Khan et al. Scr Med. 2025 Jan-Feb;56(1):1-13. DOI:10.5937/scriptamed56-52822

ORIGINAL ARTICLE

Assessing the Efficacy of Methanol Extracts of *Ocimum Sanctum* Linn. and *Ocimum Basilicum* Linn. in Diabetic Neuropathy

Shabeena Khan,[1, 2] Abhishek Bhanot,[2, 3] Divya Dhawal Bhandari[4]

1. Department of Pharmacognosy, Shree Dhanvantary Pharmacy College, Kim (Surat) Gujarat, India.
2. Department of Pharmacognosy, ASBASJSM College of Pharmacy, Bela (Ropar) Punjab, India.
3. AGM-DRA, Beta Drugs limited, Vill. Nandpur, Baddi (Solan) Himachal Pradesh, India.
4. University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India.

Citation:
Khan S, Bhanot A, Bhandari DD. Assessing the efficacy of methanol extracts of *Ocimum sanctum* Linn. and *Ocimum basilicum* Linn. in diabetic neuropathy. Scr Med. 2025 Jan-Feb;56(1):1-13.

Corresponding author:
SHABEENA KHAN
E: shabeenasabbir@gmail.com

Abstract

Background/Aim: Our nerves can be impacted by thousands of diseases and ailments, but diabetes-related neuropathy is the name given to the endocrine system issue that results in nerve damage. Herbal plants having antioxidant activity play an important role in managing diabetes and the associated complications. *Ocimum sanctum* and *Ocimum basilicum* plants have shown antihyperglycemic as well as neuroprotective activity but the effect of *Ocimum sanctum* and *Ocimum basilicum* on diabetic neuropathy (DN) has not been studied so far. So, the given manuscript was aimed to evaluate the effect of both plants by various in vitro biochemical parameters as well as *in vivo* studies.

Methods: The given manuscript describes the effect of methanol extracts of *Ocimum sanctum* and *Ocimum basilicum*, in DN induced Swiss albino mice of either sex weighing 25-35 g. Mice were divided into 6 groups viz control (I) receiving citrate buffer, diabetic (II) and test groups (III, IV, V, VI) receiving streptozotocin at a dose of 100 mg/kg to induce DN on 21st day followed by treatment of test groups (III, IV, V, VI) at a dose of 100 mg/kg and 200 mg/kg daily for 14 days, with *Ocimum basilicum* and *Ocimum sanctum*, respectively.

Results: The results showed that the methanol extracts of *Ocimum sanctum* and *Ocimum basilicum* gave significant change in weight variation, tail immersion test and blood glucose in comparison to diabetic control. In brain tissue homogenate measurement of oxidative stress by thiobarbituric acid reactive substance (TBARS), glutathione (GSH) and serum nitrite level, the methanol extract of both plants produced significant change when compared to diabetic control.

Conclusion: This study underscores the promising role of herbal adjuncts in addressing the complexities of diabetic neuropathy and warrants continued investigation into their clinical utility.

Key words: Diabetic neuropathies; *Ocimum sanctum*; *Ocimum basilicum*; Oxidative stress; Streptozotocin.

Received: 17 August 2024
Revision received: 12 October 2024
Accepted: 12 October 2024

Introduction

Diabetes is a disease that has both macro- and microvascular consequences,[1-3] the most worrisome of which is diabetic neuropathy. There are almost 40 million diabetics who have neuropathy.[4, 5] From clinical point of view, diabetic neuropathy is defined as a destructive disease of the peripheral nerve associated with nerve pain, paraesthesia or other problems associated with neurological

deficit.[6-13] Distal symmetrical sensorimotor polyneuropathy (DPN) in diabetic patients have found to reduce the quality of life specially due to the associated morbidity and mortality.[14] Hence, there is a need of secondary prevention through early diagnosis and treatment.[15] Diabetes is associated with hyperglycaemia linked oxidative stress or any defect in lipid metabolism due to increased gluconeogenesis and ketogenesis.[16]

Diabetes mellitus can cause widespread or localised damage to peripheral nerves, which is known as diabetic neuropathy. It falls into two general categories: diffuse and focused types. Diffuse neuropathies are prevalent, chronic and frequently progressive conditions. Examples of these include diabetic autonomic neuropathy (DAN) and distal symmetrical sensorimotor polyneuropathy (DPN).

Peripheral nerve mononeuropathies like median and ulnar neuropathies in diabetics closely resemble entrapment neuropathies seen in non-diabetic individuals, suggesting heightened vulnerability of diabetic nerves to compression. The most frequent cranial neuropathy involves the third cranial nerve, resulting in one-sided headache, double vision (diplopia), eyelid drooping (ptosis) and sparing of the pupil (diabetic ophthalmoplegia).[17, 18]

The plant kingdom offers significant potential for discovering new drugs and there has been growing recognition of the importance of medicinal plants in recent years. A number of medicinal plants are known to possess anti-oxidant and anti-hyperglycaemic activities, among which, *Ocimum* species has proved effective in reducing various risk factors associated with bronchitis, diarrhoea, dysentery and many more. *Ocimum sanctum* L, a perennial herbaceous plant, also known as holy Tulsi, is a member of *Lamiaecae* family. It is regarded as one of the most significant sources of medicinal compounds, with numerous secondary metabolites and essential oils that are suggested for the treatment of skin conditions, rheumatoid arthritis, bronchial asthma, dysentery, bronchitis, malaria, diarrhoea and other conditions.[19,20] According to pharmacological studies cited in this research, *Ocimum sanctum* also demonstrates anticancer, antifungal, antimicrobial, antifertility, hepatoprotective, antispasmodic, cardioprotective, antiemetic, antidiabetic, analgesic, adaptogenic and diaphoretic effects, confirming its therapeutic efficacy.[20, 21] *O sanctum* L leaf powder has also demonstrated a decrease in fasting blood glucose (21 %) and glycated protein (11 %), total cholesterol (11 %), low density lipoprotein (LDL, 14 %), very low density lipoprotein (VLDL, 16 %) and triglycerides (TG, 16 %). It has been applied to 27 individuals with type 2 diabetes.[22, 23] Sweet basil (*Ocimum basilicum* L), a renowned culinary herb in the *Lamiaceae* family, originates from India and Pakistan but is now cultivated worldwide.[24] Basil leaves have been traditionally utilised across cultures to treat various health issues including cancer, tremors, bronchitis etc.[25] These traditional uses are substantiated by pharmacological studies showing basil's efficacy in scavenging radicals, combating cancer, alleviating pain, fighting infections and modulating the immune system.[26] These bioactive effects are attributed to compounds such as phenolic acids, flavonoids, rosmarinic acid, aromatic compounds and essential oils present in *O basilicum*, such as eugenol, chavicol, linalool and β-terpineol.[27, 28] For a wide range of ailments and disorders, *O basilicum* is used in Ayurveda and traditional Chinese medicine.[29, 30] Recent studies reported decrease of blood glucose in streptozotocin-induced DM rats by adding 1 % - 2 % of *Ocimum sanctum* leaf powder.[31-35] As reported earlier, *Ocimum sanctum* Linn. and *Ocimum basilicum* Linn. possess a high level of antioxidant activity[32, 36] which is attributed to the flavonoids and phenolic compounds.[37] In case of *Ocimum sanctum* Linn. and *Ocimum basilicum* Linn, both plants have shown antihyperglycemic as well as neuroprotective activity. But the effect of *Ocimum sanctum* Linn. and *Ocimum basilicum* Linn. on DN has not been studied so far. The given manuscript has been designed to evaluate the effect of methanolic extracts of *Ocimum sanctum* Linn. and *Ocimum basilicum* Linn. on DN through *in vitro* biochemical parameters, specifically blood glucose, serum nitrite, thiobarbituric acid reactive substance (TBARS), brain protein content, reduced glutathione and *in vivo* studies too as literature shows antidiabetic activity of methanolic extracts obtained from leaves of several *Ocimum* species even at a concentration of 0.5 mg/kg than standard medication.[38]

Methods

Plant materials

The whole plant of *Ocimum sanctum* Linn. and *Ocimum basilicum* Linn. was collected from nursery

and botanical garden of NIPER, Mohali. The Plants Herbarium (*Ocimum sanctum* Linn. = SOS/11/11 and *Ocimum basilicum* Linn. = SOB/11/12) were deposited in the Department of Pharmacognosy, ASBASJSMCOP, Bela (Ropar). It was authenticated and identified as *Ocimum sanctum* Linn. and *Ocimum basilicum* Linn. by Dr AS Sandhu, (NIPER), Mohali (vide letter No ASCB/32/11/3924 dt 5/12/2011).

Preparation of extract

About 200 g of each crude powdered drug was taken in two Soxhlet apparatus (one for *Ocimum sanctum* Linn. and other one for *Ocimum basilicum* Linn.) thimble after making moderately coarse and then continuous hot Soxhlet extraction was done using a suitable solvent methanol. As reported earlier by number of researchers and even by Borah et al the yield of residue after Soxhlet extraction and evaporation have been found to be maximum with methanolic extract and also the maximum amount of bioactive phytoconstituents responsible for therapeutic potential have been found to be in methanolic extract which forms the basis for the selection of methanolic extracts for the present study.[39] Then methanol extracts are subjected to phytochemical screening for the detection of various phytoconstituents and the thin layer chromatography (TLC) profiling. Phytochemical screening and TLC profile of various extracts revealed the detection of various phytoconstituents (phenolics and flavonoids). The methanolic extracts of *Ocimum sanctum* Linn. and *Ocimum basilicum* Linn. were dissolved in 1 % solution of carboxymethyl cellulose (CMC) to prepare suspension. Streptozotocin was purchased from Sigma Aldrich (India) and dissolved in 0.1 N citrate buffer (pH 4.5). All other chemicals and reagents were of analytical grade and were freshly prepared before use. These methanolic extracts were then utilized for the biochemical characterisation, *in vitro* assay and *in vivo* study.

Pharmacognostic studies

Macroscopic (colour, shape, size, odour, taste, surface characteristics and texture), microscopic evaluation (transverse section, powder microscopy and determination of leaf constants), histochemical, physical parameters (extractive values and physiochemical) and quantitative and qualitative estimations were performed to identify different biochemical parameters of the plant extract. TLC was also performed using various solvent systems. Thin layer chromatography of methanol extract was performed which showed different R_f values using different solvent systems.

Pharmacological evaluation

In vitro studies - Antioxidant assay using DPPH (1,1-diphenyl-2-picrylhydrazyl)

Radical scavenging capacity was measured by Blois[26] method which includes dissolving extract powder in methanol to make stock solution and further diluted to obtain concentrations of 10 µg/mL, 20 µg/mL, 50 µg/ mL, 100 µg/mL, 200 µg/mL and 500 µg/mL. Decolourisation of DPPH was evaluated at 517 nm using Shimadzu spectrophotometer. The scavenging activity = A0-A1/A0*100 where A0 is absorbance of control/blank while A1 is absorbance of extract/standard.

In vivo studies

Swiss albino mice of either sex weighing 25-35 g were kept in animal house under standard conditions of light/dark cycle along with diet with free access to water. Behavioural and biochemical improvement using methanolic extract of *Ocimum sanctum* Linn. and *Ocimum basilicium* Linn. were noted in the diabetes affected mice (DN) ie group III-VI in comparison to diabetic (group II) and normal control (group I).

Experimental design

Six groups of six mice each were employed for study as: Group I: normal control including non-diabetic mice administered with 0.1 N citrate buffer; Group II: diabetic control including mice administered with single dose of STZ (100 mg/kg, intraperitoneal) and Group III-VI test group administered with single dose of STZ (100 mg/kg, intraperitoneal) to induce DN (21st day) followed by four treatment arms of Group III and IV-*Ocimum basilicium* Linn. and Group V and VI-*Ocimum sanctum* Linn. each, administered with oral dose of 100 mg/kg and 200 mg/kg, respectively after induction of DN due to STZ. Administration of treatment in the 4 arms (Group III to Group VI) was initiated for 14 days daily, in diabetic mice after 21st day of induction of DN using STZ (Figure 1).

All the mice groups were subject to behavioural changes (body weight, tail immersion test) before and after administration of respective solutions, ie 0.1 N citrate buffer for normal control, STZ (100 mg/kg, intraperitoneal only once) for Group II and test solutions for Group III to Group

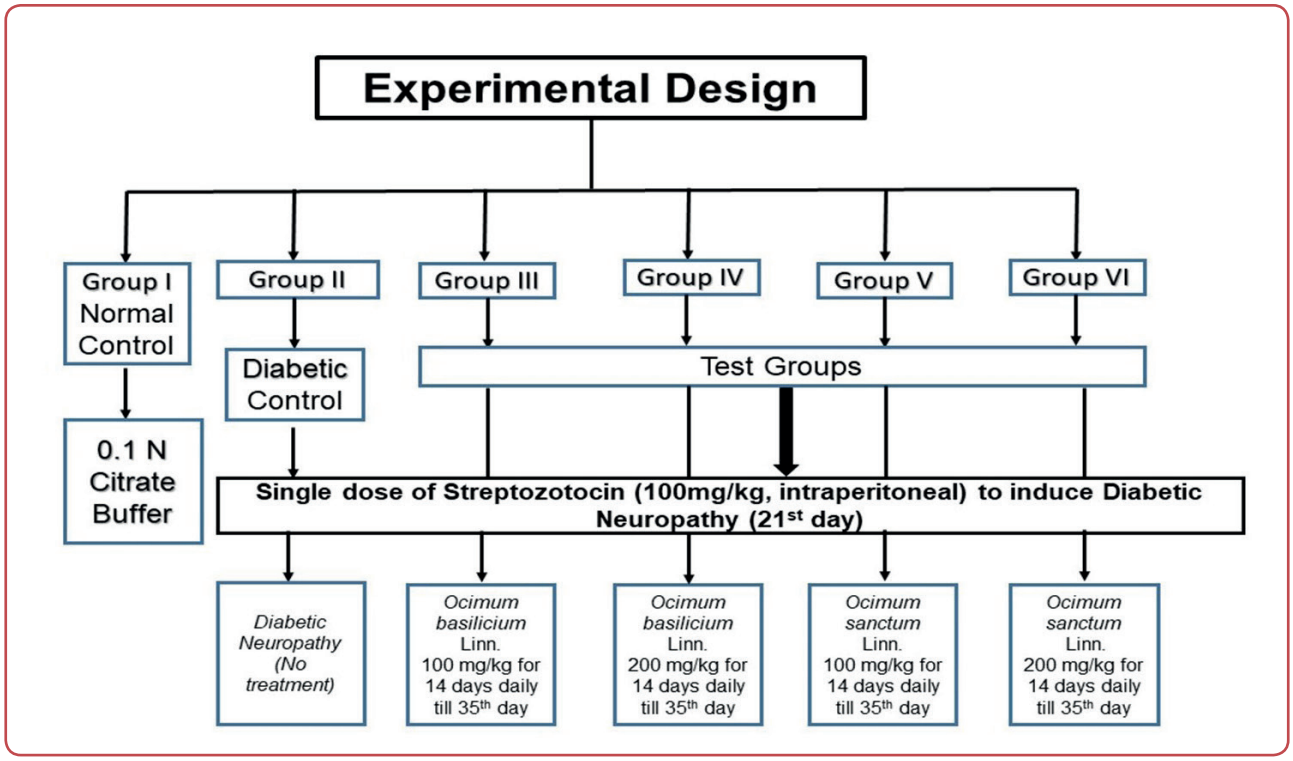

Figure 1: *Experiment design*

VI, on scheduled days ie 0, 4, 7, 14, 21, 28 and 35th day. The fasting blood glucose was noted down on 0, 4, 7, 14, 21, 28, 35th days after administration of citrate buffer. At the end of study (35th day), mice were sacrificed and the brain tissue was preserved for determination of serum nitrite, glutathione (GSH), TBARS levels.

Biochemical parameters

Various biochemical parameters were measured using blood withdrawn retro-orbitally with help of micro-capillaries. Collected blood was kept for 30 min at room temperature followed by separation of serum and plasma from blood by centrifugal techniques. Following estimations were performed at the end of study on day 35: blood glucose, serum nitrite, TBARS, brain protein content and reduced glutathione.

Statistical analysis

The results of statistical analysis were given in terms of mean ± standard error of means while behavioural results were expressed by two-way ANOVA then Bonferroni's post-test. The biochemical results, one-way ANOVA then Tukey's multiple range tests were applied. The p-value < 0.001 was statistically significant.

Results

Morphological study of leaf parts, morphological characterisation and microscopical study along with leaf constants for both plants, ie *Ocimum sanctum* Linn. and *Ocimum basilicum* Linn. is given in Figure 2, 3 and Table 1.

Additionally, powder behaviour was studied using different chemical reagents to identify presence of compositional materials including lignin, cellulose, fixed oil, starch and protein. Extractive values and ash values along with physicochemical characteristics, qualitative phytochemical analysis and quantitative analysis of phenolic and flavonoid content is presented in Table 2.

Thin layer chromatography of methanol extract was performed which showed different R_f values using different solvent systems as shown in Figure 4 and Table 3.

In vitro antioxidant activity using DPPH model was performed and the observations are described in Figure 5.

Behavioural and pharmacological activity were studied using *in vivo* model and the observations are mentioned in Figure 6. *In vitro* studies also confirmed the antioxidant activity using %

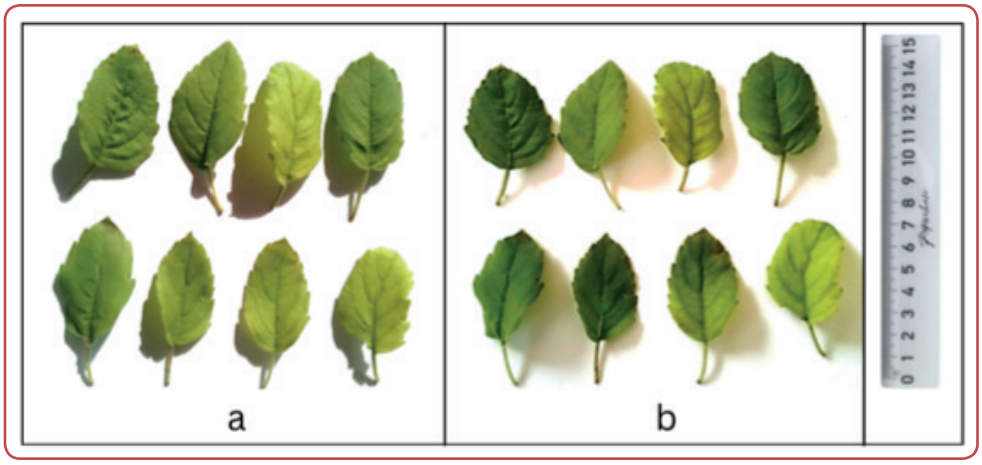

Figure 2: Morphological study of Ocimum sanctum Linn. and Ocimum basilicum Linn.

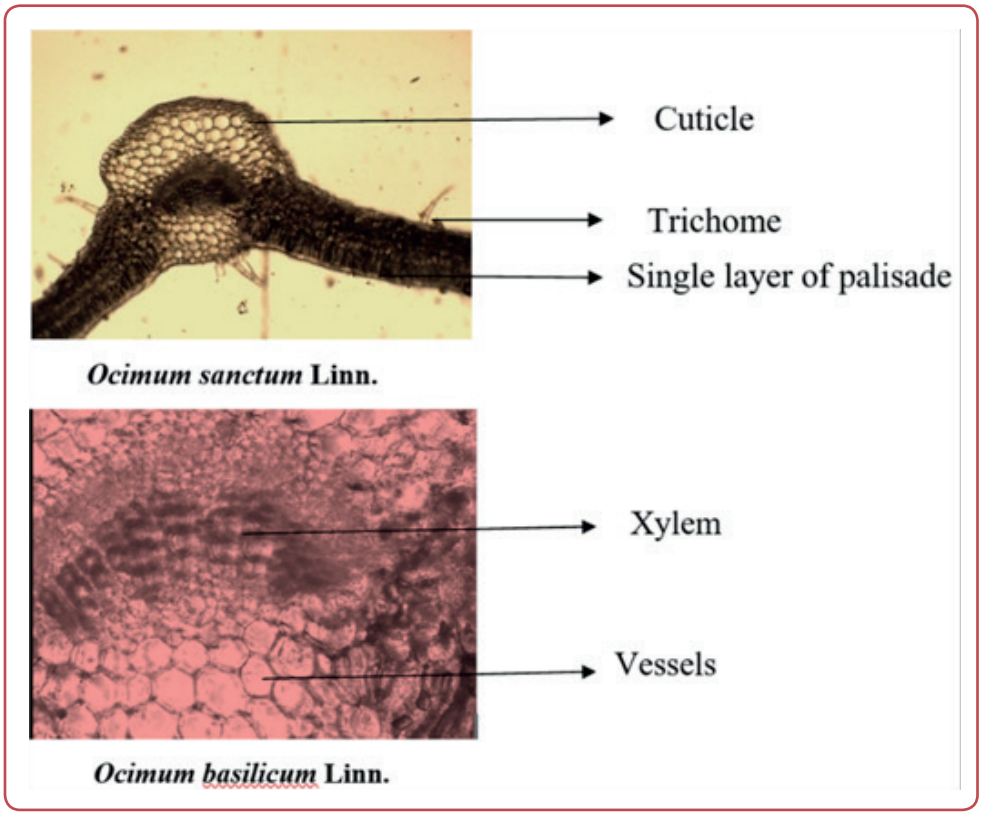

Figure 3: Figure 3: Microscopical studies (TS) of Ocimum sanctum Linn. and Ocimum basilicum Linn.

Table 1: Morphological characteristics, microscopical study and leaf constants of Ocimum sanctum Linn. and Ocimum basilicium Linn.

Parameters	*Ocimum sanctum* Linn.	*Ocimum basilicum* Linn.
a. Morphological characters		
1. Colour	Green	Green
2. Odour	Characteristic	Aromatic and characteristic
3. Taste	Slightly aromatic	Characteristic slight bitter
4. Size	3 cm (length), 2 cm (width)	16-50 mm long, 8-32 mm wide
5. Shape	Elliptical	Elliptical or ovate
6. Margin	Ovate	Entire

7. Arrangement	Alternate	Inflorescences
8. Venation	Reticulate	Decussate
9. Surface	Pubescent, hairy	Hairy
10. Apex	Acute	Acute or obtuse
11. Petiole	Slender	5-20 mm long
b. Microscopical study		
1. Trichomes	Covering and glandular trichomes	Yellowish-brown glandular trichomes
2. Stomata	Both anomocytic and diacytic type of stomata present on both surfaces	Diacytic stomata occur on both surfaces
3. Parenchyma cells	Present	Sclerenchymatous layer
4. Vascular bundle	Xylem surrounded by phloem	Lignified vessels
5. Fibres	Thin-walled fibres	Thick-walled fibres
c. Leaf constants		
1. Stomatal index of upper epidermis	10 upper epidermis	8 upper epidermis
2. Stomatal index of lower epidermis	13 lower epidermis	11 lower epidermis
3. Vein islet number	12/mm^3	10/mm^3
4. Vein termination number	5/mm^3	4/mm^3

Table 2: Powder microscopy, extractive values, ash values, physicochemical characteristics and various qualitative and quantitative parameters of Ocimum sanctum Linn. and Ocimum basilicum Linn.

Parameters	*Ocimum sanctum* Linn.	*Ocimum basilicum* Linn.
a. Powder behaviour		
1. Phloroglucinol HCl (lignin)	+	+
2. Iodine sol followed by H_2SO_4 (cellulose)	+	+
3. Sudan III (fixed oil and fat)	+	-
4. Caustic alkali + HCl (calcium oxalate)	+	+
5. Weak Iodine solution (starch)	+	+
6. Lugol's solution (protein)	+	+
7. Millon's reagent (protein)	+	-
b. Extractive values		
1. Alcohol soluble extractive value (% w/w)		
Hot extraction	3.6	4.0
Cold extraction	3.2	3.8
2. Water soluble extractive value (% w/w)		
Hot extraction	4.5	6.1
Cold extraction	4.4	4.2
c. Ash values		
1. Total ash	14 % w/w	13 % w/w
2. Acid insoluble ash	2.7 % w/w	1.6 % w/w
3. Water soluble ash	3.9 % w/w	3.7 % w/w
d. Physicochemical parameters		
1. Loss on drying	6.6 % w/w	6.5 % w/w
2. Swelling index	12.5 mL	11.4 mL
3. Foaming index	100	100
4. Moisture content	7.6 % w/w	5.5 % w/w

e. Successive extraction		
% yield (% w/w)	15.35	7.97
f. Qualitative phytochemical analysis		
1. Sterols		
Salkowski test	+	-
Liebermann Burchard's reaction	+	-
2. Tannins		
Lead acetate solution	+	+
Ferric chloride solution	+	+
Potassium dichromate	+	+
3. Flavonoids		
Shinoda test	+	+
Zn HCl test	+	+
g. Quantitative tests		
Total phenolic content*	87.5 mg	61.5 mg
Total flavonoid content**	45.26 mg	27.2 mg

**gallic acid equivalents/g of extract, **quercitin equivalent/g of extract, +: present, -: absent;*

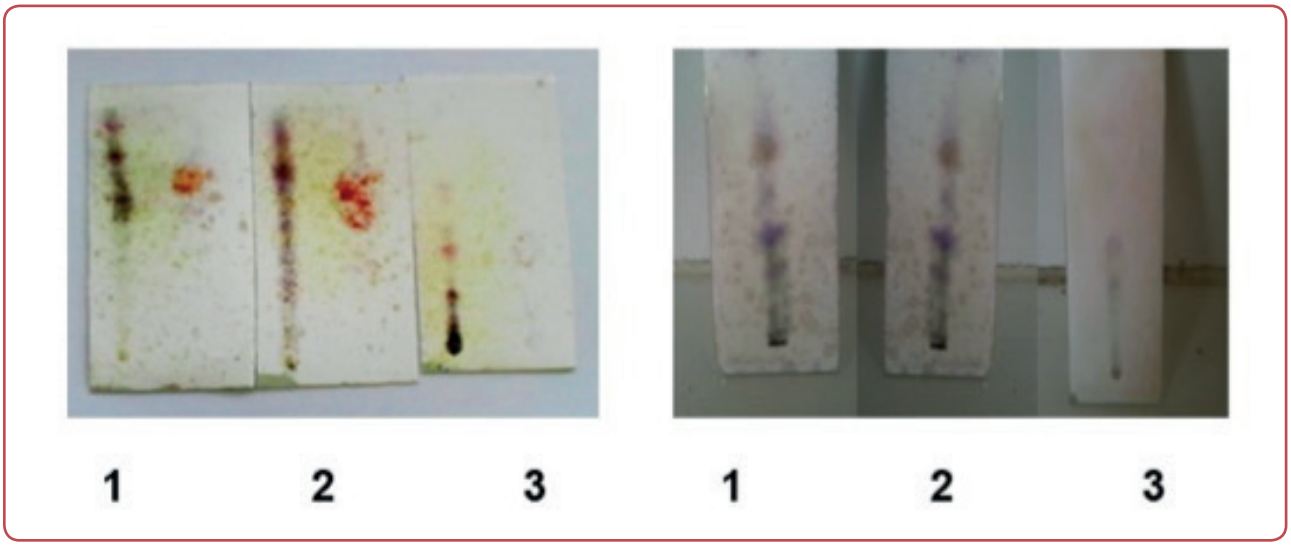

Figure 4: Thin layer chromatography (TLC) profiling of Ocimum sanctum Linn. and Ocimum basilicium Linn.

Table 3: Thin layer chromatography (TLC) study of Ocimum sanctum Linn. and Ocimum basilicium Linn.

Solvent system	Ratio	N	R_f values
***Ocimum sanctum* Linn.**			
1. Ethylacetate: toluene	9.9:0.1	3	0.8, 1.6, 3.2
2. Toluene: diethylamine: ethylacetate; methanol: chloroform;	10:2:6:2:1	3	4.9, 6.2, 7.0
3. Ethylacetate: Toluene: Formic acid	10:00.1	3	4.1, 6.0, 7.2
***Ocimum basilicum* Linn.**			
1. Ethylacetate: glacial acetic acid (GAA)	9:01	3	0.06, 0.54, 0.92
2. Chloroform: ethylaceate: GAA	3:01:01	1	0.96
3. Chloroform: methanol	9:01	4	0.92, 0.76, 0.65, 0.50

N: number of spots observed;

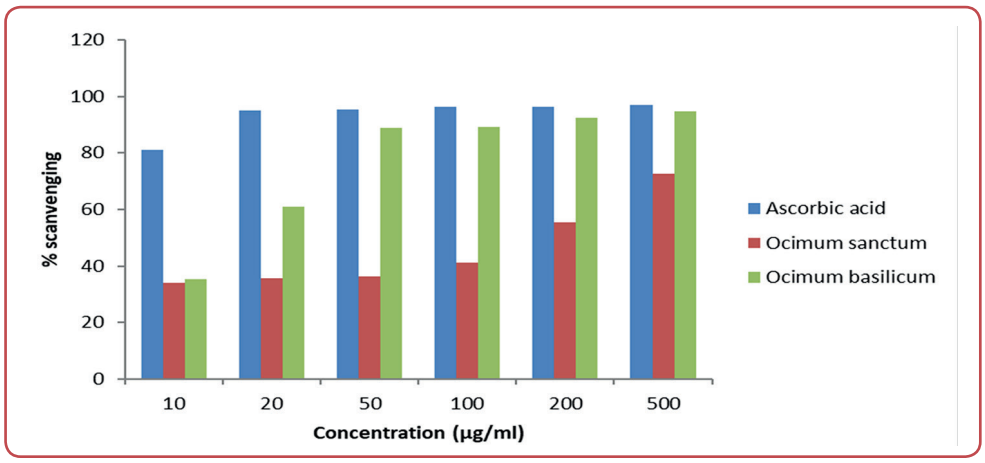

Figure 5: The anti-oxidant effect of Ocimum sanctum Linn. and Ocimum basilicum Linn. on diabetic mice

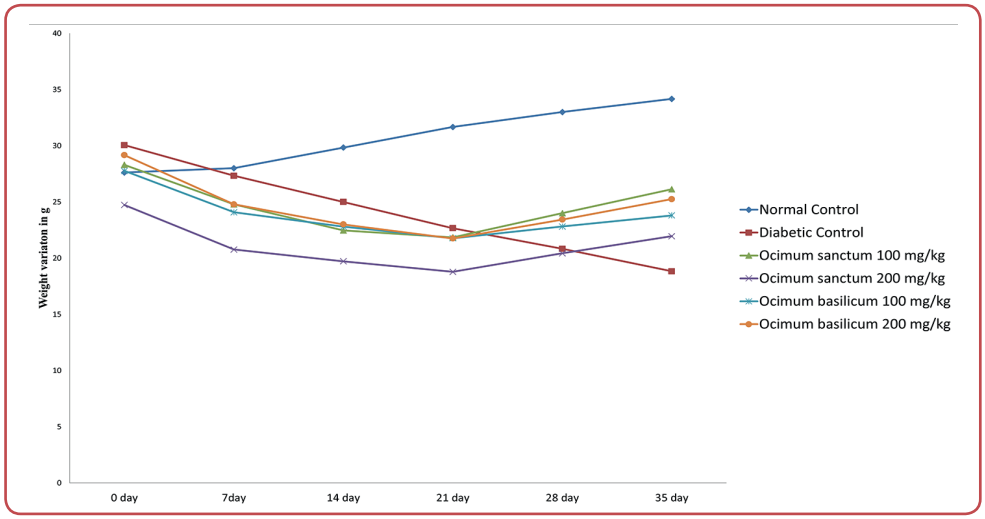

Figure 6: Effect of methanol extract of Ocimum sanctum Linn. and Ocimum basilicum Linn. on weight variation in diabetic mice

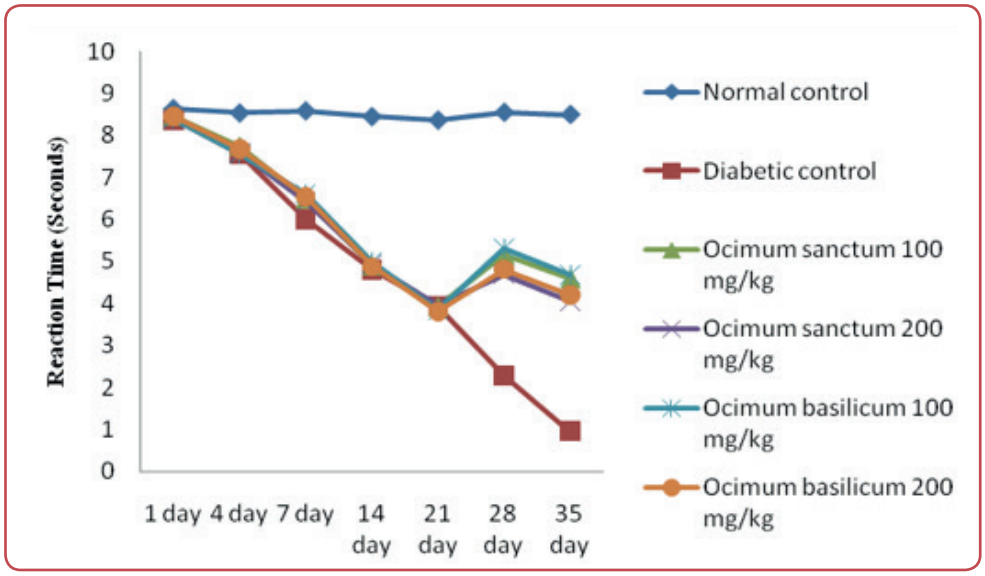

Figure 7: Effect of methanol extract of Ocimum sanctum Linn. and Ocimum basilicum Linn. on tail immersion latency time in diabetic mice

scavenging of both *Ocimum sanctum* Linn. and *Ocimum basilicium* Linn. on DPPH model where a concentration dependent increasing pattern of scavenging was observed in both the plant extracts. *In vivo* study using diabetic mice also concluded various behavioural and biochemical outcomes. Weight variation showed improvement in all the 4 treatment arms (Group III to Group VI) as a result of weight gain in contrary to weight loss observed in diabetic control group.

Increase in tail immersion latency time was observed in the 4 treatment arms in comparison to the diabetic control (Group II) which also indicated an improvement in reaction threshold and indicating possibly improvement in neuropathy as shown in Figure 7.

Significant reduction in fasting blood glucose levels in the mice population of the 4 treatment arms (Group III-VI) was observed after treatment

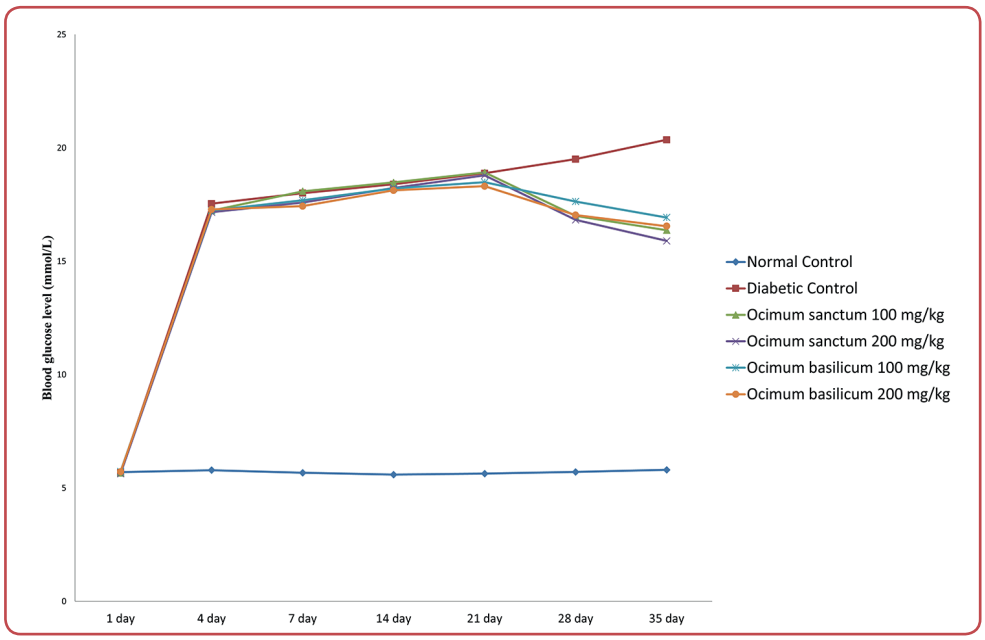

Figure 8: Effect of methanol extract of Ocimum sanctum Linn. and Ocimum basilicum Linn. on blood glucose level in diabetic mice

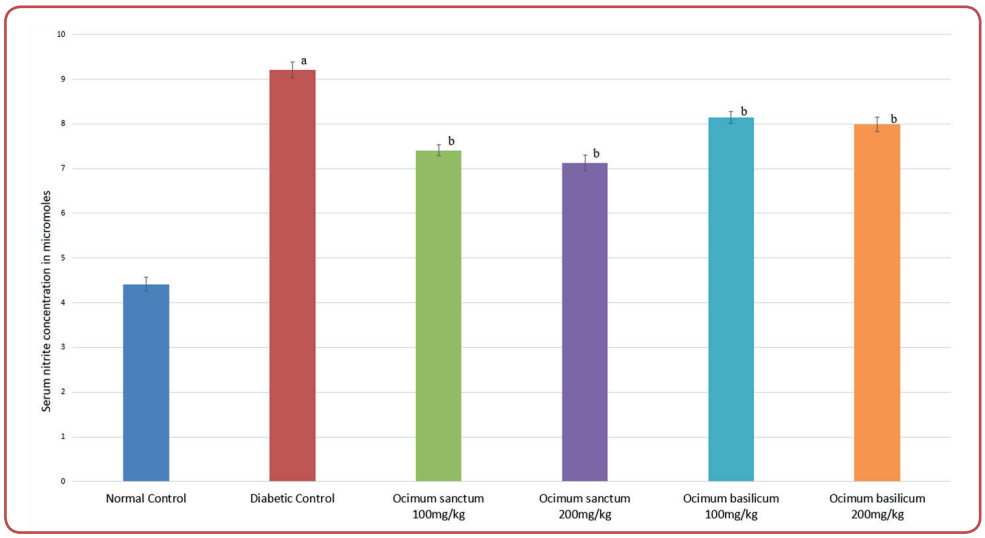

Figure 9: Effect of methanol extract of Ocimum sanctum Linn. and Ocimum basilicum Linn. on serum nitrite level in diabetic mice

Values are presented as mean ± SEM, n = 6; a = p < 0.001 vs normal control group, b = p < 0.001 vs diabetic control group, b = p < 0.01 vs diabetic control group;*

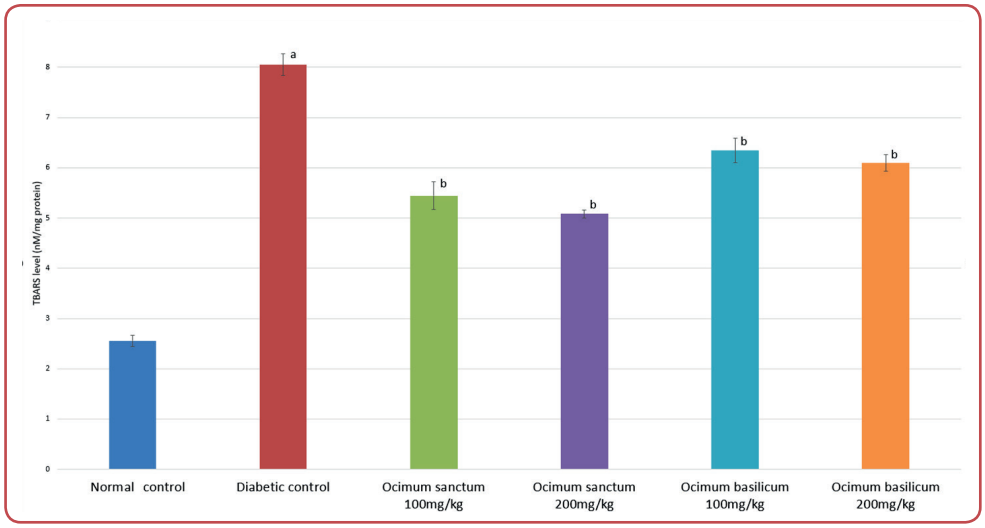

Figure 10: Effect of methanol extract of Ocimum sanctum Linn. and Ocimum basilicum Linn. on thiobarbituric acid reactive substances (TBARS) in diabetic mice

Values are presented as mean ± SEM, n = 6; a = p < 0.001 vs normal control group, b = p < 0.001 vs diabetic control group, b = p < 0.01 vs diabetic control group;*

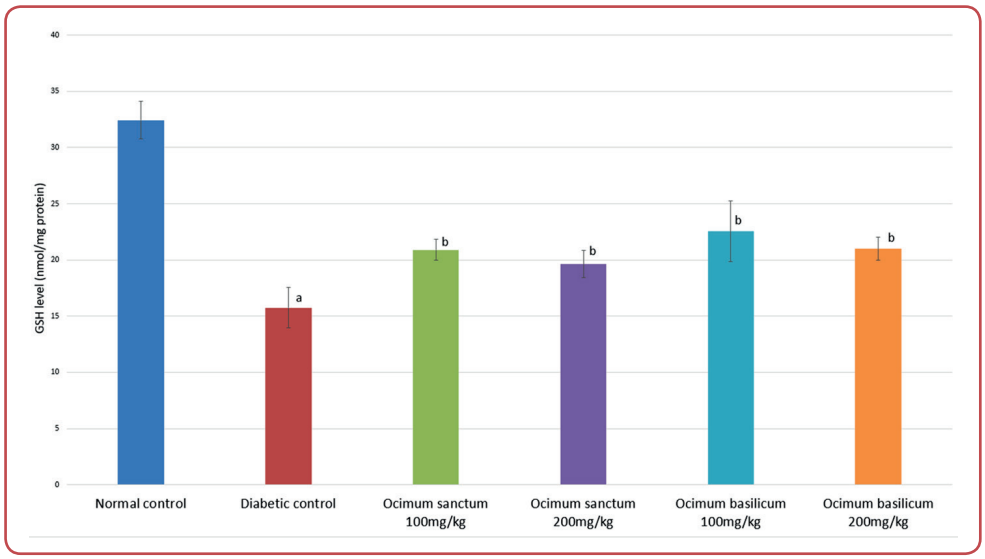

Figure 11: Effect of methanol extract of Ocimum sanctum Linn. and Ocimum basilicum Linn. on glutathione (GSH) level in diabetic mice

Values are presented as mean ± SEM, n = 6; a = p < 0.001 vs normal control group, b = p < 0.001 vs diabetic control group, b = p < 0.01 vs diabetic control group;*

intervention in comparison to diabetic control group (Group II) as shown in Figure 8.

Dose dependent significant effect was observed in the levels of serum nitrite, TBARS and GSH which showed overall improvement in comparison to the normal and diabetic control as given in Figure 9, 10 and 11, respectively. The study concluded the overall antioxidant effect along with improvement in diabetic neuropathy in the experimental methods employed.

Discussion

In the present study, the effect of methanol extracts *Ocimum sanctum* Linn. and *Ocimum basilicum* Linn. was measured on DN mice. The exact pathophysiological mechanism by which nerves are damaged in DN is controversial but prolonged hyperglycaemia is an accepted primary causative mechanism. The direct glucose toxicity in the neurons especially due to increased intracellular glucose oxidation, leads to an increase in reac-

tive oxygen species production. Hyperglycaemia induced overproduction of reactive free radical cause oxidative stress. Studies with antioxidants support the role of oxidative stress associated with diabetic neuropathy.[40] *Ocimum sanctum* Linn. and *Ocimum basilicum* Linn. possess a high level of antioxidant activity, which is attributed to the flavonoids (rutin, orientin and vicenin) and phenolic compounds (gallic acid, irsilineol, cirsimaritin, isothymusin, apigenin and rosmarinic acid and appreciable quantities of eugenol). The result showed that the methanol extract of *Ocimum sanctum* Linn. and *Ocimum basilicum* Linn. gave significant change in weight variation, tail immersion test and blood glucose when compared to diabetic control. In brain tissue homogenate, measurement of oxidative stress by TBARS, GSH and serum nitrite levels produced significant change when compared to diabetic control showing significant protection in DN by *Ocimum sanctum* Linn. and *Ocimum basilicum* Linn.

Two weeks of uncontrolled hyperglycaemia in rats has been reported to result in hyperalgesia, a symptom of diabetic neuropathy.[20] Sufficient time for intervention should be allowed. Tail dip latency was monitored to assess the degree of hyperalgesia. In this study, experimental diabetes-induced hyperglycaemia resulted in a reduction in abortion latency. The shortening of tail beat latency is related to hyperglycaemia duration. Hyperalgesia was reported after 21st day of the experimental protocol. Weight loss is not caused by neuropathy but is common in DN.

Conclusion

Diabetes mellitus remains a significant global health concern, with neuropathy posing a substantial threat to affected individuals. The underlying pathophysiological mechanisms involve prolonged hyperglycaemia and subsequent metabolic dysregulation. Early detection and management of diabetes, alongside addressing associated risk factors, are crucial in mitigating neuropathic complications. Natural antioxidants present in plant extracts offer promising avenues for therapeutic intervention. The study investigating the effects of methanol extracts of *Ocimum sanctum* Linn. and *Ocimum basilicum* Linn. in streptozotocin induced diabetic neuropathy yielded notable findings. These extracts demonstrated significant ameliorative effects on various parameters associated with neuropathy, suggesting their potential in preventing and treating diabetic neuropathy due to their high level of antioxidant activity, which is attributed to the flavonoids (rutin, orientin and vicenin) and phenolic compounds (gallic acid, irsilineol, cirsimaritin, isothymusin, apigenin and rosmarinic acid and appreciable quantities of eugenol). Notably, the methanolic extract of *Ocimum sanctum* Linn. and *Ocimum basilicum* Linn. exhibited pronounced efficacy, warranting further exploration towards clinical applications. While these findings underscore the therapeutic potential of *Ocimum sanctum* Linn. and *Ocimum basilicum* Linn. in managing diabetic neuropathy, additional clinical studies are imperative to validate these results in diabetic populations. Nonetheless, this study underscores the promising role of herbal adjuncts in addressing the complexities of diabetic neuropathy and warrants continued investigation into their clinical utility.

Ethics

The experimental protocol has been approved by Institutional Animal Ethical Committee (IAEC) (decision No 724/02/a/CPCSEA), dated 15 August 2021. All the animals have been kept and cared according to the guidelines of CPCSEA, New Delhi.

Acknowledgement

Authors would like to thank ASBASJSM College of Pharmacy, Bela, Panjab University, Shree Dhanvantary Pharmacy College, Kim and UIPS, Panjab University, Chandigarh for providing access to literature.

Conflicts of interest

The authors declare that there is no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

Author ORCID numbers

Shabeena Khan (SK):
0009-0001-7707-6260
Abhishek Bhanot (AB):
0009-0006-0060-5845
Divya Dhawal Bhandari (DDB):
0000-0002-2813-0267

Author contributions

Conceptualisation: SK
Methodology: AB, SK
Data curation: SK
Writing - original draft: SK, AB, DDB
Writing - review and editing: DDB

References

1. Hussain S, Habib A, Najmi AK. Anemia prevalence and its impact on health-related quality of life in Indian diabetic kidney disease patients: Evidence from a cross-sectional study. J Evid Based Med. 2019 Nov;12(4):243-52. doi: 10.1111/jebm.12367.
2. Hussain S, Habib A, Singh A, Akhtar M, Najmi AK. Prevalence of depression among type 2 diabetes mellitus patients in India: A meta-analysis. Psychiatry Res. 2018 Dec;270:264-73. doi: 10.1016/j.psychres.2018.09.037.
3. Hussain S, Jamali MC, Habib A, Hussain S, Akhtar M, Najmi AK. Diabetic kidney disease: an overview of prevalence, risk factors, and biomarkers. Clinical Epidemiology and Global Health. 2020. doi: 10.1016/j.cegh.2020.05.016.
4. Abbott CA, Malik RA, van Ross ER, Kulkarni J, Boulton AJ. Prevalence and characteristics of painful diabetic neuropathy in a large community-based diabetic population in the U.K. Diabetes Care. 2011 Oct;34(10):2220-4. doi: 10.2337/dc11-1108.
5. Boulton AJ, Gries FA, Jervell JA. Guidelines for the diagnosis and outpatient management of diabetic peripheral neuropathy. Diabet Med. 1998 Jun;15(6):508-14. doi: 10.1002/(SICI)1096-9136(199806)15:6<508::AID-DIA613>3.0.CO;2-L.
6. Veves A, Backonja M, Malik RA. Painful diabetic neuropathy: epidemiology, natural history, early diagnosis, and treatment options. Pain Med. 2008 Sep;9(6):660-74. doi: 10.1111/j.1526-4637.2007.00347.x.
7. WebMD, LLC [Internet]. Peripheral neuropathy and diabetes. [Cited: 21-Jul-2018]. Available at: http://www.webmd.com/diabetes/ peripheralneuropathyriskfactorssymptoms.
8. Ashok S, Ramu M, Deepa R, Mohan V. Prevalence of neuropathy in type 2 diabetic patients attending a diabetes centre in South India. J Assoc Physicians India. 2002 Apr;50:546-50. PMID: 12164406.
9. Karki DB, Yadava SK, Pant S, Thusa N, Dangol E, Ghimire S. Prevalence of sensory neuropathy in type 2 diabetes mellitus and its correlation with duration of disease. Kathmandu Univ Med J (KUMJ). 2016 Apr-Jun;14(54):120-4. PMID: 28166066.
10. Ibarra CT, Rocha Jde J, Hernández RO, Nieves RE, Leyva RJ. [Prevalence of peripheral neuropathy among primary care type2diabeticpatients].RevMedChil.2012Sep;140(9):1126-31. Spanish. doi: 10.4067/S0034-98872012000900004.
11. Khawaja N, Abu-Shennar J, Saleh M, Dahbour SS, Khader YS, Ajlouni KM. The prevalence and risk factors of peripheral neuropathy among patients with type 2 diabetes mellitus; the case of Jordan. Diabetol Metab Syndr. 2018 Feb 21;10:8. doi: 10.1186/s13098-018-0309-6.
12. Shera AS, Jawad F, Maqsood A, Jamal S, Azfar M, Ahmed U. Prevalence of chronic complications and associated factors in type 2 diabetes. J Pak Med Assoc. 2004 Feb;54(2):54-9. PMID: 15134204.
13. International Diabetes Federation. Diabetes Facts and Figures [Internet]. [Cited: 20-Jpr-2020]. Available at: https://www.idf.org/aboutdiabetes/what-is-diabetes/facts-figures.html.
14. Benbow SJ, Wallymahmed ME, MacFarlane IA. Diabetic peripheral neuropathy and quality of life. QJM. 1998 Nov;91(11):733-7. doi: 10.1093/qjmed/91.11.733.
15. Darivemula S, Nagoor K, Patan SK, Reddy NB, Deepthi CS, Chittooru CS. Prevalence and its associated determinants of diabetic peripheral neuropathy (dpn) in individuals having type-2 diabetes mellitus in rural south india. Indian J Community Med. 2019 Apr-Jun;44(2):88-91. doi: 10.4103/ijcm.IJCM_207_18.
16. Jayant SK, Srivastava N. Effect of *Ocimum sanctum* against alloxan induced diabetes and biochemical alterations in rats. Integr Obesity Diabetes 2016;2(5):1-4. doi: 10.15761/IOD.1000162.
17. Vinik AI, Mehrabyan A. Diabetic neuropathies. Med Clin North Am. 2004 Jul;88(4):947-99, xi. doi: 10.1016/j.mcna.2004.04.009.

18. Nilay D. Solanki ND, Bhavsar SK, Pandya DT. Role of phytotherapy in diabetic neuropathy and neurodegeneration: from pathogenesis to treatment. J Phytopharmacol. 2018;7(2):152-61. doi: 10.31254/phyto.2018.7209.
19. Boham BA, Kocipai AR. Flavanoids and condense tannins from leaves of Hawaiian Vaccinium vaticulatum and V. Calicinium. Pacific Sci. 1994;48(4):458-63.
20. Bonjar GHS, Nik AK, Aghighi S. Antibacterial and antifungal survey in plants used in indigenous herbal-medicine of south east regions of Iran. J Biolog Sci. 2004;4(3):405-12. doi: 10.3923/jbs.2004.405.412.
21. Doss A. Preliminary phytochemical screening of some Indian Medicinal Plants. Anc Sci Life. 2009 Oct;29(2):12-6. PMID: 22557345.
22. Rai V, Iyer U, Mani UV. Effect of Tulasi (*Ocimum sanctum*) leaf powder supplementation on blood sugar levels, serum lipids and tissue lipids in diabetic rats. Plant Foods Hum Nutr. 1997;50(1):9-16. doi: 10.1007/BF02436038.
23. Antora RA, Salleh RM. Antihyperglycemic effect of Ocimum plants: A short review. Asian Pacific J Trop Biomed. doi: 10.1016/j.apjtb.2017.07.010.
24. Rubab S, Hussain I, Khan BA, Unar AA, Abbas KA, Khichi ZH, et al. Biomedical description of Ocimum basilicum L. J Islamic Int Med Coll 2017;12(1):59–67.
25. Janick J, Simon JE. Advances in New Crops; Portland, OR, USA: Timber Press 1990; pp. 484–489.
26. Ch MA, Naz SB, Sharif A, Akram M, Saeed MA. Biological and pharmacological properties of the sweet basil (Ocimum basilicum). J Pharm Res Int. 2015;7(5):330–9. doi: 10.9734/BJPR/2015/16505.
27. Purushothaman B, Srinivasan RP, Suganthi P, Ranganathan B, Gimbun J, Shanmugam KA comprehensive review on Ocimum basilicum. J Nat Remedies 2018;18(3):71–85. doi: 10.18311/jnr/2018/21324.
28. Majdi C, Pereira C, Dias MI, Calhelha RC, Alves MJ, Rhourri-Frih B, et al. Phytochemical Characterization and Bioactive Properties of Cinnamon Basil (Ocimum basilicum cv. 'Cinnamon') and Lemon Basil (Ocimum×citriodorum). Antioxidants (Basel). 2020 Apr 29;9(5):369. doi: 10.3390/antiox9050369.
29. Duke JA, Ayensu ES. Medicinal plants of China. Michigan: Reference Publications; 1985.
30. Shakeri F, Hosseini M, Ghorbani A. Neuropharmacological effects of Ocimum basilicum and its constituents. Physiol Pharmacol 2019;23:70-81.
31. Chandrasekaran CV, Srikanth HS, Anand MS, Allan JJ, Viji MM, Amit A. Evaluation of the mutagenic potential and acute oral toxicity of standardized extract of *Ocimum sanctum* (OciBest™). Hum Exp Toxicol. 2013 Sep;32(9):992-1004. doi: 10.1177/0960327112472992.
32. Mahajan N, Rawal S, Verma M, Poddar M,Alok S. A phytopharmacological overview on Ocimum species with special emphasis on *Ocimum sanctum*. Biomed Prevent Nutr. 2013;3(2):185-92. doi: 10.1016/j.bionut.2012.08.002.
33. Pattanayak P, Behera P, Das D, Panda SK. *Ocimum sanctum* Linn. A reservoir plant for therapeutic applications: An overview. Pharmacogn Rev. 2010 Jan;4(7):95-105. doi: 10.4103/0973-7847.65323.
34. Sethi J, Sood S, Seth S, Talwar A. Evaluation of hypoglycemic and antioxidant effect of *Ocimum sanctum*. Indian J Clin Biochem. 2004 Jul;19(2):152-5. doi: 10.1007/BF02894276.
35. Suanarunsawat T, Songsak T. Anti-hyperglycemic and anti-hyperlipidemic effect of dietary supplement of white *Ocimum sanctum* L. before and after STZ-induced diabetes mellitus. Int J Diabetes Metab. 2005;13(1):18-23. doi: 10.1159/000497569.
36. Madhuri, S. Studies on oestrogen induced uterine and ovarian carcinogenesis and effect of ProImmu in rats. Ph.D. thesis, Rani Durgavati, Vishwa Vidyalaya, Jabalpur, MP, India: 2008.
37. Madhuri S, Govind P. Effect of ProImmu, a herbal drug on estrogen caused uterine and ovarian cytotoxicity. Biomed. 2010;5(1):57-62.
38. Bihari CG, Manaswini B, Panda SKSP, Tripathy SKST. Phytochemical investigation & evaluation for antidiabetic activity of leafy extracts of various Ocimum (Tulsi) species by alloxan induced diabetic model. J Pharm Res 2011;4:28-9.
39. Borah R, Biswas SP. Tulsi (*Ocimum sanctum*), excellent source of phytochemicals. IJEAB. 2018;3(5):1732-8. doi: 10.22161/ijeab/3.5.21.
40. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958;5(4):181:1199-200. doi: 10.1038/1811199a.