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IN VIVO EVALUATION STUDIES FOR ISOFLAVONES LOADED NANODROPLETS FOR DIABETIC ENCEPHALOPATHY.

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ABSTRACT:

Diabetic encephalopathy (DE) is a debilitating complication of diabetes mellitus, characterized by cognitive decline, memory impairment, and mood disturbances. Despite its significance, there is a lack of effective therapeutic strategies to address this condition. Isoflavones, a class of plant-derived compounds, have been shown to exhibit neuroprotective and antioxidant properties, making them promising candidates for the treatment of DE. In this study, we investigated the in vivo efficacy of isoflavones loaded nanodroplets (IND) for the treatment of DE. Male Sprague-Dawley rats were induced with streptozotocin to develop type 1 diabetes mellitus, followed by injection of INL or vehicle control. The rats were then evaluated for cognitive function, memory, and mood disturbances using a battery of behavioral tests. Additionally, we assessed the changes in brain morphology and biochemistry using magnetic resonance imaging (MRI) and Western blot analysis.Our results show that IND significantly improved cognitive function, memory, and mood disturbances in diabetic rats compared to vehicle control. IND also reduced oxidative stress and inflammation in the brain, as evidenced by decreased levels of malondialdehyde and tumor necrosis factor-alpha (TNF-alpha). Furthermore, IND increased the expression of brain-derived neurotrophic factor (BDNF) and reduced the expression of cleaved caspase-3, indicating enhanced neuroprotection. These findings demonstrate the therapeutic potential of IND in the treatment of DE. The nanodroplet delivery system allows for targeted and sustained release of isoflavones to the brain, which may improve their bioavailability and efficacy. Our study provides a novel therapeutic approach for the treatment of DE, and further research is needed to explore the long-term efficacy and safety of IND in human subjects.

Keywords: Diabetic Encephalopathy, Isoflavones, Nanodroplets, Cognitive Function.

INTRODUCTION:

Diabetic encephalopathy (DE) is a severe and debilitating complication of diabetes mellitus, characterized by cognitive impairment, mood disturbances, and behavioral changes. Despite its significant impact on quality of life and healthcare costs, there is currently no effective treatment available for DE. Recent studies have suggested that isoflavones, a class of plant-derived polyphenols, may have neuroprotective and cognitive-enhancing properties, making them a promising therapeutic approach for the treatment of DE.

Nanodroplets, a novel type of nanoparticles, have been shown to effectively deliver hydrophobic molecules such as isoflavones across the blood-brain barrier, a major obstacle in the treatment of central nervous system disorders. The use of nanodroplets has been demonstrated to enhance the bioavailability and efficacy of various therapeutic agents, including those used to treat neurodegenerative disorders.

In this study, we aimed to investigate the efficacy and safety of isoflavones loaded nanodroplets in an in vivo model of DE. We hypothesized that the nanodroplet delivery system would enhance the bioavailability and brain penetration of isoflavones, resulting in improved cognitive function and neuroprotection in diabetic animals. Our study will provide valuable insights into the potential therapeutic utility of isoflavones loaded nanodroplets for the treatment of DE and may pave the way for the development of novel treatments for this debilitating condition.

Methodology:

The study will be conducted in accordance with the principles of animal welfare and will involve a randomized, controlled trial design. Diabetic animals will be randomly assigned to receive either isoflavones loaded nanodroplets or a placebo control treatment. Cognitive function will be evaluated using a battery of behavioral tests, including the Morris water maze and the object recognition test. Neuroprotection will be assessed using immunohistochemical analysis and biochemical assays. Safety and tolerability will be monitored through regular monitoring of blood chemistry and hematology.

By evaluating the efficacy and safety of isoflavones loaded nanodroplets in an in vivo model of DE, this study aims to contribute to the development of novel treatments for this devastating condition. Memory, mood disturbances, oxidative stress, inflammation.

In vivo studies

Animals:

Male wistar albino rats (11-12 weeks) of weight 150-250 grams were obtained from JSS College of Pharmacy, Ooty which were selected for the pharmacokinetic and pharmacodynamic studies. The study protocol for animal studies was approved by the Institutional Animal Ethical Committee, JSS College of Pharmacy, Ooty, Tamilnadu, India. (JSSCP/OT/IAEC/14/ 2022-2023.CPCSEA; Approval no.14, 2022) and their guidelines were followed throughout the studies. The animals were housed under standard conditions of temperature (25 °C), in 12/12 h light and dark cycles, and were fed with a standard pellet diet and water ad libitum.

Induction and assessment of diabetes:

STZ-induced diabetic associated cognitive decline model: A single dosage of 55 mg/kg streptozotocin produced in citrate buffer (pH 4.4, 0.1 M) was injected intraperitoneally to induce diabetes. The age-matched control rats received an identical volume of citrate buffer. Diabetes was proven after 72 h of streptozotocin injection, the blood samples were taken by tail vein and plasma

glucose levels were assessed. The rats having fasting plasma glucose levels of more than 300 mg/dL were selected and employed for the present study. Bodyweight levels were measured before and at the end of the experiment to see the influence of S-Equol on these parameters and plasma glucose levels were measured five times during whole experimental tenure (3,10,20,30,and 36 days after the beginning of the treatment)
Animals were divided into seven groups of six animals each.

Group 1- Control group received a vehicle (DMSO).
Group 2- Diabetic received Streptozotocin (55mg/kg in i.p)
Group 3- Diabetic+ Estradiol (0.2mg/kg in i.p).
Group 4- Diabetic+ Metformin (500mg/kg in oral).
Group 5- Diabetic+ S-Equol (20mg/kg in oral).(92)
Group 6- SEND low dose (2mg/kg in i.n).
Group 7- SEND high dose (4mg/kg in i.n).

Blood glucose concentrations were checked every week during the whole experiment tenure.All the animals were treated daily for a period of the next 30 days. After the treatment period, the animals were subjected to behavioral parameters like the Morris water maze test and Memory consolidation test. The learning and memory was evaluated during day 31-36.At the end of the study, all the animals were anesthetized with Ketamine (60 mg/kg i.p.) and the rats were dissected for brain isolation. Cerebral cortex and hippocampus were dissected.The left hemispheres is separated for the estimation of acetylcholinesterase activity, antioxidant studies (ROS, MDA, GSH, SOD, Catalase) , inflammatory markers (NFκB, IL-1β, and TNF-α), and estimation of Erβ. The right hemisphere was immediately immersed in 4% formaldehyde and further subjected to histopathological analysis.

The rapid dissection of the cortex and hippocampus was done and rinsed with ice cold saline.These brain parts were homogenized separately in mixture of chilled phosphate buffer and distilled water.The nuclear debris was separated out by centrifuging the homogenates at 4600 rpm for 10min at 4oC.The supernatants thus obtained were re centrifuged at 15000rpm for 30min at 4oC to obtain the post mitochondrial supernatant.The samples of the brain and supernatant were stored at -80oC and used for futher biochemical assay.The protein content was determed followed by biochemical assays.(94)

Biochemical markers:
Blood glucose level: Glucose concentrations were measured with the Gluco one glucometer (Blood glucose Monitor, Model: BG-03) in rat tail vein blood. Glucose oxidase (GOD) oxidizes the specific substrate β-D-glucose to gluconic acid and hydrogen peroxide (H2O2) is liberated. Peroxidase (POD) enzyme acts on hydrogen peroxide to liberate nascent oxygen (O2), then nascent oxygen couples with 4- amino antipyrine and phenol to form red quinone imine dye. The intensity of the color is directly proportional to the concentration of glucose present in the plasma. The intensity of the color is measured by a colorimeter at 530 nm or green filter and compared with that of a standard treated similarly. The final color is stable for at least 2 hours if not exposed to direct sunlight. (85)

Acetylcholinesterase activity: Cholinergic dysfunction was assessed by Ach activity. The quantitative measurement of Ach levels in the cerebral cortex and hippocampus was performed. The assay mixture contained 0.05ml of supernatant, 3ml of 0.01M sodium phosphate buffer (pH 8), 0.10 ml of acetylthiocholine iodide, and 0.10 ml 5,5, dithiobis (2-nitro benzoic acid) (Ellman reagent). The change in absorbance was measured at 412 nm for 5 min. Results were calculated using a molar extinction coefficient of the chromophore ($1.36\times104\ M^{-1}\ cm^{-1}$) and expressed as a percentage of control.(85)

Behavioral parameters:

Morris water maze test: Animals were kept for spatial Morris water maze test. The device is made up of a circular water tank (180 cm in diameter and 60 cm high). A platform (12.5 cm in diameter and 38 cm high) is placed inside the tank, invisible to the rats, and filled with water kept at 282 °C at a height of 40 cm. The storage tank was situated in a large room where there have been several brightly colored cues external to the maze; these were visible from the pool and were employed by the rats for spatial orientation. The position of the cues remained unchanged throughout the study. The water maze task was administered for five consecutive days(day 31-35). The rats have trained accordingly.(85)

Memory consolidation test: The level of memory consolidation were assessed by carrying out a probe trial. The extent of memory consolidation was denoted by the time taken in the target quadrant examined after learning. The probe trial was carried out similarly to the training trial, with the hidden platform detached from the pool. The time taken in the target quadrant is considered 60 s. Each rat was positioned at a start position exactly opposite to the platform quadrant in the probe trial. Further, the number of crossings above the platform position of each rat was also recorded.(85)

Antioxidant parameters:

Reactive Oxygen Species (ROS):2',7' –dichlorofluorescein diacetate (DCFDA; Sigma Aldrich), a fluorogenic dye that measures hydroxyl, peroxyl, and other ROS activity within the cell. After diffusion into the cell, DCFDA is deacetylated by cellular esterases to a non-fluorescent compound, which is later oxidized by ROS into 2', 7' – dichloro fluorescin (DCF) which emits green fluorescence upon excitation with a blue filter. Carboxy-H2DCFDA is non-fluorescent but in the presence of ROS, when this reagent is oxidized, it becomes green fluorescent. (85)

Estimation of lipid peroxidation: Malondialdehyde (MDA) was measured as an index of lipid peroxidation according to Ohkawa with minor adjustments.(93) 0.1 ml supernatant was mixed with 1.5 ml glacial acetic acid (20%), 0.2 ml sodium dodecyl sulphate (8.1%), and 1.5 ml thiobarbituric acid (0.8 percent). The tube-containing mixture was agitated, heated for 1 h at 95 °C on a water bath, and cooled below tap water. Each tube received 5 ml pyridine and n-butanol (1:15) and 1 ml distilled water and was centrifuged at 4000 rpm for 10 min. To estimate MDA formation, the upper organic pink layer absorbance was measured at 532 nm. A calibration curve was plotted for malondialdehyde bis- (dimethoxy acetyl). The data were nmol MDA/mg protein.(93)

Estimation of reduced glutathione: The method was used to test the amount of reduced glutathione. In short, 10 percent of the post-mitochondrial supernatant was precipitated with 10 percent of sulphosalicylic acid (4%). After keeping the samples at 4 °C for at least an hour, they were spun at 1200 g for 15 minutes at 4 °C. The assay mixture contained 0.1 ml supernatant, 2.7 ml phosphate

buffer (0.1 M, pH 7.4) and 0.2 ml 5,5, dithiobis (2-nitro benzoic acid) (Ellman's reagent, 0.1 mM, pH 8.0) in a total volume of 3.0 ml. The yellow colour developed was read immediately at 412 nm.

Estimation of superoxide dismutase: The method was used to test the activity of cytosolic superoxide dismutase. The test system was made up of 0.1 mM EDTA, 50 mM sodium carbonate, and 96 mM nitro blue tetrazolium (NBT). In the cuvette, 2 ml of the above mixture was put in with 0.05 ml of post-mitochondrial supernatant and 0.05 ml of hydroxylamine hydrochloride (adjusted to pH 6.0 with NaOH). By measuring the change in optical density at 560 nm every 30 or 60 seconds for 2 minutes, the auto- oxidation of hydroxylamine could be seen.(90)

Estimation of NFκB: Activation of the NF-κB pathway may be linked to the amount of p65 in the nucleus. NF-κB free p65 in the nuclear lysate was measured with the NF-kB/p65 ActivELISA (Imgenex, San Diego, USA) kit. A sandwich ELISA is what the NF-kB ActivELISA. Anti-p65 antibody-coated plates caught free p65, and the amount of bound p65 was measured by adding a second anti-p65 antibody and an alkaline phosphatase (AKP)-conjugated secondary antibody. This was done in an ELISA plate reader at 405 nm.(85)

Estimation of TNF- α and IL-1 β: The Dialcone murine TNF-α and IL- 1β immunoassay kit's assistance and instructions were used to quantify TNF-α and IL- 1β only for research purposes. With a pre-coated murine-specific monoclonal antibody and a supplied murine-specific enzyme-linked polyclonal antibody for TNF-α and IL- 1β, the sandwich ELISA method has been used for control, standard, or test samples. The quantity of murine TNF-α and IL- 1β that was initially bound was taken into account when calculating the colour intensity, which was measured at 450 nm as the main wavelength and 620 nm as the reference wavelength. The standard curve was then used to read off the sample values. (90)(92)

Estimation of SEND on Erβ by (qRT-PCR):Total RNA was extracted with Trizol buffer (Invitrogen, USA) in which the total RNA purity and concentration was assessed by using Qubit fluorometer and reverse transcribed into cDNA preparation kit (G BIOSCIENCES, master premix for first-strand cDNA synthesis). Quantitative real-time PCR was performed with the SYBR Green Master Mix (G BIOSCIENCES, Product code-786-5062) using Light cycler 96 (Roche). All reactions were performed in triplicates and data were analysed according to ΔΔCt method (using Light Cycler 96 SW 1.1 Software). (63)

Table-4: The primer sequences.

OLIGO NAME	FORWARD		REVERSE	
	SEQUENCE (5’ ->3’)	Tm	SEQUENCE (5’ ->3’)	Tm
GAPDH	CCCACTCTTCCACCTTCG AT	59.35	GGATAGGGCCTCTCTTGC TC	61.40
ER BETA	CTCACGTCAGGCACATCA G T	59.3	TGTGAGCATTCAGCATCT CC	59.3

Gel electrophoresis: Gel electrophoresis is a way to separate and visualize fragments of mRNA. When an electric field is applied on the fragments, they move through an agarose gel matrix based on their size and charge. The electric field is generated by applying potential across an electrolyte

solution (buffer). When agar is boiled in an aqueous buffer, it breaks down and forms a gel when it cools. A 1.5% agarose gel was made in a 1x TE buffer and melted in a 90°C hot water bath. Then, the agarose that had been melted was cooled to 45oC. The gel comb was used to pour 6µl of 10 mg/mL ethidium bromide into a gel casting device. After the gel had set, the comb was taken out of it. The electrophoresis buffer was poured into the gel tank, and the gel platform was put in it so that the gel would be submerged. The samples were put on the gel, and it was run at 50 V for 30 minutes. A gel documentation device (E gel imager, Invitrogen) was used to see the stained gel. (95)

Histopathology of the cerebral cortex and hippocampus: The right hemisphere of the brain was immediately immersed in 4% formaldehyde for 18 hours; then, it was sequentially transformed into 10%, 20%, and 30% sucrose solution, for 24 hours each time. After complete dehydration, the tissue was frozen and then prepared for the frozen section. The brain was cut serially on a Leica microtome into a 20-µm-thick coronal section subjected to hematoxylin-eosin (HE) staining. After immersing in PBS for 30 minutes. All coronal sections were mounted on glass slides and stained with the routine HE technique(96).

e. Statistical analysis: Results were expressed as mean ± SEM. The intergroup variation was measured by one-way analysis of variance (ANOVA) followed by Tukey's test. Statistical significance was considered at $P<0.05$. The statistical analysis was done using GraphPad Prism Version 9.0.0.

IN VIVO:

Biochemical markers:

Effect of SEND on body weight and blood glucose level:

After the streptozotocin injection, plasma glucose levels were examined on third day and on six weeks of the experimental period. Plasma glucose levels were elevated in diabetic rats (441.2±5.543mg/dl) as compared to the control rats (117.1±1.022mg/ml

). however, after the treatment period with metformin at dose of 500mg/kg and S-Equol ND high dose 4mg\kg the blood glucose levels and body weights were both reversed in diabetic rats($p<0.05$). Chronic SEND significantly and dose dependently preserved($p<0.05$) (Table-33)

Table-1: Effect of SEND on body weight and blood glucose level (mean ± S.E.M.)

Treatment	Body weight(gm)		Plasma Glucose (mg/dl)	
	Onset of study	End of Study	Onset of study	End of Study
Control	230.0±21.77	261.4±8.548	117.1±1.022	118.1±1.022
Diabetes	240.2±24.20	140.2±4.200a	119.2±2.186	441.2±5.543 a
Diabetes + Estradiol	226.2±16.64	235.6±15.67b	115.6±1.042	270.8±7.706 b
Diabetes + Metformin	231.0±20.07	227.8±15.31b	111.8±1.097	108.5±2.857 b
Diabetes + S-Equol	248.6±5.573	239.6±16.46b	113.3±2.589	264.4±7.122 b
Diabetes + SEND(2)	230.0±22.42	222.8±10.63 b,c	112.8±3.036	265.5±5.882 b,c

Diabetes + SEND(4)	222.0±28.42	215.8±13.56 b,c	112.5±2.303	218.2±2.313 b,c

a($p<0.05$) different from control group;b($p<0.05$) different from Diabetic; c($p<0.05$) different from one another. SEND(2)-2mg/kg,SEND(4)-4mg/kg.

Effect of SEND on acetylcholinesterase activity: Acetylcholinesterase activity was elevated in cerebral cortex whereas in hippocampal acetylcholinesterase activity did not alter in the diabetic animals after the six weeks. SEND significantly and dose dependently prevented this rise in acetylcholinesterase activity($p<0.05$) (Figure-42).

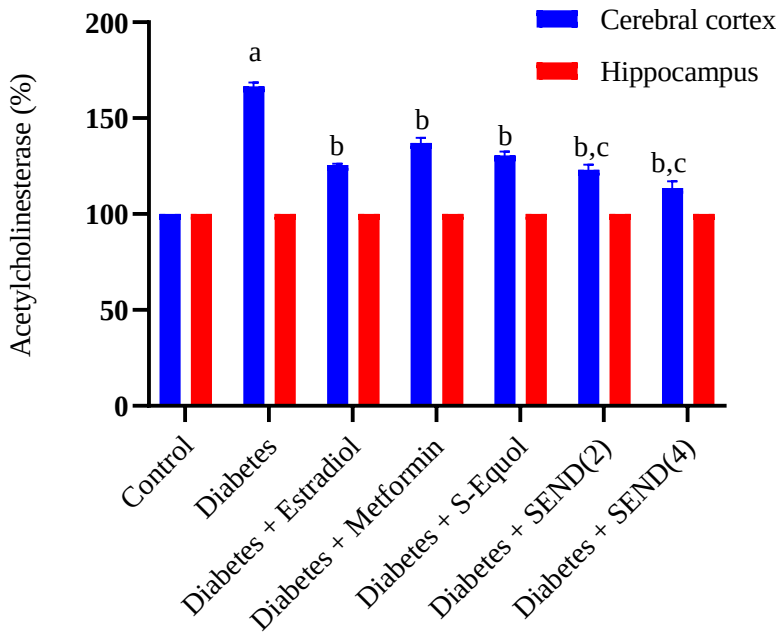


Figure 1: Effect of SEND on acetylcholinesterase activity in cerebral cortex and hippocampus of diabetic rats.

a($p<0.05$) different from control group;b($p<0.05$) different from Diabetic; c($p<0.05$) different from one another.

II. Behavioural parameters:

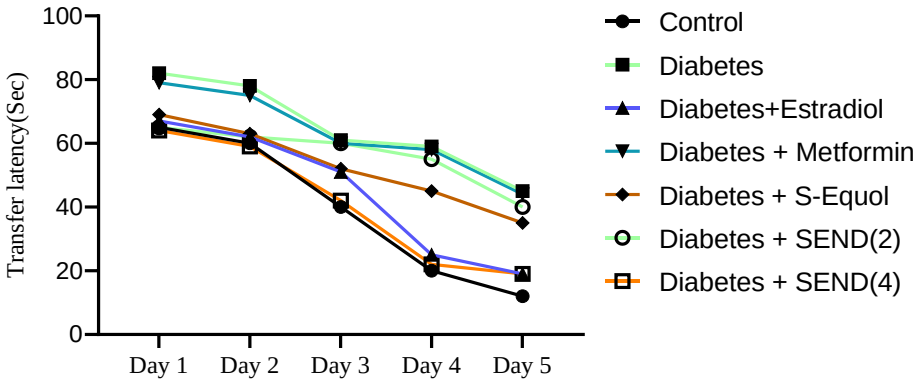
i. Effect of SEND on diabetes induced cognitive decline by Morris water maze:

The cognitive function was estimated by Morris water maze test. The mean escape latency for the trained rats decreased from 65 to 15 over the course of the 5 learning trials. There was a significant difference between control and diabetic animals($p<0.05$). Chronic SEND treatment significantly decreased mean transfer latency in diabetic animals (Fig no-43A). Diabetic animals showed a lower ability to find the platform and learn its location in the 5th day of training. This poorer performance was improved by the chronic treatment with SEND(4) as evident by decreased latency to find the platform from the 2nd day of training.

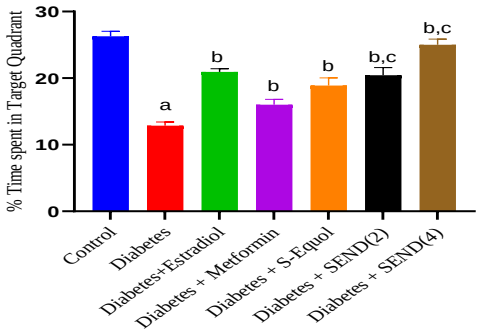
In the probe trial of the Morris water maze study, this measures how well the animals had learned and consolidated the platform location during the five days of training, animals showed a

significant difference (Fig no - 43B). The time spent in the target quadrant was significantly lower in diabetic animals as compared to the control group. The rats chronically treated with SEND spent more time in the target quadrant than the diabetic group in the probe test.

The time spent in the target Quadrant was significantly lower for the diabetes group when compared to other groups. The P-value is found to be <0.05 which shows the study is significant by one-way ANOVA by Dunnett's Multiple Comparison Test.



(A)



(B)

Figure 2: Effect of S-Equol and SEND on the performance of spatial memory acquisition phase.(A)Transfer latency (B)%Time Spent in target quadrant.

[a]($p<0.05$) different from control group;[b]($p<0.05$) different from Diabetic; [c]($p<0.05$) different from one another. A-Transfer latency B- %Time spent in Target Quadrant.

ii. **Memory consolidation test:**

Comparable results were evidenced from the previous platform crossings experiments. Diabetic rats crossed over the platform less frequently as compared to the non-diabetic control rats. S-Equol ND High dose exhibited the highest number of crossings over the platform compared to diabetic rat ($p< 0.05$).

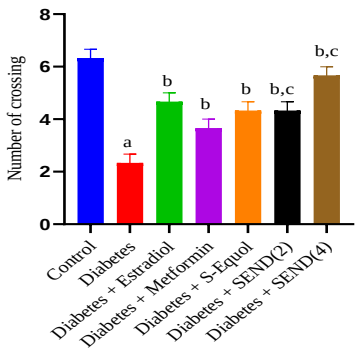


Figure 3: Effect of SEND on the number of times of crossing platform.

[a](p<0.05) different from control group;[b](p<0.05) different from Diabetic; [c](p<0.05) different from one another.

III. Antioxidant parameters:

i. Effect of SEND on ROS-Dichlorofluorescein (DCFDA): ROS-Dichlorofluorescein levels were increased significantly in the cerebral cortex and hippocampus of diabetic rats as compared to the control group(p<0.05). Chronic treatment with SEND(2) and (4) significantly decreased ROS levels in STZ-induced diabetic rats(p<0.05)(Figure 45).

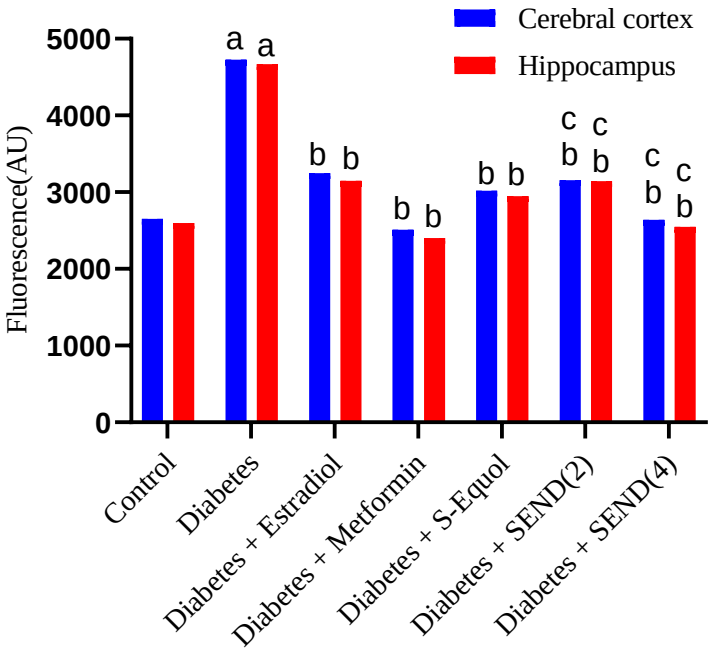


Figure 4: Effect of SEND on ROS-Dichlorofluorescein (DCFDA)

[a](p<0.05) different from control group;[b](p<0.05) different from Diabetic; [c](p<0.05) different from one another.

ii. Effect of SEND on lipid peroxidation: LPO levels were increased significantly in the cerebral

cortex and hippocampus of diabetic rats as compared to the control group($p<0.05$). Chronic treatment with SEND(2) and (4) significantly decreased LPO levels in STZ-induced diabetic rats($p<0.05$)(Figure 46).

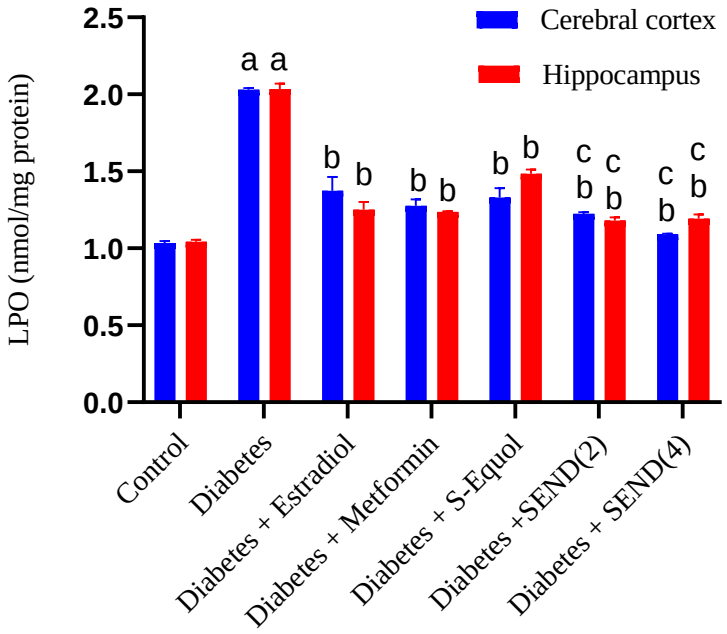
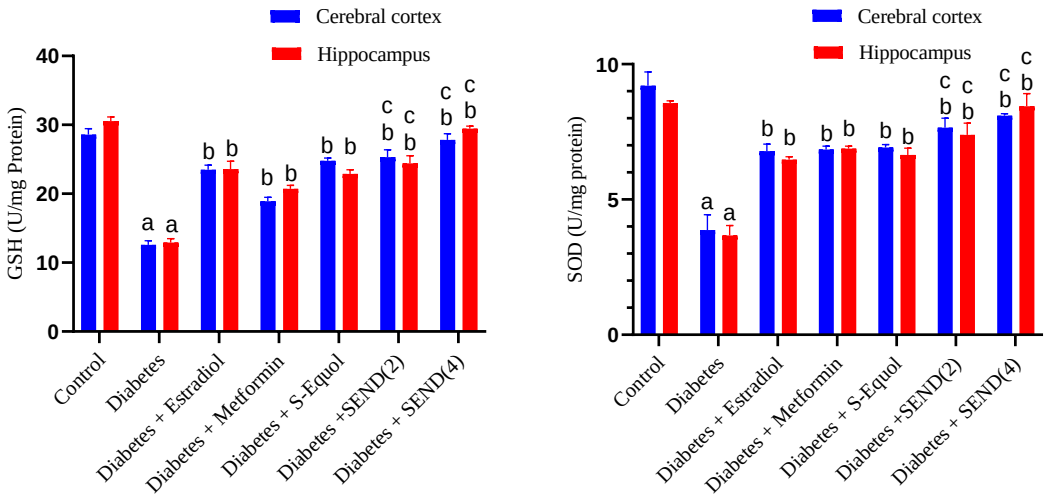


Figure 5: Effect of SEND on LPO

[a]($p<0.05$) different from the control group;[b]($p<0.05$) different from Diabetic; [c]($p<0.05$) different from one another.

iii. Effect of SEND on diabetic-induced changes in antioxidant profile**:** The reduced glutathione levels and enzyme activity of superoxide dismutase and catalase significantly decreased in the cerebral cortex and hippocampus of diabetic rats as compared to the control group($p<0.05$). Chronic treatment with SEND(2) and (4) significantly increased glutathione levels and enzyme activity of superoxide dismutase and catalase in STZ-induced diabetic rats($p<0.05$) (Figure 47).



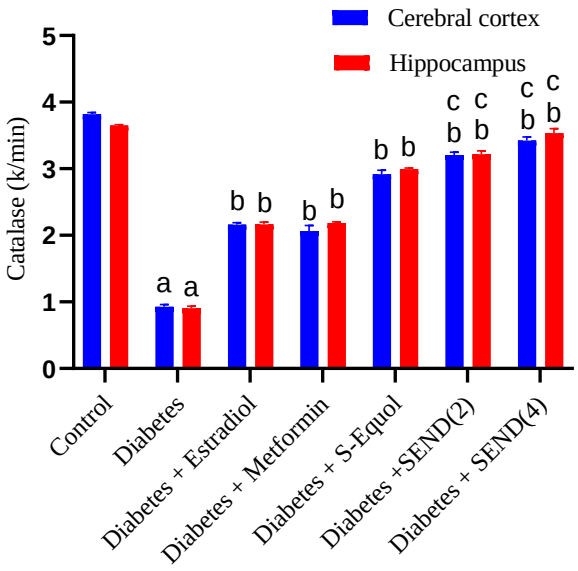


Figure 6:Effect of SEND on diabetic induced changes in GSH, SOD, Catalase

[a](p<0.05) different from control group;[b](p<0.05) different from Diabetic; [c](p<0.05) different from one another.

iv. Effect of SEND on NF-κB: NF-κB p65 subunits were significantly elevated in the cerebral cortex and hippocampus of diabetic rats. The chronic treatment of the SEND significantly (p<0.05) suppressed the elevated NF-κB in treated groups.

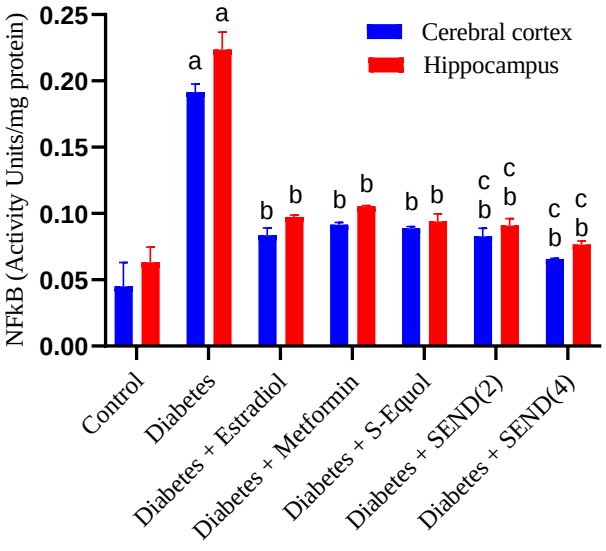


Figure 7: Effect of SEND on NFκB

[a](p<0.05) different from control group;[b](p<0.05) different from Diabetic; [c](p<0.05) different from one another.

v. Effect of SEND on TNF-α, IL-1β: The levels of TNF-α and IL-1β were elevated in the diabetic animals. Treatment with SEND Markedly and Dose dependently inhibited TNF-α and IL-1β levels in the cerebral cortex and hippocampus of STZ-induced rats (p<0.05).

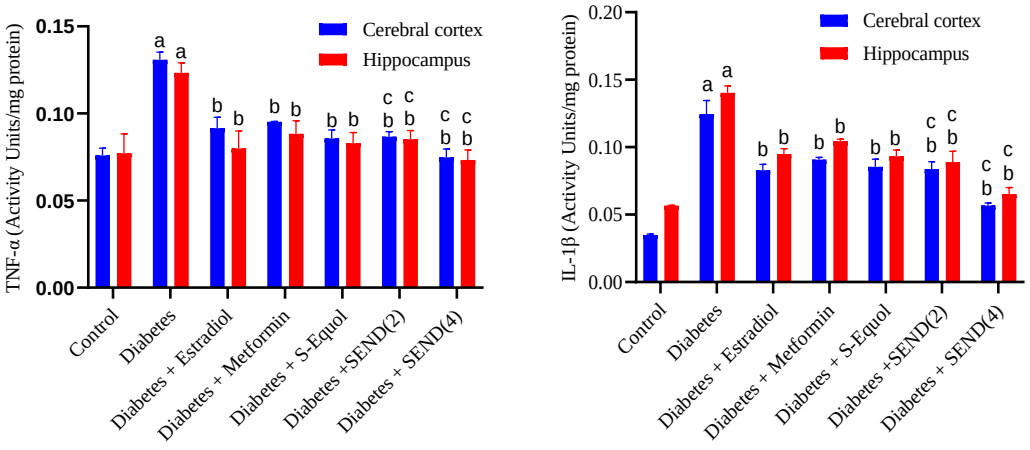
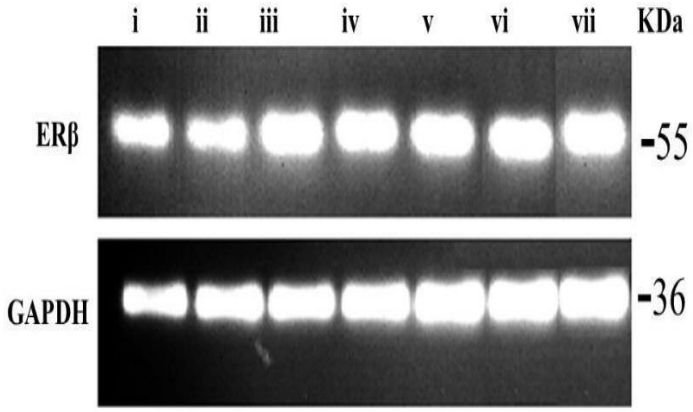


Figure 8: Effect of SEND on TNF-α, IL-1β

[a](p<0.05) different from control group;[b](p<0.05) different from Diabetic; [c](p<0.05) different from one another.

Gene expression analysis by Rt-qPCR of Erβ: The gene expression analysis was done using the RT-PCR method in ΔΔCt model the cycle threshold has been assessed the forward and reverse primers of Erβ as well as internal reference GAPDH. The expression of error beta gene evaluated in different concentrations of prepared ND. To determine to SEND formulation mediated activation of the Erβ receptor. The primer sequences used were as follows: GAPDH (forward 5'-CCCACTCTTCCACCTTCGAT- 3'; reverse 5'-GGATAGGGCCTCTCTTGCTC); Erβ (forward 5'- CTCACGTCAGGCACA TCAGT-3'; reverse 5'-TGTGAGCATTCAG CATCTCC-3').
The data is compared with the internal reference GAPDH.SEND(4) was significantly (p<0.05) increasing the gene expression as compared with other treated groups.



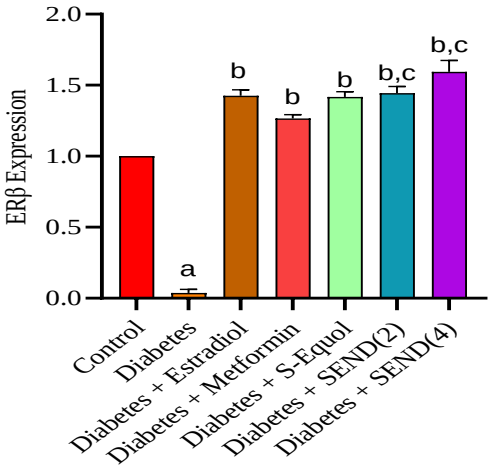
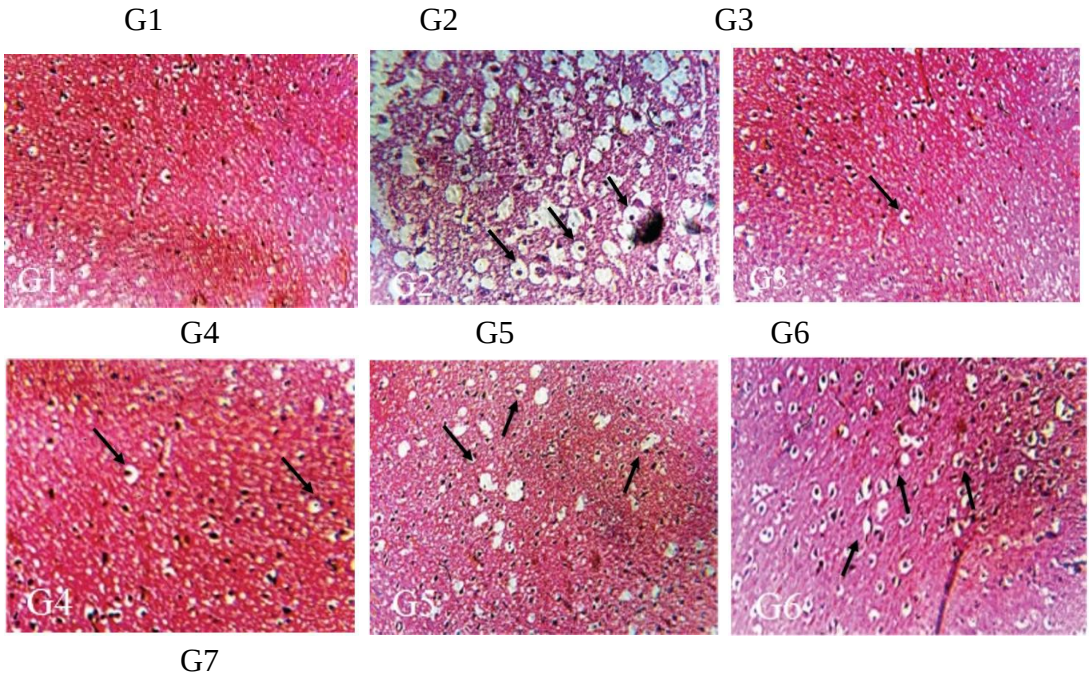


Figure 9: Gene expression analysis of SEND regulates Erβ in diabetes.
a($p<0.05$) different from control group;b($p<0.05$) different from Diabetic; c($p<0.05$) different from one another.

Histopathology of the cerebral cortex and hippocampus: Histopathology studies show that standard drugs and high-dose formulations protect the cells in the hippocampus. Histopathology studies show that Standard drug and High dose Formulation protects the cells in the hippocampus based on the inference.

Histopathology of Cerebral cortex: G1- Neuronal cells were observed. G2- In STZ- induced group perineural vacuolation, neuronophagia were found, G3-Slight neuronal shrinkage and mild microgliosis find in the cerebral cortex treated with Estradiol.G4- Slight neuronal shrinkage and Inflammatory cells can be seen in the neuropils. G5- less perineural vacuolation is observed in S-Equol treated group. G6-In SEND(2) perineural vacuolation and Mild congestion of blood vessels are observed. G7- Neuronal cells with less perineural vacuolation is observed in SEND(4) treated group.



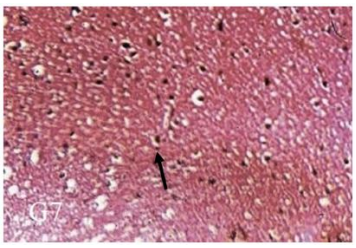


Figure 10: Histopathology of the cerebral cortex.
G1-Control; G2-Diabetes; G3-Diabetes+Estradiol; G4-Diabetes+ Metformin; G5- Diabetes+S-Equol; G5-Diabetes-SEND(2); G6-Diabetes+SEND(4).
Histopathology of Hippocampus: G1-Section from non-diabetic control showing 6– 8 compact layers of small pyramidal cells, most with vesicular nuclei. G2-a layer of large pyramidal cells shows disorganization with many apoptotic cells. The molecular layer shows apoptotic cells. Extensive damage in the cells. G3-Preservation of small pyramidal cells except for the deepest layer. G4-shrinkage and darkening of many large pyramidal cells. G5-Granular cells show less vacuolation, & molecular layer shows the normal size of cells, with widened capillaries. G6-Preservation of small pyramidal cells. G7-Preservation of small pyramidal cells. Granular cells show less vacuolation, & molecular layer shows the normal size of cells, with widened capillaries.

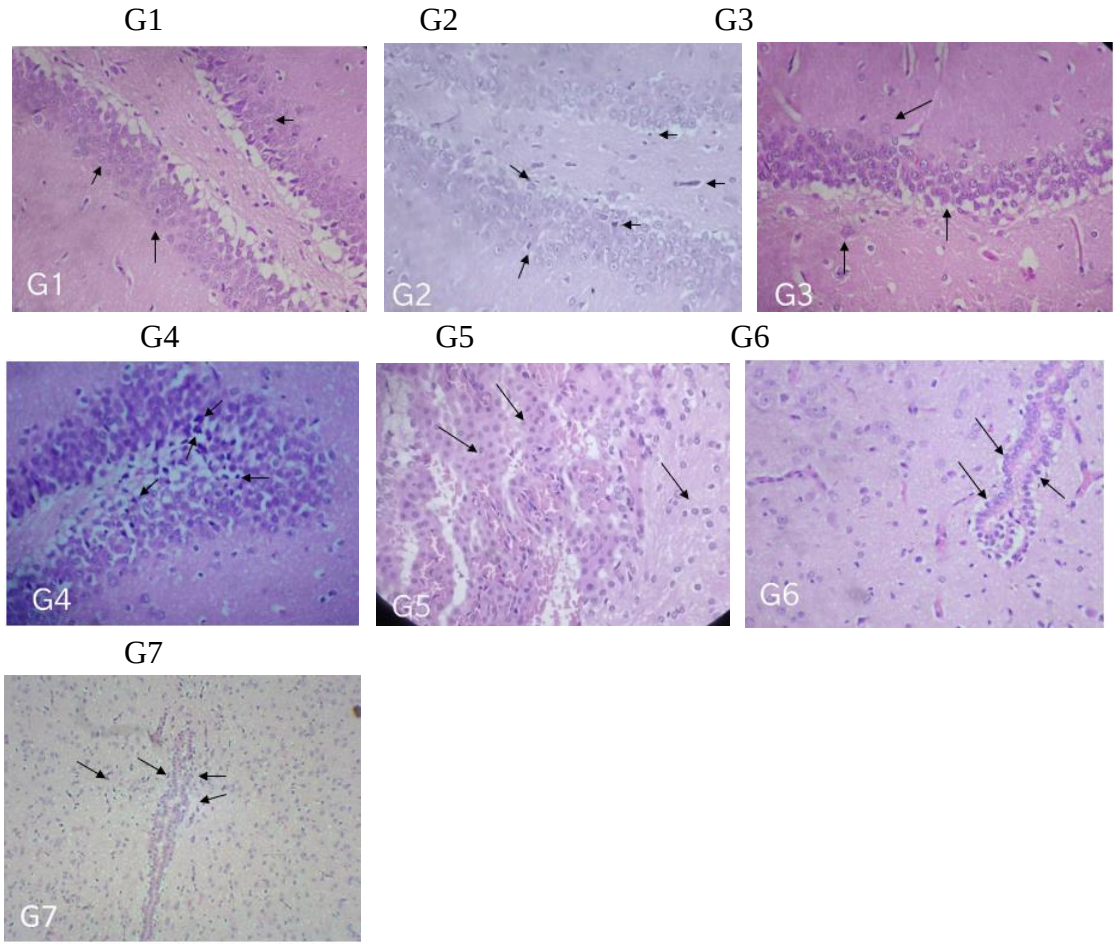


Figure 11: Histopathology of the hippocampus.
G1-Control; G2-Diabetes; G3-Diabetes+Estradiol; G4-Diabetes+ Metformin; G5- Diabetes+S-Equol; G5-Diabetes-SEND(2); G6- Diabetes + SEND(4).

8. DISCUSSION

Diabetic encephalopathy (DE) is a long-term consequence of diabetes mellitus that affects the central nervous system (CNS) and causes cognitive and motor dysfunctions, as well as postural balance problems, Chronic hyperglycemia in people with diabetes, is linked to a significant incidence of progressive dementia. Among the likely causes of this, in addition to the direct effects of Indirect consequences include changes to the cerebrovascular system as well as hyperglycemia, hypo- or hyperinsulinemia, and other conditions . Isoflavones are bioactive compounds similar to 17-β-estradiol in chemical structure with mildly estrogenic properties and are often referred to as phytoestrogen. It is also a subclass of flavonoids present in plants and has potent antioxidant properties . The behavioral, biochemical, and molecular functions of the brains of diabetic rats were examined in this study. As a result of STZ-induced diabetes, there was a significant decline in cognitive performance and a significant rise in acetylcholinesterase activity in the cerebral cortex. The drug delivery methods have various benefits, including improved patient compliance, high safety, amazing simplicity of administration, the quick beginning of the action, and less systemic exposure, so we selected the nasal route for the treatment. Based on the literature in the present study we hypothesize that isoflavone-loaded Nanodroplets may dramatically and dose- dependently improve the cognitive impairment in diabetes.

Studies from Kuhad and Chopra and by Sanchez-Chavez and Salceda have demonstrated that, while the hippocampal acetylcholinesterase activity of hyperglycaemic rats did not change, it was active in the cerebral cortex and serum of diabetic rats. In the current investigation, administration of SEND to diabetic rats reduced the rise in acetylcholinesterase activity in the cerebral cortex but did not affect hippocampal acetylcholinesterase activity.Direct glucose toxicity in neurons is particularly caused by elevated intracellular glucose oxidation, which raises the formation of reactive species. Oxidative stress appears to be a major contributor to neuronal damage in both people and diabetic rats.In the present study, we found that oxidative damage to rat synapses causes cognitive loss. we found that the cerebral cortex and hippocampus of diabetic rats had significantly lower levels of reduced glutathione, superoxide dismutase, and catalase activities. Treatment with SEND(4) in the current investigation caused the levels of brain lipid peroxides, glutathione superoxide dismutase, and catalase to revert to their baseline levels. This finding is supported by the research showing that S-Equol formulation protects against oxidative damage in diabetes mellitus.

It is known that pro-inflammatory cytokines are raised in several neuropathological conditions linked to learning and memory. According to experimental studies, tumor necrosis factor (TNF)-alpha inhibits long-term potentiation (LTP) in the dentate gyrus region of the rat hippocampus in two phases . The inflammatory marker nuclear transcription factor Kappa beta was increased in an untreated group, especially in the cerebral cortex and hippocampus. Treatment with novel formulation notably downregulated the NF-kB expression in the treated group there by it inhibited the intensity of proinflammatory cytokines such as TNFα and IL1β.The cytokines TNFα and IL1β were depleted in dose-dependent manager and the novel formulation significantly exhibited neuroprotection and decreased the neuronal deterioration in the cortex and hippocampus of the treated animal.

CONCLUSION

In this study, we have demonstrated the efficacy of isoflavones loaded nanodroplets in alleviating the symptoms of diabetic encephalopathy in a rat model. The results show that the nanodroplets significantly improved cognitive function, reduced oxidative stress and inflammation, and increased the expression of neurotrophic factors in the brain. Moreover, the nanodroplets exhibited excellent bioavailability and targetability to the brain, which is crucial for the treatment of diabetic encephalopathy. Our findings suggest that isoflavones loaded nanodroplets may be a promising therapeutic approach for the treatment of diabetic encephalopathy, and further studies are warranted to explore their potential clinical applications.

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