GMR
The Original

ANTIDIABETIC AND ORGAN-PROTECTIVE EFFECTS OF A POLYHERBAL FORMULATION IN ALLOXAN-INDUCED DIABETIC WISTAR RATS

Ranu Gupta[1], CH KVLSN Anjana Male[2]*, Subodh Dubey[3]

[1]Research Scholar, School of Pharmacy, ITM University, Gwalior, Madhya Pradesh, India
[2]Professor, School of Pharmacy, ITM University, Gwalior, Madhya Pradesh, India
[3]Dean, School of Pharmacy, Vikrant University, Gwalior, Madhya Pradesh, India

ABSTRACT
Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia and associated metabolic abnormalities. Herbal medicines have gained considerable attention due to their therapeutic efficacy and lower incidence of adverse effects. The present study evaluated the antidiabetic potential of a polyherbal formulation (PHF) in alloxan-induced diabetic rats. Diabetes was induced in Wistar rats by intraperitoneal administration of alloxan monohydrate (150 mg/kg). Animals were divided into eight groups comprising normal control, diabetic control, glibenclamide-treated standard group (10 mg/kg), and five treatment groups receiving PHF-1 to PHF-5 (500 mg/kg) orally for 21 days. Blood glucose levels were monitored on days 0, 7, 14, and 21. Biochemical parameters including lipid profile, liver enzymes, and renal markers were estimated at the end of the study. Histopathological examination of pancreatic tissues was performed. Data were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. Alloxan administration significantly increased blood glucose levels, dyslipidemia, hepatic enzyme levels, and renal biomarkers compared with normal animals. Treatment with polyherbal formulations significantly reduced hyperglycemia and improved biochemical parameters. PHF-3 and PHF-4 exhibited the greatest antihyperglycemic activity, reducing blood glucose levels from 279.27 ± 1.36 and 281.40 ± 1.27 mg/dL to 105.59 ± 1.03 and 100.04 ± 1.30 mg/dL, respectively, after 21 days. These formulations also restored serum lipid profile, liver function markers, renal function markers, body weight, and pancreatic architecture comparable to glibenclamide. The polyherbal formulation demonstrated significant antidiabetic activity in alloxan-induced diabetic rats. PHF-3 and PHF-4 exhibited superior antihyperglycemic and organ-protective effects, suggesting their potential as promising herbal therapeutic agents for diabetes management.

KEYWORDS: Diabetes mellitus, Alloxan, Polyherbal formulation, Antihyperglycemic activity, Glibenclamide, Histopathology, Wistar rats.

INTRODUCTION
Diabetes mellitus is a multifactorial metabolic disorder characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The disease is associated with disturbances in carbohydrate, lipid, and protein metabolism and may lead to severe complications including nephropathy, neuropathy, retinopathy, and cardiovascular disorders if left untreated [1-2].

Current therapeutic approaches involve the use of oral hypoglycemic agents and insulin therapy; however, long-term treatment is often associated with adverse effects, high cost, and reduced patient compliance [3-4]. Consequently, there is increasing interest in plant-based therapies and polyherbal formulations as alternative approaches for diabetes management [5].

Polyherbal formulations combine multiple medicinal plants to achieve synergistic pharmacological effects. Several medicinal plants possess antidiabetic activity through mechanisms such as stimulation of insulin secretion, enhancement of peripheral glucose utilization, inhibition of carbohydrate digestion, and antioxidant protection of pancreatic β-cells [6].

The present study was designed to evaluate the antidiabetic efficacy of polyherbal formulation tablets containing ethanolic and aqueous extracts of *Gymnema sylvestre* leaves, *Vinca rosea* whole plant, *Cinnamomum zeylanicum* bark, and *Eugenia jambolana* seeds in alloxan-induced diabetic rats using biochemical and histopathological parameters [11].

MATERIALS AND METHODS
Experimental Animals
Healthy adult Wistar rats of either sex weighing 150–200 g were used for the study. Animals were maintained under standard laboratory conditions with a 12 h light/dark cycle and had free access to standard pellet diet and water ad libitum [15]. Animals were acclimatized for one week prior to experimentation. Experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC).

Induction of Experimental Diabetes

Alloxan monohydrate was dissolved in sterile water for injection to obtain a concentration of 50 mg/mL. Experimental diabetes was induced by a single intraperitoneal administration of alloxan monohydrate (150 mg/kg body weight) following overnight fasting [7-9]. To prevent immediate hypoglycemic shock, animals received 5% glucose solution for 24 h following alloxan administration. After 72 h, fasting blood glucose levels were measured using a glucometer. Rats exhibiting blood glucose levels greater than 200 mg/dL were considered diabetic and included in the study.

Experimental Design

Animals were randomly divided into eight groups (n = 6):

Group I: Normal control; **Group II:** Diabetic control; **Group III:** Standard (Glibenclamide, 10 mg/kg); **Group IV:** PHF-1 (500 mg/kg); **Group V:** PHF-2 (500 mg/kg); **Group VI:** PHF-3 (500 mg/kg); **Group VII:** PHF-4 (500 mg/kg); **Group VIII:** PHF-5 (500 mg/kg)

Treatments were administered orally for 21 consecutive days.

Assessment of Blood Glucose Levels

Blood samples were collected from the tail vein on days 0, 7, 14, and 21. Blood glucose concentrations were measured using a digital glucometer.

Biochemical Analysis

At the end of the treatment period, blood samples were collected through retro-orbital puncture under mild anesthesia. Serum was separated by centrifugation and analyzed for: Fasting blood glucose, Total cholesterol, Triglycerides, HDL cholesterol, LDL cholesterol, SGOT, SGPT, ALP, Urea and Creatinine using standard commercial diagnostic kits.

Histopathological Studies

Pancreatic tissues were excised, fixed in 10% formalin, dehydrated, embedded in paraffin wax, sectioned (4–5 μm), stained with hematoxylin and eosin, and examined microscopically for pathological changes.

Statistical Analysis

Data were expressed as Mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test. Values of $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Effect on Blood Glucose Levels

The diabetic control group exhibited progressive elevation in blood glucose levels throughout the experimental period, confirming successful induction of diabetes. Glibenclamide treatment significantly reduced blood glucose concentrations by day 21. Among the test formulations, PHF-3 and PHF-4 demonstrated the most pronounced antihyperglycemic activity. Blood glucose levels were reduced to near-normal values by the end of the treatment period, indicating potent antidiabetic activity. PHF-1, PHF-2, and PHF-5 also produced significant reductions but were less effective than PHF-3 and PHF-4.

The observed antihyperglycemic effect may be attributed to synergistic interactions among phytoconstituents capable of improving insulin secretion, enhancing glucose uptake, and protecting pancreatic β-cells from oxidative damage.

Effect on Body Weight and Fluid Intake

Diabetic rats exhibited significant body weight loss and increased fluid intake compared with normal animals. Administration of PHF formulations improved body weight and reduced excessive water consumption. PHF-3 and PHF-4 produced the greatest restoration of body weight and normalization of fluid intake, suggesting improved metabolic control.

Effect on Hemoglobin and Urine Sugar

Alloxan-induced diabetes caused a marked decrease in total hemoglobin and persistent glycosuria. Treatment with polyherbal formulations significantly increased hemoglobin levels and reduced urinary glucose excretion. PHF-4 demonstrated the greatest improvement among all formulations, closely resembling the effect of glibenclamide.

Table 1: Effect of Administration of Feeding on Body Weight and Fluid Intake in Normal and Diabetic Rats

Group	Body Weight (g)		Fluid intake g/animal/day
	Before treatment	After treatment	
Control	192±1.08	221.5±1.39	21.41±0.47
Diabetic control	202.16±1.35	168.5±2.513	74.01±0.01
Standard	206.06±1.72**	221.72±1.16*	54.02±0.15***
PHF-1	208.13±1.03**	221.06±1.02***	58.92±0.01***
PHF-2	196.39±2.02*	212.1±1.16**	57.12±0.10***
PHF-3	204.41±2.01*	221.03±2.18***	56.17±0.39***
PHF-4	197.02±2.31**	216.06±2.12**	56.13±0.15***
PHF-5	211.29 ± 2.10*	177.30±1.12	70.11±1.20

Note: All values are expressed as mean ± S.E.M (n=6), *p < 0.05, while **p < 0.01 and ***p < 0.001 consider as significant as compared to standard and diabetic control; One-way ANOVA followed by Dunnett's multiple comparison test.

Table 2: Effect of Administration of Feeding on Total Haemoglobin and Urine Sugar in Normal and Diabetic Rats

Group	Total hemoglobin (%)		Urine sugar	
	Before treatment	After treatment	Before treatment	After treatment
Control	11.53±0.12	11.7±0.481	Nil	Nil
Diabetic control	12.82±0.11	7.69±0.744	+4	+4
Standard	13.13±0.67**	16.25±0.399***	+4	+2
PHF-1	13.09±0.23*	15.97±0.154***	+4	+2
PHF-2	13.06±0.14**	16.23±0.216***	+4	+2
PHF-3	14.06±0.19*	13.61±0.297***	+4	+2
PHF-4	13.72±0.18**	16.5±0.196***	+4	+2
PHF-5	14.23±0.16**	9.098±0.256**	+4	+4

Note: All values are expressed as mean ± S.E.M (n=6), *p < 0.05, while **p < 0.01 and ***p < 0.001 consider as significant as compared to standard and diabetic control; One-way ANOVA followed by Dunnett's multiple comparison test.

Table 3: Effect of Polyherbal Formulations on Serum Glucose Levels (mg/dL)

Group	Day 0	Day 7	Day 14	Day 21
Normal Control	92.24 ± 1.13	94.20 ± 1.24	93.39 ± 1.30	95.53 ± 1.32
Diabetic Control	285.46 ± 1.08	295.26 ± 1.06	305.80 ± 1.19	310.18 ± 1.01
Standard (Glibenclamide)	278.39 ± 1.17	180.24 ± 1.26	120.42 ± 1.25	98.81 ± 1.43
PHF-1	280.29 ± 1.18**	230.77 ± 1.37*	190.04 ± 1.16*	160.62 ± 1.55***
PHF-2	282.38 ± 1.47*	220.72 ± 1.26**	175.18 ± 1.50**	140.73 ± 1.74*
PHF-3	279.27 ± 1.36*	200.04 ± 1.25**	130.39 ± 1.14*	105.59 ± 1.03*
PHF-4	281.40 ± 1.27*	195.49 ± 1.15***	125.84 ± 1.49**	100.04 ± 1.30*
PHF-5	283.38 ± 1.48**	225.29 ± 1.87**	180.72 ± 1.26**	150.39 ± 1.15***

Note: All values are expressed as mean ± S.E.M (n=6), *p < 0.05, while **p < 0.01 and ***p < 0.001 consider as significant as compared to standard; One-way ANOVA followed by Dunnett's multiple comparison test.

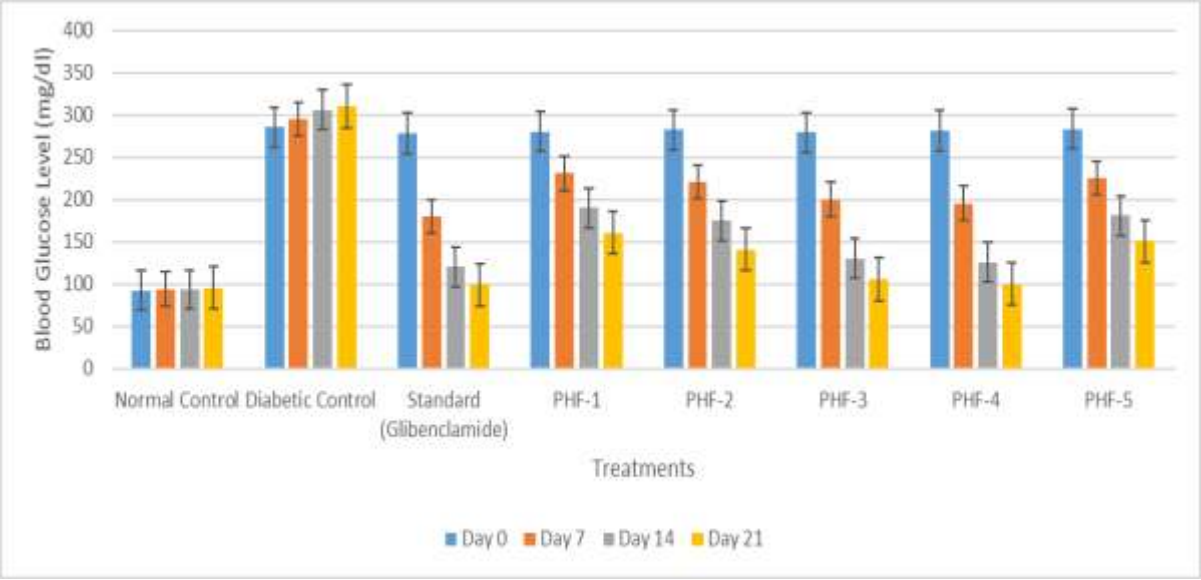

Figure 1: Blood Glucose Level in the treated group

Effect on Biochemical Parameters

The diabetic control group exhibited significant elevations in fasting blood glucose, total cholesterol, triglycerides, LDL cholesterol, SGOT, SGPT, ALP, urea, and creatinine, while HDL cholesterol levels were markedly reduced. Treatment with PHF formulations significantly improved these abnormalities. PHF-3 and PHF-4 showed the most substantial effects, restoring biochemical parameters toward normal values. These findings indicate that the formulations possess antihyperglycemic, antihyperlipidemic, hepatoprotective, and nephroprotective activities.

Table 4: Effect of Polyherbal Formulations on Biochemical Parameters

Parameter	Normal Control	Diabetic Control	Standard	PHF-1	PHF-2	PHF-3	PHF-4	PHF-5
Fasting Blood Glucose (mg/dL)	95.3 ± 0.03	310.2 ± 0.10	98.7 ± 0.14	160.9 ± 0.05	140.3 ± 0.14	105.1 ± 0.03	100.5 ± 0.03	150.5 ± 0.05
Total Cholesterol (mg/dL)	110.9 ± 0.04	220.7 ± 0.08	120.3 ± 0.15	160.4 ± 0.06	145.9 ± 0.15	125.2 ± 0.14	122.8 ± 0.14	150.5 ± 0.16
Triglycerides (mg/dL)	100.4 ± 31	210.4 ± 7	115.21 ± 0.14	155.47 ± 0.25	140.10 ± 0.15	120.04 ± 0.14	118.71 ± 0.30	148.34 ± 0.25
HDL (mg/dL)	50.2 ± 0.33	30.3 ± 0.12	48.12 ± 0.15	38.10 ± 0.12	42.39 ± 0.22	46.71 ± 0.32	47.10 ± 0.12	40.32 ± 0.42
LDL (mg/dL)	80.7 ± 0.32	160.9 ± 0.16	90.28 ± 0.30	120.82 ±0.44	105.10 ± 0.80	95.22 ± 0.30	92.17 ± 0.34	115.17 ± 0.10
SGOT (U/L)	35.9 ± 0.20	85.4 ± 0.30	40.21± 0.42	60.27 ± 0.27	52.29 ± 0.21	45.10 ± 0.20	42.25 ± 0.26	55.09 ± 0.34
SGPT (U/L)	30.4 ± 0.26	80.1 ± 0.34	38.19 ± 0.12	58.10 ± 0.60	50.45 ± 0.11	42.11 ± 0.12	40.19 ± 0.22	53.42 ± 0.22
ALP (U/L)	90.0 ± 0.31	180.1 ± 0.16	100.10 ± 0.74	130.39 ± 0.41	115.20 ± 0.41	105.01 ± 0.44	102.24 ± 0.32	120.10 ± 0.18
Urea (mg/dL)	28.2 ± 0.10	65.3 ± 0.11	32.11 ± 0.15	45.22 ± 0.45	40.61 ± 0.30	35.22 ± 0.11	33.10 ± 0.17	42.29 ± 0.29
Creatinine (mg/dL)	0.74 ± 0.05	1.82 ± 0.08	0.91 ± 0.05	1.31 ± 0.06	1.13 ± 0.05	0.95 ± 0.04	0.90 ± 0.04	1.21 ± 0.05

Note: All values are expressed as mean ± S.E.M (n=6), $p < 0.001$ consider as significant as compared to standard; One-way ANOVA followed by Dunnett's multiple comparison test.

Histopathological Evaluation

Histopathological examination of pancreatic tissues from diabetic control animals revealed inflammatory infiltration and pancreatic damage consistent with alloxan-induced β-cell toxicity. In contrast, pancreatic tissues from PHF-treated groups exhibited restoration of normal architecture. PHF-3 and PHF-4 showed nearly normal pancreatic histology with minimal inflammatory changes, indicating protection against β-cell destruction and promotion of tissue recovery. The histological findings corroborate the biochemical results and support the antidiabetic potential of the polyherbal formulation.

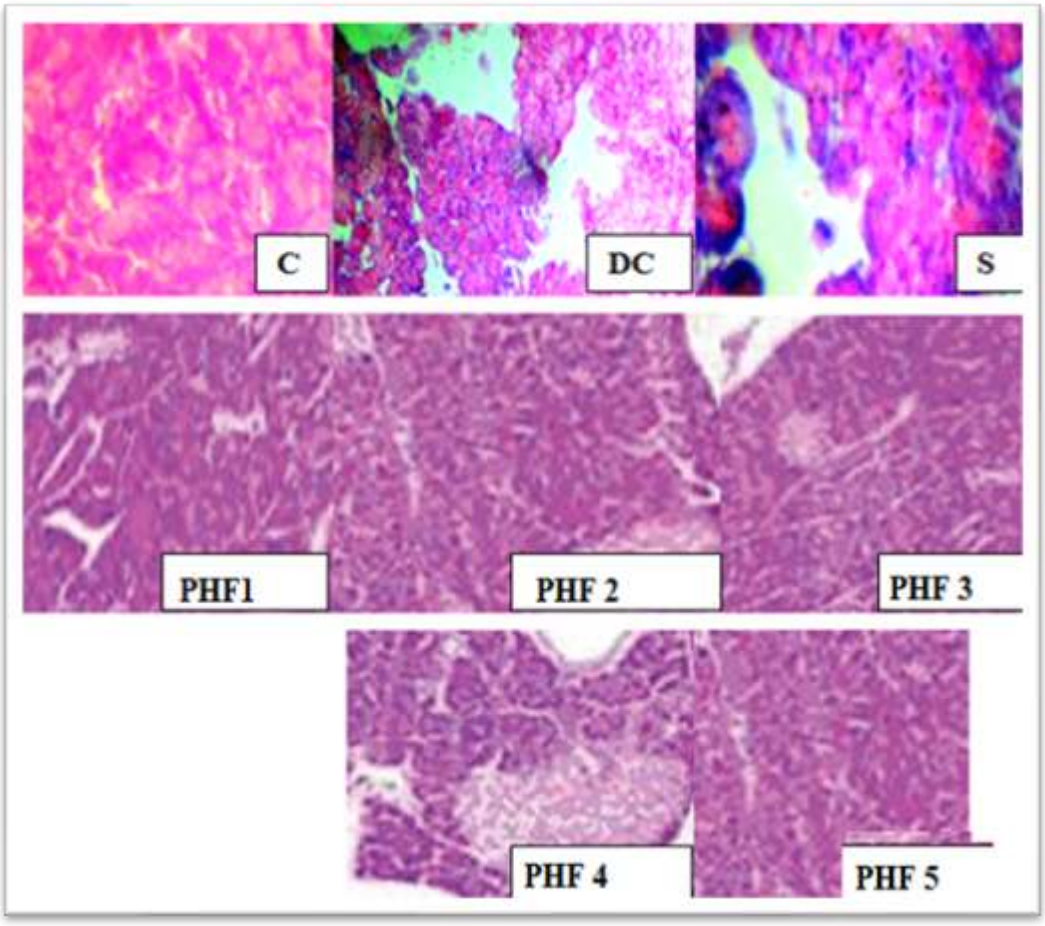

Figure 2: Histopathology of Pancreas of Treatment Group

CONCLUSION

The present investigation demonstrated significant antidiabetic activity of the polyherbal formulation containing ethanolic and aqueous extracts of *Gymnema sylvestre* leaves, *Vinca rosea* whole plant, *Cinnamomum zeylanicum* bark, and *Eugenia jambolana* seeds in alloxan-induced diabetic rats. [12] The formulation effectively reduced hyperglycemia, improved lipid abnormalities, normalized hepatic and renal biomarkers, restored body weight, and protected pancreatic tissues from alloxan-induced damage. Among all formulations, PHF-3 and PHF-4 exhibited superior therapeutic efficacy comparable to the standard drug glibenclamide. [13], [14] These findings suggest that the optimized polyherbal formulations may serve as promising candidates for further preclinical and clinical development as herbal antidiabetic agents [10].

REFERENCES

1. American Diabetes Association. Classification and diagnosis of diabetes. Diabetes Care. 2024;47(Suppl 1):S20-S42.
2. Jain A, Chhajed M, Saluja MS, Dwivedi S, Patel B. Treatment of Diabetes with Indian Herbs and Herbal Medicines: A Review. International Journal of Pharmacy & Life Sciences. 2023 Mar 1;14(3).
3. Vyas A, Mahajan S, Dwivedi S, Koka SS, Darwhekar GN. Natural Products Targeting GLP-1: Insights into Herbal Therapy for Insulin Resistance and Beta-Cell Function. International Journal of Drug Discovery and Pharmacology. 2026 Jan 27;5(1):100001.
4. Chawla A, Chawla R, Jaggi S. Microvascular and macrovascular complications in diabetes mellitus. Indian J Endocrinol Metab. 2016;20(4):546-551.
5. Mukherjee PK. Quality Control and Evaluation of Herbal Drugs. New Delhi: Elsevier; 2019.
6. Modak M, Dixit P, Londhe J, Ghaskadbi S, Devasagayam TPA. Indian herbs and herbal drugs used for the treatment of diabetes. J Clin Biochem Nutr. 2007;40(3):163-173.
7. Szkudelski T. The mechanism of alloxan and streptozotocin action in β-cells of the rat pancreas. Physiol Res. 2001;50(6):537-546.
8. Gautam GK, Vidhyasagar G, Dwivedi SC, Dwivedi S. Anti-diabetic activity of aqueous and methanolic extract of Abutilon muticum. Int J Pharm Teach Pract. 2013;4(1):522-6.
9. Dwivedi S, Chandekar A, Tripathi A, Mishra R, Chhajed M, Sharma PK, Dubey A. In-Vivo Anti-diabetic activity of *Leonotis nepetaefolia* (L.) R. Br. Root in Alloxan induced Diabetic Model.
10. Dwivedi S, Chaturvedi P. From Tradition to Biochemical Innovation: Phytobioactive Compounds in Modern Neuropharmacology. International Journal of Pharmacology. 2026 Jan 31;22(1).

11. Nadesakanthan, S., Sanjeewa, A., Uluwaduge, I., Peiris, P., Meedin, F., & Bandara, S. (2023). Oral Health Protection Through an Ayurveda Poly Herbal Formulation- An In Vitro Study. *Journal of Ayurvedic Herbal and Integrative Medicine*, *3*(1), 1–10. https://doi.org/10.29121/jahim.v3.i1.2023.26
12. Darbar, S., Agarwal, S., Saha, S., & Banerjee Mukherjee, M. (2024). Ameliorative Effect of Polyherbal Formulation for Restoring Liver Health: A Systematic Review. *Journal of Ayurvedic Herbal and Integrative Medicine*, *4*(1), 71–83. https://doi.org/10.29121/jahim.v4.i1.2024.53
13. Malik, S. (2025). Molecular Breeding Approaches for Yield Enhancement in Legumes, SARPS: Journal of Advanced Research in Plant Science, 1(1), 8-11. https://doi.org/10.29121/SARPS.v1.i1.2025.3
14. Umakanth, N., Gogineni, R., Madan Mohan Rao, K., Revanth Reddy, B., SivaKrishna, K., Ramakrishna, Y., & Rao, M. C. (2025). Lightning–Meteorology Relationships Over Sri Lanka and Indonesia: A Machine Learning Approach. *International Journal of Engineering Science Technologies*, *9*(3), 14–26. https://doi.org/10.29121/ijoest.v9.i3.2025.691
15. Do, A. T., & Mazumdar, A. (2025). Integrating The Full Range Leadership Model In Vietnam's Pharmaceutical Industry: A Systematic Review Of Cultural And Organisational Fit. International Journal of Engineering Science Technologies, 9(3), 38–51. https://doi.org/10.29121/ijoest.v9.i3.2025.701