Effect of rutin on early diabetic neuropathy in experimental animals

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ABSTRACT

The aim of the present study was to explore the effect of rutin on early diabetic neuropathy in experimental animals. Streptozotocin (55 mg/kg, i.p.) was administered to different groups of rats. Four weeks after injection of streptozotocin, rutin (50 and 100 mg/kg, p.o.), metformin (200 mg/kg, p.o.) and sertraline (30 mg/kg, p.o.) were administered in diabetic rats (n=5) for three weeks. The antidiabetic activity of rutin was studied by measuring serum glucose, serum triglyceride and glycosylated hemoglobin. Thermal hyperalgesia, formalin induced hyperalgesia, cold allodynia, walking function test, tumour necrosis factor- alpha, plasma antioxidant enzymes like SOD, NO, MDA, GSH, catalase levels and sciatic nerve axonal degeneration were performed to assess the extent of neuropathy. Four weeks after a single dose intraperitoneal injection of streptozotocin (55 mg/kg), rats exhibited significant hyperalgesia along with increased serum glucose and decreased body weights as compared with control rats. Treatment of rutin (50 and 100 mg/kg; p.o.) given for 3 weeks starting from the 4th week of streptozotocin injection significantly lowered biochemical changes and delayed paw and tail withdrawal latency in hyperalgesia and allodynia respectively. Preventive treatment of rutin significantly improved paw flinching response in formalin induced hyperalgesia. Rutin also inhibited the level of serum TNF-α. Rutin restored antioxidant enzymes NO level and SOD, GSH, CAT level activities in diabetic rats. Rutin has shown beneficial effect in preventing the progression of early diabetic neuropathy in rats.

Keywords: Rutin, Diabetic Neuropathy, hyperalgesia, antioxidant, TNF-α.

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INTRODUCTION

Diabetes is a global health problem and its prevalence is set to increase to 366 million worldwide by the year 2025 [1]. Persistent hyperglycemia in diabetic patients despite appropriate therapeutic measures leads to several complications including retinopathy, nephropathy and neuropathy. Diabetic neuropathy is the most common complication affecting more than 50% of the diabetic patients. Etiology of diabetic neuropathy is complex and multifactorial. Over the past 25 years, animal experiments and in vitro studies have identified biochemical pathways likely to be important in the development of diabetic complications and have led to possible approaches to treatment. All of these pathways are related to the metabolic and/or redox state of the cell. Pathways which are mainly driven by metabolism are increased flux of glucose to polyol pathway; increased hexosamine shunt; excess/inappropriate activation of protein kinase C isoforms; accumulation of advanced glycation end products; impaired neurotrophic support; activation of poly (ADP-ribose) polymerase (PARP); impaired and decrease in nerve myoinositol content. While each pathway may be injurious alone, collectively they cause an imbalance in the mitochondrial oxidation-reduction state of the cell and lead to excessive formation of reactive oxygen species [2-4].

Diabetic pain manifests itself as a variety of symptoms, such as muscular aches, burning or tingling sensations, cutaneous hyperesthesia and loss of cold, heat and vibration sensation [5]. Nerve degeneration occurs typically in a distal-to-proximal gradient, so that the distal part of the extremities (hands and feet) are especially sensitive to neuropathic pain. Various kinds of behavioral testing in STZ-induced diabetic rats have been reported. Mainly

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thermal hyperalgesia [6], have been found in the paw thermal response test. The thermal tail flick test seems to show heterogeneous results [7]. Mechanical hyperalgesia in response to application of increasing pressure to the paw is reported frequently [8]. Tactile allodynia is another possible manifestation of diabetes neuropathy [9]; light touch is perceived as painful [7]. Hyperalgesia produced by the injection of the chemical irritant formalin into the paw is intensified in the diabetic rat [10]. Besides these behavioral changes, painful diabetes neuropathy has a range of other manifestations, such as impaired motor and sensory conduction velocity and nerve blood flow [11].

Tight and stable glycemic control is the only established method of slowing the relentless progression of the neuropathic state and most patients eventually show some form of neuropathy. In addition to glycemic control, treatments such as anticonvulsants [12], antidepressants [13], ion channel blockers [14], N-methyl-D-aspartic acid (NMDA) receptor antagonists [15], and opioids [16] merely provide symptomatic relief and do not modify the course of the disease. Anticonvulsants and antidepressants are the most common treatment options, but evidence of the long-term effects of these drugs is still lacking [17]. Reactive oxygen/nitrogen species and inflammatory cytokines exert diverse actions implicated in diabetic neuropathy, from development to the initial stages of diabetes to progression and to late stages of neuropathic pain. The recognition of these molecules as significant pathogenic factors in this complication provides new therapeutic targets to be exploited.

Renewed interest has been observed in recent years on the multiple activities of natural molecules. Rutin is one of the most commonly found flavonol glycosides in the human diet. The rutin exhibit a wide variety of biological activities, including antiviral, antibacterial, anti-inflammatory, anti-carcinogenic, and antioxidant actions [18]. Rutin exerts its anti-hyperglycemic effect by improving glucose tolerance, enhancement of insulin release, insulin binding affinity and in addition to decreasing the activity of gluconeogenic and glycogenolytic enzymes [19]. Strong evidence for a cerebral neuroprotective and memory enhancing action of ruitn also comes from recent experiments [20]. In another study Khan *et al* showed the neuroprotective role of rutin (Vitamin P), indicate that rutin attenuates ischemic neural apoptosis by reducing the expression of p53, preventing morphological changes and increasing endogenous antioxidant enzymatic activities. Thus, rutin treatment may represent a novel approach in lowering the risk or improving the function of ischemia reperfusion brain injury related disorders. However, the role of rutin in diabetic neuropathy in human subjects or animal studies has not been investigated so far. Therefore, the present study was designed to investigate the effect of rutin on diabetic neuropathy.

MATERIALS AND METHODS

Animals
Sprague Dawley rats of either sex weighing between 180-230g were obtained from National Institute of Bioscience, Pune. Animals were housed into groups of three under standard laboratory conditions of temperature 25 ± 1 ℃ free access to water and standard laboratory feed (Amrut feed, Pune, India). All experiments were carried out between 0900 and 1700 h. The experimental protocols were approved by Institutional Animal Ethics Committee (SCOP/IAEC/Approval/20010-11/07) and care of the animals was carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Drugs and reagents
Streptozotocin and rutin were purchased from Sigma (St. Louis, MO, USA). Metformin and sertraline were obtained as gift sample (Cipla Pharmaceuticals, India). Glucose oxidase–peroxidise (GOD/POD) diagnostic Kit was purchased from Biolab, Mumbai and other chemicals used were of analytical grade (Qualigens, India) and purchased from local supplier.

Induction and assessment of diabetes
A single dose of 55 mg/kg streptozotocin prepared in citrate buffer (pH 4.4, 0.1 M) was injected intraperitoneally to induce diabetes. The age-matched control rats received an equal volume of citrate buffer and used along with diabetic animals. Diabetes was confirmed after 48h of streptozotocin injection, the blood samples were collected through tail vein and plasma glucose levels were estimated by enzymatic GOD-PAP (glucose oxidase peroxidase) diagnostic kit method. The rats having plasma glucose levels more than 250 mg/dl [21]. were selected and used for the present study. Streptozotocin (STZ), a β-cytotoxin, increases pancreatic islet O-linked protein glycosylation in a dose dependent, irreversible fashion and also inhibits GlcNAcase, the enzyme that removes O-GlcNAc from proteins which accounts for its diabetogenic toxicity [22]. Body weight and plasma glucose levels were measured before and at the end of the experiment to see the effect of rutin on these parameters.

Treatment schedule
After a basal recording of nociceptive reaction at 4th week of streptozotocin injection, control and diabetic rat were randomly selected and divided in six groups of 5-6 animals each. Four weeks after the diabetic induction treatment

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with rutin was given for three weeks (5th, 6th and 7th weeks).

Group I: [NC] Normal control rats (Distilled water 5 ml/kg, *p.o.*)
Group II: [DC] Diabetic control rats (Streptozotocin 55 mg/kg, i.*p.*)
Group III: [DC + METFORMIN] Diabetic rats received Metformin (200 mg/kg/day, *p.o.*)
Group IV: [DC + SERTRALINE] Diabetic rats received Sertraline (30 mg/kg/day, *p.o.*)
Group V: [DC + RUTIN] Diabetic rats received Rutin (50 mg/kg/day, *p.o.*)
Group VI: [DC + RUTIN] Diabetic rats received Rutin (100 mg/kg/day, *p.o.*)

Starting from the 5th week after STZ injection till 8th week, the control and diabetic control groups received vehicle. Rutin was freshly prepared by dissolving in distilled water. The animals were sacrificed under deep anesthesia, blood was collected by retro-orbital plexus and plasma separated. Plasma was used for TNF-α assay, nitric oxide, superoxide dismutase, catalase and reduced glutathion. The samples were stored at -80 °C until processed for biochemical estimations.

Assessment of plasma glucose level
Plasma glucose was estimated using glucose oxidase peroxidase method [23].

Assessment of body weight
Body weights were measured pre-treatment (i.e. after four weeks of induction of diabetes) and post-treatment (i.e. after seven weeks of induction of diabetes) by using digital weighing balance.

Estimation of biochemical parameters
Serum triglyceride level
The samples were analyzed for plasma Triglyceride (total) levels using Triglyceride solution kit provided by Biolab Diagnostics Pvt. Ltd., Mumbai, India.

Glycosylated haemoglobin
Blood was collected by puncturing retro-orbital plexus under mild ether anesthesia by using fine glass capillary in epindorff tubes. Serum was separated by centrifugation and buff coat (packed blood cells) was taken, Wash the packed cells by using normal saline at least six times. Added ¼ part of distilled water to the packed cells and ¼ part of CCl_4, shake vigorously and centrifuged for 20 minutes at 3000 rpm. Aspirated Hb concentration to 10 g/dL by using normal saline. 2.0 ml of hemolysate was used for next part of the procedure. 1.36 ml of hemolysate and was added to 0.64 ml of normal saline and mixed. 2ml hemolysate, 1ml of oxalic acid reagent was mixed well. Kept in boiling water bath for one hour (cover the tube using cotton). Test tube was cool to room temperature and added 1ml of TCA reagent. Mixed thoroughly centrifuged at 3000 rpm. 2.0ml of supernatant was added to 0.5 ml thiobarbituric and mixed well. Kept at 37°C for 40 minutes. Reading was taken as blank (2ml) distilled water and 0.5ml of thiobarbituric acid) at 443nm.

Estimation of TNF-α level
TNF-α level was measured in the serum at the end of study, i.e., at 7th weeks after STZ injection and 3th week after rutin administration. TNF-α was estimated using rat TNF-α kit (Thermofisher scientific). It is a solid phase indirect enzyme linked immuno-sorbent assay (ELISA) using a microtitre plate reader at 450 nm. Concentrations of TNF-α calculated from plotted standard curves. TNF-α levels were expressed as means ± SEM.

Estimation of nociception
Assessment of thermal hyperalgesia
Tail-immersion (hot water) test
Tail of rat was immersed in a hot water bath (52.5 ± 0.5 °C) until tail withdrawal (flicking response) or signs of struggle were observed (cut-off 12 s). Shortening of the tail withdrawal time indicates hyperalgesia [24].

Hot-plate test
The hyperalgesic response on the hot-plate is considered to result from a combination of central and peripheral mechanisms [24]. In this test, animals were individually placed on a hot-plate (Eddy's Hot-Plate) with the temperature adjusted to 55 ± 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 cut-off time was 10 s in order to avoid damage to the paw.

Estimation of cold allodynia
Cold allodynia was evaluated after seven weeks of diabetes in all diabetic rats, with tail immersion test. The tail-flick latency of each animal was determined by immersing the tail into the cup filled with water that had a constant temperature of 10°c and recording the tail withdrawal latency (in s, cut of time: 15 s) with manual stop watch. A

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shortened duration of immersion indicates allodynia [25].

Estimation of formalin test
Formalin evoked flinching was evaluated after seven weeks of induction of diabetes in all diabetic rats. Rats were restrained manually and formalin (50 μl of 0.5% solution) injected sub-dermally into the hind paw dorsum. Rats were then placed in an observation chamber and flinching behaviours counted in 1 min blocks every 5 min for 1 h. The sum of flinches was grouped to highlight specific phases of the test [26].

Estimation of walking function test
The walking function test was performed after seven weeks of induction of diabetes. The device used for the walking test was a rod of 6 cm diameter and 1m long, maintained horizontally 40 cm above a table. The rod was graduated in order to allow the measurement of the distance covered by the animals. Three trials per session were performed. For each trial (60 s maximum), each rat was placed at an extremity of the rod, and the time needed to walk the 1 m distance was recorded. If the animal fall down or be unable to walk the 1 m distance, 60 s were credited. For each animal, the mean duration of the three trials was calculated and retained as the characteristic value [27].

Estimation antioxidant parameters
Nitric oxide
500 μl of Greiss reagent (1:1 solution of 1% sulphanilamide in 5% phosphoric acid and 0.1% napthaylamine diamine dihydrochloric acid in water) was added to 100 μl of plasma and absorbance was measured at 546 nm [28]. Nitrite concentration was calculated using a standard curve for sodium nitrite and expressed as ng/ mg of protein.

Catalase
CAT activity was measured based on the ability of the enzyme to break down H_2O_2. The method of employed in the assay of CAT activity [29]. 20 μl of plasma added to 980 μl of the assay mixture containing 900 μl of 10 mmol/L of H_2O_2, 50 μl of Tris HCI buffer (pH 8.0) and 30 μl of distilled water. The rate of decomposition of H2O2 was monitored spectrophotometrically at 240 nm and expressed as µg/ mg of protein.

Reduced glutathione
Reduced glutathione levels were estimated based on the ability of the SH group to reduce 5,5'-dithiobis- (2-nitrobenzoic acid) to form 1 mole of 2-nitro-5- mercaptobenzoic acid per mole of SH. The method of Sedlak and Lindsay, 1968 was employed in the determination of GSH levels (11). To 0.5 ml of plasma, 1.5 ml of 0.2 mol/L Tris HCI buffer (20 mmol/L EDTA, pH 8.2), 0.1 ml of 0.01 mol/L of 5, 5'-dithiobis-(2-nitrobenzoic acid) and 7.9 ml of methanol were added. The mixture was incubated at 37ºC with occasional shaking for 30 minutes. The mixture was then centrifuged at 3,000 g 15 minutes and the absorbance of the supernatant was determined at 412 nm. The GSH concentrations of the samples were derived from the standard curve prepared using known amounts of GSH. GSH levels are expressed as ng/mg protein.

Superoxide dismutase
To 100 μl plasma, 1ml of sodium carbonate (1.06 gm in 100 ml water), 0.4 ml of 24 mM NBT and 0.2 ml of EDTA (37 mg in 100 ml water) was added and zero minute reading was taken at 560 nm. Reaction was initiated by addition of 0.4 ml of 1mM Hydroxylamine hydrochloride, incubated at 25°C for 5 minutes and the reduction of NBT was measured at 560nm and expressed as µg/mg of protein [30].

Histopathology study of sciatic nerve
Rat sciatic nerve were isolated and fixed in 10% neutral formalin solution, embedded in paraffin and cut into sections of 3-4 µm thickness, which were stained with haematoxylin and eosin. Each sample was examined by light microscopy.

RESULTS

Table- I: Effect of three weeks repeated dose treatment of rutin on body weight, Serum triglyseride, Glycosylated haemoglobin, TNF-α in diabetic rats

| Groups	Body weight (gm)	Serum triglyseride (mg/dl)	Glycosylated haemoglobin (%)	TNF-α (pg/ml)
Normal Control	218.00 ± 08.60	88.19 ± 3.76	4.06 ± 0.22	72.46 ± 04.53
Diabetic Control	145.00 ± 06.45	234.80 ± 4.18[@]	7.83 ± 0.47[@]	262.40±30.50[@]
DC + Metformin	200.00 ± 08.99	116.50± 4.66[#]	5.20 ± 0.33[#]	143.20 ± 14.09[#]
DC + Sertraline	202.50± 06.79	169.20 ± 3.61[#]	5.29 ± 0.41[#]	117.60 ± 07.67[#]
DC + Rutin(50)	202.00 ± 15.30	173.70± 4.80[#]	6.14 ± 0.25**	130.60 ± 14.38[#]
DC + Rutin(100)	208.00 ± 07.34	118.50 ± 6.02[#]	5.38 ± 0.26[#]	98.80 ± 09.91[#]
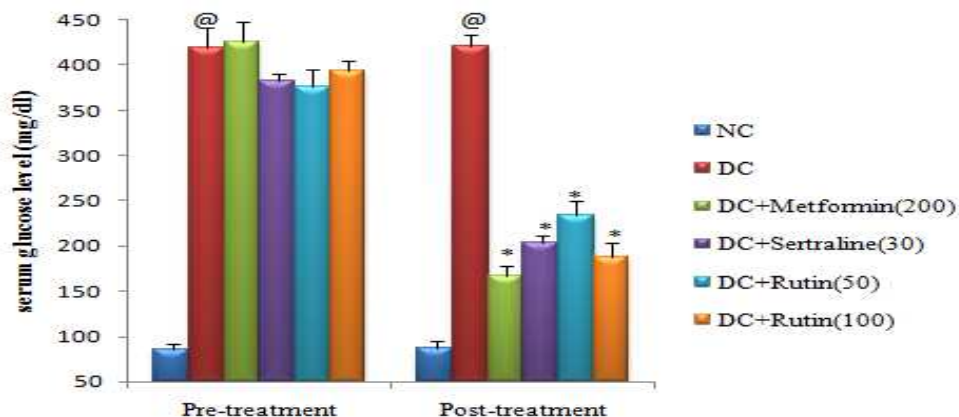
Fig- I: Effect of three weeks repeated dose treatment of rutin on serum glucose level in diabetic rats

Table- II: Effect of three weeks repeated dose treatment of rutin on water immersion test, Hot plate, walking function test, cold allodynia in diabetic rats

| Groups	water immersion test (Reaction time in sec)	Hot plate (Reaction time in sec)	walking function test	cold allodynia
Normal Control	10.2 ± 0.48	7.6 ± 0.24	13.80 ± 1.49	10.80 ± 0.58
Diabetic Control	$4.4 \pm 0.28^@$	$1.8 \pm 0.37^@$	$51.20 \pm 3.84^@$	$4.50 \pm 0.50^@$
DC + Metformin	$8.0 \pm 0.50^\#$	$7.0 \pm 0.31^\#$	$26.60 \pm 2.99^\#$	$8.40 \pm 0.40^\#$
DC + Sertraline	$8.6 \pm 0.64^\#$	$7.4 \pm 0.40^\#$	$20.60 \pm 2.13^\#$	$9.25 \pm 0.95^\#$
DC + Rutin(50)	$7.0 \pm 0.31^{**}$	$6.4 \pm 0.54^\#$	41.10 ± 2.56	$7.00 \pm 0.44^*$
DC + Rutin(100)	$8.2 \pm 0.37^\#$	$7.2 \pm 0.83^\#$	$30.20 \pm 2.78^\#$	$8.60 \pm 0.40^\#$
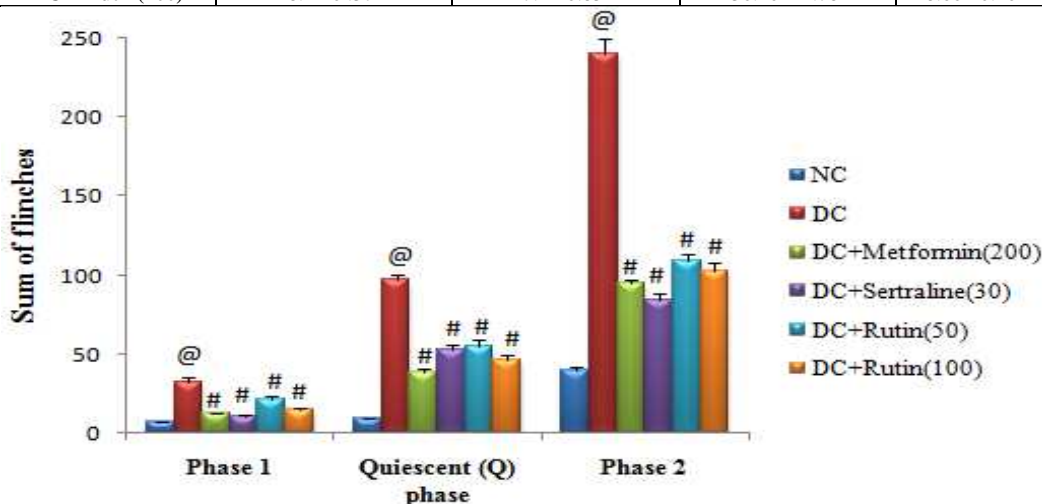
Fig- II: Effect of three weeks repeated dose treatment of rutin on hyperalgesia using formalin test in diabetic rats
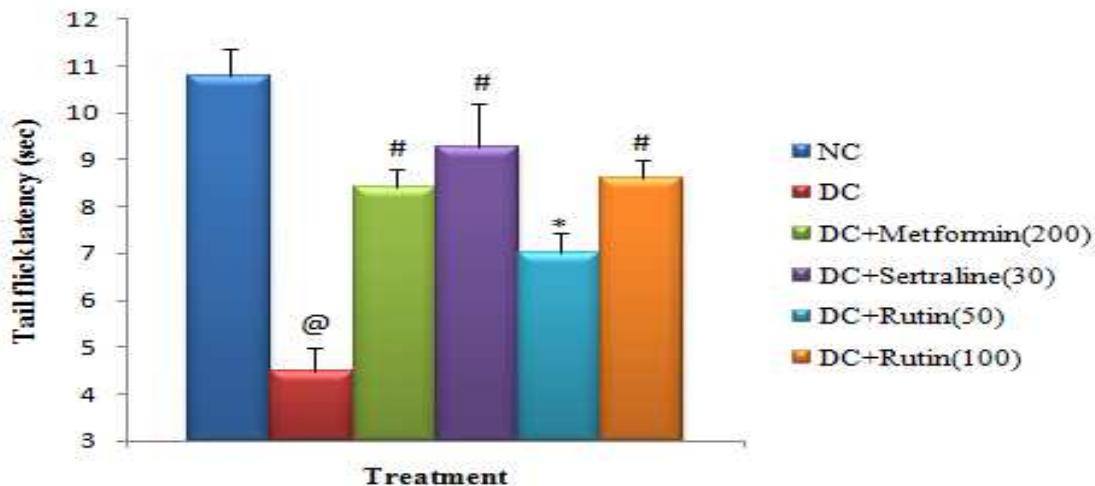

Fig- III: Effect of three weeks repeated dose treatment of ruitn on cold allodynia using water immersion test (10^0 C) in diabetic rats

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Table- III: Effect of three weeks repeated dose treatment of rutin on antioxidant parameters in diabetic rats

Groups	NO (ng/kg)	Catalase (ug/mg)	GSH (ug/kg)	SOD(ug/kg)
Normal Control	129.80 ± 5.65	41.95 ± 2.40	17.29 ± 0.89	76.22 ± 1.93
Diabetic Control	$239.80 \pm 8.71^@$	$23.87 \pm 4.01^@$	$07.27 \pm 0.44^@$	$43.02 \pm 1.51^@$
DC + Metformin	$139.30 \pm 7.99^\#$	$35.00 \pm 1.99^*$	$14.66 \pm 1.06\#$	$69.23 \pm 1.68^*$
DC + Sertraline	$153.00 \pm 7.49^\#$	$32.63 \pm 1.21^*$	$11.72 \pm 1.01^{**}$	$62.22 \pm 1.99^*$
DC + Rutin(50)	$180.70 \pm 5.03^\#$	32.80 ± 1.70	$12.18 \pm 0.96^{**}$	$62.07 \pm 2.05^*$
DC + Rutin(100)	$149.70 \pm 4.79^\#$	$35.34 \pm 2.29^*$	$15.17 \pm 0.56^\#$	$69.80 \pm 1.88^*$

n= 5, values are mean $\pm$ SEM; $^@$ P < 0.001 compared to Normal Control group (ANOVA followed by Dunnetts test)
* P < 0.05, ** P < 0.01, $^\#$ P < 0.001 compared to Diabetic Control group (ANOVA followed by Dunnetts test)
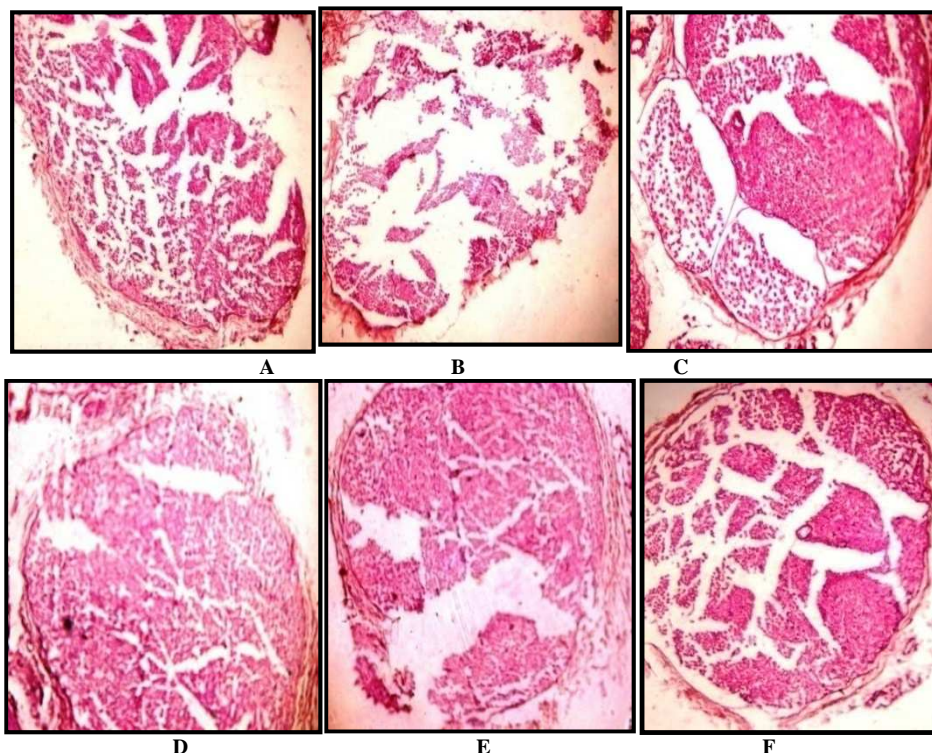

Fig- IV: Effect of three weeks repeated dose treatment of rutin on histopathological study of transverse section of sciatic nerve in diabetic rats at 100X (A) Normal control (NC), (B) Diabetic control (DC), (C) DC + Metformin (200), (D) DC + Sertraline (30), (E) DC + Rutin (50) and (F) DC + Rutin (100) group respectively

DISCUSSION

Rutin as one of the most commonly found flavonol glycosides in the human diet. It exhibits a wide variety of biological activities, including antiviral, antibacterial, anti-inflammatory, anti-carcinogenic, and antioxidant actions [18]. Hence, considering antidiabetic and antioxidant activity of rutin, the present investigation was under taken to evaluate efficacy of rutin on early diabetic neuropathy in experimental animals.

Streptozotocin is the most common substance used for the induction of diabetes in the rodents. The cytotoxic action of streptozotocin is mediated by reactive oxygen species, liberation of the toxic amounts of NO, alkylation and damage of DNA which result in rapid destruction of pancreatic β-cells [31]. In the present study intraperitoneal injection of STZ (55 mg/kg) produced significant elevation in serum glucose level and exhibited decrease in body weight. The present study has demonstrated diminutions in serum glucose concentration and an increase in the body weight in diabetic rats treated with rutin (50 and 100 mg/kg). This could be the result of improved glycemic control produced by rutin. In addition, flavonoids exert their effect either by promoting the entry of glucose into cells, thus stimulating glycolytic enzymes, glycogenic enzymes, reducing glycogen breakdown, depressing gluconeogenic enzymes or by inhibiting the glucose-6-phosphatase in the liver, consequently reducing the release of glucose in the blood [32].

Hypercholesterolemia and hypertriglyceridemia have been reported to occur in diabetic rats [33]. The elevated levels of plasma triglycerides during diabetes were significantly depleted with the treatment of rutin, metformin and sertraline. We also observed increase in the level of glycosylated haemoglobin (HbA_{1C}) in diabetic control group rats which might be due to the presence of excessive amounts of blood glucose. During diabetes, the excess of

glucose present in blood reacts with the haemoglobin to form HbA_{1C} which has been found to be increased over a long period of time in diabetes mellitus [34]. In the present study, three weeks treatment of rutin significantly ($P <$ 0.05) decreased level of glycosylated haemoglobin (GHb %).

Clinical application of specific agents that suppress production and/or activity of TNF-α may inhibit the development and exacerbation of chronic diabetic complications [35] reported that TNF- α and interleukins, which play a crucial role in the pathogenesis of nerve degeneration, activated p38 mitogen-activated protein kinase is phosphorylated by these cytokines, suggesting that it may play an important role in pain transmission and nerve degeneration. We have observed a marked rise in the release of inflammatory cytokines TNF- α in the diabetic rats. Rutin have recently been shown that it reduces reactive oxygen species produced by receptor activator of NF-κB ligand (RANKL) and its inhibitory effect results from reduced levels of TNF-α.

Neuropathic pain is a common symptom of diabetic neuropathy; hence we studied the effect of rutin on sensori-motor alterations in experimental diabetic rats. Assessment of behavioural responses to external stimuli in diabetic animal provides valuable information regarding the mechanisms of abnormal sensation and pain associated with diabetes. In the present study STZ-induced diabetic rats showed similar alteration of nociceptive threshold to tail immersion test and hot plate test which is in agreement with previous study [36]. Seven weeks diabetic rats demonstrated reduced latencies in both hot and cold immersion tests and hot plate test. Reduced tail-flick latencies and paw withdrawal latencies were partially corrected by three weeks treatment of rutin. Unmyelinated C fibers are responsible for pain and A-delta fibers for carrying temperature, crude touch and pricking pain sensations. Both hot as well as cold sensation was altered in present study which indicates damage of these respective fibers. Since rutin significantly increased tail flick latency in tail immersion test and paw withdrawal latency in hot plate test, it strongly suggests the role of rutin in protecting unmyelinated C fibers and myelinated A-delta fibers.

The formalin test is used to investigate spinal sensitization in animals and allows investigation of sensory processing beyond peripheral nociceptive pathways [37]. All Phases of the formalin test in normal rats is driven by spinal prostaglandin release [38], while the increased flinching in diabetic rats has been attributed to elevated COX-2 protein and prolonged prostaglandin release [39]. After three weeks repeated dose treatment with rutin (50 and 100 mg/kg) showed most prominent effect during Phase Q and Phase 2 of the formalin test. Metformin and sertraline also showed significant decreased in flinching response in formalin test.

The use of walking track analysis provides a non-invasive method of assessing the functional status of the sciatic nerve during the regeneration process, because proper walking requires coordinated function involving sensory input, motor response, and cortical integration. The beam-walk apparatus has been used to assess sensorimotor deficits [40]. Diabetic animals significantly increased latency time of animal to travel 1 m distance in walking function test as compared to normal control. Three weeks repeated dose treatment of rutin (50 and 100 mg/kg) significantly reduced latency time as compared to diabetic control group.

Excessive local levels of NO during inflammation may damage axons and growth cones [41]. NO combines with superoxide to form peroxynitrite, which rapidly causes protein nitration or nitrosylation, lipid peroxidation, DNA damage and cell death and has direct toxic effects on the nerve tissue leading to neuropathic pain [42]. Total nitric oxide, an indicator of nitrosative stress, is increased in the experimental model of diabetic neuropathy [21]. Rutin treatment at 50 and 100 mg/kg doses attenuated the increased nitric oxide levels, might be its iNOS inhibitory potential [43].

Hyperglycemia is reported to induce oxidative stress through multiple pathways such as redox imbalances secondary to enhanced aldose reductase activity [44], increased advanced glycation end products[45], altered protein kinase C activity, especially b-isoforms [46], prostanoid imbalances and mitochondrial overproduction of superoxide [47]. We observed a significant increase in catalase and reduction in endogenous antioxidant enzyme activity in diabetic rats. In our study, rutin restored various biochemical parameters such as catalase, reduced glutathion, superoxide dismutase and nitric oxide level activities in diabetic rats. Rutin has recently been reported to preserve the activity of antioxidant enzymes and lysosomal membrane which may be referred to its role in modulating the levels of H_2O_2 and O_2 [48].

Histopathological study of sciatic nerve of diabetic rats showed significant derangement of nerve cells. Rutin 50 and 100 mg/kg significantly protected nerve from structural alteration induced by diabetes.

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CONCLUSION

In conclusion, it can be said that rutin not only attenuated the diabetic condition but also reversed neuropathic pain through modulation of oxidative–nitrosative stress and inflammatory cytokine release in the diabetic rats. Thus, rutin may find a clinical application to treat neuropathic pain in the diabetic patients.

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