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Research Article

Comparative Antidiabetic Activity of Neomarica Gracilis and Codiaeum Variegatum Leaf Extracts in Streptozotocin-Induced Diabetic Rats

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This study compared the antidiabetic activity and safety of hydroalcoholic leaf extracts of Neomarica gracilis and Codiaeum variegatum, formulated individually and as a 1:1 polyherbal suspension. Leaves were extracted with 70% ethanol by Soxhlet extraction, and the extracts were evaluated for yield, phytochemical constituents, total phenolic and flavonoid contents, and suspension quality. Acute oral toxicity was assessed according to OECD 423, while diabetes was induced in male Wistar rats using streptozotocin (50 mg/kg), followed by 21 days of treatment with glibenclamide or herbal formulations at 200 mg/kg. The extract yields were 8.06% and 8.50% for N. gracilis and C. variegatum, respectively. C. variegatum showed higher total phenolic (44.92 mg GAE/g) and flavonoid (44.25 mg RE/g) contents than N. gracilis. All formulations remained physically stable during the 30-day observation period, and no mortality was observed up to 2000 mg/kg in the acute toxicity study. Among the tested preparations, the polyherbal suspension demonstrated the most favourable overall response, particularly with respect to blood glucose, lipid parameters and pancreatic histopathological changes. These findings suggest that combining N. gracilis and C. variegatum may provide enhanced antidiabetic potential compared with their individual formulations.

Keywords: Neomarica gracilis, Codiaeum variegatum, polyherbal suspension, diabetes mellitus, streptozotocin, phytochemicals, phenolics, flavonoids.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder in which persistent hyperglycaemia is accompanied by disturbances in lipid and protein metabolism. Oxidative stress and progressive tissue injury contribute to the complications associated with prolonged metabolic dysregulation. The supplied thesis identifies herbal preparations as a research area of interest and emphasizes simultaneous assessment of efficacy, safety and formulation quality. [1,2] N. gracilis and C. variegatum were selected for comparative investigation. Preliminary screening in the thesis detected alkaloids, glycosides, carbohydrates, proteins/amino acids, flavonoids and phenolic compounds in both extracts; saponins, triterpenoids and steroids were additionally detected in C. variegatum. [3,4] The study therefore moved from extract preparation and phytochemical characterization to pharmaceutical suspension development and evaluation, acute oral toxicity, streptozotocin-induced diabetes, 21-day treatment and pancreatic histopathology. The stated objective was to compare the two individual formulations and their combined preparation against a standard antidiabetic drug. [3,5]

2. MATERIALS AND METHODS

2.1 Ethical approval, animals and housing

Animal experiments were reported as approved by the Institutional Animal Ethics Committee (CCSEA approval PBRI/IAEC/21042026/008). Male Wistar albino rats aged 2–3 months were maintained under

standard conditions with a 12-h light/dark cycle, standard chow and water, after acclimatization.

2.2 Extraction and phytochemical analysis

Leaves were dried, powdered and extracted separately in a Soxhlet apparatus using 70% ethanol (ethanol:water 70:30) for approximately 6–8 h or until the siphoning solvent became colourless. Filtrates were concentrated under reduced pressure below 50°C, dried and stored at 4–8°C. Yield was calculated as extract weight divided by starting plant-material weight ×100. [5] Qualitative screening covered alkaloids, glycosides, carbohydrates, proteins/amino acids, flavonoids, tannins/phenolics, saponins, triterpenoids and steroids using standard colour/precipitation reactions. [6] TPC was determined by the Folin–Ciocalteu method using gallic acid standards (10–100 µg/mL), 10% Folin–Ciocalteu reagent and 7.5% sodium carbonate; absorbance was measured at 765 nm and expressed as mg GAE/g extract. TFC used rutin standards (10–100 µg/mL), 2% aluminium chloride and absorbance at 415 nm, expressed as mg RE/g. [6,7]

2.3 Suspension formulation and evaluation

Table 1: Composition of Herbal Suspensions Formulation

Name of Ingredient	Formulation I (*Neomarica gracilis*)	Formulation II (*Codiaeum variegatum*)	Formulation III (Polyherbal suspension)
Neomarica gracilis Leaf Extract	4.0 g	—	2.0 g
Codiaeum variegatum Leaf Extract	—	4.0 g	2.0 g
Sodium Carboxymethyl Cellulose (CMC)	2.0 g	2.0 g	2.0 g
Tween 80 (Wetting Agent)	0.1%	0.1%	0.1%
Methyl Paraben (Preservative)	0.2%	0.2%	0.2%
Sucrose	10 g	10 g	10 g
Sorbitol	5 g	5 g	5 g
Distilled Water	q.s. to 100 mL	q.s. to 100 mL	q.s. to 100 mL

The thesis formulation table specifies the above composition; the polyherbal formulation contains equal extract quantities. Physical stability was followed for 30 days at 25 ± 2°C. pH, viscosity and sedimentation volume were measured using the procedures described in the thesis. [8,9]

2.4 Acute toxicity and diabetes induction

OECD 423 acute oral toxicity testing used doses of 5, 50, 300 and 2000 mg/kg, with clinical observations during the first 30 min, first 24 h and daily for 14 days. Diabetes was induced by freshly prepared STZ (50 mg/kg) in 0.1 M citrate buffer, pH 4.5, after overnight fasting; fasting blood glucose was checked after 72 h. [10]

2.5 Experimental groups and outcomes

Group	Treatment	Dose
I	Normal control	1 mL distilled water, oral
II	Diabetic control	STZ 50 mg/kg
III	Glibenclamide	10 mg/kg/day, oral
IV	F-I: N. gracilis	200 mg/kg, oral
V	F-II: C. variegatum	200 mg/kg, oral
VI	F-III: polyherbal	200 mg/kg, oral; 1:1 extracts

Treatment continued for 21 days. Body weight and blood glucose were assessed on days 1, 3, 7, 14 and 21. Serum was obtained by centrifugation at 2500 rpm for 10 min at 25°C. TG, TC and HDL were measured; VLDL was calculated as TG/5 and LDL as TC − (HDL + VLDL). Pancreas was fixed in 10% neutral

buffered formalin, processed through graded ethanol/xylene, embedded in paraffin, sectioned at 4–5 µm and stained with H&E. Islet architecture, β-cell preservation, degeneration, necrosis and inflammatory changes were evaluated microscopically. The available thesis does not state group-wise sample size, exact inferential statistical test or p-values. Accordingly, this manuscript retains the reported mean ± variability values but does not invent statistical tests or significance levels.

3. RESULTS

3.1 Extract yield and phytochemical profile

Table 2: Percentage Yield of plant material

Plant	Material	Extract	Yield
N. gracilis	300 g	24.18 g	8.06%
C. variegatum	350 g	29.75 g	8.50%

Table 3: Phytochemical analysis of hydroalcoholic leaf extracts of N. gracilis C. variegatum

Constituent group	N. gracilis	C. variegatum
Alkaloids	+	+
Glycosides	+	+
Carbohydrates	+	+
Proteins/amino acids	+	+
Flavonoids	+	+
Tannins/phenolics	+	+
Saponins	−	+
Triterpenoids/steroids	−	+

C. variegatum yielded slightly more extract. Both extracts shared the major screened constituent groups, whereas saponins and triterpenoid/steroidal reactions were additionally positive in C. variegatum.

3.2 Quantitative phytochemicals and formulation quality

Table 4: Total Phenolic Content (TPC) and Total Flavonoids content (TFC) estimation in hydroalcoholic leaf extracts of N. gracilis and C. variegatum

Extract	TPC (mg GAE/g)	TFC (mg RE/g)
N. gracilis	26.52	25.25
C. variegatum	44.92	44.25

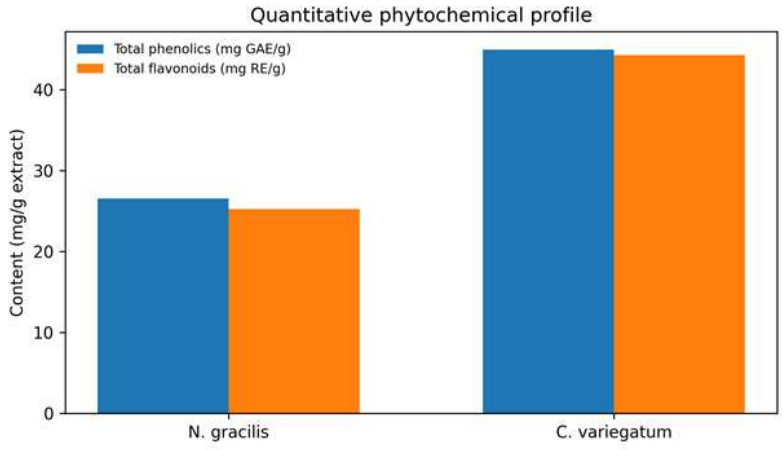

Figure 1. Total phenolic and flavonoid contents.

The gallic-acid calibration curve reported in the thesis was y = 0.0025x + 0.1107 (R^2 = 0.9884). C. variegatum showed the higher phenolic and flavonoid values.

Table 5: pH, Viscosity, Sedimentation volume and Spreadability test

Parameter	F-I	F-II	F-III
Stability	Stable, 30 d	Stable, 30 d	Highly stable, 30 d
pH	6.4 ± 0.03	6.5 ± 0.02	6.3 ± 0.04
Viscosity (cP)	850 ± 15	780 ± 12	880 ± 18
Sedimentation F	0.78 ± 0.02	0.74 ± 0.03	0.82 ± 0.02

All formulations remained stable for 30 days; F-III had the highest sedimentation-volume value and viscosity.

3.3 Acute toxicity and in-vivo response

Dose	Observed outcome in all formulations
5 mg/kg	No mortality; general observations normal
50 mg/kg	No mortality; general observations normal
300 mg/kg	No mortality; mild changes in selected observations
2000 mg/kg	No mortality; mild changes in selected observations

No mortality was observed at any tested dose. The thesis interpreted the findings as an LD_{50} greater than 2000 mg/kg, while noting mild changes at the two higher doses.

Table 6: Effect of Formulation I (*Neomarica gracilis*) Formulation II (*Codiaeum variegatum*) and Formulation III (polyherbal combination) on Body weight (gm) of the rats

Body weight (gm)						
Groups	**Treatments**	**1 day**	**3 days**	**7 days**	**14 days**	**21 days**
Group 1	Normal control	244.10 ± 0.251	246.05 ± 0.243	241.20 ± 0.219	239.10 ± 0.205	238.05 ± 0.238
Group II	Diabetic control (Streptozocin) 50 mg/kg	253.85 ± 0.198	258.10 ± 0.176	263.25 ± 0.185	268.60 ± 0.220	272.45 ± 0.240
Group III	Glibenclamide (10 mg/kg)	246.80 ± 0.230	248.45 ± 0.215	245.10 ± 0.190	245.75 ± 0.200	242.20 ± 0.210
Group IV	Formulation I (*Neomarica gracilis*)	259.75 ± 0.220	263.30 ± 0.250	258.90 ± 0.310	263.20 ± 0.180	261.00 ± 0.230
Group V	Formulation II (*Codiaeum variegatum*)	253.60 ± 0.240	257.80 ± 0.200	253.50 ± 0.160	256.40 ± 0.150	255.10 ± 0.210
Group VI	Formulation III (Polyherbal suspension)	249.90 ± 0.230	253.40 ± 0.210	249.00 ± 0.180	251.60 ± 0.190	248.20 ± 0.250

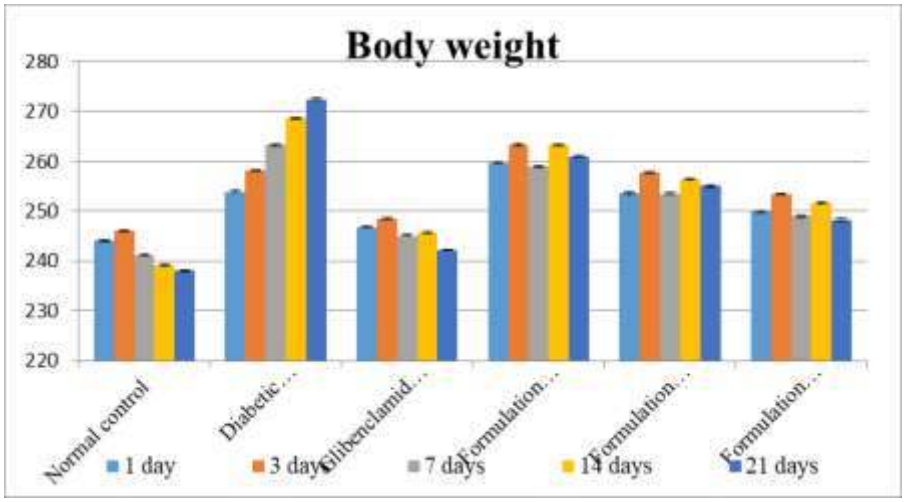

Figure 2. Effect of Formulation I (Neomarica Gracilis) Formulation II (Codiaeum Variegatum) And Formulation III (Polyherbal Combination) On Body Weight of The Rats

The diabetic control showed a progressive increase in body weight. F-III remained closer to the normal and standard groups than the individual herbal formulations. [11,12]

Table 7: Effect of test samples of Formulations on Blood Glucose Level in experimental rats

Blood Glucose Level (gms)						
Groups	**Treatments**	**1 day**	**3 days**	**7 days**	**14 days**	**21 days**
Group 1	Normal control	87.09 ± 0.194	86.47 ± 0.210	88.99 ± 0.336	87.99 ± 0.196	89.78 ± 0.271
Group II	Diabetic control (Streptozocin) 50 mg/kg	156.89 ± 0.181	179.89 ± 0.262	199.78 ± 0.240	239.67 ± 0.308	258.91 ± 0.230
Group III	Glibenclamide (10 mg/kg)	94.07 ± 0.228	93.78 ± 0.106	95.89 ± 0.498	92.91 ± 0.289	95.80 ± 0.095
Group IV	Formulation I (*Neomarica gracilis*)	105.69 ± 0.097	103.91 ± 0.603	108.99 ± 0.203	112.91 ± 0.237	109.47 ± 0.210
Group V	Formulation II (*Codiaeum variegatum*)	110.99 ± 0.371	112.79 ± 0.275	109.45 ± 0.222	118.23 ± 0.258	116.79 ± 0.360
Group VI	Formulation III (Polyherbal suspension)	99.85 ± 0.220	98.10 ± 0.200	99.05 ± 0.180	98.20 ± 0.250	102.05 ± 0.210

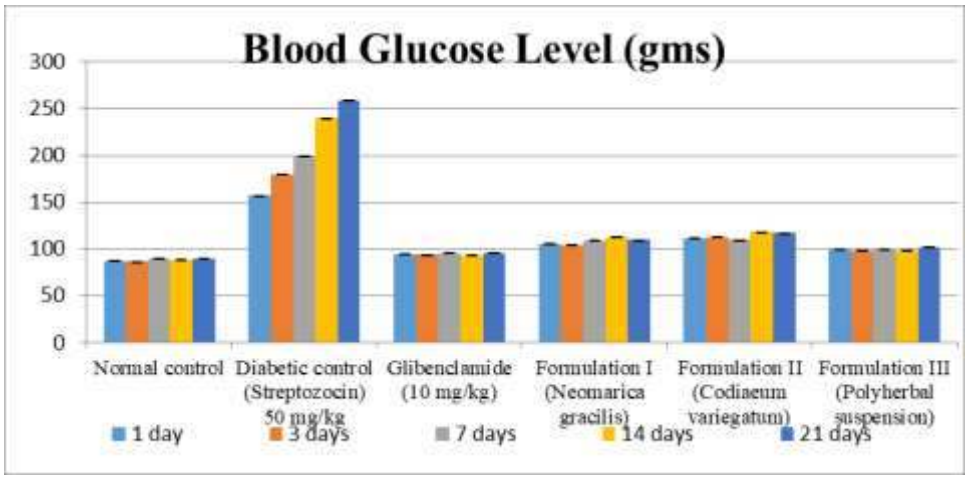

Fig 3: Effect of Formulations on Blood Glucose Level of the rats

The diabetic control increased progressively, whereas all herbal treatments restrained the rise. F-III showed the lowest herbal values across the reported time points and remained comparatively close to glibenclamide. The source table labels the unit as "gms"; this manuscript deliberately does not substitute a conventional unit without source confirmation.

3.4 Biochemical and histological findings

Table 8: Effect of test samples of Formulations on Biochemical Parameters in experimental rats

Treatment Groups	TG (g/L)	TC (g/L)	HDL (g/L)	LDL (g/L)
Normal control	58.90 ± 1.18	80.45 ± 0.92	46.10 ± 0.30	22.90 ± 0.78
Diabetic control (Streptozocin) 50 mg/kg	172.80 ± 2.10	183.90 ± 2.65	19.85 ± 0.58	118.40 ± 1.70
Glibenclamide (10 mg/kg)	63.20 ± 1.50	84.10 ± 1.45	41.10 ± 0.35	27.40 ± 0.92
Formulation I (*Neomarica gracilis*)	79.40 ± 1.88	99.20 ± 2.02	22.20 ± 0.44	46.10 ± 1.20
Formulation II (*Codiaeum variegatum*)	74.60 ± 1.92	96.10 ± 2.08	27.80 ± 0.50	39.10 ± 1.22
Formulation III (Polyherbal suspension)	68.80 ± 1.55	91.20 ± 1.48	34.90 ± 0.40	36.20 ± 1.05

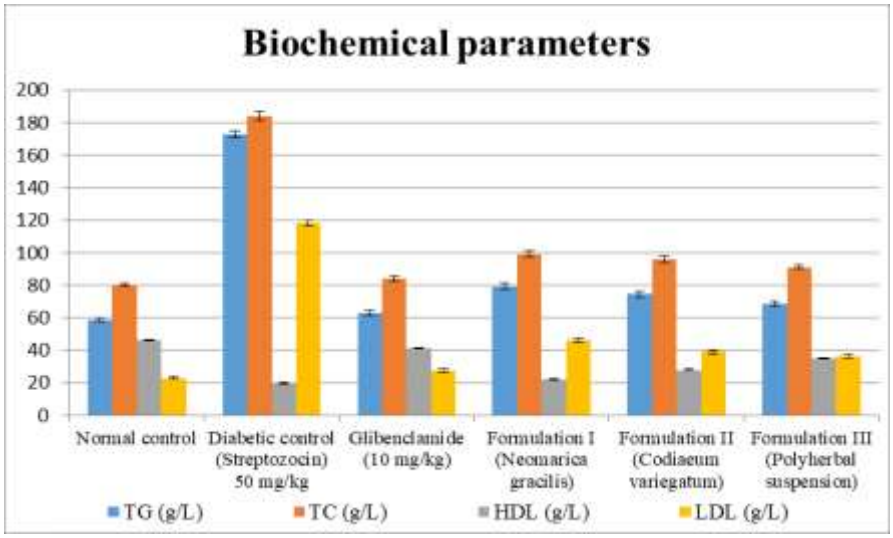

Fig4: Graphical representation of effect of Formulations on Biochemical Parameters of the rats

Diabetes produced an adverse lipid pattern. F-III showed the strongest overall improvement among the herbal groups, with lower TG, TC and LDL and higher HDL than the individual formulations.

3.4 Histopathological Examination of Pancreas

Pancreatic microscopy showed preserved islets in the normal group, marked degeneration in the diabetic control, substantial restoration after glibenclamide, moderate improvement with F-I, better preservation with F-II and the most prominent herbal architectural recovery with F-III.

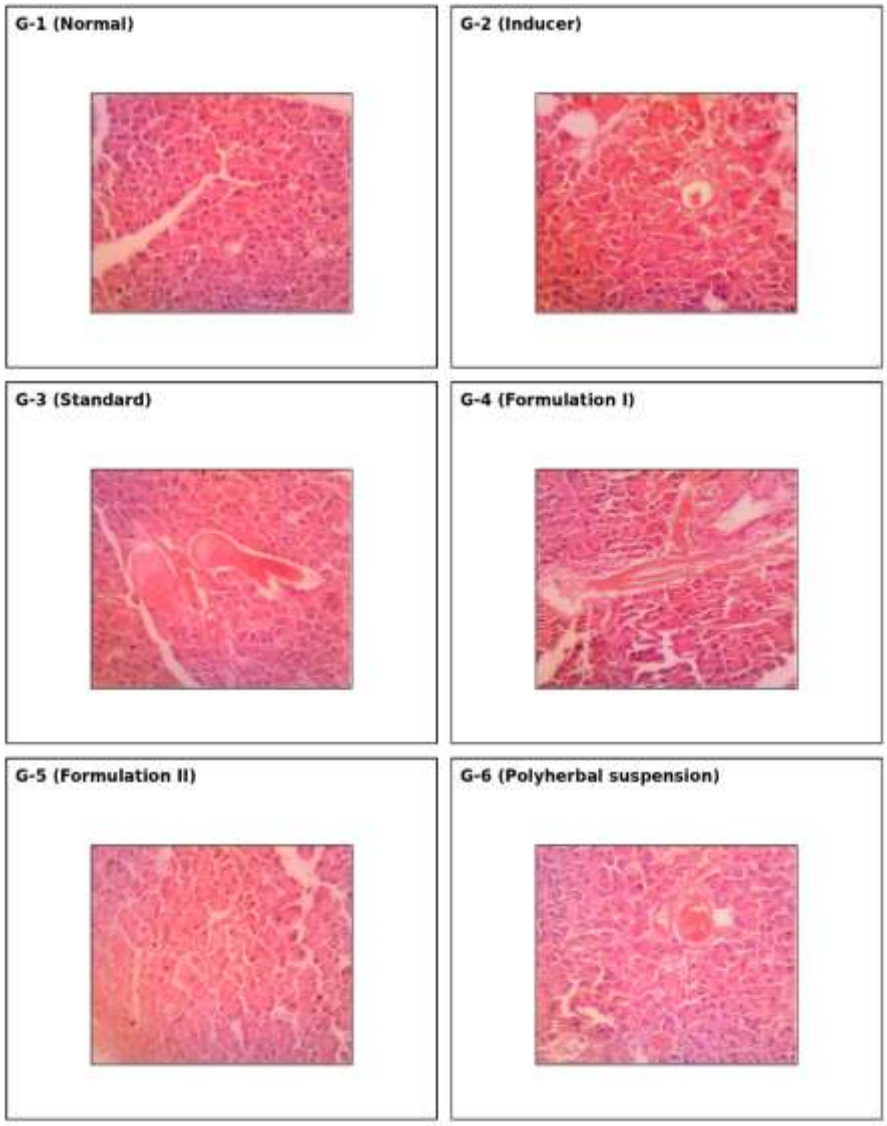

Figure 5: Histopathological Examination of Pancreas

Pancreatic microscopy showed preserved islets in the normal group, marked degeneration in the diabetic control, substantial restoration after glibenclamide, moderate improvement with F-I, better preservation with F-II and the most prominent herbal architectural recovery with F-III.

G-1 (Normal): Normal pancreatic architecture with well-defined and intact islets of Langerhans and normal cellular arrangement.

G-2 (Inducer): Marked degeneration and disorganization of the islets of Langerhans with noticeable pancreatic tissue alterations.

G-3 (Standard): Considerable restoration of pancreatic architecture with improved organization of the islets and reduced degenerative changes.

G-4 (Formulation I): Moderate improvement in islet structure with reduced cellular degeneration compared with the inducer group.

G-5 (Formulation II): Improved pancreatic architecture with better preservation and organization of the islets.

G-6 (Polyherbal suspension): Greater preservation of pancreatic architecture and islet organization, showing the most prominent improvement among the herbal treatment groups.

Overall, the histological findings indicate that the treatments, particularly the polyherbal suspension, provided protective effects against diabetes-induced pancreatic damage

DISCUSSION

The findings form a consistent comparative pattern. C. variegatum had both a slightly higher extraction yield and substantially greater measured phenolic/flavonoid content. This does not by itself establish causality, but it provides a plausible compositional distinction between the two extracts. The broader qualitative profile of C. variegatum, including saponins and triterpenoid/steroidal reactions, further differentiates it from N. gracilis. [3,4,6,7] Formulation performance was satisfactory across all preparations. The polyherbal suspension did not show evidence of impaired physical stability and had the highest reported sedimentation volume and viscosity. The combination therefore retained acceptable pharmaceutical characteristics while allowing the pharmacological effects of the two extracts to be evaluated together. [8,9] The in-vivo data provide the strongest evidence for the comparative advantage of F-III. The diabetic-control glucose value increased from 156.89 to 258.91 across the study, while F-III remained close to 100–102 in the reported numerical scale. F-I and F-II also showed clear improvement but were less consistent with the standard group. The lipid findings followed the same direction, with F-III showing the best herbal HDL value and lower TG, TC and LDL than the individual preparations. [11,12,13] The pancreatic findings provide structural support for the biochemical pattern. Greater preservation of islet organization in F-III suggests that the combined preparation was associated with less apparent STZ-related pancreatic damage than the individual herbal formulations. The thesis attributes the stronger combined effect to complementary or synergistic phytoconstituent actions; this remains a hypothesis because the present work did not directly measure molecular mechanisms, insulin secretion or individual active constituents. [12,13] The findings demonstrate that all tested formulations possessed antidiabetic potential, with Formulation III (polyherbal suspension) showing superior overall activity. Its better performance in controlling body weight, reducing hyperglycemia, and improving lipid abnormalities suggests a possible synergistic or complementary effect of *Neomarica gracilis* and *Codiaeum variegatum*. These findings support the potential use of the combined formulation for further investigation as a herbal antidiabetic preparation.

CONCLUSION

N. gracilis and C. variegatum hydroalcoholic leaf extracts were successfully formulated as individual and combined suspensions. C. variegatum showed higher phenolic and flavonoid contents, while all formulations demonstrated acceptable short-term physical stability and no mortality during acute toxicity testing up to 2000 mg/kg. In streptozotocin-induced diabetic rats, all herbal preparations improved glucose and lipid outcomes, with the 1:1 polyherbal suspension showing the strongest overall response and the most favourable pancreatic histological appearance. Overall, the study supports the potential of the *Neomarica gracilis–Codiaeum variegatum* polyherbal formulation as a promising herbal antidiabetic preparation. However, further studies involving detailed mechanistic investigations, long-term toxicity assessment, dose optimization, and clinical evaluation are required to establish its therapeutic efficacy and safety.

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