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Original article

Effect of Clitoria ternatea herbal tea on management of *diabetic neuropathy*

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Abstract:

Objective: *The purpose of this study was to assess the impact of supplementing with butterfly pea flower extract on changes in diabetic polyneuropathy.*

Materials and Methods: *A comparative study involving 101 patients with diabetic polyneuropathy was conducted, in which 33 participants were allocated to one of three groups: DIAB-N, DIAB-N (CT), DIAB-N (PGB). The DIAB-N (CT) group received a daily dose of blue tea made from 5 grams of C. ternatea flower extract for duration of 12 weeks. End of study serum levels of 3-nitrotyrosine, IL-6, and NLR ratio were measured to evaluate the potential preventive effects of C. ternatea flower extract on diabetic polyneuropathy in individuals with type-2 diabetes.*

Result: *The group suffering from diabetic polyneuropathy (DIAB-N) displayed markedly higher concentrations of 3-nitrotyrosine and IL-6, alongside a significantly lower NLR ratio ($p<0.001$). When compared to the control group, the introduction of blue tea led to a notable decrease in serum levels of 3-nitrotyrosine and IL-6, as well as an increase in the NLR ratio, indicating a statistically significant difference ($p<0.001$).*

Conclusion: *blue tea supplementation is beneficial in improvement of quality of life in diabetic polyneuropathy patients.*

Keywords: *Clitoria ternatea herbal tea, diabetic neuropathy*

Introduction:

Diabetes affects 6% of the world's population and is caused by insulin resistance and deficiency, resulting in high blood sugar levels (hyperglycemia). Hyperglycemia affects arteries and contributes to the mortality rate associated with diabetes. Complications of diabetes include both macrovascular and microvascular issues, with diabetic neuropathy being a significant concern.[1] By 2030, 472 million people worldwide will have diabetes, with 236 million having diabetic peripheral neuropathy (DPN).[2] DPN, a sensory neuropathy, damages both small and large fibers, causing negative symptoms like touch loss and heat/cold, and positive ones like hypersensitivity and paradoxical pain. It begins with small sensory fibers. DPN treatment involves glucose control, lifestyle modifications, analgesics, and neuroprotective drugs. First-line treatment is Duloxetine-Amytriptyline-Pregabalin-Gabapantin-Venlafaxine, followed by opioid.[4]

Unfortunately the recommended pharmacological treatments are suboptimal. Pregabalin is a common drug used to treat neuropathy, but high doses are not recommended due to side effects. It has proven effective in diabetic neuropathy treatment, but has limitations due to side effects and drug interactions, especially in elderly patients.[5]

Prolonged hyperglycemia in type 2 diabetes increases oxidative stress, leading to reactive oxygen species (ROS)

production. Mitochondria and NADPH oxidase are key sources of ROS, causing diabetes complications. Antioxidants are considered therapeutic compounds for diabetic neuropathy treatment.[6]

Since the human body lacks excessive antioxidant reserves to counteract free radical activity, antioxidant therapy can be considered for patients with DM and dyslipidemia.[15] Natural products, compared to chemical drugs, offer the advantage of minimal side effects and high safety.[7] One such product is the extract derived from Clitoria ternatea or the butterfly pea flower (CTE), which has been recognized for its antioxidant, anti-inflammatory, anti-diabetic, anti-dyslipidemia, antibiotic, and liver-protective properties. This claim is further supported by Talpate et al., (2013) who demonstrated significant anti-hyperglycemic effects of CTE at 200 and 400 mg/kg BW.[8] These doses resulted in reduced Fasting Serum Glucose (FSG) levels, decreased Nitric Oxide levels, and increased SOD and CAT activities.

Down the literature, few studies highlighted only preliminary evaluation of Clitoria ternatea in diabetic polyneuropathy and anti-oxidant activity in animal study. There was no documentation on molecular mechanism underlying neuro-productivity of Clitoria ternatea herbal tea *in diabetic* polyneuropathy. Purpose of the study to evaluate ameliorate effect of Clitoria ternatea herbal tea on liver function, kidney function and protein oxidative stress and *Neutrophil- Leukocyte ratio* in diabetic neuropathy as same as standard drug Pregabalin.

MATERIALS AND METHODS

Subjects

The study aims to recruit adult participants aged 20-60 from the General Medicine OPD at Koonaseema Institute of Medical Science and Research Foundation, based on criteria such as diagnosis of Type 2 Diabetes Mellitus, stable antidiabetic treatment, painful distal symmetrical and sensorimotor polyneuropathy, diabetic neuropathy, and related endocrine disorders, among others. Participants with Type 1 diabetes, pregnant or lactating, and those with related endocrine disorders are excluded from the study.

Study design:

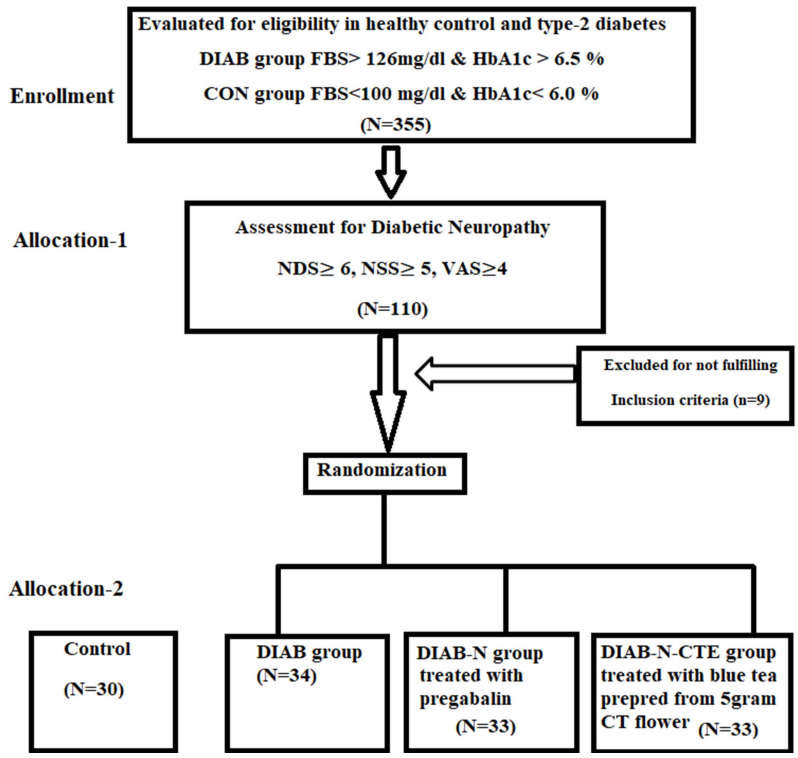
This was a comparative study conducted on type-2 diabetes patients from September 2022 to March 2023 at General Medicine outpatient department, Konaseema institute of medical sciences and research foundation (KIMS&RF) located in Konaseema district in Andhra Pradesh. After being evaluated for study eligibility, control and diabetic subjects were found to meet the inclusion criteria. As per study flowchart (Fig.1) A baseline measure of diabetic polyneuropathy was assessed as per author Akter et al., (2023).[9] The study involved 101 patients with diabetic polyneuropathy, randomly assigned to one of three treatment groups: Metformin alone, Metformin combined with butterfly pea supplement or Metformin with pregabalin. The sample size was 110, with a 5% error rate and 80% statistical power. However, due to staffing issues, surgeries, non-compliance, asthma treatment, and nine patients with diabetic polyneuropathy, follow-up was not completed.

Ethics:

The protocol was approved by the Institution Committee of Ethics in Human Research, which is a division of the Konaseema Institute of Medical Sciences & Research Foundation, in accordance with Indian Council of Medical Research regulations (Ref No. IEC/PR/2021:114). Each patient who wished to participate in the research signed a written informed permission form after being informed.

Plant preparation and extraction:

Butterfly pea, also known as Clitoria ternatea L., is a popular plant in southern India, used for drinking and god-worship. It has been recognized as a herbarium voucher at Dr. Y.S.R. Horticulture University (Voucher Number: 78/2021), and an herbal tea can be made from 5 grams of flowers in 150 ml of drinking water.



Figuer.1. Study flow diagram.

Study procedure:

This study is planned as a comparative study consisting of four groups. CON Group (control) thirty healthy adults' voluntaries; DIAB Group diabetic subjects received only Metformin: DIAB-N Group diabetic Neuropathy patients received metformin (N=34); DIAB-N(PRAGB) Group diabetic Neuropathy patients received metformin and Pragabaline 150mg twice orally (N=33): DIAB-N(CT) Group diabetic Neuropathy patients received metformin and *Clitoria ternatea* flower extraction (N=33) for 12 weeks. End of the study period DIAB-N (PGB) Group and DIAB-N (CT) Group was subjected with neuropathy assessment.

Statistical Analysis:

Descriptive statistics were accessed and results are expressed as anarithmetic mean ± standard error. Statistical significance between the control and experimental group was assessed by using statistical procedure one-way ANOVA, followed by the Tukey-Kramer multiple comparison tests. $P < 0.05$ was considered significant.

Result:

A total 110 diabetic neuropathy patient (NDS ≥ 6, NSS ≥ 5, VAS ≥ 4), 30 healthy non-diabetic subjects were selected to subjected to evaluate the anti- neuropathy potential of *Clitoria ternatea* flower extract (blue tea beverage) against to diabetic polyneuropathy. Nine participants withdrew from the research because of reasons that were not associated with the research (fig.1); remaining hundred and one participants were carried out study their clinical and demographic characteristics are presented in Table 1. The liver marker enzyme and kidney marker tests did not reveal any adverse events following the consumption of blue tea beverages.

Total patient	**CON**	**DIAB**	**DIANB-N**	**DIAB-N(PRGB)**	**DIAB-N(CT)**
Age	38.5±9.56	44.5±8.55	45.72±8.5	45.54±8.22	46.34±8.73
Male	60%	70%	57%	52%	54%
Female	40%	30%	43%	48%	46%
FBS (mg/dl)	91.66±10.3	252.97±62.2[a]**	259.5±55.36[b]*	213±67.46[b]*	162.26±67.46[b]*
HbA1c(%)	5.40±0.25	7.86±0.73[a]**	8.13±0.67[b]*	7.12±0.54[b]*	6.9±0.54[b]*
Hb gm/dl	14.5± 3.3	15.1± 3.7	14.8± 2.3	15.3± 2.9[c]**	12.4± 2.6
Hypertension (%)	-	56%	50%	53%	50%
ALT (IU/L)	25.97±5.22	31.87±6.96	29.26±6.68	30.03±6.6 [a]*	28.03±6.71
AST (IU/L)	23.10±4.00	26.76±4.97	23.97±4.28	25.88±4.8 [a]*	24.27±4.31
ALP (IU/L)	136.76±26.61	128.47±29.7	119.57±23.73	133.66±32.5 [a]*	128.46±29.69
Creatinine (mg/dl)	0.96±0.26	1.64±0.32	1.33±0.36	1.3±0.36 [a]*	1.24±0.34
Urea (mg/dl)	25.55±6.27	29.05±5.28	21.93±7.5	23.55±7.5 [a]*	20.16±6.32

Table.1-: Illustration of demographic characteristics in control and diabetic experimental subjects.

In the table, the results represent the arithmetic mean with ± predictable error of thirty experimental subjects. The statistical drift of the study was set at p value less than 0.01* or p value less than 0.001**. ANOVA between the experimental groups was performed and mentioned in the bar diagram as a symbol of **a**-compared with the control group mean; and **b**-compared with the mean of diabetic polyneuropathy group; c-compared with the mean of pregabalin treated group.

Blue tea beverage attenuates Diabetic polyneuropathy in type-2 diabetic patients.

The study found significant differences in diabetic polyneuropathy scores (DPN) on NDS, NSS, and VAS between diabetic and control groups (P<0.001; Fig. 2 & Table 2), indicating worse quality of life symptoms due to diabetes complications. The research revealed a notable reduction in diabetic polyneuropathy scores (DPN) within the blue tea-treated group DIAB-N (CT) when contrasted with the baseline group DIAB-N. Findings show there was a significant difference between the blue tea therapy group compared with the standard drug pragabaline-treated group (P<0.001; Fig. 2, & Table 2). It shows that optimum anti-neuropathy activity was obtained with blue tea prepared from 5 grams of *Clitoria ternatea* flower.

Variables	Baseline	Blue tea therapy (N=33)	Pregabalin (N=33)	P value
NDS	6.53±0.26	4.54±0.23[b]**	5.12±0.24[b]**	<0.001
NSS	5.44±0.28	3.53±0.28[b]**	4.22±0.26[b]**	<0.001
VAS	4.43±0.29	2.64±0.24[b]**	3.19±0.26[b]**	<0.001

Table 2. **A comparison of DPN between diabetic patients in the two groups.**

In the table, the results represent the arithmetic mean with ± predictable error of thirty experimental subjects. The statistical drift of the study was set at p value less than 0.01* or p value less than 0.001**. ANOVA between the experimental groups was performed and mentioned in the bar diagram as a symbol of **a**-compared with the control group mean; and **b**-compared with the mean of diabetic polyneuropathy group.

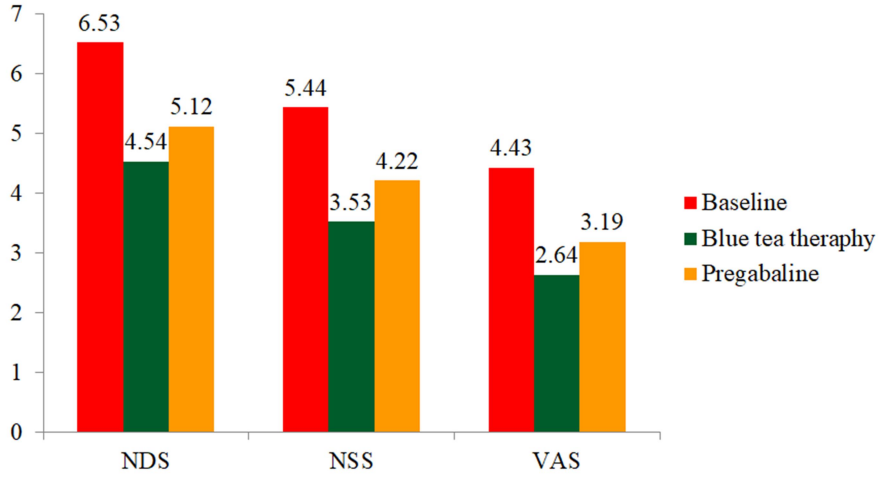


Figure 2. A comparison of DPN between diabetic patients in the study groups.

Blue tea beverage attenuates hyperalgesia and neuropathic pain Diabetic polyneuropathy in type-2 diabetic patients.

The results of the Subjective Peripheral Neuropathy Screen Questionnaire (SPNSQ) for each visit revealed a significant decrease from 80% of the Baseline DIAB-N to 41% at the end of treatment with blue tea DIAB-N (CT), as compared to the standard drug pragabalin-treated group DIAB-N (PRGB), which showed a 60% decrease in hyperalgesia symptoms after 12 weeks of treatment ($P<0.001$; Fig. 3 & Table 3). The average SPNSQ score for neuropathic pain also decreased from 67% at the Baseline DIAB-N to 38% at the end of treatment with blue tea DIAB-N (CT), exhibiting a decrease of 45% ($P<0.001$; Fig. 3 & Table 3). These findings demonstrate that the blue tea, derived from five grams of Clitoria ternatea flower, exhibits superior anti-neuropathy activity.

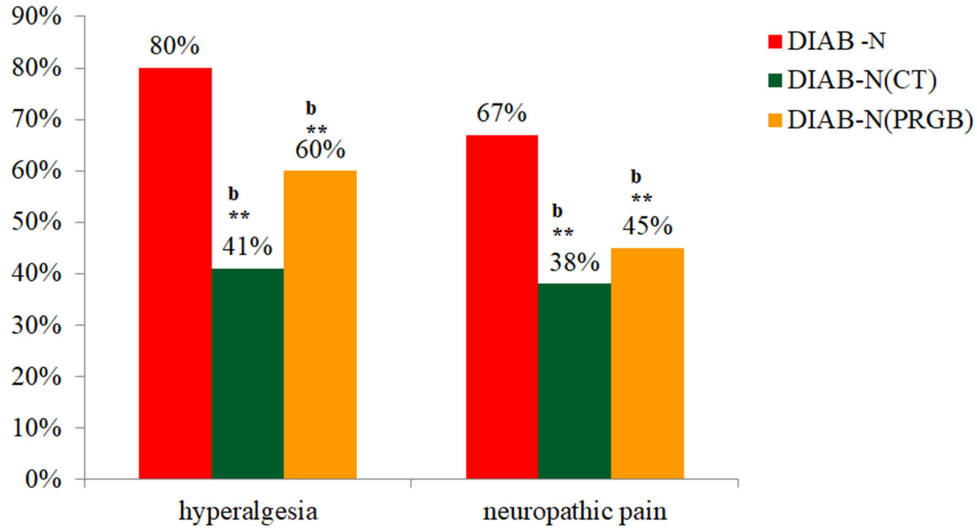


Figure 3. Effect of blue tea on hyperalgesia and neuropathic pain in diabetic polyneuropathy patients

Blue tea potential use in treating diabetes patients' nitrosative stress

Findings revealed that, in comparison to healthy human subjects, diabetes mellitus patients, diabetic neuropathy patients had significantly higher serum 3-Nitrotyrosine level ($p<0.001$). Since an increase in 3-Nitrotyrosine is a defining feature of Nitrosative stress, an increase in protein 3-Nitrotyrosine is thought to be the most accurate biomarker of protein oxidation. The function of 3-Nitrotyrosine in diabetic patients receiving blue tea beverage has also been identified. According to our unique data, patients with DPN treated with *Clitoria ternatea* flower extract had significantly lower 3-Nitrotyrosine content than patients without diabetes ($p<0.001$; Fig.4).

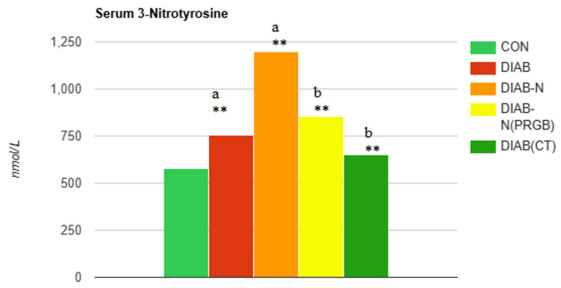
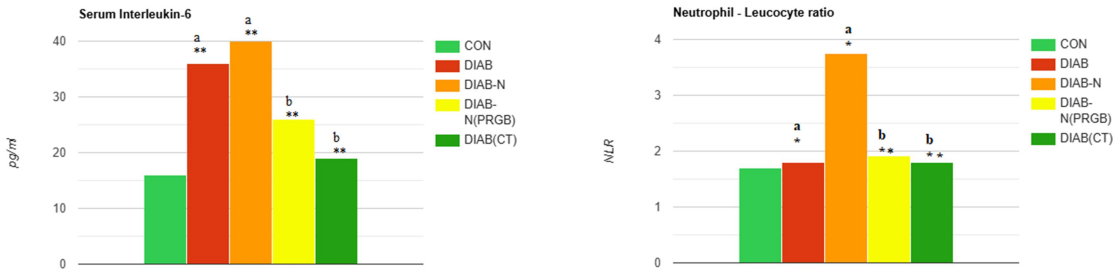


Figure 4. Effect of blue on tea serum 3-nitrotyrosine level in diabetic polyneuropathy in type-2 diabetic patients.

The results represent the arithmetic mean with ± predictable error of thirty experimental subjects. The statistical drift of the study was set at p value less than 0.01* or p value less than 0.001**. ANOVA between the experimental groups was performed and mentioned in the bar diagram as a symbol of **a**-compared with the control group mean; and **b**-compared with the mean of diabetic polyneuropathy group.

Effect of blue tea therapy on inflammatory markers in diabetic neuropathy

Between the diabetic neuropathy group and the control group, the serum IL-6 level of inflammation showed a significant average difference ($p<0.001$), demonstrating how changes in inflammation brought on by diabetes complications are affected by changes in nervous system, which are indicative of a serum IL-6 level in type 2 diabetic patients having noticeably worse quality-of-life symptoms. Treatment with blue tea resulted in a statistically significant ($p<0.001$; Fig.5A) decrease from baseline to at the end of treatment. The patient undergoing blue tea therapy had lowering IL-6 level compared to the patients taking pregabalin ($p<0.001$). This illustrates the relationship between inflammation and diabetic polyneuropathy. In terms of routine laboratory parameters, mean *Neutrophil- Leukocyte ratio* levels were significantly reduced in blue tea treated group ($p<0.001$; Fig. 5B & Table 2) compare with DIAB-N (diabetic neuropathy). In addition, statistically decrease NLR ratio compare to pregabalin treated group ($p<0.05$).



A **B**

Figure 5. (A&B) Effect of blue tea on inflammatory markers in diabetic polyneuropathy in type-2 diabetic patients.

A-IL-6 level; B- neutrophils- lymphocytes ratio**.** The results represent the arithmetic mean with ± predictable error of thirty experimental subjects. The statistical drift of the study was set at p value less than 0.01* or p value less than 0.001**. ANOVA between the experimental groups was performed and mentioned in the bar diagram as a symbol of **a**-compared with the control group mean; and **b**-compared with the mean of diabetic polyneuropathy group.

DISCUSSION:

Clitoria ternatea, a neuroprotective plant in India, has been confirmed in Ayurvedic literature and FDA approval for food additives in Thailand and Japan.[10] The study aims to investigate the potential benefits of blue tea from Clitoria ternatea flower on neuropathic symptoms and laboratory parameters in diabetic neuropathy patients.

According to the results of the current investigation, Chusak C et al., (2018) observed no negative effects on markers of renal or hepatic function.[11] According to our research, patients with diabetic neuropathy have a significantly lower quality of life it reflected in score of DPN than subjects in good health (fig.2 & table.2; $p<0.001$). Low anti-oxidant levels, glucose autooxidation, and excess glycosylated proteins in diabetics can lead to nerve damage and microvascular complications, causing peripheral neuropathy, the most common secondary complication of diabetes.[12]

The study found that blue tea supplementation significantly improved the quality of life in diabetic neuropathy patients with type-2 diabetes, reducing dysesthesia and improving sensory function, compared to standard pregabalin treatment (Fig.3 & table.3; $p<0.001$). Our study proposes a novel treatment strategy for diabetic neuropathy, focusing on sensory function loss, a key factor in lower limb issues and gait irregularities, contrasting current pharmacological treatments.

Blue flowers of Clitoria ternatea contain anthocyanins, water-soluble antioxidant pigments derived from polyhydroxylated glycosides, which effectively drain nitatossive and reactive oxygen species due to their molecular structure.[13] Pregabalin blocks calcium entry and prevents neuropathic pain, whereas anthocyanins reduce the peripheral nervous system's reliance on free radicals, which contribute to non-communicable diseases and complications.[14]

3-Nitrotyrosine, a marker for oxidative stress, is a product of tyrosine modification under peroxynitrite, a mechanism crucial for complications in diabetes mellitus. Diabetes mellitus is a mild inflammatory condition triggered by oxidative stress, with increased 3-Nitrotyrosine levels linked to disease onset and progression. In our study found increased 3-nitrotyrosine levels in diabetic polyneuropathy patients, similar to previous research and potential biomarkers of peripheral diabetic neuropathy.[15]

This is the first study to look at how Clitoria ternatea affects on serum 3-nitrotyrosin levels in people with type 2 diabetes in a clinical setting. The present results corroborate those of other authors, who showed that Clitoria ternatea flower extract has been shown to lower protein carbonyl contents, 3-Nitrotyrosine level and regulate oxidative stress. Furthermore, Clitoria ternatea flower extract increased postprandial plasma antioxidant status in overweight and obese subjects [16], Therefore, these changes may also account for the decreased 3-nitrotyrosine, the nitrosative stress end product, in diabetic individuals experiencing neuropathic stress. The possible mechanism to attenuate Reactive Nitrogen specious product 3-Nitrotyrosine level in Clitoria ternatea treated group due to antioxidant potential of anthocyanins content in flower extract.

This study investigates the link between serum IL-6 concentration and diabetic peripheral neuropathy (DPN) in type-2 diabetic patients. IL-6 is involved in DPN pathophysiology (Chanda et al., 2022), leading to neuroinflammation and pain.[17] Higher levels of TNF-α and IL-6 are linked to distal sensorimotor polyneuropathy DSPN progression, promoting free radical formation and dysesthesia. These findings match our results. Chronic inflammation promotes free radical formation, dysesthesia, and abnormal DPN scores (fig. 3 and table. 3).

The study's IL-6 data demonstrated the amazing impact that supplementing with blue tea beverages had on type-2 diabetic patients' diabetic neuropathy. Blue tea supplementation significantly reduces inflammation and serum IL-6 levels in type-2 diabetic patients. S et al., (2023) report that consuming 1 g of Clitoria ternatea flower extract reduced inflammation and IL-6 levels.[18] Anthocyanins, rich in water-soluble antioxidants, may reduce inflammation and lower IL-6 levels.

Immune system exacerbation in chronic low-grade inflammatory disorders like diabetes is often exacerbated by reactive oxygen and nitrogen species, leading to a high neutrophil-to-lymphocyte ratio in diabetic neuropathy,

retinopathy, and nephropathy. The study revealed that the group with diabetic polyneuropathy had a significantly higher mean NLR value compared to the group with diabetes (Fig. 5; p <.001). The study reveals that individuals with type 2 diabetes who have higher NLRs are more likely to experience issues, including reduced lymphocyte and increased neutrophil counts, and that the NLR value can also serve as a marker for diabetic polyneuropathy in addition to the DPN score.

The study investigates the impact of Clitoria ternatea tea on hematology parameters, specifically neutrophil-leukocyte ratio, in diabetic polyneuropathy patients, finding significant improvement in neuropathy (Figure 5; p <.001). The study suggests that blue tea beverage supplementation may reduce NLR due to the rich content of Anthocyanins in Clitoria ternatea flowers[9], which have immunomodulatory and antioxidant properties, potentially reducing immune system exacerbation in diabetic neuropathy.

The study shows that blue tea consumption in diabetic polyneuropathy patients reduces pain scores, improves dysesthesia and sensory function, and decreases reactive nitrogen and inflammatory markers. Compared to pregabalin, blue tea supplementation offers advantages like no drug advisory reaction. Clitoria ternatea may suppress polyneuropathy and improve oxidative stress and inflammatory status in type-2 diabetic patients. Further research with larger sample sizes and different doses is recommended.

Limitations of the study:

This study had a number of drawbacks. Our inability to perform long-term patient follow-ups was our first obstacle. Furthermore, the administration technique adopted in this study required extraction preparation wherever possible. This is a conventional way; we do not use the modern drug delivery system because it makes drug administration simpler. To confirm these results and offer more thorough information, future clinical trials with bigger sample numbers and more thorough evaluations are necessary. It's possible that this discrepancy affected the study's findings.

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