463 ± 43.92***	+ 8.47
A.indica leaves exttract	250 mg/kg	274 ± 39.24	268 ± 16.73	232.8 ± 18.93	240.6 ± 59.18*	222 ± 45.16*	- 18.97
A.indica leaves extract	500 mg/kg	362 ± 45.61	349 ± 14.92	305 ± 68.87*	262 ± 68.79**	274 ± 59.61**	- 24.30
A.indica seeds extract	100 mg/kg	296 ± 64.33	268 ± 57.81	273 ± 61.94	228 ± 52.37*	234 ± 18.92*	-20.94
A.indica seeds extract	200 mg/kg	398 ± 61.37	376 ± 17.86	318 ± 21.15*	292 ± 73.28**	286 ± 23.46**	-28.14

***p <0.001 as compared to normal control. **p <0.01 as compared to diabetic control. *p<0.05 as compared to diabetic control.

Table 2: Effect of different *A.indica* extract on Blood glucose level in alloxan induced diabetic mice during prolonged treatment:

GROUPS	DOSES	Blood Glucose Level (mg/dl) (mean ± S.D.)				
		Pre treatment	Post treatment			
		0 days	7 days	14 days	21 days	
					(Mean± S.D.)	%
Normal control	Distilled water	112± 17.82	115.6 ± 8.73	121 ± 9.35	116 ± 7.47	+3.57
Diabetic control	Distilled water	371 ± 74.93***	402 ± 68.31***	429 ± 58.73***	440 ± 83.44***	+18.59
A.indica leaves exttract	250 mg/kg	312 ± 42.32	293 ±62.78	268 ± 97.94*	224 ±59.82**	- 28.20
A.indica leaves extract	500 mg/kg	392 ± 23.61	316 ±54.93*	276 ± 83.38**	238 ±28.79**	-39.28
A.indica seeds extract	100 mg/kg	367 ± 27.34	329 ± 57.8	283 ± 61.97*	258 ± 21.74**	-29.70
A.indica seeds extract	200 mg/kg	388 ± 33.63	307 ± 17.81*	245 ± 21.56**	225 ± 18.86**	-42.01

***p <0.001 as compared to normal control.**p <0.01 as compared to diabetic control.*p<0.05 as compared to diabetic control

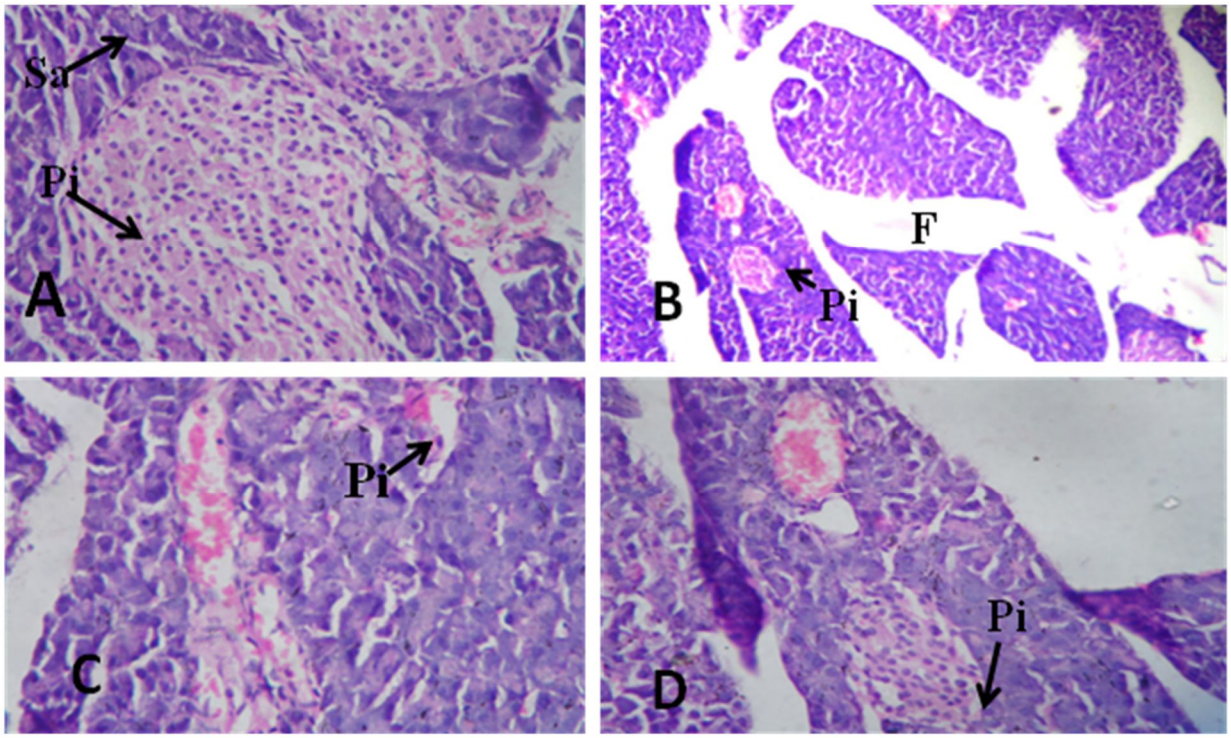


Figure 1: Effect of oral administration of a.indica leaves and seeds extract (21days)on diabetic mice. A- Normal control, B- Diabetic control, C- *A.indica* leaves treated group, D- *A.indica* seeds treated group.

IV. DISCUSSION

Diabetes is a serious metabolic disorder with micro and macrovascular complications that results in significant morbidity and mortality [6, 22, 25]. Alloxan induces diabetes in a different animal species by damaging the insulin secreting pancreatic β-cell[21].

In the present study, the toxic effect of ethanolic extract of A.indica was measured. Our previous finding revealed a well defined role of the ethanolic extract of *A.indica* in lowering blood glucose level in alloxan induced diabetic mice. In this study, it was observed that the leaf and seeds extract of *A.indica* possess significant dose-dependent blood glucose lowering activity. Single doses of the leaf extract (250 and 500mg/kg b.wt) and seeds extract (100 and 200 mg/kg b.wt) in diabetic mice produced dose dependent percentage reduction in fasting glucose level. The percentage reduction was maximal after 6 hrs of administration. The results are consistent with Sonia and srinivasan[20].

In addition, a dose dependent Percentage reduction in fasting blood glucose level was observed in the diabetic mice with different *A.indica* extract in the repeated dose effect study. The percentage reduction in fasting blood glucose level on diabetic induced albino mice after daily treatment for 21 days were 28.20% (250 mg/kg of *A.indica* leaves extract), 39.28% (500 mg/kg of *A.indica* leaves extract), 29.70% (100 mg/kg of *A.indica* seeds extract) and, 42.01% (200 mg/kg of *A.indica* seeds extract). These findings were also comparable with earlier works reported by Murty, et al.[14] in dogs and Khosla, et al.[10] in diabetic rabbits. Each extract of *A.indica* also improved oral glucose tolerance. *A.indica* extract caused a significant reduction in blood glucose level in both single dose and repeated dose study on diabetic albino mice. This indicates that the extract may contain bioactive compounds which probably act by increasing insulin release or by enhancing the uptake and utilization of glucose peripherally.

V. CONCLUSION

The results of this study showed that *A.indica* leaves and seeds extract reduced blood glucose levels in alloxan induced diabetic albino mice when given orally. On the other hand, *A.indica* has a favourable effect to inhibit the histopathological changes of the pancreas in alloxan induced diabetes.

VI. ACKNOWLEDGEMENT

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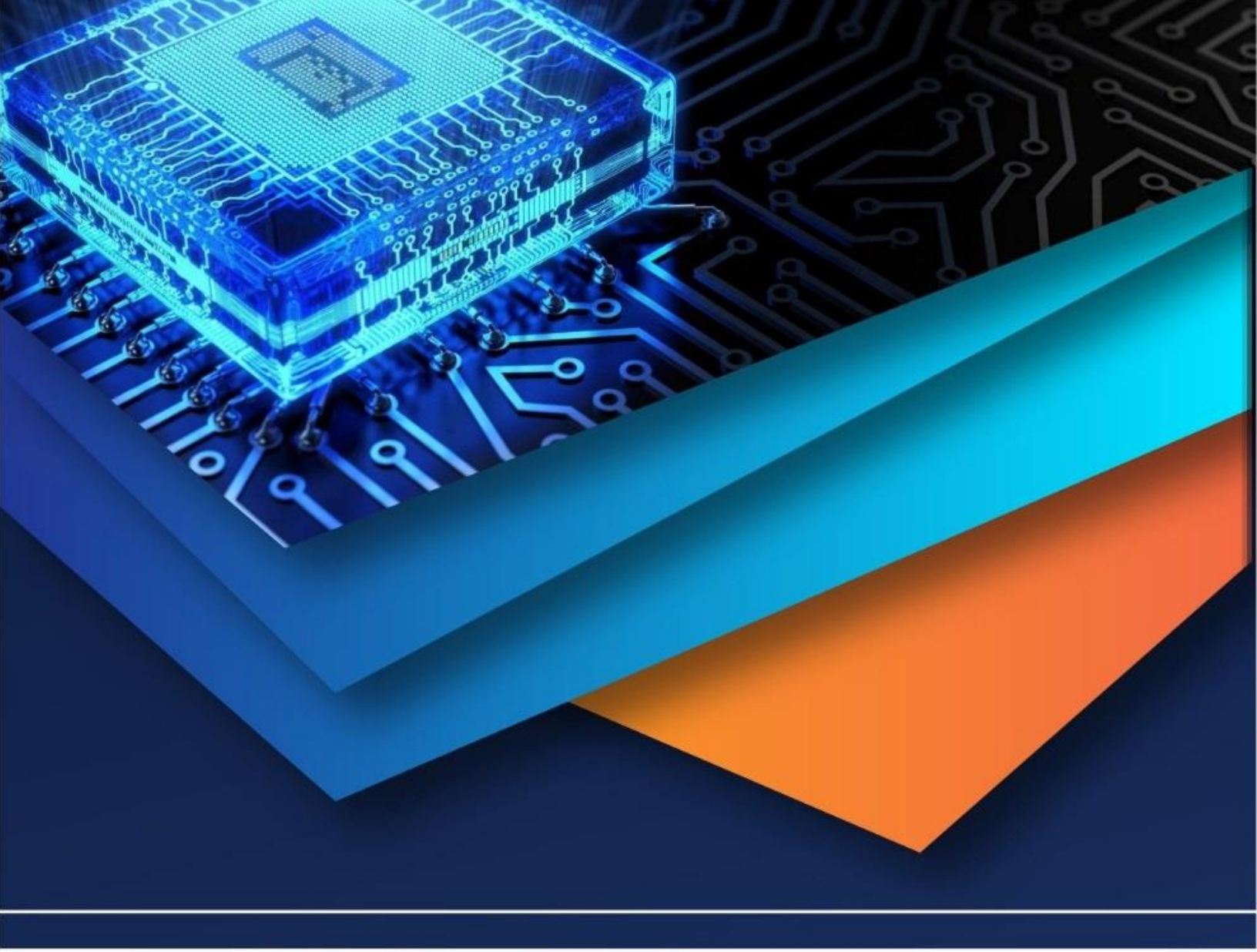

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