International Journal of Pharmacology

ISSN 1811-7775

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Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2022.374.387

Research Article
Curcumae Ameliorates Diabetic Neuropathy in Streptozotocin Induced Diabetic Rats via Alteration of Gut Microbiota

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Abstract

Background and Objective: Diabetes Mellitus (DM) is a metabolic dysfunction with various symptoms characterized via induces the hyperglycemia effect along with alteration of fat, protein and carbohydrate metabolism. The current experimental study exhibited the neuroprotective effect of curcumae against streptozotocin (STZ) induced Diabetic Neuropathy (DN) via alteration of gut microbiota. **Materials and Methods:** Intraperitoneal injection of STZ (70 mg kg^{-1}) was used for the induction of DM. Blood glucose level, insulin, body weight and biochemical parameters were estimated at end of the protocol. Mechanical withdrawn threshold and motor nerve conduction velocity were estimated. The composition of faecal gut microbiota was estimated. **Results:** Curcumae significantly ($p<0.001$) increased the level of plasma insulin, body weight and declined the body weight. Curcumae significantly ($p<0.001$) declined the level of Creatine Kinase (CK), Aspartate Aminotransferase (AST) and Lactate Dehydrogenase (LDH) at dose-dependently. Curcumae significantly ($p<0.001$) reduced the Mechanical withdrawn threshold and motor nerve conduction velocity. Curcumae significantly ($p<0.001$) suppressed the level of MDA and boosted the GSH, SOD level. It also down-regulated the level of inflammatory cytokines in the brain, liver, heart and kidney. Curcumae also improved gut dysbiosis in DM rats by suppressing potential pathogenic species while enriching prebiotic species. **Conclusion:** Curcumae has an anti-diabetic impact and alters the gut microbiota linked with DM phenotypes and ROS generation levels.

Key words: Diabetes mellitus, curcumae, antioxidant, inflammatory, gut microbiota

Citation: Liu, Y., B. Huang, Z. Zhu and T. Zheng, 2022. Curcumae ameliorates diabetic neuropathy in streptozotocin induced diabetic rats via alteration of gut microbiota. Int. J. Pharmacol., 18: 374-387.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes Mellitus (DM) is considered as the major chronic metabolic disorder characterized via hyperglycemia which further led to the induction of series of complications such as neurodegenerative disorder, pancreatic diseases, liver diseases, cardiovascular diseases and blindness[1,2]. It is well developed the relationship between diabetes and neurodegenerative diseases includes Alzheimer's disease[3]. Advanced Glycation End Products (AGEPs) do not accumulate in the brain but they have been found in senile plaques, where they can reduce the solubility of proteins like Tau proteins and amyloid (A). Because insulin was proven to boost memory in a few modest experiments, few investigations supported the idea of brain insulin resistance[4,5]. Compared with those without induction of diabetes, those with the diseases have a 1.2-1.5 fold greater rate of reduction in cognitive function. Various epidemiological and clinical examinations have shown that pathophysiological features of neuropathic disease and diabetes are comparable to each other, which showed the complicated and related mechanisms such as oxidative stress, insulin resistance and inflammation[3,4]. In addition, impaired insulin signalling in brain tissue may impair the ability of neurons to self-repair, thereby accelerating the progression of neurodegenerative disease. Clinical evidence for a successful cure for diabetics with cognitive impairment is scarce[3,6].

The deposition of amyloid β(Aβ) peptide as well as neurofibrillary tangles hyperphosphorylated tau protein that is commonly observed in brain cells are linked to the weakening of cognitive functions in patients, such as memory deficits and behavioural damages[7]. Previous studies have shown that during the early stages of Alzheimer's disease, instabilities in various phases of cellular metabolism appeared to be clinically significant in disease, such as increased insulin resistance in the brain, decreased glucose utilisation and energy metabolism[8,9]. Even the exact etiology of AD is unknown but some researches showed that the excessive generation of free radicals may lead to triggering the neuronal weakening in the AD. Free radicals and Reactive Oxygen Species (ROS) induces the alteration in the cellular function as well as structure, during the AD case[9,10]. For instance, over-expression of Aβ and inflammation, energy deficiency, myocardial dysfunction and hyperphosphorylated tau protein, all these parameters are responsible for boosting ageing as well as age-related neurodegenerative dysfunction[10,11]. As a result, free radical scavengers and antioxidants have been proposed as therapeutic medications for delaying and reversing the pathogenic progression of neurodegenerative disorders[12,13]. As a result, finding a medicine that is both anti-diabetic and cognitively protective is crucial.

Diabetes mellitus, particularly type II, is a chronic disease characterized by a disruption in glucose metabolism, which increases the risk of specific consequences such as retinopathy, vascular pathology and central neuropathy[14-16]. Recent studies have focused on central neuropathy, which refers to the damage of neurons and can lead to cognitive impairment. Diabetic central neuropathy was originally assumed to be caused by hyperglycemia's effect on the brain's structural or functional domains[17,18]. Hyperglycemia can cause neuronal development damage and maturation as well as increase amylin production, leading to AD[18,19]. Glycemic variability has been discovered to be a substantial underlying cause of diabetic central neuropathy in a recent study. Furthermore, there is significant evidence that the gut microbiota plays a key role in the pathophysiology of type 2 diabetes and that gut SCFA-producing bacteria influence hippocampus neurogenesis, casting doubt on the underlying mechanism originally described[17,18,20]. In a mouse model of Alzheimer's disease, systemic broad-spectrum combinatorial antibiotic therapy reduces neurodegenerative pathology. Furthermore, insulin resistance is a well-known indication of type II diabetes and is thought to be a potentially key component of Alzheimer's disease and associated dementias[17,18]. These results show that the cause of diabetic central neuropathy is still unknown.

The gut microbiota, which is made up of billions of bacteria and interacts with the host via neuroendocrine, immunological and neurological pathways[18]. These pathways are regarded as the microbiota-gut-brain axis. Previous research has suggested that gut bacteria influence brain development, behaviour and function via the axis[21]. In a recent experiment, faeces from people with Autism Spectrum Disorder (ASD) were transplanted into germ-free mice, resulting in autistic behaviours and alternative splicing of ASD-related genes in the brains[20,21]. Microbiota changes are increasingly being viewed as a possible target for the development of new therapeutic interventions for several nervous disorders, including Parkinson's Disease (PD), depression, ageing and Alzheimer's disease[21].

The microbiota-gut-brain axis has long been thought to be a potential target for the therapy of central nervous system diseases. This study examined the effect of curcumae on central neuropathy and gut microbiota in type 2 diabetic rats for the first time in this experimental investigation.

MATERIALS AND METHODS

Study area: The animal study was carried out from January- February, 2021 in the Baoji Third Hospital, Baoji, 721000, China.

Chemical: Curcumae and streptozotocin were purchased from Sigma Chemicals (St. Louis, USA). All the chemical and reagents used in the current experimental study was procured from the Sinopharm Chemical Reagent Beijing Co, Ltd. (Beijing, China).

Animals: Sprague Dawley (SD) (5 weeks old, male rats) was used for the current experimental protocol. For the current experimental protocol, the rats were received from the Laboratory Animal Center of the Institute. The rats were kept in the standard environmental (20±5°C, 60% relative humidity and 12 hrs light and dark cycle). The rats have received the standard pallet diet (10% Kcal, 20% protein, 10% fat, 70% carbohydrate, 3.85 kcal g^{-1}) and water *ad libitum*. The current procedure received approval from the Institutional Animal Ethical Committee to control and supervise animal experiments.

Drug treatment: Intraperitoneal injection of 70 mg kg^{-1} streptozotocin was used for the induction of diabetes. The rats were acclimatized for 4 weeks and estimation of fasting blood glucose levels[22]. The rats were having a fasting blood glucose level >16.0 mmol L^{-1} was considered type 2 diabetes.

Generation of diabetic rats: After successful induction of diabetes, the rats were divided into four groups of ten rats each as follows:

Group I : Normal control group (NC)
Group II : STZ induced diabetic group (DM)
Group III : DM+CU (1.25 mg kg^{-1})
Group IV : DM+CU (2.5 mg kg^{-1})
Group V : DM+CU (5 mg kg^{-1})
Group VI : DM+sitagliptin

The rats have received the oral administration of above mention treatment once a day till 4 weeks. DM group rats were treated with an equal volume of pure water. All groups of rats received a sufficient quantity of food and water every day until the end of the experimental protocol.

Mechanical Withdraw Threshold (MWT): Von Pain Measurement Instrument was used for the estimation of MWT (IITC Life Science, Woodland Hills, CA, USA). The rats were individually placed in a plastic cage with mesh (1 cm 2 perforations) and acclimated for 15 min. The centre of the planar was vertically stimulated with the electronic Von Frey probe after acclimation, making it appear somewhat S-shaped and the paw withdrawal reaction was assessed. A positive reaction was described as a fast-flinching reaction that occurred shortly after stimulation and pressure values (gram) were registered. Physical activity-induced paw withdrawal was not identified as a positive reaction. The experimental protocol was repeated 3 times at an interval of 15 min and finally mean value was recorded.

Motor Nerve Conduction Velocity (MNCV): At end of the experimental protocol MNCV was estimated in the sciatic nerve of the terminally anaesthetized rat. Evoked potential equipment and electromyogram instrument was used for the estimation of electromyograms from the plantar foot muscles in the response to stimulation (15-30 mA<0.1 ms pulses) at the Achilles tendon and sciatic notch. The nerve length between the two stimulation locations was calculated (*ex vivo*). MNCV was calculated using the latencies of compound M waves and the Eq.[23]:

$$\text{MNCV}\left(\frac{\text{m}}{\text{s}}\right) = \frac{\text{Distance between stimulation sites}}{\text{Latencyo of M wave (Sciatic notch)} - \text{Latency of M wave (Achilles tendon)}}$$

Preparation of brain homogenate and biochemical parameter estimation: The blood samples were collected from the abdominal aorta in the pre-incubated test tubes and the serum was kept at -80°C. Followed the behavioural tests, the rats were sacrificed via using the excess diethyl ether and successfully isolated the brain tissue via decapitation protocol. Biochemical parameters were estimated using the obtained different tissues (brain, heart, liver and kidney). A homogenate (10%) solution was made from the different tissues by weighing them, homogenizing them in ice-cold saline preparation, centrifuge at 1000 rpm for 5 min at 4°C to remove debris and then preparing a supernatant aliquot for malonaldehyde determination. The remaining pellet was again centrifuged at 10000 g for 30 min at 4°C and Post Mitochondrial Supernatant (PMS) was obtained for the estimation of catalase (CAT), glutathione (GSH) and superoxide dismutase (SOD) using the standard kits following the manufacture instruction Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Faecal DNA extraction and sequencing: Total DNA was extracted from frozen faeces using a commercially available kit following the manufacturer's instructions (QIAamp Fast DNA Stool Mini Kit, Qiagen). The V3-V4 region of the 16S rRNA genes was amplified using the 341F/806R primer combination (for 341F, 5'-CCTACGGGNGGCWGCAG-3', for 806R, 5'-GACTAC

HVGGGTATCTAATCC-3'). A QIA quick PCR purification kit was used to purify the amplified PCR products. For optimum pair-end sequence readings, DNA sequencing was performed using the Illumina MiSeq instrument with the barcoding sequence kit (version 3). The quality of sequencing data was checked using Fast QC. Following the successful clearing of the Phix sequence, the Mothur software was used to further process the selected high-quality sequences. Tags containing a large number of undefined bases and homo-polymers as well as tags that were outside of the planned range, were eliminated. The tag was then aligned to SILVA 119, 16S rRNA gene sequences to ensure that the tags had the proper alignment region and locations. A pre-clustering technique was utilised for additional denoising and chimeric sequences were removed based on UCHIME's prediction. The Ribosomal Database Project's (RDP) Nave Bayesian Classifier was trained with an 80% pseudo-bootstrap confidence score on the RDP 16S rRNA gene training set (version 10) to assign significant taxonomic ranks. If sequences designated as Archaea, Eukaryota, chloroplasts or mitochondria were not classified to the kingdom stage, they were eliminated. Finally, sequences were sorted into groups related to their taxonomy and assigned to operational taxonomic units at a similarity level of 97% (OTUs).

Statistical analysis: The current experimental study's data is presented in the form of mean SD. GraphPad Prism was used to estimate statistical significance by employing *post hoc* testing. Statistical significance was defined as $p>0.05$.

RESULTS

Blood glucose, insulin and body weight: During diabetes, the increased blood glucose level and reduced insulin and body weight are commonly observed. Figure 1 showed the effect of curcumae on glucose, insulin and body weight. STZ treated rats exhibited the boosted glucose level and curcumae treatment significantly ($p<0.001$) suppressed the level of blood glucose level in Fig. 1a. Figure 1b showed the suppressed level of FINS and curcuame treatment significantly ($p<0.001$) improved the level of FINS. Figure 1c displayed the body weight of different group rats. Normal rats showed the normal pattern to increase the body weight as compared to another group. STZ induced DN rats exhibited the decreased body weight due to expansion of disease and curcuame treated rats showed an improvement in body weight.

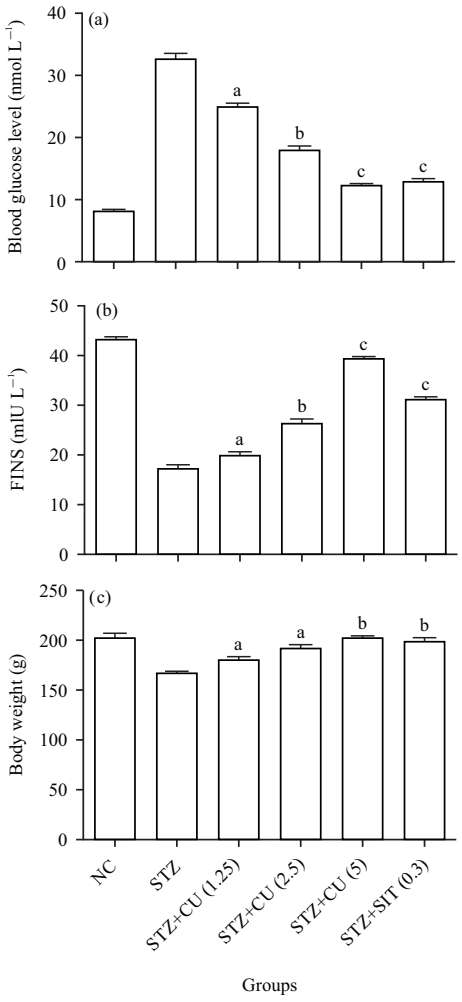

Fig. 1(a-c): Effect of different treatment groups on (a) Blood glucose level, (b) FINS and (c) Body weight
Mean±SD, one-way ANOVA followed by *post hoc* testing. Where *p<0.05, **p<0.01 and ***p<0.001

Mechanical withdrawn threshold and motor nerve conduction velocity: During the neurology dysfunction, the activity of mechanical withdrawn threshold and motor nerve conduction velocity. Figure 2a showed the reduced mechanical withdrawn threshold and curcumae treatment significantly boosted the mechanical withdrawn threshold activity. Figure 2b demonstrated the decreased motor nerve conduction velocity after the STZ treatment and curcumae treatment significantly improved the motor nerve conduction velocity.

LDH, AST and CK: The cardiac parameters such as CK, AST and LDH are boosted during diabetes. A similar result was observed in this experimental study. STZ induced group rats demonstrated the ameliorate level of LDH and curcuame

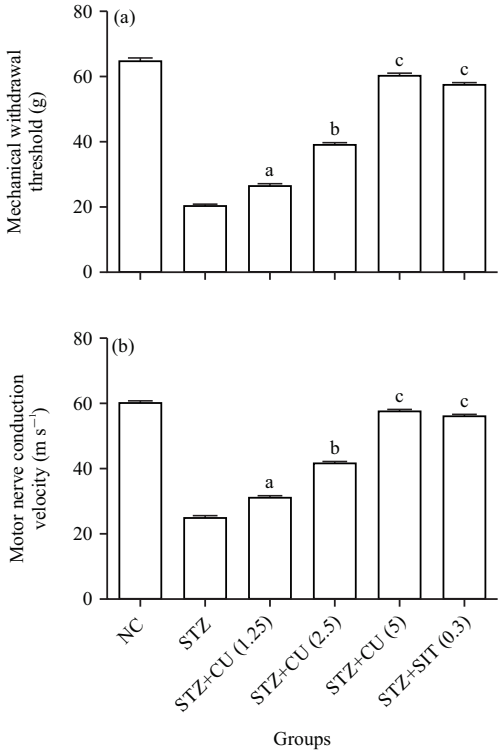

Fig. 2(a-b): Effect of different treatment groups on (a) Mechanical withdrawn threshold and (b) Motor nerve conduction velocity
Mean±SD, one-way ANOVA followed by *post hoc* testing. Where *p<0.05, **p<0.01 and ***p<0.001

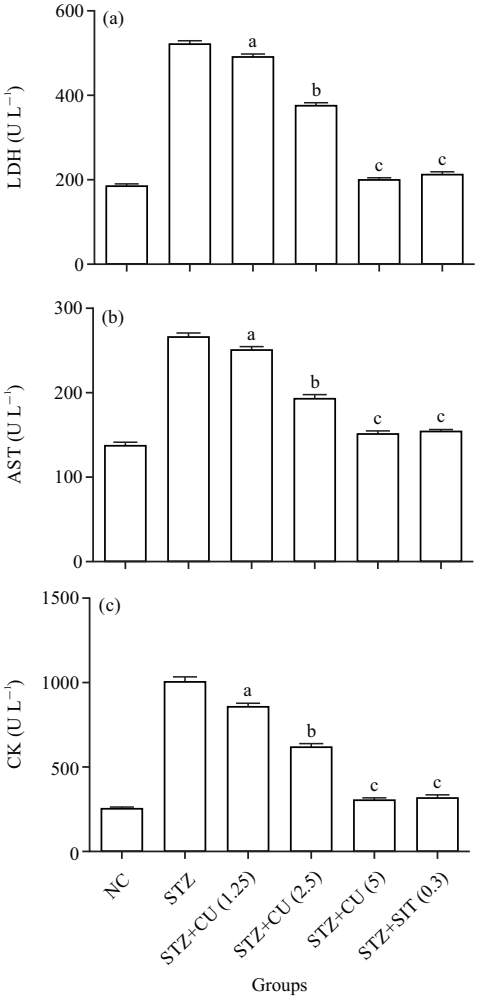

Fig. 3(a-c): Effect of different treatment groups on (a) LDH, (b) AST and (c) CK
Mean±SD, one-way ANOVA followed by *post hoc* testing. Where *p<0.05, **p<0.01 and ***p<0.001

treatment significantly suppressed the level of LDH in Fig. 3a. A similar pattern was observed at the AST level. Figure 3b showed the increased level of AST in the STZ induced DN rats and curcumae treatment significantly ($p<0.001$) suppressed the level of AST. Figure 3c demonstrated the enhanced level of CK in the STZ induced DN rats and curcuame treatment significantly ($p<0.001$) suppressed the level of CK almost near to the normal level.

Lipid parameters: During diabetes, alteration of lipid was observed and a similar result was observed in this experimental study. STZ induced rats displayed the ameliorated level of TC and curcumae treatment significantly suppressed the level in Fig. 4a. STZ induced DN rats exhibited the boosted level of LDL and curcumae treatment significantly ($p<0.001$) reduced the level in Fig. 4b. TG level boosted in the STZ induced DN group rats and curcumae treatment significantly suppressed the level of TG in Fig. 4c. A similar result was observed in the VLDL, STZ induced DN rats exhibited the increased level of VLDL and curcumae treated rats exhibited the reduced level in Fig. 4d. STZ induced DN rats exhibited a reduced level of HDL and curcumae treated rats exhibited an increased level of HDL in Fig. 4e.

Cytokines: Inflammatory reaction plays an important role in the expansion of diabetes and its complications. Diabetic neuropathy increased the level of the inflammatory cytokine and boost brain injury. The level of cytokines such as TNF-α, IL-1β and IL-6 increased in the brain tissue of STZ induced diabetic neuropathy rats in Fig. 5a. The cytokines level such as TNF-α, IL-1β and IL-6 augmented in the kidney tissue of STZ induced diabetic neuropathy rats in Fig. 5b. The cytokines such as TNF-α, IL-1β and IL-6 up-regulated in the heart tissue of STZ induced diabetic neuropathy rats in Fig. 5c. The level of cytokines such as TNF-α, IL-1β and IL-6 enhanced in the liver tissue of STZ induced diabetic neuropathy rats in Fig. 5d.

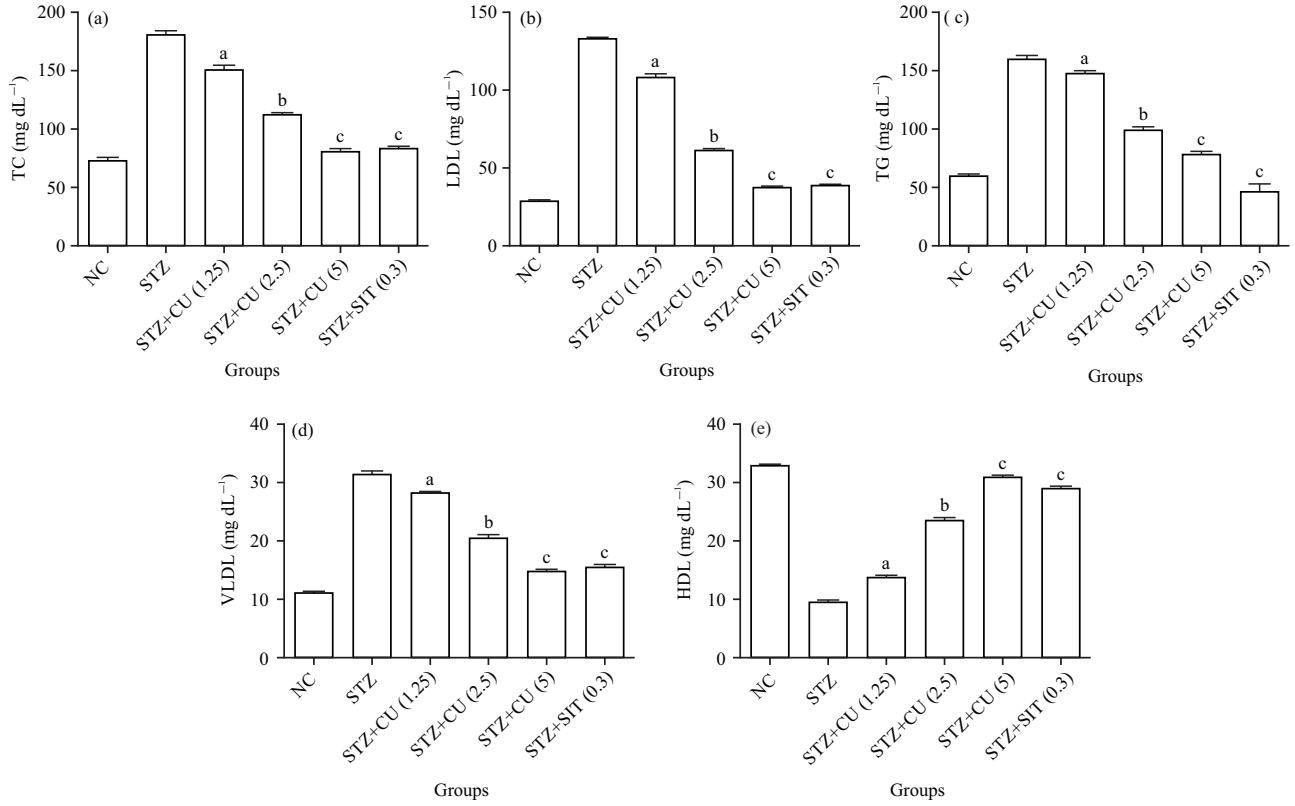

Fig. 4(a-e): Effect of different treatment groups on (a) TC, (b) LDL, (c) TG, (d) VLDL and (e) HDL

Mean±SD, one-way ANOVA followed by *post hoc* testing. Where, *p<0.05, **p<0.01 and ***p<0.001

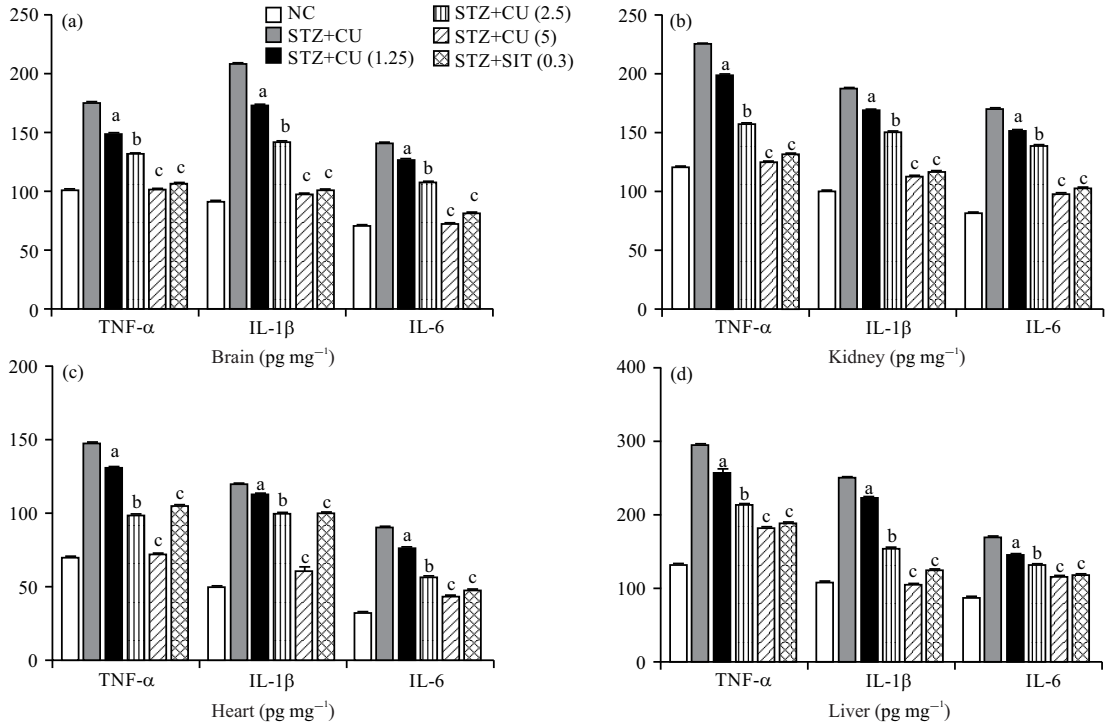

Fig. 5(a-d): Pro-inflammatory cytokines parameters effect on, (a) Brain, (b) Kidney, (c) Heart and (d) Liver

Mean±SD, one-way ANOVA followed by *post hoc* testing. Where, *p<0.05, **p<0.01 and ***p<0.001

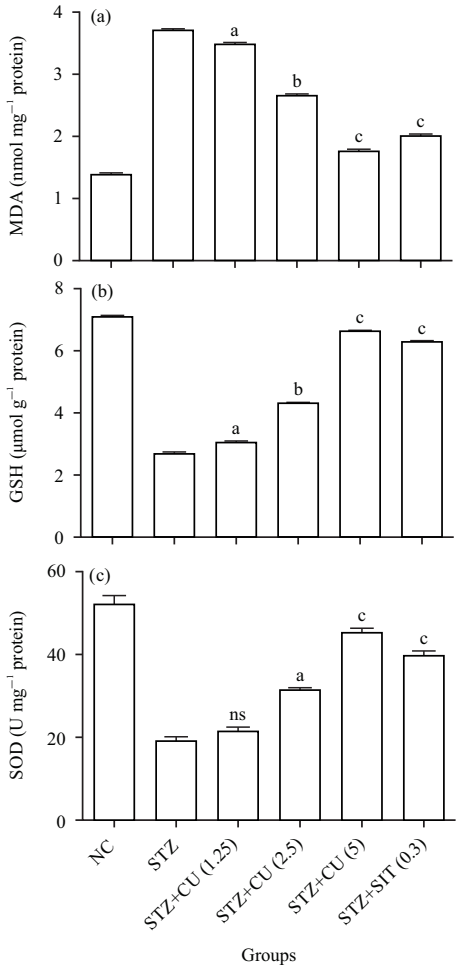

Fig. 6(a-c): Effect of antioxidant parameters in brain tissue, (a) MDA, (b) GSH and (c) SOD
Mean±SD, one-way ANOVA followed by *post hoc* testing. Where, *p<0.05, **p<0.01 and ***p<0.001

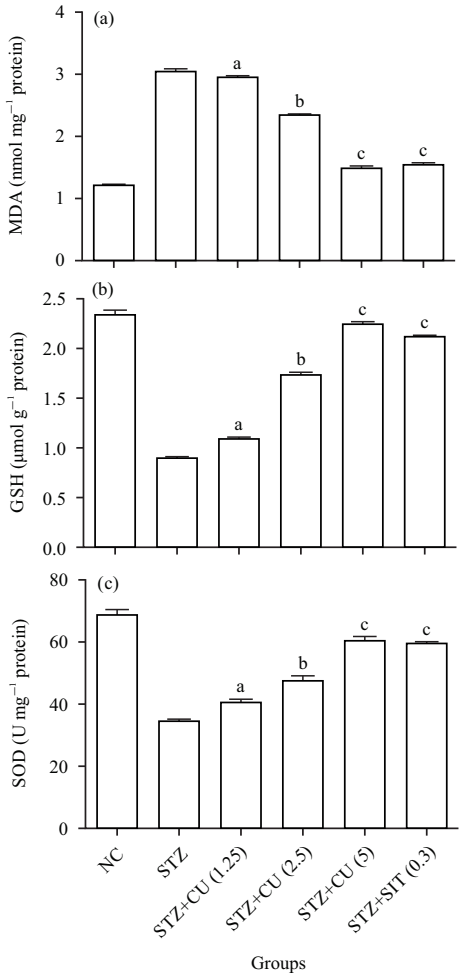

Fig. 7(a-c): Effect of antioxidant parameters in kidney tissue, (a) MDA, (b) GSH and (c) SOD
Mean±SD, one-way ANOVA followed by *post hoc* testing. Where, *p<0.05, **p<0.01 and ***p<0.001

Curcumae treatment significantly (p<0.001) suppressed the level of inflammatory cytokines in Fig. 5(a-d), respectively.

Antioxidant in different tissue: Oxidative stress plays an important role in the development of diabetes and its complications. During diabetic neuropathy, increased the level of free radicals, which further boost the level of oxidative stress in tissue. STZ induced rats showed the augmented level of MDA and decreased level of GSH, SOD (Fig. 6c) in the brain tissue in Fig. 6(a-c), respectively. STZ induced diabetic neuropathy rats treated with Curcumae significantly (p<0.001) reduced the MDA level and boosted the GSH and SOD level. Sitagliptin treated rats significantly (p<0.001) suppressed the level of MDA and enhanced the level of GSH and SOD.

In the kidney, STZ induced rats exhibited the boosted level of MDA in Fig. 7a and suppressed level of GSH and SOD in Fig. 7(b, c). Curcumae and Sitagliptin treated rats significantly (p<0.001) suppressed the MDA level and boosted the GSH and SOD level.

In the heart tissue, the antioxidant level was similar observed. STZ induced rats showed the increased level of MDA in Fig. 8a and suppressed level of GSH in Fig. 8b and SOD in Fig. 8c and curcumae treatment altered the level of endogenous antioxidant.

The level of MDA in Fig. 9a boosted and suppressed GSH and SOD in Fig. 9b and c level was reduced in the STZ induced diabetic neuropathy rats. Curcumae and Sitagliptin treated rats significantly (p<0.001) modulated the level of endogenous antioxidant parameters.

Microbial structure among different conditions: To investigate the underlying mechanism of curcumae, faecal

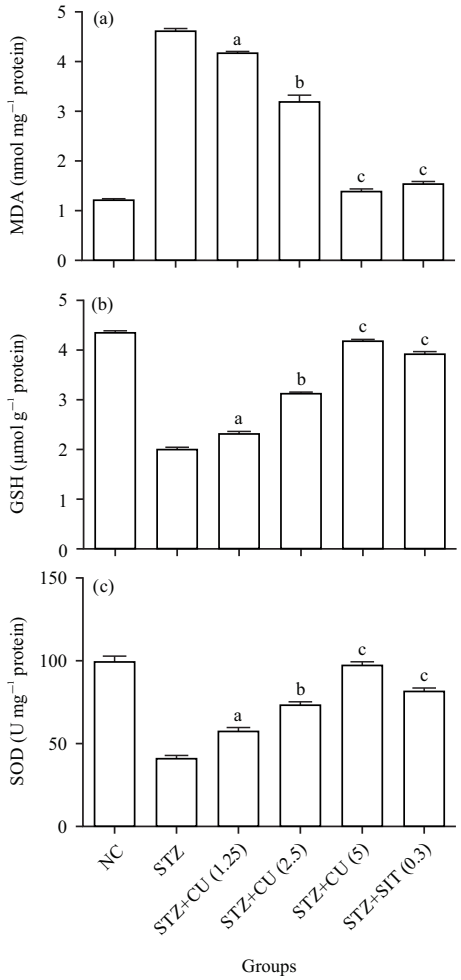

Fig. 8(a-c): Effect of antioxidant parameters in heart tissue, (a) MDA, (b) GSH and (c) SOD
Mean±SD, one-way ANOVA followed by *post hoc* testing. Where, *p<0.05, **p<0.01 and ***p<0.001

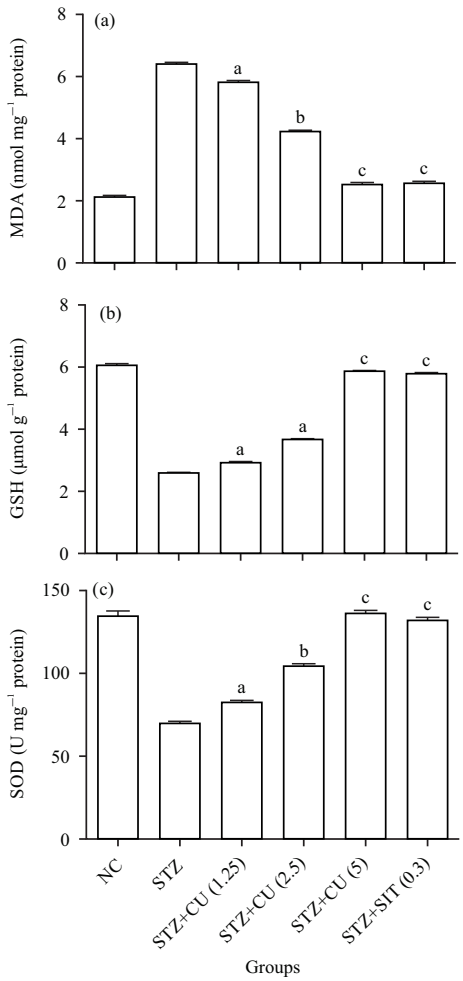

Fig. 9(a-c): Effect of antioxidant parameters in liver tissue, (a) MDA, (b) GSH and (c) SOD
Mean±SD, one-way ANOVA followed by *post hoc* testing. Where, *p<0.05, **p<0.01 and ***p<0.001

16s rRNA gene sequencing and correlate with the composition of gut microbiota in normal, diabetic and treated groups. Figure 10 showed the gut microbiota composition of all group rats. Firmicutes was the most prominent phylum of the control group, followed via Proteobacteria, Bacteroidetes, Actinobacteria, Candidatus Saccharibacteria and Verrucomicrobia. Figure 10 demonstrated the effect of curcumae on the composition of overall gut microbiota. Figure 10a showed the composition of all gut microbiota of each sample at the phylum level. Figure 10a showed the reduced relative abundance of *Streptococcaceae* and increased relative abundance of *Porphyromonadaceae* in STZ induced DN rats and curcumae treated rats exhibited the up-regulation in the relative abundance of *Streptococcaceae* and decreased relative abundance of *Porphyromonadaceae*. Figure 10b demonstrated the relative abundance of Bacteroidetes, Candidatus Saccharibacteria, Firmicutes, Proteobacteria and Spirochaetes of all group rats. STZ induced DN rats showed the increased relative abundance of *Bacteroidetes* and reduced relative abundance of firmicutes. Curcumae treatment considerably reduced the relative abundance of *Bacteroidetes* and increased the relative abundance of Candidatus Saccharibacteria, Firmicutes, Proteobacteria and Spirochaetes. Figure 10c demonstrated the average relative abundance of Bacteroidetes, Candidatus Saccharibacteria, Firmicutes, Proteobacteria and Spirochaetes. Table 1 showed the genus identified in the faeces of rats. Table 1 showed the increased relative abundance of *Alistipes, Anaerotruncus, Desulfovibrio, Flavonifractor, Helicobacter, Lachnospiraceae* unclassified, *Lachnospiraceae* uncultured, *Lactococcus, Oscillibacter, Prevotella, Ruminococcaceae*

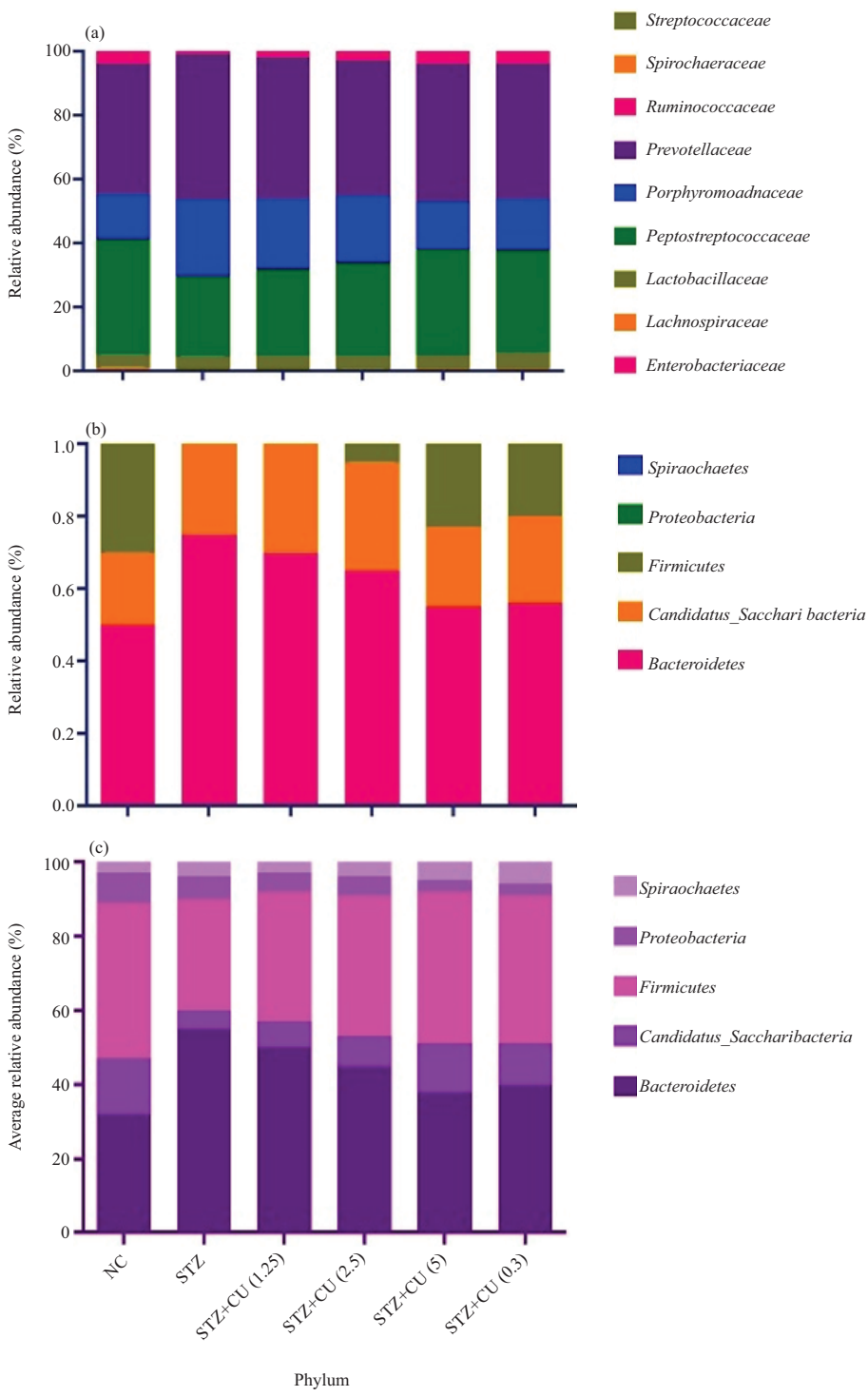

Fig. 10(a-c): Gut microbiota composition, (a) Relative abundance phylum, (b) Relative abundance phylum (%) and (c) Average relative abundance

Mean±SD, one-way ANOVA followed by *post hoc* testing. Where, *p<0.05, **p<0.01 and ***p<0.001

incertae sedis, *Ruminococcaceae unclassified*, *Ruminococcaceae uncultured*, *Ruminococcus* and increased relative genus abundance of *Akkermansia*, *Allobaculum*, *Anaerostipes*, *Bacteroides*, *Bacillus*, *Blautia*, *Bifidobacterium*, *Coprococcus*, *Collinsella*, *Fusobacterium*, *Faecalibacterium*, *Lachnospiraceae incertae sedis*,

Table 1: Number of reads assigned to each genus identified in the faeces of rats

	Groups					
Genus	NC	STZ	STZ+CU (1.25 mg kg^{-1})	STZ+CU (2.5 mg kg^{-1})	STZ+CU (5 mg kg^{-1})	STZ+SIT (0.3 mg kg^{-1})
Alistipes	16.54±1.93	32.34±2.32	28.76±1.38*	24.56±2.34**	17.64±2.93***	18.45±1.83***
Akkermansia	394.34±12.34	0±0	123.45±8.76***	203±10.23***	345.3±12.34***	333.4±9.34***
Anaerotruncus	35.3±2.12	104.56±1.83	90.34±2.84*	68.54±3.23**	40.3±2.12***	47.6±1.93***
Allobaculum	41.2±1.93	9±1.12	11.2±1.83*	16.3±2.11**	37.2±2.34***	35.43±1.39***
Anaerostipes	303.4±6.54	1±.02	43.4±1.55***	111±1.45***	281.3±4.56***	254.4±3.24***
Bacteroides	3123.0±234	246±12.3	843.3±34.2***	1392.4±39.8***	2783.4±123.4***	2654.2±112.9***
Bacillus	19.34±1.02	4.53±0.83	7.65±0.95*	10.54±1.23**	16.94±1.47***	15.4±1.93***
Blautia	1412.3±45.4	10.34±1.92	158.3±2.45***	556.3±11.5***	1111.3±35.7***	1043.3±32.4***
Bifidobacterium	83.4±1.34	9.12±0.83	15.65±1.93*	37.87±2.83**	76.54±3.43***	70.32±4.32***
Coprococcus	467.3±8.75	12.34±1.93	65.78±2.47*	194.3±3.21**	432.4±8.76***	411.3±6.83***
Collinsella	131.3±3.45	0±0	9.75±1.83***	43.5±2.43***	125.4±6.54***	121.2±5.47***
Desulfovibrio	201.94±6.53	432.3±5.68	409.3±4.56*	334.5±6.89**	234.3±6.53***	245.3±5.92***
Fusobacterium	11.34±1.93	0±0	1.34±0.94**	5.43±0.93***	10.34±2.31***	9.1±1.93***
Flavonifractor	30.34±1.83	143.45±4.67	132.3±3.45*	90.3±4.23**	35.65±3.21***	41.45±2.56***
Faecalibacterium	847.0±6.54	3.43±0.63	54.5±1.93**	256.4±4.89***	654.3±4.56***	543.3±4.09***
Helicobacter	34.5±1.83	76.54±2.83	68.4±3.04*	52.5±3.45**	35.4±3.21***	39.5±2.19***
Lachnospiraceae incertae sedis	983.0±8.45	212.5±4.36	287.3±2.45*	453.5±6.78**	874.5±9.83***	854.3±8.78***
Lachnospira	74.3±2.34	10.31±1.83	17.65±1.89*	38.4±2.04**	65.6±3.12***	60.3±2.83***
Lachnospiraceae unclassified	654.4±6.57	1434.3±14.56	1234.5±13.45*	993±7.43**	712.3±7.65***	734.3±6.54***
Lachnospiraceae uncultured	1343.0±12.45	5124±35.67	4534.3±30.4*	3234.4±26.5**	1834.3±21.45***	2032±22.94***
Lactococcus	4.23±1.83	18.45±2.34	16.43±2.12*	13.2±1.74**	5.6±0.83***	9.23±1.92***
Lactobacillus	745.3±25.4	423.3±21.4	485.8±23.2*	603.3±25.4**	734.3±24.3***	701.7±23.3***
Marvinbryantia	89.3±6.54	24.3±2.89	30.1±2.90*	45.6±3.12**	76.9±4.82***	70.2±3.45***
Oscillospira	49.02±2.35	0±0	5.94±0.93***	18.8±1.34***	42.9±2.84***	40.1±2.91***
Oscillibacter	7.12±0.83	374.5±19.49	333.4±17.45*	213.4±15.46**	15.41±1.21***	23.45±1.93***
Parabacteroides	259.3±15.43	80.2±5.62	102.1±4.35*	176.3±5.12**	245.3±5.83***	222±4.32***
Parasutterella	71.34±1.74	20.1±1.21	28.2±1.56*	45.1±2.12**	68.4±2.95***	64.3±2.04***
Phascolarctobacterium	1454.3±78.9	20.3±0.93	234.3±12.35***	732.3±24.93***	1345±45.4***	1234±34.8***
Peptostreptococcaceae incertae sedis	265.4±7.54	21.34±1.83	38.7±2.83*	102.3±4.67**	234.6±7.54***	222.1±6.54***
Prevotella	1234.0±15.43	3111±25.67	3001±24.54*	2083±23.45**	1343.3±12.67***	1531±13.68***
Ruminococcaceae incertae sedis	154.3±5.67	487.3±9.43	443.3±8.92*	341.3±7.93**	189.3±8.45***	201.2±5.43***
Roseburia	901.3±12.93	145.3±4.52	187.8±5.84*	354.3±6.52**	854.2±10.8***	832.1±9.34***
Ruminococcaceae unclassified	139.2±4.32	198.3±5.41	185±4.38*	171.2±4.32**	145.3±3.89***	154.2±4.32***
Ruminococcaceae uncultured	201.3±6.78	1543±10.93	1345.1±11.35*	897.3±8.94**	245.2±6.34***	301.2±5.32***
Ruminococcus	16.5±1.92	256.3±8.3	243.1±6.83*	168.3±5.89**	32.2±1.89***	43.5±1.74***
Sutterella	114.5±4.35	1±0.06	5.3±0.83***	39.7±1.89***	104.3±3.56***	92.3±3.89***
Turicibacter	343.4±9.65	3.41±0.51	36.5±1.83**	123.1±4.83***	302.3±8.76***	300.1±6.54***

Values (Means±SD, n = 5) with different letters (*, **, ***) within a line are significantly different at the 5%

Lachnospira, *Lactobacillus*, *Marvinbryantia*, *Sutterella*, *Turicibacter*, *Oscillospira*, *Parabacteroides*, *Parasutterella*, *Phascolarctobacterium Roseburia, Peptostreptococcaceae incertae sedis* in STZ induced group rats and curcumae treated rats significantly altered the relative abundance of a different genus.

DISCUSSION

In the current investigation, the neuroprotective effect of curcumae on diabetes-induced cognitive impairment in rats, which is a widely used protocol in the expansion of classical DN phenotypes such as reduced body weight, hyperglycemia, reduced MWT and moderated MNCV. In this experimental study, curcumae upgraded the DN phenotypes via boosting the MWT. Moreover, curcumae reduced the BGL and boosted the body weight in the DN rats, which may remind that curcumae could replace the hypoglycemic drugs in the clinical application but it may play a significant role as an antioxidant stress adjuvant for antidiabetic drugs in DN patients. STZ was used to generate diabetic models in which the pancreatic β-cells were damaged and blood glucose levels were quickly enhanced. Also, various doses of the curcumae and ways of drug administration such as gavage, time solvent, might be responsible for the various effects on the blood glucose level.

Curcumae ability to ameliorate DN symptoms may be attributed to more than just its neuroprotective effect via lowering ROS generation in DN rats. Various studies have

shown that oxidative stress increases and decrease antioxidant levels during diabetes[24,25]. The metabolic syndrome is characterized by long-term hyperglycemia and increased ROS intake, which leads to increased oxidative stress and NADPH oxidase overactivation[26]. In this experimental study, STZ induced DPN rats showed the increased level of MDA in the brain, heart, kidney, liver and reduced level of GSH, SOD in the brain, heart, kidney, liver.

The current investigation was designed and scrutinized the effect of curcumae in diabetes-induced memory impairment (learning and memory) in experimental rats. Type 2 diabetic rats showed a significant impairment in the memory that was demonstrated with behavioural parameters such as passive avoidance, MWM and EPM test.

Neuro-disorder is a common problem with diabetes mellitus (type 2). One of the most prevalent consequences of diabetes is diabetic central neuropathy, which involves neuron destruction and can lead to dementia[27,28]. Curcumae already confirm their antidiabetic effect against diabetes but its diabetic neuropathy effect has not been explored. Furthermore, the effects of curcumae on diabetic central neuropathy and the gut environment are still unknown[29]. Current experimental work is the first attempt to scrutinize the diabetic neuroprotective effect via altering the gut microbiota and protecting the hippocampal neuronal injury in STZ induced diabetic rats. Curcumae may reduce the diabetic central neuropathy effect by stimulating the microbiota-gut-brain axis, according to our present research.

It is well known that diabetic central neuropathy induces neuronal injury and brain structural and physiological alteration induced via diabetes[3]. Diabetes has been linked to the progressive induction of cognitive impairment, which eventually leads to Alzheimer's disease[3,4]. Some pieces of evidence have shown that type 2 diabetes induces apoptosis in the hippocampal neurons via estrogen receptors PI3/Akt pathway[30,31]. Previous study has suggested that microbial dysbiosis plays a key role in the aetiology of Alzheimer's Disease (AD) and cognitive impairment and that faecal microbial translocation reversed the pathophysiology of AD[3,32]. In the meantime, microbiota dysbiosis played a significant role in the pathological process of diabetic cognitive impairment.

The gut microbiota is made up of the collective genome of microorganisms in the gastrointestinal (GI) tract (100 trillion) and these gut microbiotas play a crucial role in altering the health of hosts[33]. Intestinal microbes affect the central nervous system via enteroendocrine, autonomic and central nervous systems as well as the production of numeric metabolites and Microbial Associated Molecular Patterns (MAMPs) produced by the microbiota. A microbiota-gut-brain axis has been proposed[34,35]. Previous researches demonstrated that the dysfunction of gut microbiota is involved in the expansion and function of impairment of the central nervous system[34,36]. Few investigations demonstrated that microbiota dysbiosis was linked with behavioural and cognitive dysfunction in rodents. The microbiota-gut-brain axis is thought to be a key target for preventing and treating Central Nervous System (CNS) disorders and diseases[37,38].

The current goal in this experiment is to see if curcumae therapy helps to restore the gut microbiota to its normal condition. In this experiment, we discovered that curcumae administration alters microbial diversity when compared to diabetic rats, which is consistent with earlier research[39]. The result demonstrated that the gut microbiota of the rats largely depends on the *Bacteroidetes* and *Firmicutes* phyla. Diabetic rats showed a considerable shift of the gut microbiota with the reduction of the *Candidatus Saccharibacteria* and *Firmicutes* percentage and also boosted the proportion of *Spirochaetes* and *Bacteroidetes* phyla as compared to normal control[40]. Additionally, the ratio of *Bacteroidetes*: *Firmicutes* was boosted in the diabetic group and curcumae treated group rats showed the reduction in the ratio of *Bacteroidetes*: *Firmicutes*. Diabetic rats showed the alteration of *Lactobacillaceae, Ruminococcaceae, Enterobacteriaceae* and *Prevotellaceae* as compared to the normal control group rats. Among *Firmicutes* phylum, *Ruminococcaceae* family was a major utilizer of the plant polysaccharides and its enrichment might be counteracting the expansion of autoimmune diabetes[41]. The *Lactobacillacea* family was boosted after the curcumae treatment as compared to STZ induced diabetic rats. Curcumae treatment showed a higher abundance of *Ruminococcaceae* as compared to the STZ induced DM rats. According to previous research, *Lactobacillus* species can metabolise tryptophan into indole 3 aldehyde, which binds to the aryl hydrocarbon receptor (AhR) in immune cells' gut. Indole is a ligand for AhR, which may cause intestinal cells to secrete glucagon-like peptides[42]. Previous investigations showed that the diabetic rats enrichment of *Enterococcaceae* and *Enterobacteriaceae. Enterococcaceae* and *Enterobacteriaceae* (pro-inflammatory micro-organism in the gut) level was boosted in the diabetic patients[42,43]. They may also contribute to the increase in inflammatory levels in the host, promoting the development of insulin resistance. Previous research has found a larger abundance of *Enterobacteriaceae* (gram-negative bacteria) in diabetic patients, which could be linked to increased intestinal permeability regardless of glucose tolerance.

Short Chain Fatty Acids (SCFAs), such as butyric acid, propionic acid and acetic acid are significant metabolic

products of gut bacteria dietary fibre degradation in the colon[44]. SCFAs bind to the G Protein-Coupled Receptor (GPCR), causing the enteroendocrine hormone peptide YY (PYY) to be secreted by the gut epithelium L cells, limiting motility and increasing energy harvest from the meal[45]. SCFA-GPR interactions have been shown to allow direct signalling from the stomach to the central nervous system. Microglia, the brain's resident macrophages, rely on the gut microbiota for maturation and function and SCFAs and GPR were required to maintain microglia homeostasis and the integrity of the blood-brain barrier in rodents[45]. Recently, the research found that a high-fructose diet caused gut dysbiosis with decreased SCFA, resulting in impaired colonic epithelial barrier impairment, induction of neuro-inflammation in the hippocampal and loss of neuronal in rodents and neurodegeneration changes that could be protected by curcumae treatment[44]. These findings demonstrate that curcumae medication protects against changes in neurological function, which could explain curcumae's neuroprotective effect on hippocampus neuron loss caused by type 2 diabetes by activating the microbiota-gut-brain axis.

CONCLUSION

Curcumae significantly reduced the glucose level and boosted the body weight and insulin level. It's also reduced the LDH, CK and AST levels along with suppression of mechanical withdrawn threshold and motor nerve conduction velocity. Curcumae considerably increased the level of HDL and reduced the level of VLDL, LDL, TG and TC. Curcumae showed the neuroprotective effect via reduction of mechanical withdrawn threshold and motor nerve conduction velocity, suppressed the antioxidant parameters in the brain, liver, heart and kidney, reduced the level of inflammatory cytokines in the brain, liver, heart and kidney and altered the gut microbiota related with DM phenotypes.

SIGNIFICANCE STATEMENT

This study is novel and explores the neuroprotective effect of Curcumae against STZ induced diabetic neuropathy in rats. Brain injury is a common complication occurring due to diabetes. Curcumae treatment considerably suppressed the blood glucose level and improved insulin. Curcumae considerably suppressed the antioxidant and cytokines levels in the different tissue (brain, liver, kidney and heart). Curcumae significantly altered the level of gut microbiota. This study helps the researcher to uncover the critical complication associated with diabetic neuropathy. Thus a new beneficial therapy on diabetic neuropathy occurred during diabetic Mellitus.

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