COMBINED EFFECT OF AQUEOUS EXTRACT OF *CURCUMA LONGA* LINN. WITH METFORMIN IN DIABETES INDUCED NEUROPTAHIC PAIN IN RATS

ROMAN ADHIKARI, JYOTHI Y*, DEEPIKA BORA, VAMSEE VEENA A

Department of Pharmacology, Krupanidhi College of Pharmacy, Bengaluru - 560 036, Karnataka, India.
Email: jokiran05@gmail.com

ABSTRACT

Objectives: A number of studies previously conducted have shown that the progression of severity of diabetic neuropathy relates the increased oxidative stress due to hyperglycemia describing the probable underlying mechanism as changes in the anti-oxidant enzyme level leading to several vascular and neuronal changes. The present investigation was carried out to observe the combined effect of aqueous extract of *Curcuma longa* Linn. (Curcumin [CUR]) with metformin (Met) in delaying of diabetes-induced neuropathic pain in rats.

Methods: The high-fat diet (HFD) treated male Sprague–Dawley (SD) rats were induced diabetic by a single intra-peritoneal injection of streptozocin (35 mg/kg). Eddy's hot plate and hot water tail immersion test were the models used to assess the neuropathic pain. The HFD treated and diabetic rats were treated with Met (500 mg/kg), CUR (300 mg/kg) and a combination of low dose of Met with CUR, followed by the oral glucose tolerance test before and after the treatment on both normal and diabetic rats. After 7 weeks of treatment thiobarbituric acid reactive substances, superoxide dismutase, catalase, and glutathione levels were also estimated in the sciatic nerve homogenate of diabetic rats and compared with the control, followed by a supporting histopathological examination of sciatic nerve.

Results: Diabetic rats treated with *C. longa* Linn. and Met alone and in combination showed a significant improvement in control of blood glucose level and restoration of anti-oxidant enzyme levels. There was a significant improvement in the hyperalgesia (p<0.001) due to diabetic neuropathy and also the reaction time to the nociceptive stimuli in neuropathic pain models was increased after the treatment.

Conclusion: Curcumin has a property of protecting the islets β cells, decrease the insulin resistance and decrease the oxidative stress. Hence the use of Curcumin along with the Oral Hypoglycemic Agents could be useful in preventing the complications associated with Diabetes when subjected to prolonged treatment.

Keywords: Anti-oxidant, Curcumin, Diabetes, Metformin, Neuropathy, Streptozocin.

INTRODUCTION

Diabetes mellitus has become one of the most common causes for microvascular and macrovascular complications [1] and this prevalence is surprisingly increasing with the growing population, aging, and urbanized lifestyle, contributed by the several other risk factors such as obesity, hypertension, and familial history of the disease [2]. A particularly draining complication of diabetes is diabetic peripheral neuropathy (DPN) accounting for significant Morbidity disposing the individual to ulcerations in feet and loss of the anterior extremities [3].Currently, available treatments of neuropathic pain rarely reach the symptomatic relief from pain without any effect on the underlying causes; hence a drug with curative analgesic effects along with the symptomatic treatment is desired for the treatment [4]. Qualitative management of diabetes with oral hypoglycemic agents (OHAs) and insulin requires a routine administration of these agents and offer numerous side effects [5]. Metformin (Met) a commonly used OHA acts by suppressing the gluconeogenesis and glycogenolysis thereby stimulating the glucose utilization in the skeletal muscles. The risk of hypoglycemia although is less than that of other OHAs in Met, chances of hypoglycemia and regularly occurring lactic acidosis may get raised when used in higher doses [6,7].

Different naturally available compounds could be advantageous with possible therapeutic action and could be resourceful for the treatment of secondary complications of diabetes, if the hypothesized therapeutic properties of the natural compounds are properly validated and clinically practiced [8]. *Curcuma longa* Linn. also called as turmeric, derived from the rhizomes of the plant is commonly used as the Indian spice, belongs to a perennial member of zingiberaceae family and is usually cultivated in India and South East Asia [9]. Curcumin (CUR), the

primary constituent of turmeric responsible for its yellow color, along with its various pharmacological properties has been demonstrated to show potent anti-oxidant [10] and anti-inflammatory [11] properties.

DPN is usually characterized by various interconnected pathways dependent on oxidative/nitrosative stress, advanced glycation/ lipoxidation, poly ADP-ribose polymerase activity, and endoplasmic reticulum stress that activate pro-inflammatory that in turn has potential adverse effects on neurons and Schwann cells [12]. CUR has been shown to be a powerful anti-oxidant that inhibits the lipid peroxidation in rat liver microsomesby maintaining the activities of anti-oxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) peroxidase at higher levels [13]. A study conducted showed the ability of CUR in decreasing the plasma glucose and plasma insulin in type 1 diabetes [5]. CUR improves the insulin resistance in muscles mostly by increasing the oxidation of fatty acid and glucose and lowers plasma glucose, and lipids [14].

Hence, this study aimed to delay the neuropathy pain associated with uncontrolled diabetes, by reducing the dose of Met and combining with CUR, an herbal drug that possesses anti-oxidant and anti-diabetic activity.

METHODS

This study was performed in Postgraduate Studies and Research Center, Krupanidhi College of Pharmacy, Bengaluru, Karnataka, India. The Aqueous extract of *C. longa* Linn. (Batch No. CUR/13002) was obtained from Green Chem. Industry, Bangalore, as a free sample. The powdered form of CUR so obtained was suspended in 5% of carboxymethyl

cellulose (CMC) and orally fed to the animals during the treatment period.

Experimental animals

The experiments were performed on male Sprague–Dawley (SD) rats weighing 200-250 g obtained from an inbred colony in the Animal House, Department of Pharmacology, Krupanidihi college of Pharmacy Karnataka, Bangalore. These animals were kept in an environment with controlled temperature (25°C), humidity (45-75%) and 12:12 hrs light:dark cycle, as per the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals. All the rats were provided with normal pellet diet and water *ad-libitum*, prior to the dietary manipulation. The experimental protocol was approved by the Institutional Ethical Committee (KCP/IAEC-0001/2013-14).

Induction of diabetes mellitus

The animals were fed with a high-fat diet (HFD) for 2 weeks before the initiation of the experiment.

Diabetes was induced by a single intra-peritoneal injection streptozotocin (STZ) dissolved in 0.5 M/l citrate buffer (pH: 4.4) at a dose of 35 mg/kg, after the overnight fasting. Following by STZ injection, the animals were then fed with 5% w/v glucose solution for 5-6 hrs in order to prevent initial drug-induced hypoglycemic mortality. The rats with non-fasted plasma level ≥300 mg/dl were considered diabetic. The blood samples were collected from the tail vein and blood glucose was checked using a glucose diagnostic kit (Accu-Check). HFD was continued throughout the study period [15].

Oral glucose tolerance test (OGTT) [16]

The OGTT was performed in overnight fasted (18 hrs) on normal control and diabetic rats. Glucose (2 g/kg p.o.) was fed 30 minutes after the administration of drugs. Blood was withdrawn from the tail vein under light ether anesthesia at 0, 30, 60, and 120 minutes of glucose administration. Glucose levels were determined using Accu-Chek active glucose strips by using Accu-Chek glucometer manufactured by Roche.

OGTT was performed both before and after the treatment on the following group of animals:

Group 1: Normal control, rats received saline/vehicle
Group 2: Aqueous extract of *C. longa* Linn. (300 mg/kg) [17]
Group 3: Met (500 mg/kg) [18]
Group 4: Aqueous extract of *C. longa* Linn. (300 mg/kg) [17] + Met.

Treatment protocol

Rats were divided into nine groups (n=6) and the treatment with the respective drugs was given for 7 weeks. The drugs and the plant extract were suspended in 0.5% w/v of CMC.

Group 1: Normal control, rats received saline/vehicle
Group 2: Diabetic control (DC) (HFD/Low dose STZ)
Group 3: HFD control rats receive HFD
Group 4: HFD fed rats treated with aqueous extract of *C. longa.* (300 mg/kg) [17]
Group 5: Diabetic (HFD/low-dose STZ) Rats treated with aqueous extract of *C. longa* (300 mg/kg) [17]
Group 6: HFD fed rats treated with Met (500 mg/kg) [18]
Group 7: Diabetic (HFD/low-dose STZ) rats treated with Met (500 mg/kg) [18]
Group 8: HFD rats treated with extract of *C. longa* (300 mg/kg) [17] + Met
Group 9: Diabetic (HFD/low-dose STZ) rats treated with extract of *C. longa* (300 mg/kg) [17] + Met.

The normal animals without diabetes were taken to observe the effect of HFD on animals and to see whether the treatment affects the blood glucose level, body weight and normal intake of food and water.

Assessment of food and water intake

Food and water intake was assessed every day from each group of animals and calculated as a daily intake as the average intake of 6 animals. Body weights were taken twice a week for each group of animals. Since the diabetic animals required an excess of water and had frequent urination the bedding was changed daily.

Estimation of blood glucose

The blood was withdrawn through the tail vein, and the glucose levels were estimated using glucose dye oxidoreductase reactive strips and glucometer (Accu-check, Roche Diagnostics USA).

Estimation of anti-oxidant enzymes

At the end of the treatment, animals were sacrificed by cervical dislocation and sciatic nerve was immediately isolated from the upper part of the axotomized nerve. Tissue homogenate was prepared with 0.1 M tris-HCl buffer (pH=7.4,) and supernatant of homogenate was employed to estimate the SOD [19], CAT [20], thiobarbituric acid reactive substances (TBARS) [21], and reduced GSH [22].

Experimental neuropathic models

Hot plate test (Eddy's) [23]

In this test, animals were individually placed on a hot plate (Eddy's hot plate) with the temperature adjusted to 55°C±1°C. The latency to the first sign of paw licking or jump response to avoid the heat was taken as an index of the pain threshold; the cutoff time was taken as 15 seconds in order to avoid damage to the paw. The responses were recorded 1-week post STZ injection.

Tail flick test [24]

5 cm of the tail of a rat was immersed in warm water kept constant at 50°C±5°C. The reaction time was the time taken by the rat to deflect their tail. The latent period of the tail-flick was taken as the index of anti-nociception and was determined before and at 15, 30, 45, and 60 minutes after the administration of drugs. The maximum reaction time was fixed at 15 seconds.

Statistical analysis

Values were expressed as ± standard error of mean. The pharmacological effect was analyzed by using one-way analysis of variance followed by Dunnett's test. The differences between groups were considered to be significant at ($p < 0.05$).

Histopathological analysis

Samples of sciatic nerve were kept in the fixative solution (10% formalin) and cut into 4 μmm thickness. Staining was done by using hematoxylin and eosin. Nerve cross sections were analyzed under light microscope (×400) for axonal degeneration and vascular defects.

RESULTS

Feed and water consumption were significantly increased in DC group when compared with the normal control group. DC group had a significant reduction in body weight when compared with the control group, whereas the HFD control had an increased body weight when compared to the normal. The feed and water intake was higher in HFD control than the HFD group treated with CUR and Met. HFD fed animals treated with CUR, Met and their combination showed decreased body weight when compared to the HFD control group.

Blood glucose levels

There was a significant increase in the serum glucose levels of the DC group during the course of the study. The HFD control group showed increased blood glucose referring the impaired glucose tolerance, however, HFD animals on treatment with Met, CUR, and their combination showed almost similar blood glucose levels similar to the normal control group (Table 1). The group treated with CUR+Met (COMBI) showed significant control in serum glucose levels as compared with CUR group and Met group.

OGTT after treatment

CUR ($p < 0.01$) and COMBI ($p < 0.01$) showed a significant reduction in blood glucose level when compared with the DC group (Fig. 1).

Effect of CUR and its combination with Met in sciatic nerve anti-oxidant enzymes (Table 2)

CUR (27.35 u/mg, p<0.05), COMBI (28.89 u/mg, p<0.05) treated group showed a significant increase in the SOD levels when compared to the DC group. CUR (2.20 u/mg, p<0.05), COMBI (2.12 u/mg, p<0.01) showed a significant increase in TBARS level when compared to the DC group. GSH levels in CUR (62.42 u/mg, p<0.05), COMBI (69.71 u/mg, p<0.05) were increased when compared to the DC group. CUR (1.21 u/mg, p<0.05), COMBI (1.55 u/mg, p<0.01) treated showed a significant increase in CAT level when compared to the DC group.

Effect of CUR and its combination with Met on hot plate test (Table 3)

There was a significant difference in the reaction time for nociceptive stimuli in the normal and diabetic group during the treatment period. Diabetic group progressively showed a decrease in the reaction time as compared to the normal control group as the severity of diabetes was increasing. Treatment with CUR showed improvement in the reaction time. There was a significant increase in the reaction time in the group treated with CUR (7.86 seconds, p<0.001) alone and combined with Met (8.02 seconds, p<0.001) at the end 7th week of the treatment period when compared with the DC group.
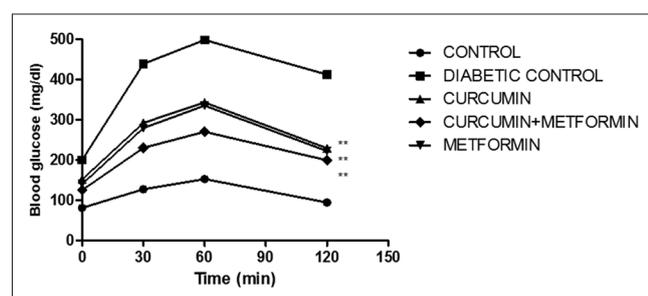
Fig. 1: Oral glucose tolerance test after treatment. All values are represented as mean ± standard error of mean, n=6, **p<0.01, ***p<0.001 when groups were compared with the diabetic control group

Effect of CUR and its combination with Met on hot water immersion test (Table 4)

Normal control and diabetic groups were evaluated for tail flick latency test by hot water tail immersion test. DC group showed a decrease in the tail flick latency as compared to normal control group, which indicates severe hyperalgesia. Treatment with CUR and Met alone and in combination showed an increase in the tail flick latency as treatment progressed (Fig. 2).

Histopathology

The sciatic nerve of diabetic rats developed severe pathological changes as compared with the groups treated with CUR and its combination with Met. The myelin fibers were well preserved and axons were not degenerated into treatment groups, whereas the DC group showed multifocal lesions and thickened hyaline endoneurial vessels.

DISCUSSION

Diabetes-induced neuropathic pain is considered as one of the major complication of diabetes mellitus [25]. Studies suggest that hyperglycemia leads to the toxicity of neurons due to increased glucose oxidation, leading to increased reactive oxygen species that may be controlled by the treatment with anti-oxidants [26-28].

The anti-oxidant activity of CUR has been shown to be more powerful as of α-tocopherol in inhibiting lipid peroxidation [29]. CUR inhibits the overexpression of nitric oxide synthase [30] and activation of nuclear factor kappa B7 [31], protects the islets against streptozocin (STZ) induced oxidative stress by scavenging free radicals and improves the life of the β-cell and hence increases the insulin secretion [32]. Hence, in our study, we hypothesized the anti-oxidant and anti-diabetic activity of CUR in delaying the diabetes-induced neuropathic pain in rats.

The HFD treated male SD rats were induced diabetes by intra-peritoneal administration of STZ (35 mg/kg). Initially, rats were pre-treated with HFD for two weeks and continued with the same diet followed by the treatment with CUR (300 mg/kg) and in combination of low dose of Met with CUR for the period of seven weeks. OGTT was performed to show the relationship between the orally loaded

Table 1: The effect of aqueous extract of *Curcuma longa* Linn. alone and in combination with Met on blood glucose level

Blood glucose (mg/dl)

Weeks/groups	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
Normal control	111±2.13	115±3.34	108±2.32	113±4.34	110±2.45	117±2.54	115±2.34
DC	271±2.35	291±2.6	312±3.40	343±2.34	363±4.56	380±3.19	420±3.45
HFD control	123±3.78	126±4.54	131±2.48	136±5.56	148±2.56	151±4.98	158±5.12***
HFD CUR	119±3.45	123±5.57	128±4.76	124±4.90	124±6.12	123±5.65	120±7.76***
HFD Met	120±2.34	125±4.45	131±5.65	128±2.34	125±6.89	120±7.65	118±2.34***
HFD COMBI	123±2.56	126±2.65	129±4.46	124±4.67	120±4.67	118±6.78	118±6.76***
Diabetic CUR	265±4.65	272±3.45	268±5.67	254±5.54	230±3.6	200±4.45	187±5.89
Diabetic+Met	278±3.46	258±5.67	237±7.68	220±5.89	198±6.78	170±4.67	151±5.09
Diabetic+COMBI	274±5.56	248±6.89	215±4.67	196±6.8	167±4.56	154±7.89	137±2.10*

All values are represented as mean±SEM, n=6, *p<0.05, ***p<0.001 when all groups were compared with the DC group. CUR: Curcumin, Met: Metformin, HFD: High-fat diet, SEM: Standard error of mean, DC: Diabetic control

Table 2: The effect of aqueous extract of *Curcuma longa* Linn. alone and in combination with Met in anti-oxidant enzyme levels of sciatic nerve

Groups	SOD (U/mg of protein)	CAT (U/mg of protein)	GSH (mmol/mg of protein)	TBARS (nmol/mg of protein)
Normal control	32.24±3.21	1.85±0.06	78.45±2.01	1.55±0.07
DC	12.3±2.1	0.45±0.09	40.54±2.41	4.89±0.08
CUR	27.35±2.41*	1.21±0.07*	62.42±1.98*	2.20±0.09*
Met	19.67±1.45	0.91±0.10	52.89±2.34	3.2±0.1
COMBI	28.89±1.12*	1.55±0.07**	69.71±1.65*	2.12±0.07**

All values are represented as mean±SEM, n=6, *p<0.05, ***p<0.001 when all groups were compared with the DC group. CUR: Curcumin, Met: Metformin, SOD: Superoxide dismutase, CAT: Catalase, GSH: Glutathione, TBARS: Thiobarbituric acid reactive substances, SEM: Standard error of mean, DC: Diabetic control

Table 3: The effect of aqueous extract of *Curcuma longa* Linn. alone and in combination with Met on Eddy's hot plate

Reaction time (seconds)

Groups	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
Normal control	8.55±0.17	8.78±0.22	8.31±0.15	9.01±0.44	8.90±0.26	8.34±0.42	9.50±0.32
DC	5.67±026	5.21±0.15	5.10±0.17	4.76±0.15	4.45±0.08	4.01±0.14	3.87±0.08
CUR	5.87±0.12	6.29±0.10	6.81±0.11	7.32±0.09	7.49±0.06	7.61±0.13	7.86±0.13***
Met	5.32±0.14	5.89±0.05	6.01±0.10	6.23±0.13	6.76±0.21	6.91±0.05	7.04±0.10
COMBI	5.89±0.21	6.45±0.16	7.01±0.11	7.34±0.12	7.71±0.14	7.97±0.13	8.02±0.17***

All values are represented as mean±SEM, n=6, *p<0.05, ***p<0.001 when all groups were compared with the DC group. CUR: Curcumin, Met: Metformin, SEM: Standard error of mean, DC: Diabetic control

Table 4: The effect of aqueous extract of *Curcuma longa* Linn. alone and in combination with Met on tail immersion test

Reaction time (seconds)

Groups	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
Normal control	8.55±0.17	8.78±0.20	8.31±0.15	9.01±0.25	8.90±0.24	8.34±0.26	9.50±0.18
DC	5.67±0.06	5.21±0.12	5.10±0.14	4.76±0.17	4.45±0.16	4.01±0.20	3.87±0.25
CUR	5.87±0.10	6.29±0.11	6.81±0.16	7.32±0.09	7.49±0.08	7.61±0.12	7.86±0.08***
Met	5.32±0.29	5.89±0.23	6.01±0.25	6.23±0.19	6.76±0.11	6.91±0.17	7.04±0.24**
COMBI	5.89±0.12	6.45±0.12	7.01±0.08	7.34±0.16	7.71±0.07	7.97±0.11	8.02±0.19**

All values are represented as mean±SEM, n=6, *p<0.05, ***p<0.001 when all groups were compared with the DC group. CUR: Curcumin, Met: Metformin, SEM: Standard error of mean, DC: Diabetic control
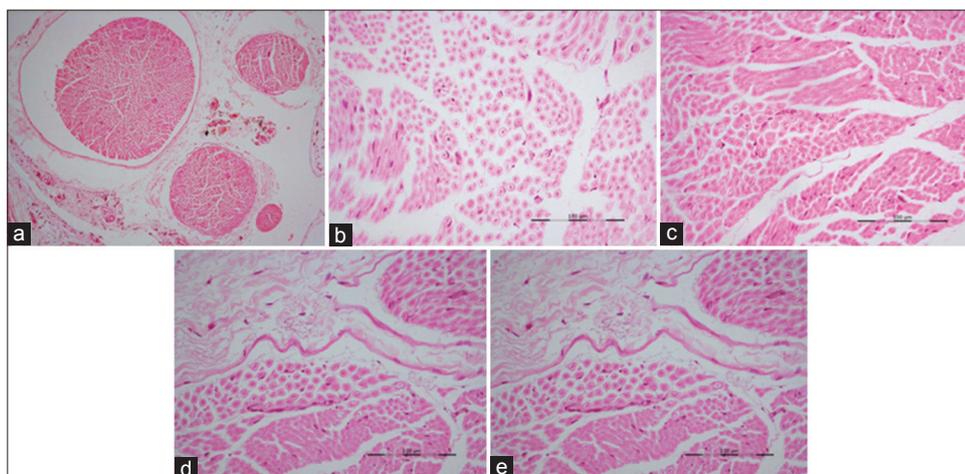
Fig. 2: (a) Normal control: Myelinated fiber density is well preserved. There is no axonal degeneration, and the fibers are compactly arranged. The endoneurial vessel is not thickened. (b) Diabetic control: Multifocal loss of both large and small myelinated fibers. Small myelinated fiber loss is more prominent than large diameter fiber loss. Thickened and hyalinized endoneurial vessel. (c) Metformin (Met) treatment: The endoneurial vessels are normal in morphology. Intact myelination but blood vessels are not included. Some regenerating clusters are seen. (d) Curcumin (CUR) treatment: Mild loss in the small myelinated fiber density. Few regenerating clusters are seen. The endoneurial vessel is not thickened. (e) CUR and Met treatment: Well-preserved myelinated fiber density. There is no axonal degeneration, and the fibers are compactly arranged. The endoneurial vessel is not thickened

glucose with Insulin. In our study, SD rats fed with HFD did not show the normal glucose tolerance reporting the insulin resistance. Initially, STZ administration increased the blood glucose level, decreased the body weight and after the treatment with CUR and combination with low dose of Met, it showed a significant improvement in controlling the blood glucose and body weight. The control in blood glucose might be due to the β-cell protecting activity [33] and ability to decrease the insulin resistance by CUR [34]. Superoxides produced due to the oxidative stress in diabetes might be responsible for vascular and Neuropathic complications [35]. SOD converts the superoxide radicals to H_2O_2 and O_2 and GSH further breaks H_2O_2 to water molecules. In this study, we examined the anti-oxidant enzyme level in sciatic nerve homogenate and it showed a decreased level of TBARS (CUR - 2.20 u/mg, p<0.05; COMBI - 2.12 u/mg, p<0.01) and increased level of SOD, (CUR - 27.35 u/mg, p<0.05; COMBI - 28.89 u/mg, p<0.05), GSH (CUR - 62.42 u/mg, p<0.05), COMBI - 69.71 u/mg, p<0.05), and

CAT (CUR - 1.21 u/mg, p<0.05;COMBI - 55 u/mg, p<0.01) comparative to DC Group indicating the anti-oxidant activity.

Pain aroused due to the injury in peripheral nerves is characterized by increased response to painful stimuli (hyperalgesia) and pain in response to a non-painful stimulus (allodynia) [36]. Previous studies reported the higher pain threshold response of STZ treated diabetic rats in the hot plate test [37]. Treatment with CUR, Met and combination showed an improvement in hyperalgesia and reported an increased response to paw withdrawal time Eddy's hot plate test (p<0.001) and tail-flick latency test in the hot water immersion test. The improvement in hyperalgesia might be due to the anti-oxidant property and property of inhibiting the NO and tumor necrosis factor (TNF)-α release [27]. Hence, combination of aqueous extract of CUR with low doses of Met might be useful in preventing the complication of type 2 diabetes when subjected to long-term treatment.

The metabolic insult of diabetes may directly affect the neuronal tissue similar to neurodegenerative changes precipitated by compromised nerve vascular supply [12]. The histopathological report supports the neuronal degeneration observed sciatic nerve of in DC group. CUR, its combination treated animals showed stable sciatic nerve stability than the DC group.

CONCLUSION

Uncontrolled blood glucose level leading to hypoglycemia, oxidative stress, and end-organ complications can ultimately become fatal. A number of commercially available OHAs though are effective in controlling the blood glucose, long-term use of the higher doses reports severe side effects. Hence, herbal drugs possessing anti-diabetic and anti-oxidant properties are desired for long-term treatment in order to reduce the dose of commonly used OHA and avoid their side effects. The basic pathophysiology of diabetes involves the destruction of β-cell or its improper functioning in secreting insulin due to unwanted metabolic changes, hence β cells become more compromised to the stimuli that demands excess insulin for the body. CUR has a property of protecting the islets β-cell, decrease the insulin resistance and decrease the oxidative stress. Hence, the use of CUR along with the OHA could be useful in preventing the complications associated with Diabetes when subjected to prolonged treatment.

ACKNOWLEDGMENTS

REFERENCES

1. Hsiao CJ, Cherry DK, Beatty PC, Rechtsteiner EA. National ambulatory medical care survey: 2007 summary. Natl Health Stat Report 2010;(27):1-32.
2. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: Global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res Clin Pract 2011;94(3):311-21.
3. Nicolucci A, Carinci F, Cavaliere D, Scorpiglione N, Belfiglio M, Labbrozzi D, *et al.* A meta-analysis of trials on aldose reductase inhibitors in diabetic peripheral neuropathy. The Italian Study Group. The St. Vincent Declaration. Diabet Med 1996;13(12):1017-26.
4. Mizoguchi H, Watanabe C, Yonezawa A, Sakurada S. New therapy for neuropathic pain. Int Rev Neurobiol 2009;85:249-60.
5. Jin L, Xue HY, Jin LJ, Li SY, Xu YP. Antioxidant and pancreas-protective effect of aucubin on rats with streptozotocin-induced diabetes. Eur J Pharmacol 2008;582(1-3):162-7.
6. Bodmer M, Meier C, Krähenbühl S, Jick SS, Meier CR. Metformin, sulfonylureas, or other antidiabetes drugs and the risk of lactic acidosis or hypoglycemia: A nested case-control analysis. Diabetes Care 2008;31(11):2086-91.
7. Al-Abri SA, Hayashi S, Thoren KL, Olson KR. Metformin overdose-induced hypoglycemia in the absence of other antidiabetic drugs. Clin Toxicol (Phila). 2013;51(11):444-7.
8. Galuppo M, Giacoppo S, Bramanti P, Mazzon E. Use of natural compounds in the management of diabetic peripheral neuropathy. Molecules 2014;19(3):2877-95.
9. Ammon HP, Wahl MA. Pharmacology of *Curcuma longa*. Planta Med 1991;57(1):1-7.
10. Kunchandy E, Rao MN. Oxygen radical scavenging activity of curcumin. Int J Pharm 1990;58:237-40.
11. Jurenka JS. Anti-inflammatory properties of curcumin, a major constituent of *Curcuma longa*: A review of preclinical and clinical research. Altern Med Rev 2009;14(2):141-53.
12. Fagerberg SE. Studies on the pathogenesis of diabetic neuropathy. IV. Angiopathia diabetica vasae nervorum. Acta Med Scand 1957;159(1):59-62.
13. Reddy AC, Lokesh BR. Studies on spice principles as antioxidants in the inhibition of lipid peroxidation of rat liver microsomes. Mol Cell Biochem 1992;111(1-2):117-24.
14. Na LX, Zhang YL, Li Y, Liu LY, Li R, Kong T, *et al.* Curcumin improves insulin resistance in skeletal muscle of rats. Nutr Metab Cardiovasc Dis 2011;21(7):526-33.
15. Kumar NP, Annamalai AR, Thakur RS. Antinociceptive property of *Emblica officinalis* Gaertn (Amla) in high-fat diet-fed/low dose streptozotocin induced diabetic neuropathy in rats. Indian J Exp Biol 2009;47:737-42.
16. Bergman RN, Finegood DT, Ader M. Assessment of insulin sensitivity *in vivo*. Endocr Rev 1985 Winter;6(1):45-86.
17. Ali Hussain HE. Hypoglycemic, hypolipidemic and antioxidant properties of combination of *Curcumin* from *Curcumin longa,Linn* and partially purified product from *Abroma Augusta,Linn*. in streptozocin induced diabetes induced nephropathy. Indian J Clin Biochem 2002;17(2):33-43.
18. Pushparaj P, Tan CH, Tan BK. Effects of *Aberrhoa bilimbi* leaf extract on blood glucose and lipids in streptozotocin-diabetic rats. J Ethnopharmacol 2000;72(1-2):69-76.
19. Elastner EF. Inhibition of nitrite formation from hydroxyl ammonium chloride. A simple assay for super oxide dismutase. Anal Biochem 1976;70(2):616-20.
20. Link EM. The mechanism of pH-dependent hydrogen peroxide cytotoxicity *in vitro*. Arch Biochem Biophys 1988;265(2):362-72.
21. Gelvan D, Saltman P. Different cellular targets for Cu- and Fe-catalyzed oxidation observed using a Cu-compatible thiobarbituric acid assay. Biochim Biophys Acta 1990 14;1035(3):353-60.
22. Mahboob M, Rahman MF, Grover P. Serum lipid peroxidation and antioxidant enzyme levels in male and female diabetic patients. Singapore Med J 2005;46(7):322-4.
23. Hargreaves K, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 1988;32(1):77-88.
24. Wang YX, Bowersox SS, Pettus M, Gao D. Antinociceptive properties of fenfluramine, a serotonin reuptake inhibitor, in a rat model of neuropathy. J Pharmacol Exp Ther 1999;291(3):1008-16.
25. Yagihashi S, Yamagishi S, Wada R. Pathology and pathogenetic mechanisms of diabetic neuropathy: Correlation with clinical signs and symptoms. Diabetes Res Clin Pract 2007;77 Suppl 1:S184-9.
26. Pittenger GL, Malik RA, Burcus N, Boulton AJ, Vinik AI. Specific fiber deficits in sensorimotor diabetic polyneuropathy correspond to cytotoxicity against neuroblastoma cells of sera from patients with diabetes. Diabetes Care 1999;22(11):1839-44.
27. Andrade P, Visser-Vandewalle V, Del Rosario JS, Daemen MA, Buurman WA, Steinbusch HW, *et al.* The thalidomide analgesic effect is associated with differential TNF-a receptor expression in the dorsal horn of the spinal cord as studied in a rat model of neuropathic pain. Brain Res 2012;1450:24-32.
28. Greene DA, Stevens MJ, Obrosova I, Feldman EL. Glucose-induced oxidative stress and programmed cell death in diabetic neuropathy. Eur J Pharmacol 1999 30;375(1-3):217-23.
29. Jovanovic SV, Steenken S, Borne CW, Simic MG. H-atom transfer is a preferred anti-oxidant mechanism of curcumin. J Am Chem Soc1999;121:9677-81.
30. Spinas GA. The dual role of nitric oxide in islet beta-cells. News Physiol Sci 1999;14:49-54.
31. Weber WM, Hunsaker LA, Roybal CN, Bobrovnikova-Marjon EV, Abcouwer SF, Royer RE, *et al.* Activation of NFkappaB is inhibited by curcumin and related enones. Bioorg Med Chem 2006;14(7):2450-61.
32. Meghana K, Sanjeev G, Ramesh B. Curcumin prevents streptozotocin-induced islet damage by scavenging free radicals: A prophylactic and protective role. Eur J Pharmacol 2007;577(1-3):183-91.
33. Seo KI, Choi MS, Jung UJ, Effect of curcumin supplementation on blood glucose, plasma insulin, and glucose homeostasis related enzyme activities in diabetic db/db mice. Mol Nutr Food Res 2008;52(11):995-1004.
34. Jang EM, Choi MS, Jung UJ, Kim MJ, Kim HJ, Jeon SM, *et al.* Beneficial effects of curcumin on hyperlipidemia and insulin resistance in high-fat-fed hamsters. Metabolism 2008;57(11):1576-83.
35. Kuhad A, Chopra K. Lycopene ameliorates thermal hyperalgesia and cold allodynia in STZ-induced diabetic rat. Indian J Exp Biol 2008;46(2):108-11.
36. Bridges D, Thompson SW, Rice AS. Mechanisms of neuropathic pain. Br J Anaesth 2001;87(1):12-26.
37. Sharma S, Kulkarni SK, Agrewala JN, Chopra K. Curcumin attenuates thermal hyperalgesia in a diabetic mouse model of neuropathic pain. Eur J Pharmacol 2006;536(3):256-61.