

## Original Article

# Imatinib Alleviates Neurological Deficits and Brain Injury After Transient Focal Cerebral Ischemia Associated With Comorbid Type 2 Diabetes

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## Abstract

The present study was planned to investigate the effects of imatinib, a selective tyrosine kinase inhibitor against focal cerebral ischemia/reperfusion (I/R) injury associated with comorbid type 2 diabetes (T2D). Focal cerebral I/R injury was induced by middle cerebral artery occlusion (MCAO) for 2 h followed by 22 h of reperfusion in T2D rats. These diabetic rats were treated with imatinib (10 and 30 mg/kg, i.p.) after MCAO and evaluated for functional (neurological score, rota rod and grip strength) and histological (cerebral infarct and edema volume) outcomes. TUNEL and immunohistochemistry assays were also performed for detecting the apoptotic DNA fragmentation and in-situ induction of CHOP, an endoplasmic reticulum (ER) stress specific pro-apoptotic factor, respectively. Imatinib (10 and 30 mg/kg) significantly alleviated various neurological deficits and reduced the brain infarct volume. Imatinib also significantly decreased TUNEL positive cells and CHOP immunoreactivity. These findings demonstrate the neuroprotective effects of imatinib in diabetic stroke model which may partly be due to attenuation of ER stress and apoptotic cell death.

## Introduction

Stroke remains the second leading cause of mortality and major cause of permanent disability worldwide (1). Presently, recombinant tissue plasminogen activator (rtPA) remains the only US-FDA approved reperfusion drug therapy for acute ischemic stroke

(2). Several neuroprotective agents found to be effective in preclinical stroke models have subsequently met with translational failures in human trials (3, 4). Stroke Therapy Academic Industry Round Table (STAIR) have recently recommended that the experiments should also be performed in animal models of stroke along with co-morbidities like diabetes, hypertension, and hypercholesterolemia for improving the chance of translational success of therapeutic agents from the bench to bed side (5). The pathophysiology of ischemic stroke is very complex and is associated with early and late phase processes such as blood brain barrier (BBB) disruption, neuroinflammation, apoptosis, neurovascular repair and regeneration. Recent investigations have highlighted the role of different

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tyrosine kinases (receptor and non-receptor TKs) in various pathophysiological processes of many neurological disorders including stroke (6).

Imatinib mesylate (Gleevec or STI571) is a selective tyrosine kinase inhibitor presently used in the clinics especially for the treatment of malignancies such as chronic myeloid leukemia (CML) and gastrointestinal stromal tumors (GISTs). Imatinib is reported to inhibit Bcr-Abl, c-Kit oncoproteins and also block the activity of platelet-derived growth factor receptor- $\alpha$  (PDGFR) and non-receptor wild-type cellular c-Abl tyrosine kinases (7). Beyond its well-established potential as anticancer agent, imatinib has recently garnered attention owing to its beneficial effects on variety of other neurological and metabolic disorders (8-17). Of interest, imatinib improves various stroke outcomes in healthy rodent models of ischemic and hemorrhagic stroke (16, 18, 19). Imatinib has been shown to increase the therapeutic time window by reducing the cerebrovascular permeability and hemorrhagic complications associated with delayed administration of thrombolytic rtPA during the treatment of ischemic stroke (18, 20). These CNS effects of imatinib have yet been demonstrated in healthy rodent animal models of stroke and largely attributed to its preservation of BBB integrity following PDGFR- $\alpha$  inhibition at neurovascular unit *in vivo* (18, 20, 21). Intriguingly, Han et al. reported that imatinib improves blood glucose level in diabetic mice via attenuating endoplasmic reticulum (ER) stress-induced pancreatic beta cell apoptosis and peripheral (liver and adipose tissue) insulin resistance (22). However, its plausible effects and mechanisms interfering with ER stress and apoptosis in brain parenchyma during acute ischemic stroke associated with comorbid diabetes have not been explored yet. Briefly, insults viz. cerebral ischemia, hypoxia, nutrient deprivation cause perturbation in protein folding processes resulting in accumulation of unfolded proteins in ER, the condition termed as ER stress. ER stress triggers unfolded protein response (UPR) which is initially protective, marked by up-regulation of various molecular chaperones and foldases that in turn help in proper protein folding and restore ER homeostasis. In case of severe and persistent stress, UPR fails to protect cells and ultimately initiates irretrievable apoptotic cell death

via many apoptotic signaling pathways including the induction of proapoptotic transcription factor such as CCAAT/enhancer binding protein homologous protein (CHOP) (23). ER-stress mediated apoptosis plays a major role in pathophysiology of cerebral ischemia associated with or without comorbid diabetes (24, 25).

The present study is therefore sought to determine the plausible beneficial effects of imatinib on functional and histological outcomes in the clinically relevant animal model of acute ischemic stroke associated with comorbid type 2 diabetes as per STAIR criteria and further explore its underlying mechanism if any via modulating brain ER stress and apoptosis.

## Materials and Methods

### Chemicals

Imatinib mesylate was obtained as a gift sample from Sun Pharmaceuticals Industries Ltd., Mumbai, India. Streptozotocin, hematoxylin, diaminobenzidine (DAB) and poly-L-lysine were procured from Sigma, St. Louis, MO, USA. 2,3,5 - triphenyltetrazolium chloride (TTC) (HiMedia Laboratories Ltd, Chandigarh) and nylon 3-0 monofilaments (ETHILON, NW3328, Johnson and Johnson Pvt. Ltd, Mumbai) were purchased locally. The primary antibody against CHOP (BioVision Ltd., USA) and its detection was performed using Vecta stain ABC kit (Vector Labs, Burlingame, CA, USA). Fluorescein-FragEL™ DNA fragmentation detection kit was purchased from Calbiochem, USA. All other chemicals were of analytical grade and purchased from commercial suppliers.

### Animals

Male Sprague-Dawley rats (110-140 g) were procured from central animal facility of the National Institute of Pharmaceutical Education and Research (NIPER), S.A.S. Nagar. The total numbers of 60 rats were used in this study. The animals were maintained under standard environmental conditions such as temperature (22°-26°C), relative humidity (40-70%) and dark/light cycle (12:12 h) till the termination of

the experiment. The animals were acclimatized to the laboratory environment for at least 3 days prior to the initiation of the experiment. Rats were housed initially in groups or later individually after surgery in polypropylene cages. The rats were allowed free access to standard rodent pellet feed (Ashirwad Industries, Chandigarh) during acclimatization period or subsequently high fat diet (HFD) along with purified water *ad libitum*. The study protocol was approved by the institutional animal ethics committee (IAEC) and carried out in accordance with the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Ministry of Environment & Forests, Govt. of India laid on animal care and use.

#### Induction of type 2 diabetes in rats

Type 2 diabetes was induced in rats by combination of high fat diet (HFD) and low dose of streptozotocin (STZ) treatment as described elsewhere (26). The rats were fed with HFD for two weeks followed by intra-peritoneal (i.p.) injection with single low dose of STZ (35 mg/kg dissolved in citrate buffer, pH 4.4.) to induce diabetes. HFD was continued to animals till the termination of the experiment. Blood samples were analyzed for plasma glucose using commercially available colorimetric kit (Accurex India Pvt Ltd, Mumbai, India) at the end of 4 weeks of dietary manipulation. Only those rats with plasma glucose of  $\geq 300$  mg/dl at the end of 4 weeks i.e. two weeks after STZ injection were only considered diabetic and included in the study.

#### Experimental design and drug treatment

The diabetic rats were randomly allocated to four experimental groups namely i) diabetic sham ii) Diabetic I/R + phosphate buffered saline (PBS, pH-7.2, 2 ml/kg) as vehicle control group iii) diabetic I/R + imatinib (10 mg/kg) and iv) diabetic I/R + imatinib (30 mg/kg). Imatinib was dissolved in PBS and administered as single dose through i.p. route immediately within two minutes after middle cerebral artery occlusion (MCAO) to the treatment groups where as diabetic control and sham groups were given vehicle PBS only as described below. The treatment doses of imatinib were selected based on the earlier study (21, 27).

#### Induction of focal cerebral ischemia/reperfusion in rats

Transient focal cerebral ischemia was performed in rats by employing MCAO method as described by Longa and co-workers (28). The diabetic rats were prior administered with atropine sulphate (0.5 mg D kg, i.p.). General anaesthesia was induced in these rats by 5 % halothane followed by its maintenance with 1 % halothane in a mixture of O<sub>2</sub>/N<sub>2</sub>O (30:70) using inhalational anaesthesia apparatus (Harvard Apparatus, Kent, UK). The right common carotid artery (CCA) was exposed through midline incision. The external carotid artery (ECA) was isolated and ligated. The internal carotid artery (ICA) was traced out and separated from vagus nerve. 3-0 nylon filament coated with poly- L-lysine was introduced through ECA lumen through a puncture and then gently advanced from ECA to ICA lumen up to 21 mm from the bifurcation of CCA or till the resistance was felt. After two hours of recovery, the animals were anaesthetized again and the filament were pulled back to allow reperfusion. In the sham-operated group, the filament was only inserted into ECA but not advanced. Rectal temperature was continuously monitored and maintained at  $37.0 \pm 0.5^\circ\text{C}$  using a feedback-controlled homoeothermic blanket system (Harvard Apparatus, Eden Bridge, Kent, UK). The site of surgery was sutured. Post operative care such as, the application of antiseptic (povidone iodine) on incision wound to prevent infection and subcutaneous administration of sterile isotonic saline solution to reduce dehydration, were performed on rats following surgery. After 22 h of reperfusion, the rats were evaluated for various functional and histological outcome measures as described below.

#### Assessment of functional outcome

##### Neurological Score

The neurological deficits were observed after 22 h of reperfusion and scored based on its severity on five-point scale (28). It comprises the scores of 0=no neurological deficits, 1=failure to extend the fore paw fully, 2= circling if pulled by tail, 3=spontaneous circling and 4= did not walk spontaneously and had depressed level of consciousness. Evaluations were performed by an investigator blinded to the treatment groups.

### Rota rod

The rats were subject to evaluation on motor coordination according to the previous report (29). Briefly, each rat was subject to baseline trial on accelerating rota rod (IITC Life Science Inc, CA, USA) in which spindle speed was set increasing from 4 to 40 rpm spanning over 3 min prior to MCAO occlusion. These rats were again subjected to test trial after 22 h of reperfusion. The latency to fall (s) of the rat from the rota rod was recorded within 3 min which was considered as cut-off time.

### Grip strength

Fore limb grip strength of the animals as a measure of neuromuscular function was assessed using grip strength meter (Columbus Instruments, Ohio, USA) as per the earlier method (30). The rats were allowed to seize the metal bar with their forelimbs and gently dragged backward in the horizontal plane. The moment the rat's grip in terms of force applied on the bar released was recorded as the peak tension (g). The maximum value out of three successful trials was taken as the final outcome.

### Assessment of histological outcome

#### Estimation of brain infarct and edema volume

After 22 h of reperfusion, the rats were killed and their brains were isolated and placed immediately at  $-10^{\circ}\text{C}$  for 30 minutes. The brains were then sectioned into coronal sections with the help of brain matrices. The sections were incubated in 2% 2, 3, 5-triphenyltetrazolium chloride (TTC) at  $37^{\circ}\text{C}$  for vital staining. After 15 min of staining, the sections were washed in PBS and 10% formal PBS was added to the sections and fixed overnight. Sections were scanned using HP scanner connected to the compatible PC and the infarct area was measured using image analysis software (Scion Image). Edema correction of infarct volume was performed using the formula, volume correction = (infarct volume  $\times$  contralateral volume)  $\div$  ipsilateral volume. The volumes of both hemispheres were calculated from which the edema volume was derived by subtracting the contralateral volume from the ipsilateral volume (31).

### TUNEL Assay

Terminal deoxynucleotidyl transferase mediated dUTP nick end labelling (TUNEL) assay was performed to detect the extent of DNA fragmentation or apoptotic cell death in  $5\ \mu\text{m}$  paraffin-embedded brain section in the cortical peri-infarct or penumbral region after 22 h of reperfusion as described earlier (25). TUNEL positive cells were observed in the ipsilateral cortical penumbral region under fluorescent microscope (Leica, Germany) and images were acquired using CCD camera (Leica DC 300F, Germany). The numbers of TUNEL positive cell were counted using the image analysis software 'Leica Qwin' (Leica, Germany) and represented as percentage of total cell population.

### Immunohistochemistry

Immunohistochemistry (IHC) analysis for the *in situ* expression of ER stress pro-apoptotic protein CHOP was performed using Vecta stain ABC kit as described elsewhere (32). For the detection of CHOP, the primary antibody against CHOP (1: 200) was used and its specific labelling was detected using DAB as a substrate. The sections were counterstained with haematoxylin and later observed under light microscope (Leica Microsystems, Germany) over ipsilateral penumbral region and images were acquired with the CCD camera. Images were randomly captured from the ipsilateral regions of a brain section of rats from different groups. Immunoreactivity of CHOP was quantitatively measured as percent dark brown stained immunopositive cells as compared to total cells counted.

### Statistical analysis

All the results are expressed as Mean  $\pm$  S.E.M except neurological score where it is expressed as median. Jandel Sigma Stat Version 2 software was used for statistical analysis. For the multiple comparisons, one way analysis of variance (ANOVA) was used followed by post hoc analysis using Tukey's test. Neurological score is expressed as median and was analyzed using Kruskal-Wallis one way analysis of variance on ranks test followed by post-hoc Dunn's multiple comparison test or Mann-Whitney test.

Differences were considered to be significant if  $p < 0.05$ .

## Results

### Imatinib alleviates functional neurological deficits

Feeding of HFD and low dose STZ (35 mg/kg) injection in rats produced significant ( $p < 0.001$ ) increase in blood glucose level ( $401.3 \pm 7.8$  mg/dl) after 4 weeks of dietary manipulation as compared to their basal level ( $109.5 \pm 2.4$  mg/dl). MCAO for 2 h followed by 22 h of reperfusion displayed significant impairment in various functional outcome parameters as evident from significant ( $p < 0.001$ ) increase in neurological deficit score, decrease in neuromuscular grip strength and rota rod falling latency in T2D rats as compared to sham-operated rats. However, the acute treatment with imatinib (10 and 30 mg/kg i.p) significantly ( $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$ ) improved the functional recovery of these neurological deficits as observed on neurological score (Fig. 1), rota rod (Fig. 2) and grip strength (Fig. 3) paradigms. There were no significant changes in body temperature, body weight and blood glucose level of diabetic animals between vehicle and treatment groups (data not shown).

### Imatinib reduces brain infarct volume

T2D rats following I/R manifested significantly larger

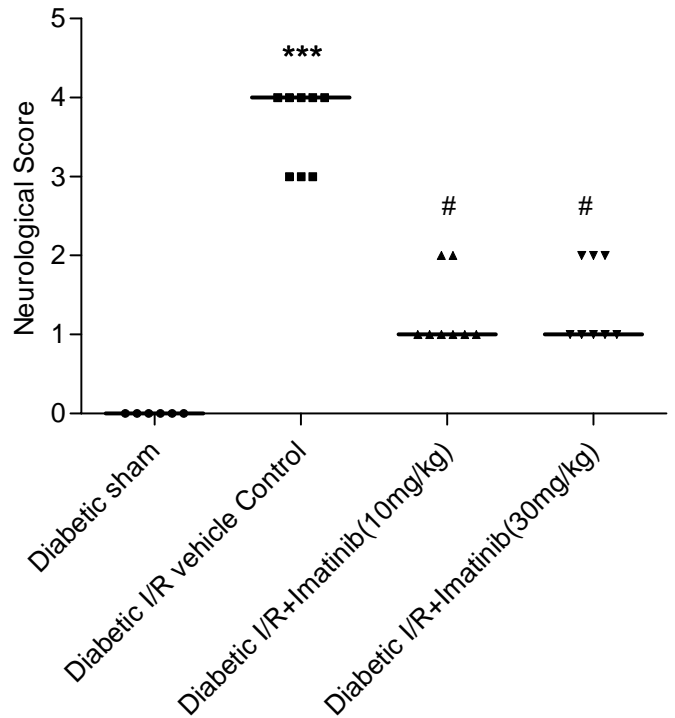


Fig. 1: Effect of Imatinib on neurological score in focal cerebral I/R injury associated with diabetes in rats. The values are expressed as median,  $n=6-8$ , \*\*\* $p < 0.001$  vs. diabetic sham, # $p < 0.05$  vs. diabetic I/R vehicle control.

brain infarct ( $p < 0.001$ ) and edema volumes as compared to sham-operated rats. Imatinib (10 and 30 mg/kg i.p.) treatment markedly ameliorated brain damage associated with significant reduction in brain infarct volume (Fig. 4) as compared to vehicle-treated

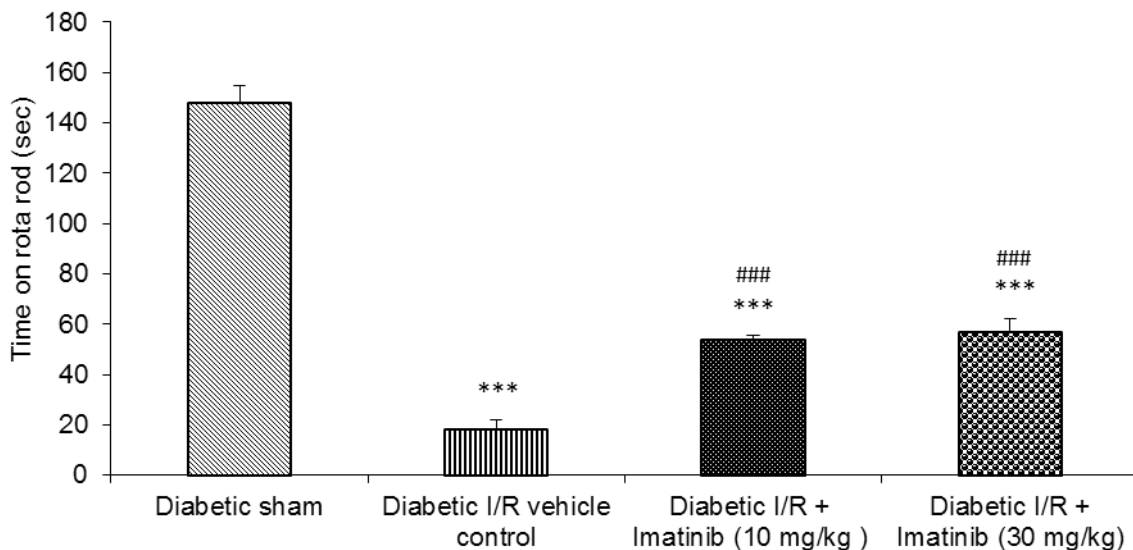


Fig. 2: Effect of Imatinib on falling latency from rota rod in focal cerebral I/R injury associated with diabetes. The values are expressed as mean  $\pm$  SEM,  $n=6-8$ , \*\*\* $p < 0.001$  vs. diabetic sham, ### $p < 0.001$  vs. diabetic I/R vehicle control.



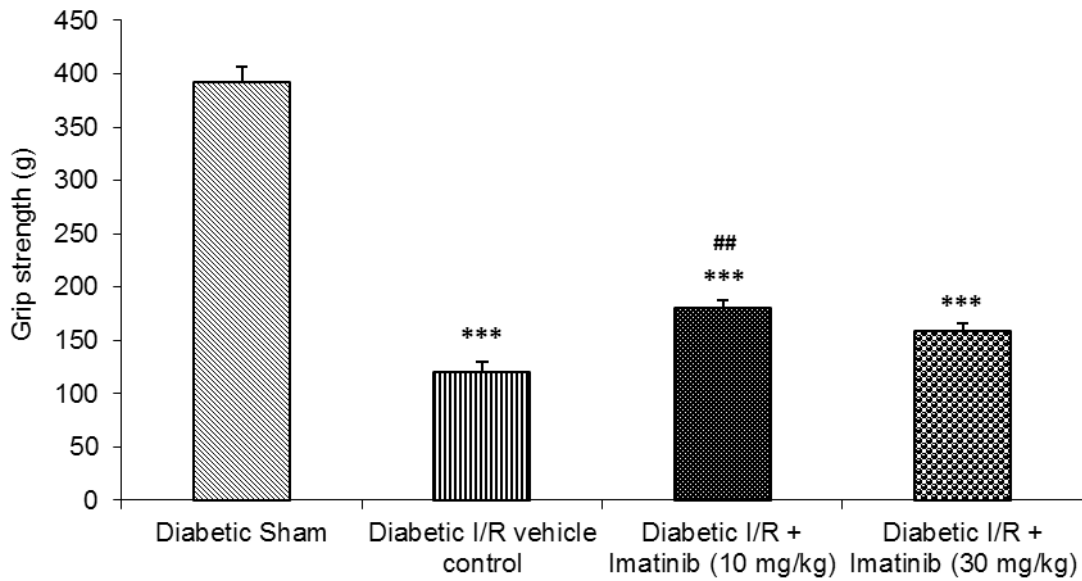


Fig. 3 : Effect of Imatinib on grip strength in focal cerebral I/R injury associated with diabetes. The values are expressed as mean±SEM, n=6-8, \*\*\*p<0.001 vs. diabetic sham, ##p<0.01 vs. diabetic I/R vehicle control.

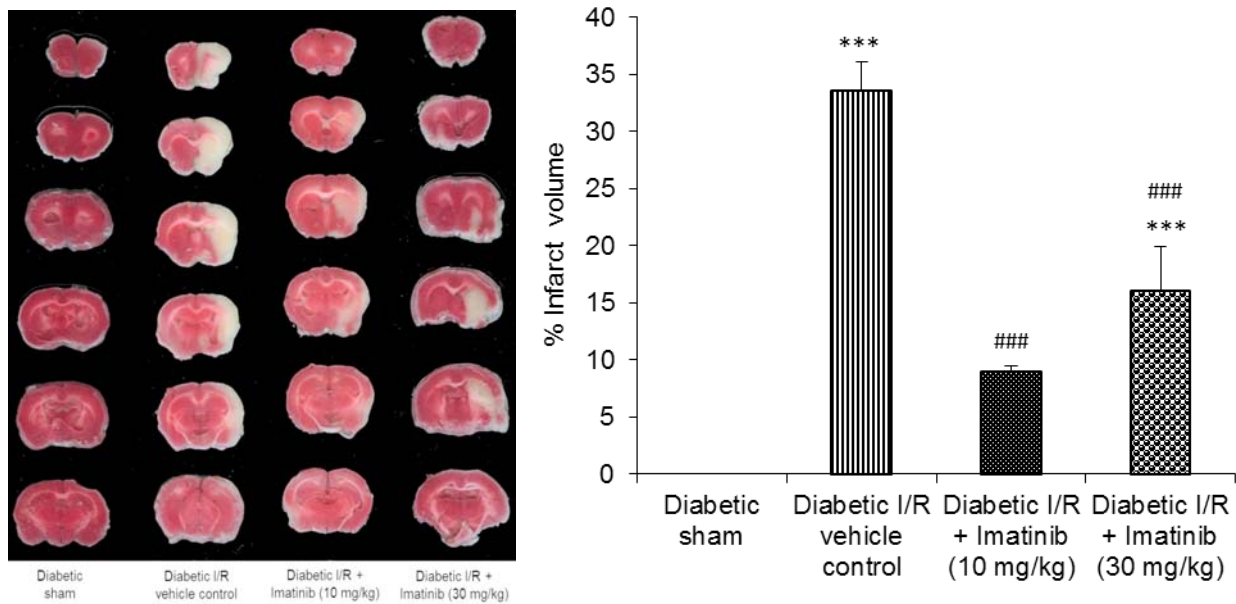


Fig. 4 : Effect of Imatinib on cerebral infarct volume in focal cerebral I/R injury associated with diabetes. The left image indicates the representative TTC stained brain coronal sections of various vehicle and imatinib (10 and 30 mg/kg)-treated groups where as the right graph represents percent cerebral infarct volume. The values are expressed as mean±SEM, n=6-8, \*\*\*p<0.001 diabetic sham, ###p<0.001 vs. diabetic I/R vehicle control.

diabetic I/R control group. Low dose of imatinib (10 mg/kg) exhibited more percent inhibition of infarct volume than its high dose (30 mg/kg) treatment, though this difference was statistically non-significant. However, imatinib (10 and 30 mg/kg) treatment did not evoke statistically significant

reduction in brain edema volume (14.3±1.4% and 17.2±4.5%) as compared to vehicle treatment (16.1±2.1%), respectively. As the optimum and maximum percent reduction of infarct volume was observed with 10 mg/kg dose of imatinib itself, further mechanistic studies were performed with imatinib (10 mg/kg) only.

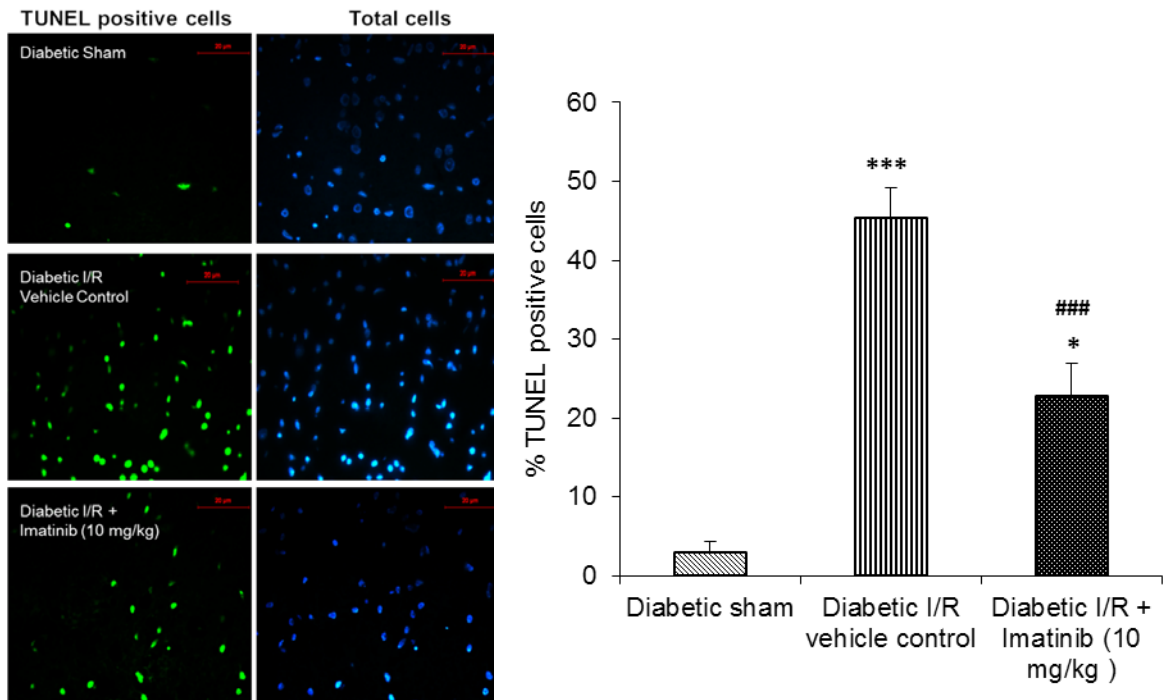


Fig. 5 : Effect of Imatinib on apoptotic DNA fragmentation after focal cerebral I/R injury associated with diabetes. The left panel shows the representative photomicrographs (field dimension 0.21 mm x 0.15 mm) showing TUNEL positive cells against DAPI stained total cells in ipsilateral cortical penumbral region of paraffin embedded brain sections of diabetic sham and diabetic I/R vehicle control and imatinib (10 mg/kg), respectively. The right panel indicates the quantitative representation of % TUNEL positive cells against total cell population. The values are expressed as mean±SEM (n=3-4). \*\*\*p<0.001 and \*p<0.05 vs. diabetic sham group, ###p<0.001 vs. diabetic I/R vehicle control group. The images were taken at 40X and the micron bar = 20 µm, DAPI (nuclear stain) - 4'-6-diamidino-2-phenylindole.

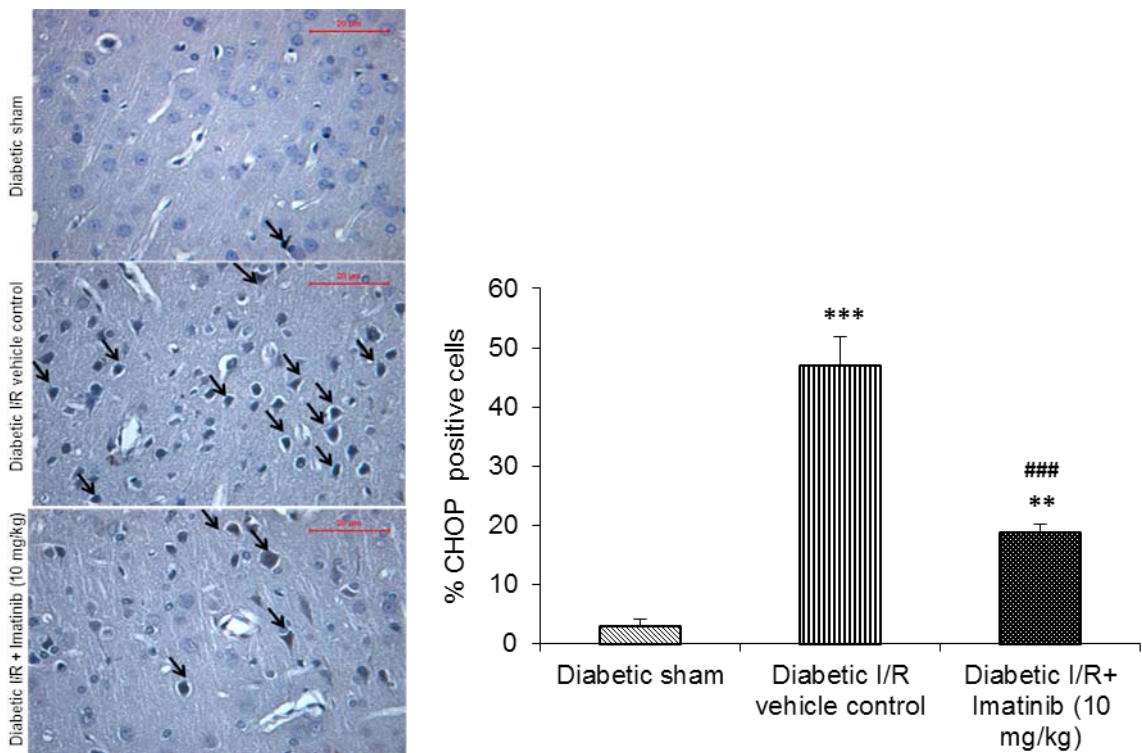


Fig. 6 : Effect of Imatinib on CHOP immunoreactivity in focal cerebral I/R injury associated with diabetes. The left images show the representative photomicrographs of immunopositive CHOP cells where the right graph denotes the quantitative representation of % CHOP immunopositive cells against total cell population. The values are mean±SEM (n=3-4). \*\*\*p<0.001 and \*\*p<0.01 vs. diabetic sham, ###p<0.001 vs. diabetic I/R vehicle control. The images were taken at 40X and the micron bar =20 µm. Black arrows represents the CHOP immunopositive cells.

### **Imatinib decreases apoptotic DNA fragmentation**

T2D rats after I/R exhibited significant ( $p < 0.001$ ) increase in apoptotic DNA fragmentation as shown by increased TUNEL positive cells in ipsilateral cortical penumbral brain region at 22 h of reperfusion. TUNEL positive cells were however rarely detected in diabetic sham-operated rats. Treatment with imatinib (10 mg/kg) significantly ( $p < 0.001$ ) decreased TUNEL positive cells as compared to vehicle treatment following I/R (Fig. 5).

### **Imatinib abrogates ER stress pro-apoptotic CHOP immunoreactivity**

The diabetic rats after I/R challenge exhibited significant increase in CHOP positive cells in ipsilateral penumbral region as compared to sham-operated group of animals. The treatment with imatinib significantly abrogated CHOP immunoreactivity ( $p < 0.001$ ) in penumbral cortical region as evidenced from the reduction in CHOP positive cell population as compared to vehicle-treated diabetic I/R control group (Fig. 6).

## **Discussion**

The present study demonstrates the beneficial effect of imatinib, a selective tyrosine kinase inhibitor in experimental focal ischemic stroke associated with comorbid type 2 diabetes via mitigating ER stress and apoptosis. Recent experimental evidences have shown that tyrosine kinases play important role in pathophysiology of stroke that can be explored as a promising new target for its treatment (6). The young and healthy rodent models of stroke are frequently used by the investigators for testing the neuroprotective effects of the agents. These animal models, however, do not closely represent the clinical situation where the stroke develops due to the presence or progression of some underlying risk factors or comorbid conditions (5). Diabetes is a one of the major risk factors and common comorbidities found among stroke patients (33). Thus, in the present study, we used the rat model of ischemic stroke with comorbid diabetes for evaluating the neuroprotective effects of imatinib, selective TK inhibitor in an effort to improve the translational

success as per STAIR criteria. As the animal experiments encompassing the dose-response phenomenon are more likely to translate successfully to humans (3, 34), we therefore planned to investigate the dose-dependent effects of imatinib (10 and 30 mg/kg) against ischemic stroke. Further, this work distinctly differs as far as exploring the mechanism of imatinib with respect to ER stress and apoptosis in ischemic stroke is concerned.

Initially, T2D was induced in rats by the combination of HFD and low dose STZ treatment. Feeding of HFD has been reported to induce characteristic features of insulin resistance in normal animals. Low dose of STZ (35 mg/kg) injection after two weeks of HFD feeding in these insulin resistant rats induced overt hyperglycemia. It is consistent with the earlier study and is widely considered as an appropriate animal model representing the natural history and clinical manifestations of human T2D (26). These rats were then subjected to MCAO surgery for 2 h followed by 22 h of reperfusion which simulates transient focal cerebral ischemia or ischemic stroke in clinical situation (28). The diabetic rats after I/R exhibited significant impairment in both functional (neurological deficits) and histological (brain infarction and edema) outcomes as compared to sham-operated animals which is in agreement with earlier reports (25, 35). Administration of imatinib (10 and 30 mg/kg) significantly improved the functional recovery of various neurological deficits upon assessment with neurological score, rota rod and grip strength tests. Assessment for the functional recovery of neurological deficits after stroke is considered as an important outcome measure in clinical trial as the ultimate goal of stroke treatment is restoration of neurological functions (3). It was also accompanied with improved histological outcome in terms of significant reduction in brain infarction post 2/22 h of I/R thus suggesting its potential neuroprotective effects. Of note, we here in demonstrate that the beneficial effects of imatinib against ischemic stroke can be best achieved with its dose as low as 10 mg/kg itself. This is in contrast to the previous animal studies on healthy rodent models in which the authors demonstrated its effect only with its single high or corresponding maximum indicated human dose of imatinib (100 or 200 mg/kg) meant for cancer treatment, without exploring



the possibility of its beneficial effects at lower doses against ischemic stroke, a distinctly different pathology. Improvement in stroke outcomes by low dose of imatinib is further likely to reduce the adverse effects or toxicities (eg. cardiotoxicity) associated with its high dose treatment as demonstrated in some other investigations (22, 36, 37). Altogether, the present data clearly support and substantiate the beneficial effects of imatinib in ischemic stroke which agrees with earlier studies on healthy rodent models of stroke (16, 18). Its beneficial effects have yet been reported at its high dose (>100 mg/kg) due to its inhibitory action on PDGFR- $\alpha$  and subsequent alleviation of BBB disruption and neuroinflammation (16, 18). However, in the current study, we did not observe any significant reduction in brain edema volume with imatinib (10 and 30 mg/kg), suggesting of the other mechanisms underlying its neuroprotective effects. Han et al. earlier demonstrated that imatinib induces remission of diabetes by alleviating ER stress and pancreatic beta cell apoptosis in animal models of diabetes (22). Therefore, we explored the possibility whether it exerts its protective effects via alleviating ER stress and apoptosis in brain parenchyma following cerebral I/R if any. There are three distinct ER transmembrane proteins such as PKR (RNA dependent protein kinase)-like endoplasmic reticulum kinase (PERK), inositol-requiring enzyme 1 (IRE-1) and activating transcription factor 6 (ATF-6), the activation of these is responsible for various cell survival and death mechanisms under ER stress conditions. Especially during severe and persistent ER stress associated with focal cerebral I/R, protective UPR fails to prevent the cells from undergoing apoptotic cell death which is largely considered to be mediated via activation of ER stress pathways involving downstream proapoptotic factor CHOP (38, 39). The results of TUNEL and immunochemistry assays have revealed that there is significant increase in apoptotic cell death in parallel with the enhanced expression of CHOP in cortical penumbral region in vehicle-treated diabetic I/R rats. These results are in consistent with earlier reports (25, 40). TUNEL positive cells are well-characterized element of DNA fragmentation or apoptotic cell death observed especially in peri-infarct zone which plays a pivotal role in pathophysiology of stroke (41). Reduction in TUNEL positive cells after treatment

with imatinib in this penumbral region, a target site of most of the neuroprotective agents, further corroborates its neuroprotective and anti-apoptotic potential. The effect was accompanied with abrogation of CHOP immunoreactivity compared with vehicle-treated diabetic I/R rats, suggesting its action via mitigating ER stress-mediated apoptosis. Although exact molecular mechanisms of CHOP is not known, it has been reported to induce apoptotic cell death via transcriptional regulation of various pro- and anti-apoptotic genes including *DOC* (downstream of CHOP), *GADD34*, *ERO1 $\alpha$* , *BIM*, *TRB3* (tribbles-related protein3) and anti-apoptotic *BCL-2* (B-cell lymphoma-2) genes (42). However, it is not clear how imatinib reduces CHOP and ER-stress associated-apoptotic cell death which needs further investigation. However, the previous findings have shown that c-Abl kinase, a target of imatinib, plays an important role in the initiation and mediation of ER stress response. c-Abl kinase is also located in ER membrane which gets translocated to mitochondria during ER stress, induces cytochrome c release and subsequently apoptotic cell death (43, 44). ER stress-induced apoptosis is also attenuated in c-Abl-deficient cells (43). It is therefore hypothesized that the inhibition of c-Abl by imatinib may be involved in alleviation of ER-induced apoptotic cell death in cortical penumbral region after focal cerebral I/R. However, the protective action of imatinib through down-regulation of ER stress pathway involving PERK activation leading to apoptosis as observed in resistant CML cannot be ruled out (45). There are some reports indicating that chronic treatment with imatinib improves blood sugar levels in CML patients with comorbid diabetic conditions (8) as well as in experimental models of diabetes (9, 10, 12, 22). However, the beneficial effects of imatinib on brain damage observed in the current study following improvement in hyperglycemia if any was ruled out as there was no significant reduction in plasma glucose on its acute dose administration. However, futuristic investigations with chronic treatment of imatinib might unfold its therapeutic benefits not only on focal ischemic stroke but also against underlying comorbid type 2 diabetes.

In conclusion, we have here in demonstrated the beneficial effects of imatinib in ischemic stroke

associated with comorbid type 2 diabetes which may in part result from attenuation of ER stress and apoptotic cell death in rats. Based on the foregoing observations, it is further stated that imatinib treatment might be a viable neuroprotection approach for the treatment of acute ischemic stroke associated with comorbid diabetes.

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## Conflict of interest

Vishal Sachan, Krishnamoorthy Srinivasan, Shyam S Sharma hereby declare that they have no conflict of interest. All experimental procedures involving animals were approved by institutional animal ethics committee and strictly followed the applicable national and institutional guidelines on laboratory animal care and use.

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