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Investigation of Phytochemical Screening, *in vivo* Antidiabetic, and Neuroprotective Potential of *Stephania glabra* L. Tuber and *Clerodendrum phlomidis* L. Leaves

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Abstract

Diabetes, depression, and Alzheimer's disease share an intricate relationship, wherein chronic metabolic dysregulation serves as a common underlying factor contributing to both neurodegenerative and metabolic dysregulation outcomes. The tuber of *Stephania glabra* L. and the leaves of *Clerodendrum phlomidis* L. are traditionally recognised for their rich phytochemical composition and are believed to possess antidiabetic, antidepressant, and anti-Alzheimer's potential. Therefore, the present investigation focused on subacute toxicity studies (500–2000 mg/kg) confirm that the ethanolic tuber extracts of *S. glabra* L. and leaves of *C. phlomidis* L. are non-toxic and harmless at 100, 200, and 500 mg/kg, exhibiting notable antidiabetic activity, evidenced by improved glycemic control and enhanced restoration of endocrine pancreatic Islets. The plant extracts demonstrated anti-Alzheimer's activity, as evidenced by Cognitive assessment tools, including the Morris Water Maze and Y-Maze. Antidepressant potential was confirmed by a significant reduction of Stagnation (Immobility Time) in the Tail Suspension and Porsolt Swim Test. The findings indicate that the extracts demonstrated notable antidiabetic, antidepressant, and anti-Alzheimer's effects, with increasing efficacy observed at 100, 200, and 400 mg/kg. Treatment improved glycemic control, cognitive performance, and reduced depressive-like behaviours. These findings highlight their therapeutic promise in metabolic and neurodegenerative disorders.

Major Findings: Ethanolic extracts of *S. glabra* tuber and *C. phlomidis* leaves were phytochemically rich. The extracts significantly reduced blood glucose levels and improved cognitive behaviors, indicating therapeutic potential against neuroendocrinal disorders.

Keywords: Alzheimer's, Antidepressant, Diabetes, Pancreases and Streptozotocin

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1. Introduction

Type 2 Diabetes Mellitus (T2DM) remains a significant public health concern, with its complications severely affecting both patient well-being and healthcare resources. According to WHO estimates for 2021, the global number of people with Type 2 Diabetes Mellitus (T2DM) was 592 million, and this figure is projected to rise by 10% by 2045. This increase is expected to lead to a surge in serious complications, including cognitive impairments, mood and psychiatric disorders, cardiovascular issues, kidney dysfunction, and vision impairment. India, with over 438 million affected individuals, ranks among the highest in diabetes prevalence, reflecting a critical need for improved early diagnosis, preventive strategies, and equitable access to long-term care services to reduce disease progression and associated burdens[1]. *S. glabra* L. is a medicinal plant known for its diverse therapeutic potential. It has demonstrated significant antidiabetic activity, aiding in the regulation of blood glucose levels[2,3]. *C. phlomidis* L. are well-recognised medicinal plants with substantial therapeutic value, particularly in the management of neuroendocrine complications. The selected plant parts exhibit strong antidiabetic activity by enhancing insulin regulation, promoting β-cell regeneration, and

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improving glucose metabolism[4]. Their antidepressant potential is evident through reduced immobility time and modulation of key neurotransmitters. At the same time, their anti-Alzheimer's effects are demonstrated by improved escape latency, enhanced spontaneous alternation, and strong antiamyloid neuroprotection. Traditionally, both plants have been used to treat neuroendocrine disorders, anxiety, wounds, liver complications, allergic reactions, microbial infections, oxidative stress, and gastrointestinal disturbances[5,6]. The tuber of *S. glabra* L. is particularly rich in isoquinoline alkaloids and polyphenolic compounds that stimulate insulin secretion, enhance peripheral glucose uptake, and mitigate hyperglycemia-induced metabolic disturbances. Beyond metabolic benefits, the tuber also provides notable neuroprotective, antidepressant, and anti-Alzheimer's effects that support cognitive performance, neuronal stability, and emotional balance, establishing *S. glabra* L. as a promising phytotherapeutics candidate for integrated metabolic and neurological care. Likewise, *C. phlomidis* L. has been widely studied for its antidiabetic, neuroprotective, nephroprotective, antioxidant, and endocrine properties. Its leaves contain abundant flavonoids, terpenoids, and phenolic compounds that regulate blood glucose levels, reduce diabetic complications, and enhance neuronal function[7,8]. These bioactive constituents also exhibit strong cognitive-enhancing and neuroprotective effects, helping to preserve neuronal integrity and potentially delay the progression of Alzheimer's disease. Collectively, *S. glabra* L. and *C. phlomidis* L. represent valuable natural resources with significant potential for the development of plant-based therapeutic interventions for complex metabolic and neurological disorders[9].

The selected plant parts are notably rich in isoquinoline alkaloids, including berberine, palmatine, jatrorrhizine, magnoflorine, cycleanine, and tetrandrine, which have been reported to exhibit antidiabetic, neuroprotective, and antidepressant activities through mechanisms such as modulation of glucose metabolism, inhibition of acetylcholinesterase, and attenuation of neuroinflammation[10,11]. Moreover, flavonoids like quercetin, kaempferol, apigenin, and rutin are present and recognised for their strong antioxidant and anti-inflammatory effects, which are instrumental in reducing oxidative stress, a key contributor to the development of both diabetes and Alzheimer's disease. The plant also contains significant levels of phytosterols (stigmasterol and β-sitosterol) and triterpenoids (lupeol and β-amyrin), compounds that contribute to its anti-inflammatory and lipid-lowering effects. Furthermore, secondary metabolites such as 1-chloroundecane, 1-hexadecanol, and phenolic derivatives, including 2,4-di-tert-butylphenol, 3,5-Bis(tert-butyl) and 4-[1-(1-methyl-indol-3-yl) ethyl] phenol, enhance the plant's pharmacological profile through their potential antimicrobial, antioxidant, and neuroprotective activities. Collectively, the synergistic interaction of these phytochemicals underpins the traditional use of the tuber of *S. glabra* and *C. phlomidis* leaves highlights its potential as a new therapeutic agent for metabolic and neurobehavioral disorders[12,13]. This study investigates the therapeutic potential of ethanolic extracts from *S. glabra* L. tuber and *C. phlomidis* L. leaves for the management of diabetes, depression, and Alzheimer's disease. Antidepressant activity was demonstrated through behavioural models, like the Tail Suspension Test (TST) and Forced Swim Test (FST), indicating modulation of central monoaminergic pathways. Furthermore, the anti-Alzheimer potential was confirmed via the Morris water maze and Y-maze tests, which revealed evidence of enhanced spatial learning and memory retention. Subacute toxicity studies established the safety profile of the extracts at therapeutic doses, with no observable adverse effects[14,15]. These findings not only validate traditional medicinal claims but also advance the pharmacological understanding of these botanicals as promising candidates for the integrated management of metabolic and neurodegenerative disorders.

2. Materials and Methods

2.1 Collection

Tuber of *S. glabra* L. and *C. phlomidis* L. leaves were poised from the Nallamala, Tirupati, Andhra Pradesh, for the present study. The selected plant parts were initially shade-dried for 15 days at a temperature (35+2°C), following which representative specimens were mounted on herbarium sheets and labelled with relevant information, including habitat, collection site, and floral characteristics. The specimens were submitted to the Department of Botany, Sri Venkateswara University, Tirupati, for taxonomic authentication, voucher No. 1198 and 0964 confirmed by an expert botanist. Subsequently, the authenticated plant parts were thoroughly washed, shade-dried for an additional two weeks, and then pulverised into a fine powder for further phytochemical and pharmacological analysis.

2.2 Phytochemical Extraction

Powdered tuber of *S. glabra* L. and leaves of *C. phlomidis* L. (500 g) were subjected to cold maceration with ethanol in a 1:2 (w/v) ratio for a period of seven days at room temperature with occasional stirring. After extraction, the mixtures were passed through Whatman No. 1 filter paper, and the resulting solutions were concentrated at 40°C under reduced pressure using a rotary evaporator to produce semi-solid residues[16,17]. The extracts were labelled as ethanolic tuber extract of *S. glabra* L. (EETSG) and ethanolic leaves extract of *C. phlomidis* (EELCP), respectively. Both extracts were stored in a vacuum desiccator at a temperature (35+2°C) until further preliminary phytochemical screening was performed following standard qualitative procedures. The ethanolic plant parts extracts were then evaluated for *in vivo* antidiabetic activity, along with cognitive impairment and antidepressant activity.

2.3 Phytochemical Screening

Qualitative phytochemical assessment was performed using standard qualitative methods to identify major bioactive constituents. The analysis revealed the presence of flavonoids, alkaloids, phytosterols, tannins, carbohydrates, saponins, and phenolic compounds in the extracts[18,19].

2.4 Experimental Animals

Albino Rats (150-180g) from Mahavir Enterprises, Hyderabad, were acclimatised for 48 hours before experimentation. They were housed under a 12-h light and dark cycle and given a regular diet. The study of approval was permitted by the institutional animal ethics committee of Hindu College of Pharmacy, Guntur, approval No. IAEC-HOCP/2022/06.

2.5 Chronic *in vivo* Antidiabetic Study

To evaluate the antidiabetic activity, albino Wister rats (Male-180-200g) were weighed and divided into nine groups (n = 6). The selected ethanolic extracts were administered for 28 days. Diabetes was induced in groups II to IX by a single Intravenous (IV) injection of Streptozotocin (55 mg/kg, *i,p*). Group I assisted as the normal control and received distilled water, while Group II assisted as the negative diabetic control. Groups III to V received ethanolic tuber extract of *S. glabra* L. (EETSG) at doses of 100, 200, and 400 mg/kg, respectively, and groups VI to VIII received ethanolic extract of *C. phlomidis* leaves (EELCP) at the same doses. Group IX received Glibenclamide (2.5 mg/kg) as the standard antidiabetic drug. The blood glucose levels are detected by a semi-auto analyser at 340nm[20,21].

2.6 Chronic *in vivo* Antidepressant Activity

To evaluate the antidepressant activity, albino Wister rats (Male-180-200 g) were weighed and divided into nine groups (n = 6). Depression was induced in Groups II to IX using reserpine (0.2 mg/kg, *I.p.*) for 15 days to establish an animal model of depression. Group III to Group V received *S. glabra* L. tuber ethanolic extract (100, 200 and 400 mg/kg) and Group VI to XIII received *C. phlomidis* L. leaves ethanolic extract at the same doses, while Group IX received imipramine (15 mg/kg/day, orally) as the standard reference drug. The evaluation of immobility time in the TST and the forced swim test was performed to determine the antidepressant-like effects of the test extracts[22].

2.7 Chronic *in vivo* Anti-Alzheimer's Activity

For the assessment of anti-Alzheimer activity, all rats were first induced with cognitive impairment by administering Aluminum chloride (150 mg/kg orally) for 28 days. Group III to group V received *S. glabra* L. tuber ethanolic extract (100, 200 and 400 mg/kg) and group VI to XIII received *C. phlomidis* L. leaves ethanolic extract at the same doses. Group IX received Piracetam (200 mg/kg/day, orally) as the standard drug. Cognitive function was evaluated using the Morris Water Maze. and Y-Maze test[23].

2.8 Chronic *in vivo* Pancreatic Histopathological Study

Pancreatic tissues were preserved in 10% formalin, embedded in paraffin wax, and then sectioned into slices of 4 to 5 µm thickness. Tissue sections were deparaffinized using xylene and rehydrated through a series of graded alcohols and stained with haematoxylin (0.5%) and eosin (1%), then dehydrated again in ascending alcohols (70%) and cleared in xylene solution. The slides were mounted and examined under a light microscope (100x) for histopathological changes[24,25].

2.9 Statistical Analysis

All values are presented as mean ± SEM, and data were analysed by one-way ANOVA. All experiments were conducted in triplicate to ensure reliability.

3. Results

3.1 Phytochemical Extraction and Screening

The phytochemical ethanolic extraction of *S. glabra* L. tuber 25% and *C. phlomidis* L. leaves 30%. These extracts were subjected to phytochemical screening, analytical profiling of bioactive constituents, and *in vivo* evaluation for antidiabetic, antidepressant and anti-Alzheimer activities. The classification of identified phytochemical groups is presented in Table 1.

3.2 Subacute Toxicity Study

The test extracts were administered orally to female Wistar albino rats at doses ranging from 100 mg/kg to 2000 mg/kg. No mortality or adverse effects on behavioural, psychological, physiological, respiratory, excretory, or neural functions were observed on days 1, 7, and 14 [26]. The lethal dose 50% (LD_{50}) was estimated to be greater than 2000 mg/kg, indicating the extracts are relatively non-toxic and well-tolerated, as presented in Table 2.

Table 1. Phytochemical screening of ethanolic test extracts

Category	Tuber of *S. glabra*	Leaves of *C. phlomidis*
Carbohydrates	YES	NO
Alkaloids	YES	YES
Flavonoids	YES	YES
Proteins and amino acids	YES	YES
Terpenoids	YES	YES
Phenolic compounds	YES	YES
Tannis	YES	NO
Phytosterols	YES	YES

3.3 Chronic *in vivo* Antidiabetic Study

The chronic *in vivo* antidiabetic study, *S. glabra* L. tuber ethanolic extract (EETSG), demonstrated strong antidiabetic potency, as evidenced by significant dose-dependent reductions in blood glucose levels and reduced blood glucose by 39.2%, 54.0%, and 61.1% at doses of 100, 200, and 400 mg/kg, respectively. Similarly, *C. phlomidis* leaves Ethanolic Extract (EELCP) produced reductions of 41%, 55%, and 68% at the same dose levels. Notably, 400 mg/kg nearly matched the efficacy of the standard antidiabetic drug Glibenclamide (2.5 mg/kg), which achieved a 71% reduction. These results confirmed that ethanolic extracts have antidiabetic properties[27] presented in Table 3.

3.4 Chronic *in vivo* Antidepressant Activity

The chronic *in vivo* antidepressant study, *S. glabra* L. tuber and *C. phlomidis* leaves ethanolic extracts administered at a dose of 400 mg/kg, demonstrated significant antidepressant-like activity in validated behavioural models[28]. Both

Table 2. Sub-acute oral toxicity studies of ethanolic test extracts

Extracts	Dose (mg/kg)	Changes	No. of days observed			Remarks	Deaths
			1	7	14		
Ethanolic tuber of *S. glabra* L.	100	1. Behavioural 2. Psychological 3. Physiological 4. Respiratory 5. Excretory 6. Neural	--	--	--	Absent	No Death
	200		--	--	--	Absent	No Death
	400		--	--	--	Absent	No Death
	800		--	--	--	Absent	No Death
	1000		--	--	✓	Absent	No Death
	2000		--	--	✓	Absent	No Death
Ethanolic leaves of *C. phlomidis* L.	100	Behavioural 2. Psychological 3. Physiological 4. Respiratory 5. Excretory 6. Neural	--	--	--	Absent	No Death
	200		--	--	--	Absent	No Death
	400		--	--	--	Absent	No Death
	800		--	--	--	Absent	No Death
	1000		--	--	✓	Absent	No Death
	2000		--	✓	✓	Absent	No Death

Table 3. Chronic Antidiabetic study of ethanolic test extracts by the Streptozotocin method

Groups	Blood Glucose Levels (mg/dl)					Glycemic Index
	1st day	7th day	14th day	21st day	28th day	
Normal Control (Water)	96 ± 0.2	97 ± 0.3	97 ± 0.2	96 ± 0.6	97 ± 0.1	--
Diabetic Control (Water)	250 ± 0.1	277 ± 0.2	311 ± 0.3	345 ± 0.1	382 ± 0.2	- 52
EETSG 100 mg/kg	270 ± 0.4	246 ± 0.1	220 ± 0.3*	192 ± 0.2*	160 ± 0.1**	+ 40
EETSG 200 mg/kg	250 ± 0.2	219 ± 0.1*	185 ± 0.2**	148 ± 0.1**	110 ± 0.9**	+ 56
EETSG 400 mg/kg	272 ± 0.1	220 ± 0.3*	176 ± 0.5**	134 ± 0.2**	98 ± 0.1**	+ 63
EELCP 100 mg/kg	285 ± 0.2	260 ± 0.2	231 ± 0.3*	204 ± 0.1*	168 ± 0.3*	+ 41
EELCP 200 mg/kg	239 ± 0.1*	201 ± 0.1**	169 ± 0.2**	136 ± 0.1*	106 ± 0.1**	+ 55
EELCP 400 mg/kg	338 ± 0.1*	276 ± 0.1**	219 ± 0.2**	161 ± 0.2**	108 ± 0.1**	+ 68
Glibenclamide 2.5 mg/kg	355 ± 0.1*	303 ± 0.2**	228 ± 0.1**	161 ± 0.1**	101 ± 0.2**	+ 71

All values are mean ± SEM (n = 6), with significance determined by one-way ANOVA and Tukey's post-hoc test (*p<0.05, **p<0.01).

Table 4. Chronic antidepressant study of test extracts by TST

Groups	Duration of Immobility (Seconds)					Δ Immobility
	1st day	7th day	14th day	21st day	28th day	
Normal control	155 ± 0.2	156 ± 0.8	156 ± 0.1	153 ± 0.8	153 ± 0.4	--
Negative control	255 ± 0.7	270 ± 0.7	287 ± 0.1	305 ± 0.4	322 ± 09	--
EETSG 100 mg/kg	256 ± 0.1	240 ± 0.1	225 ± 0.9*	209 ± 0.6*	194 ± 0.6*	65%
EETSG 200 mg/kg	266 ± 0.6	247 ± 0.7*	223 ± 0.1	204 ± 0.7**	185 ± 0.4**	74%
EETSG 400 mg/kg	251 ± 0.7	231 ± 0.3*	211 ± 0.5	192 ± 0.3**	171 ± 0.8**	88%
EELCP 100 mg/kg	258 ± 0.5	241 ± 0.7	225 ± 0.3*	209 ± 0.7*	193 ± 0.7*	66%
EELCP 200 mg/kg	254 ± 0.6	235 ± 0.6*	223 ± 0.4*	202 ± 0.6*	183 ± 0.1**	75%
EELCP 400 mg/kg	251 ± 0.1	232 ± 0.2*	212 ± 0.7*	193 ± 0.4**	174 ± 0.3**	85%
Imipramine 15 mg/kg	256 ± 0.3	233 ± 0.4*	210 ± 0.7*	187 ± 0.9**	163 ± 0.2**	97%

All values are mean ± SEM (n = 6), with significance determined by one-way ANOVA and Tukey's post-hoc test (*p<0.05, **p<0.01).

Table 5. Chronic antidepressant study of test extracts by Forced Swim Test (FST)

Groups	Duration of Immobility (Seconds)					Δ Immobility
	1st day	7th day	14th day	21st day	28th day	
Normal control	146 ± 0.2	145 ± 0.2	144 ± 0.4	145 ± 0.4	143 ± 0.1	--
Negative control	246 ± 0.3	257 ± 0.1	269 ± 0.2	280 ± 0.7	296 ± 0.3	--
EETSG 100 mg/kg	252 ± 0.4	233 ± 0.6	212± 0.1	194 ± 0.9*	178 ± 0.4*	66%
EETSG 200 mg/kg	248 ± 0.2	229 ± 0.2	209 ± 0.7*	187 ± 0.4*	168 ± 0.8**	76%
EETSG 400 mg/kg	241 ± 0.1	222 ± 0.3	202 ± 0.9**	180 ± 0.3*	161 ± 0.9**	83%
EELCP 100 mg/kg	244 ± 0.4	228 ± 0.2	213 ± 0.7	197 ± 0.4*	180 ± 0.9*	64%
EELCP 200 mg/kg	243± 0.3	224 ± 0.6*	206 ± 0.6*	188 ± 0.1**	169 ± 0.8**	75%
EELCP 400 mg/kg	247 ± 0.2	228 ± 0.9*	207 ± 0.3**	185 ± 0.6**	161 ± 0.4**	83%
Imipramine 15 mg/kg	242 ± 0.6	219 ± 0.7*	194 ± 0.4**	172 ± 0.7**	150 ± 0.6**	97%

All values are mean ± SEM (n = 6), with significance determined by one-way ANOVA and Tukey's post-hoc test (*p<0.05, **p<0.01).

ethanolic test extracts reduced immobility time in the TST to 88% and 85%, compared to 97% observed with the standard antidepressant imipramine (10 mg/kg). Similarly, in the Forced Swim Test (FST), immobility was reduced to 83% for both extracts, closely matching the effect of imipramine (10 mg/kg). These effects were found to be dose and time-dependent, presented in Tables 4 and 5.

3.5 Chronic *in vivo* Anti-Alzheimer's Activity

The chronic anti-Alzheimer's study showed that in the Morris Water Maze test, the *S. glabra* L. tuber ethanolic extract reduced escape latency at 200 and 400 mg/kg to 15 and 11 seconds, while the *C. phlomidis* L. leaves ethanolic extract reduced it to 16 and 12 s at the same doses, indicating significant improvement. Both extracts at 400 mg/kg produced effects close to the standard piracetam 9 s. In the Y-Maze test, both extracts at 200 and 400 mg/kg increased spontaneous alternation to 75% and 87% and 75% and 87%, respectively, demonstrating dose-dependent memory enhancement[29]. At 400 mg/kg, both extracts showed performance comparable to piracetam (93%), as presented in Tables 6 and 7.

3.6 Chronic *in vivo* Pancreatic Histopathological Study

Histopathological evaluation of endocrine pancreatic tissues demonstrated a significant reduction of islet size and number, along with prominent necrosis in diabetic control. In contrast, treatment with tuber of *S. glabra* (EETSG) and *C. phlomidis* (EELCP) leaves ethanolic extracts produced marked, dose-dependent β-cell restoration. At 100 mg/kg, both extracts induced mild

Table 6. Chronic Antialzheimer' s study of test extracts by the Morris Water Maze test

Groups	Escape Latency Time (Seconds)					Latency Index
	1st day	7th day	14th day	21st day	28th day	
Normal control	25 ± 0.8	26 ± 0.9	24 ± 0.6	25 ± 0.9	24 ± 0.4	--
Negative control	68 ± 0.6	58 ± 0.4	67 ± 0.4	79 ± 0.4	92 ± 0.7	--
EETSG 100 mg/kg	55 ± 0.4	45 ± 0.6	34 ± 0.6	26 ± 0.9*	19 ± 0.4*	65%
EETSG 200 mg/kg	57 ± 0.2	47 ± 0.2	36 ± 0.7	25 ± 0.4*	15 ± 0.8**	73%
EETSG 400 mg/kg	56 ± 0.9	44 ± 0.3	33 ± 0.9*	23 ± 0.3**	11 ± 0.9**	80%
EELCP 100 mg/kg	51 ± 0.7	42 ± 0.6	34 ± 0.7	26 ± 0.7*	17 ± 0.6*	66%
EELCP 200 mg/kg	57 ± 0.4	47 ± 0.7	35 ± 0.2*	22 ± 0.6*	16 ± 0.7**	71%
EELCP 400 mg/kg	57 ± 0.3	45 ± 0.8	32 ± 0.6	19 ± 0.4	12 ± 0.6**	78%
Piracetam 200 mg/kg	54 ± 0.9	46 ± 0.3	33 ± 0.4*	19 ± 0.8**	09 ± 0.3**	83%

All values are mean ± SEM (n = 6), with significance determined by one-way ANOVA and Tukey's post-hoc test (*p<0.0 5, **p<0.01).

Table 7. Chronic Antialzheimer' s study of ethanolic extracts by the Y-Maze test

Group	Spontaneous Alternation Performance Percentage					Memory index
	1st day	7th day	14th day	21st day	28th day	
Normal control	90 ± 0.8	91 ± 0.9	78 ± 0.6	83 ± 0.5	91 ± 0.5	--
Negative control	46 ± 0.7	44 ± 0.7	38 ± 0.5	30 ± 0.7	25 ± 0.8	- 36%
EETSG 100 mg/kg	44 ± 0.7	48 ± 0.6	51 ± 0.9	65 ± 0.4*	66 ± 0.6*	+55%
EETSG 200 mg/kg	53 ± 0.4	56 ± 0.2	66 ± 0.8*	70 ± 0.9*	75 ± 0.9**	+65%
EETSG 400 mg/kg	62 ± 0.3	68 ± 0.2	75 ± 0.4*	82 ± 0.4*	87 ± 0.6**	+75%
EELCP 100 mg/kg	44 ± 0.7	46 ± 0.6	51 ± 0.5*	62 ± 0.8*	66 ± 0.6*	+54%
EELCP 200 mg/kg	48 ± 0.7	52 ± 0.9	64 ± 0.2*	68 ± 0.2*	75 ± 0.2**	+62%
EELCP 400 mg/kg	57 ± 0.8	62 ± 0.5	68 ± 0.8*	76 ± 0.9*	87 ± 0.9**	+70%
Piracetam 200 mg/kg	66 ± 0.4	76 ± 0.3*	81 ± 0.6*	87 ± 0.6*	93 ± 0.6**	+80%

All values are mean ± SEM (n = 6), with significance determined by one-way ANOVA and Tukey's post-hoc test (*p<0.05, **p<0.01).

Table 8. Chronic in vivo Histopathological study

Groups	Islet size	Islet Number	Necrosis	Remarks
Normal Control	Normal	Normal	Absent	NIL
Diabetic Control	Reduced	Decreases	Prominent	Deteriorated
EETSG 100 mg/kg	Slightly Recovered	Slightly Increases	Mild	Partial Recovery
EETSG 200 mg/kg	Moderately Recovered	Moderately Increases	Rare	Moderate Restoration
EETSG 400 mg/kg	Normal	Normal	Absent	NIL
EELCP 100 mg/kg	Slightly Recovered	Slightly Increases	Mild	Partial Recovery
EELCP 200 mg/kg	Moderately Recovered	Moderately		
Increases	Rare	Moderately		
Restoration				
EELCP 400 mg/kg	Normal	Normal	Absent	NIL
Glibenclamide 2.5 mg/kg	Normal	Normal	Absent	NIL

recovery, while 200 mg/kg doses resulted in moderate restoration with minimal necrosis. Remarkably, at 400 mg/kg, both test extracts fully normalised islet morphology and eliminated necrotic changes, comparable to the standard control[30] presented in Table 8.

4. Discussion

The tuber of *S. glabra* L. and *C. phlomidis* leaves ethanolic extracts have various phytochemical constituents such as flavonoids, amino acids and Proteins, glycosides, alkaloids, Terpenoids, and phenolics, which contribute significantly to its antidiabetic, antidepressant and anti-Alzheimer's potential, which plays a crucial role in preventing neuroendocrinal stress[31]. The tuber of *S. glabra* L. and *C. phlomidis* leaves, a phytochemically rich medicinal plant part, has shown potential in the management of depression and Alzheimer's disorders. Behavioural studies demonstrated significant cognitive-enhancing effects in experimental models, as evidenced by improved learning, memory, and overall cognitive function.

The subacute toxicity assessment of the ethanolic tuber extract of *S. glabra* L. and the ethanolic leaf extract of *C. phlomidis* L. demonstrated that the selected doses of 100, 200, and 400 mg/kg were well tolerated throughout the 14-day study period. No mortality or significant behavioural, physiological, respiratory, psychological, or excretory alterations were observed at these doses. Even at higher exploratory doses up to 2000 mg/kg, only mild and transient neural or excretory changes were noted on day 14, with no indications of systemic toxicity[32].

The *S. glabra* L. tuber, rich in diverse phytochemicals, has demonstrated substantial pharmacological potential in the management of diabetes and neurodegenerative disorders. Its antidiabetic activity is supported by improved glucose regulation, accompanied by a notable increase in pancreatic islet number and size, as well as associated neuroprotective effects. The antidepressant-like activity was evidenced through behavioural assessments, particularly the reduction in immobility duration, indicating a favourable influence on depressive-like behaviour. Furthermore, its anti-Alzheimer's potential is reflected in observed memory-enhancing effects and improvements in cognitive performance. Collectively, these multifaceted actions highlight the broad therapeutic relevance of *S. glabra* L. tuber and its promise as a valuable candidate for integrative treatment strategies[33,34].

The leaves of *C. phlomidis* L., enriched with a broad range of bioactive phytochemicals, have demonstrated significant therapeutic potential in the management of diabetes. Their antidiabetic efficacy is supported by improved glycaemic control and notable restoration of pancreatic architecture, including an increase in islet number and size. In addition, the plant shows strong neuroprotective and psychotropic activities, which are clearly reflected in its behavioural effects. Its antidepressant action is evidenced by a significant reduction in immobility duration, indicating improved mood and reduced depressive-like behaviour. The plant also demonstrates meaningful cognitive benefits, shown by a shorter escape latency time and an increase in spontaneous alternation performance, both of which confirm enhancements in learning, memory, and overall cognitive function. These results collectively support the

therapeutic potential of *C. phlomidis* leaves and highlight their value as a promising multitarget approach for managing neuropsychiatric disorders[35].

The results of the chronic antidiabetic study indicate that repeated administration of the *S. glabra* tuber and *C. phlomidis* leaves methanolic extracts (200 and 400 mg/kg) led to a significant and sustained reduction in blood glucose levels over the treatment of 28 days. This consistent glycemic index improvement suggests that the extract possesses reliable antidiabetic activity suitable for long-term management[36]. The effect became more pronounced with time, indicating a potential stabilising action on glucose metabolism[36.] The observed glucose-lowering effect may be attributed to enhanced modulation of glucose regulatory pathways, although the exact mechanism remains to be elucidated. These findings support the potential of the extract as a candidate for chronic antidiabetic therapy. Further studies are recommended to explore dose dependency, mechanism of action, and clinical relevance[37].

Tuber of *S. glabra* L. *and C. phlomidis* leaves ethanolic extracts exhibited significant antidepressant like activities, as demonstrated by a reduction in immobility time in the behaviour tests like FST and TST, a validated preclinical model for assessing depressive behaviour. The extracts, particularly at higher doses, produced effects comparable to the standard drug imipramine, suggesting a possible involvement of monoaminergic mechanisms in its mode of action[38,39]. The absence of hyperactivity during the test indicates that the reduced immobility was not due to nonspecific motor stimulation but rather reflects genuine antidepressant potential. Phytochemical constituents of the tuber of *S. glabra* L. and leaves of *C. phlomidis* L., such as flavonoids, alkaloids, proteins, terpenoids, tannins, phytosterols and phenolic compounds, are known to influence central neurotransmission and may contribute to the observed behavioural effects. These findings are consistent with mental health disorders and align with earlier reports on its neuropharmacological profile[40]. The results support its potential as a natural antidepressant agent; however, further studies are required to isolate the active principles and elucidate their specific mechanisms of action through neurochemical and molecular approaches.

The current study highlights the neuroprotective potential of *S. glabra* and *C. phlomidis* in experimental models of anti-Alzheimer disease. Both extracts significantly improved cognitive performance, as demonstrated by enhanced spatial memory and learning in the Y-maze and Morris water maze tests. The observed effects may be attributed to the presence of neuroactive phytoconstituents such as stepharine and palmatine in *S. glabra*, and flavonoids and phenolic compounds in *C. phlomidis* L., which have been reported to modulate neurotransmission and protect neuronal integrity[41,42]. These findings support the traditional use of the tuber of *S. glabra* and *C. phlomidis* L. leaves in neurological disorders and underscore their potential as multi-target agents in the management of Alzheimer's disease. Further phytochemical, mechanistic, and clinical investigations are warranted to validate these preliminary findings and identify active constituents responsible for the observed effects[43]. R. K. Shaikh *et al.* (2022) reported that tuber of *Hylocereus undatus* fruits possess notable neuroprotective effects in experimental models of Alzheimer's disease[44]. In the present investigation, similar results were obtained, with both plant extracts significantly improving cognitive performance in the Y-maze and Morris water maze tasks. These behavioural improvements suggest enhancement in spatial memory and learning capacity. Compared to disease controls, treated animals showed reduced latency and increased spontaneous alternation, indicating a reversal of Alzheimer-like deficits. Such findings validate traditional claims of these plants in memory-related disorders. The consistency across studies strengthens the credibility of their therapeutic potential[45,46]. It also highlights the growing relevance of plant-based agents in neurodegenerative disease research. Reena Deshmukh *et al.* (2025) reported the cognitive-enhancing effects of *Swertia chirata* leaves extract in neurodegenerative models[47]. Our study corroborates this, with Leaves of *C. phlomidis* L. extract significantly improving memory and learning behaviours in Alzheimer's rats. The phytochemical constituents, particularly flavonoids and phenolics, may exert neuroprotection through free radical scavenging and synaptic modulation. These compounds are known to attenuate oxidative stress and neuronal damage, common features in Alzheimer's pathology. Improvement in behavioural outcomes suggests that the test extracts support cognitive resilience. Such findings support the traditional use of leaves of *C. phlomidis* L. in memory-related disorders. Its multi-target activity makes it a strong candidate for further neuropharmacological investigation[48,49]. Baranowska-Wójcik *et al.* (2025) highlighted the importance of multi-targeted phytotherapeutics in complex disorders like Alzheimer's disease[50]. Our findings support this view, showing that

S. glabra and *C. phlomidis* possess both behavioural and biochemical efficacy in reversing Alzheimer-like symptoms. Their phytochemical complexity likely contributes to broad-spectrum neuroprotective effects, including antioxidant, anti-inflammatory, and cholinergic modulation. These results justify further exploration of these plants in preclinical and clinical studies[51,52]. These findings pave the way for integrating botanical extracts into modern neurotherapeutic development. BO Ajiboye *et al.* (2024), *Lannea egregia* leaves exhibit significant neuroprotective properties through their alkaloid-rich profile[53]. In line with these findings, the present study revealed that the tuber of *S. glabra L.* extract improved spatial memory and learning in Alzheimer's-induced animal models. The Y-maze and Morris water maze tests showed significant behavioural improvement in treated groups compared to controls. These results suggest the involvement of neurorestorative mechanisms. Such behavioural outcomes support traditional claims of tuber of *S. glabra L* and *C. phlomidis* L. leaves in cognitive enhancement[54]. The data align with previously reported CNS-modulating properties of isoquinoline alkaloids like Stephanie. These observations strengthen the pharmacological basis for its use in Alzheimer's disease.

5. Conclusion

The tuber extract of *S. glabra* and the leaves extract of *C. phlomidis* demonstrated potent *in vivo* antidiabetic, antidepressant, and anti-Alzheimer's activities. Behavioural and biochemical analyses confirmed their multifaceted therapeutic potential. The observed effects are attributed to the rich presence of alkaloids, flavonoids, and phenolic compounds. These findings support their traditional medicinal use and pharmacological relevance.

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7. References

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Author Contributions

Dr. Ravi Parimi conceptualized and supervised the study. Dr. K. E. Pravallika developed the methodology and conducted the experimental work. Jetti Amos Babu performed data analysis and prepared the original draft. All authors contributed to reviewing and editing the manuscript and approved the final version.

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