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Flavonoid Rich Fraction of Helicteres Isora Fruits Ameliorate Streptozotocin and High Fat Diet Induced Diabetic Neuropathy in Sprague Dawley Rats

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ABSTRACT

The aim of the present study was to explore the effect of flavonoid rich fraction of Helicteres isora (FRFHI) fruits on diabetic neuropathy in experimental animals. A partial insulin resistance was induced in rats by 3 weeks of high fat diet regimen and confirmed with glucose tolerance test and high triacylglycerol levels. After 3 weeks of high fat diet regimen, streptozotocin (35 mg/kg; i.p.) was administered to different groups of rats. 10 days after STZ injection, FRFHI (75, 150 and 300 mg/kg) were administered in diabetic rats (n=6) for three weeks. At the end of the treatment body weight, biochemical parameters, behavioural parameters and antioxidant systems were noted along with histopathological examinations. FRFHI restored the body weight as compared to diabetic control rats and significantly lowered biochemical changes. FRFHI showed significant delay in paw and tail withdrawal latency in hyperalgesia and allodynia respectively. Improved walking function was also observed with 3 weeks of FRFHI treatment as compared to diabetic control rats. The antioxidant systems namely MDA, SOD, NO and GSH were also restored significantly with FRFHI treatment at dose levels of 75, 150 and 300 mg/kg. Histopathological examination showed FRFHI 150 and 300 mg/kg treated rats markedly restored the structural alterations as compared to diabetic control rats. FRFHI has shown beneficial effect in preventing the progression of diabetic neuropathy.

Keywords: Diabetic neuropathy, Streptozotocin, High fat diet, FRFHI

INTRODUCTION

According to International Diabetes Federation, an estimated 381 million people had diabetes and its prevalence is increasing rapidly with greatest increase expected to occur in Asia and Africa [1]. The current global scenario suggests that 50% of patients develop peripheral neuropathy 25 years after the initial diagnosis of diabetes mellitus. The prevalence of peripheral diabetic neuropathy ranges from 10% to 20% of patients with diabetes and in those with diabetic neuropathy it ranges from 40% to 50% [2].

Diabetic neuropathy describes diabetes-associated changes in the all peripheral nerves including pain fibres, motor neurons and the autonomic nervous system [3]. It is characterised by paresthesia (numbness), allodynia, hyperalgesia (lower pain threshold), and dysesthesia (loss of pain sensations) [4]. Several factors are responsible for the development of diabetic neuropathy involving complex mechanisms at molecular level as increased sorbitol and fructose, advanced glycation end products, Reactive oxygen species and Inappropriate activation of protein kinase C [5]. The available pharmacotherapy for effective management of diabetic neuropathy includes opioids, NSAIDs, antidepressants and anticonvulsants are widely used, but the degree of relief from pain are limited because of their partial effectiveness and potential toxicities [6]. Thus, inspite of these options being available the treatment paradigms are very much limited suggesting a need to develop more effective and specific treatment approaches [7]. It is indisputable from the present scenario that herbal and polyherbal formulations are gaining extensive attention and is choice of treatment to attain the desired therapeutic effect owing to lesser side effects and cost effective therapy [8]. Different extracts of *Helicteres isora* (L) reported to display various pharmacological properties such as antimicrobial, antidiabetic, anti-cancer, anti-diarrheal and antioxidant [9]. Streptozotocin (STZ) and high fat diet model involves a combination of a diet high in fat content contributing to hyperinsulinemia, insulin resistance and/or glucose intolerance followed by treatment with the beta-cell toxin STZ, which results in structural and functional

modifications in sciatic nerve by inducing nerve degeneration, demylination, oxidative stress and apoptosis [10,11]. Thus the present study was undertaken to investigate the effect of flavonoid rich fraction of *Helicteres isora* fruits in streptozotocin and high fat diet induced diabetic neuropathy in male Sprague Dawley rats.

MATERIAL AND METHODS

Drugs and chemicals

Streptozotocin was purchased from Enzo Life Sciences (UK). The dried fruits of *Helicteres isora* (L.) were collected from Western Ghats of Konkan region, Maharashtra, India. The diagnostic kits were obtained from Biolab (Mumbai, India), Crest systems (Goa, India) and Robonik (Navi Mumbai, India). All required chemicals were of laboratory grade obtained from local suppliers of Pune.

Preparation of plant Extract (Methanolic extract)

Dried fruits were coarsely powdered and flavonoids were extracted in two steps, firstly with methanol: water (9:1) and secondly with methanol: water (1:1) using sufficient solvent, left for six to twelve hours, filtered and concentrated by using rotary evaporator [12]. Flavonoid rich fraction of fruits of *Helicteres isora* was obtained in the yield of 0.3% w/w.

Experimental animals

Thirty male Sprague Dawley rats were procured from National Institute of Biosciences, Pune. The animals were housed in polypropylene cages with paddy husk as bedding. The animals were maintained under standard laboratory conditions at temperature 23 ± 2ºC with relative humidity 55 ± 10 % under 12 h light and 12 h dark cycle throughout the experiment in the animal house of Sinhgad college of Pharmacy, Pune. The animals were fed with standard pellet diet and water ad libitum. All the experimental procedures and protocols used in this study were reviewed and approved (SCOP/IAEC/2016-17/01/204) by the Institutional Animal Ethics Committee (IAEC) of Sinhgad College of Pharmacy, Pune.

Experimental procedure

Thirty male Sprague Dawley rats (200-250 g) were randomly divided into control (n=6) and diabetic neuropathy model (n=24). All the experimental rats were fed on high fat diet except control rats for the duration of 3 weeks. 3 weeks later, insulin resistance and development of partial hyperglycemia was assessed by performing oral glucose tolerance test and measuring the triacylglycerol levels and body weight. After the establishment of insulin-resistance the diabetes was induced in high fat diet fed rat's intraperitoeal injection of streptozotocin at a dose of 35 mg/kg (dissolved in 0.1 mol/L citrate buffer, pH 4.5). Rats with fasting blood glucose levels ≥ 250 mg/dl were included for further study [13].

10 days after the induction of diabetes and stabilization of blood glucose levels, diabetic rats were divided into diabetic control and diabetic rats daily treated with FRFHI orally at doses of 75, 150 and 300 mg/kg for a period of three weeks [14]. The test drug suspension was prepared by using 2% gum acacia. At the end of the treatment the effect of FRFHI on diabetic neuropathy was assessed by following parameters.

Body weight

The body weight was recorded weekly during 3 weeks of high fat diet regimen as well as FRFHI treatment using electronic balance.

Biochemical estimations

On last day of study, Blood was collected by puncturing retro-orbital plexus under mild ether anaesthesia by using fine glass capillary in epindorff tubes. Blood was centrifuged for 20 min at 5000 rpm at 37°C and serum was separated for the biochemical estimation. All the analysis was completed within 24 h of sample collection. Biochemical parameters such as blood glucose [15], HDL cholesterol [16], triacylglycerol [17], total protein [18] were determined using Chemstar Biochemistry Analyser.

Estimation of behavioural parameters [13]

Assessment of thermal hyperalgesia (Eddy's hot plate method)

The nociceptive threshold for heat was an index for thermal hyperalgesia. Eddy's hot plate, which is an instrument used to assess thermal sensitivity. The plate was preheated and maintained at a temperature of 55 ± 1.0°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.

Assessment of cold allodynia (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 shortened duration of immersion indicates allodynia.

Assessment of locomotor activity (Actophotometer test)

Each animal was observed for a period of 5 min in a square closed field area (30 × 30 × 30 cm) equipped with 6 photocells in the outer wall. Interruptions of photocell beam (locomotor/exploratory action) of rats were recorded by digital counter.

Assessment of walking function (Beam walk test)

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.

Estimation of oxidative stress

At the end of experiment all the rats were sacrificed by cervical dislocation and sciatic nerve was removed, washed and weighed. Whole nerve was rinsed with ice cold saline (0.9% sodium chloride) and homogenized by making 20 mg of the tissue per ml in chilled phosphate buffer (pH 7.4). The homogenates were centrifuged at 800 g for 5 min at 4°C to separate the nuclear debris. The supernatant thus obtained was centrifuged at 10500 g for 20 min at 4°C to get the supernatant. The supernatant was used to determine the concentration of malondialdehyde (MDA) [19], reduced glutathione (GSH) [20], nitric oxide (NO) [13] and superoxide dismutase activity (SOD) [21].

Histopathological examination of sciatic nerve by Hematoxylin-Eosin staining

The isolated tissue was preserved in 10% buffer formalin for 24 h. Nerve samples were embedded in paraffin. Four-micrometre paraffin sections were stained with hematoxylin-eosin (H/E). HE stained sample was observed under light microscope (100 x).

Statistical analysis

All the data were expressed as the mean ± S.E.M (n=6). Data were subjected to one-way analysis of variance (ANOVA) followed by the Tukey's multiple comparison test. $P<0.05$ was considered as minimum level of significance. Data was analysed using computerized GraphPadPrism version 5.0.

RESULTS

Effect of 3 weeks of high fat diet regime on induction of partial insulin resistance

As shown in Figure 1, rats fed with high fat diet were observed with significant gain in the body weight on day 7 ($P<0.001$) and more on day 14 and 21 ($P<0.001$ and $P<0.001$; respectively) as compared to control rats fed with standard chow diet. HFD fed rats were also failed to normalize the elevated blood glucose and remains significantly higher post 60 and120 min ($P<0.001$ and $P<0.01$ respectively) of oral glucose load as compared to control rats (Figure 2). However, control rats showed normalized blood glucose levels post 120 min. Moreover, serum

triacylglycerol levels was found significantly higher in the three weeks of high fat diet fed rats as compared to control rats (P<0.001) (Figure 3).

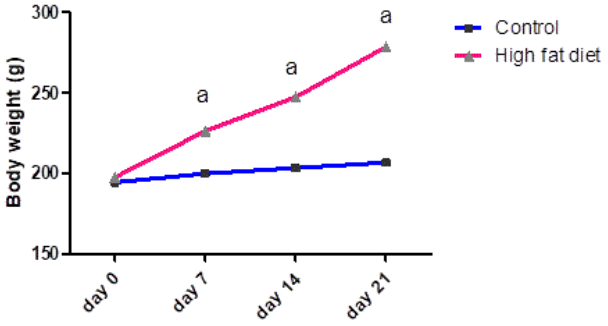

Figure1: Effect of 3 weeks of high fat diet on body weight; aP<0.001 Vs Normal control

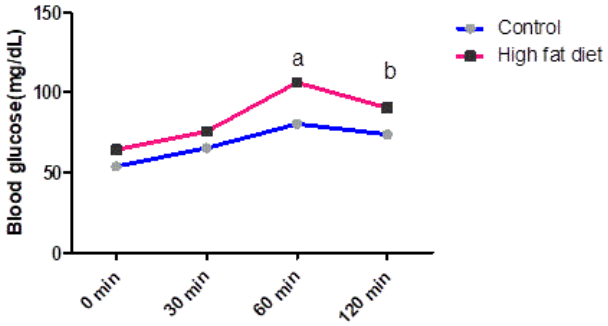

Figure 2: Effect of 3 weeks of high fat diet on glucose tolerance (OGTT); aP<0.001 and bP<0.01 Vs Normal control

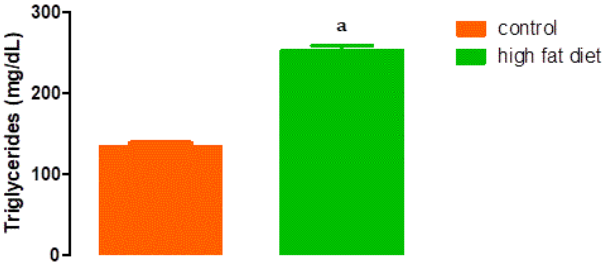

Figure 3: Effect of 3 weeks of high fat diet on serum triacylglycerol levels; aP<0.001 Vs Normal control

Effect on body weight

Significantly decreased body weight was noted in non-treated diabetic rats in comparison with control rats. (P<0.01, P<0.001 and P<0.001 respectively) Diabetic rats treated with FRFHI at doses 75, 150 and 300 mg/kg significantly restored the body weight. However, FRFHI at dose of 150 and 300 mg/kg significantly increased the body weight (P<0.001 and P<0.001 respectively) as compared to FRFHI 75 mg/kg treated diabetic rats (Table 1).

Table 1: Effect of 3 weeks treatment of FRFHI on body weight; [a]P<0.001 , [b]P<0.01 Vs Normal control, & P<0.001, [#]P<0.05 Vs Diabetic control, [%]P<0.001, [$]P<0.05 Vs FRFHI (300)

Groups	Body weight (g)			
	Day 0	Day7	Day 14	Day 21
Control	194.33 ± 5.79	199.66 ± 5.14	203.16 ± 4.96	206.50 ± 4.97

Diabetic control	186.00 ± 6.02	174.66 ± 1.76^{b}	161.50 ± 2.48^{a}	150.16 ± 1.44^{a}
FRFHI (75)	198.33 ± 2.96	201.16 ± 2.83$^{\&\$}$	202.83 ± 2.60$^{\&\%}$	197.16 ± 15.39$^{\&\%}$
FRFHI (150)	203.33 ± 2.87$^{\#}$	208.50 ± 2.51$^{\&}$	217.33 ± 1.42$^{\&}$	227.33 ± 0.88$^{\&}$
FRFHI (300)	204.16 ± 2.13$^{\#}$	218.66 ± 1.74$^{\&}$	229.83 ± 1.30$^{\&}$	245.33 ± 2.20$^{\&\$}$

Effect on biochemical parameters

A significant increase in blood glucose, serum triacylglycerol, HDL cholesterol and total protein levels were observed in non-treated diabetic control rats when compared to control rats (P<0.001). Daily oral administration of FRFHI for 3 weeks in diabetic rats at doses 75, 150 and 300 mg/kg significantly reduced the elevated blood glucose, serum triacylglycerol, HDL cholesterol and total protein when compared with diabetic control rats. Further a significant reduction was also noted in rats treated with FRFHI 300 mg/kg as compared to FRFHI 75 and 150 mg/kg treatment (P<0.001 and P<0.001; respectively) (Table 2).

Table 2: Effect of FRFHI on biochemical parameters; ^{a}P<0.001 Vs Normal control, $^{\&}$P<0.001, $^{@}$P<0.01, $^{\#}$P<0.05 Vs Diabetic control, $^{\%}$P<0.001, $^{\$}$P<0.05 Vs FRFHI (300)

Groups	Blood glucose (mg/dl)	TG (mg/dl)	HDL Cholesterol (mg/dl)	Total protein (mg/dl)
Control	90.17 ± 4.672	135.7 ± 4.047	61.33 ± 3.68	7.583 ± 0.35
Diabetic control	473.5 ± 4.387^{a}	325.7 ± 12.95^{a}	268.2 ± 14.56^{a}	3.067 ± 0.14^{a}
FRFHI (75)	390.5 ± 9.422$^{\&\%}$	294.7 ± 7.753$^{\%}$	252.5 ± 1.43$^{\%}$	3.883 ± 0.094$^{\#\%}$
FRFHI (150)	314.0 ± 3.120$^{\&\%}$	242.3 ± 5.175$^{\&\%}$	224.0 ± 3.06$^{@\%}$	4.400 ± 0.10$^{\&\%}$
FRFHI (300)	261.8 ± 10.22$^{\&}$	184.7 ± 6.510$^{\&}$	116.3 ± 9.24$^{\&}$	5.717 ± 0.12$^{\&}$

Effect on behavioural parameters

Thermal hyperalgesia and cold allodynia

Diabetic neuropathy resulted in a significant development of thermal hyperalgesia and cold allodynia noted by decrease in paw withdrawal and tail withdrawal threshold respectively as compared to normal rats (P<0.001). FRFHI treatment at the doses 150 and 300 mg/kg significantly improved paw withdrawal (P<0.05 and P<0.001) and tail withdrawal (P<0.01 and P<0.001) latency in experimental animals when compared to diabetic non-treated rats. Further, FRFHI (300 mg/kg) significantly increased paw withdrawal (P<0.001 and P<0.05) and tail withdrawal (P<0.001 and P<0.01) latency when compared to FRFHI 75 and 150 mg/kg treated rats (Figures 4 and 5)

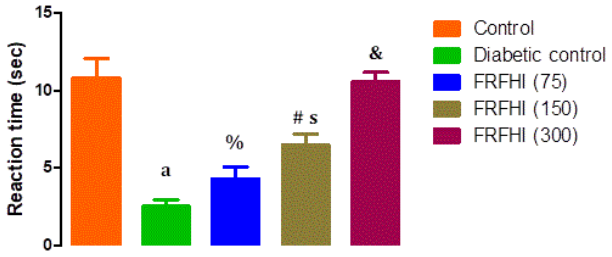

Figure 4: Effect of FRFHI on Thermal hyperalgesia; ^{a}P< 0.001 Vs Normal control, & P< 0.001, $^{\#}$P<0.05 Vs Diabetic control, $^{\%}$P<0.001, $^{\$}$P<0.05 Vs FRFHI (300)

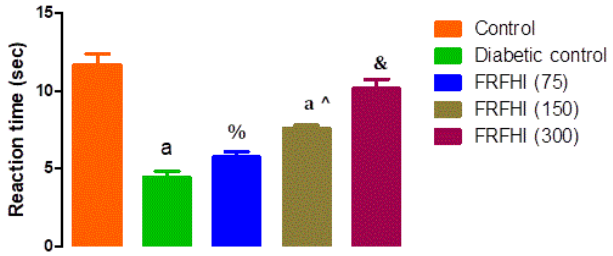

Figure 5: Effect of FRFHI on Cold allodynia; [a]P< 0.001 Vs Normal control, [&] P<0.001, [@]P<0.01 Vs Diabetic control, [%]P<0.001, ^P<0.01 Vs FRFHI (300)

Walking function

Diabetic control rats were observed with significant increase in duration for travelling 1 meter distance when compared to normal control rats (P<0.001). Oral FRFHI treatment at the doses 75, 150 and 300 mg/kg showed significant improvement in shortening of time in experimental animals when compared to diabetic non-treated rats (P<0.05, P<0.001 and P<0.001 respectively) (Figure 6).

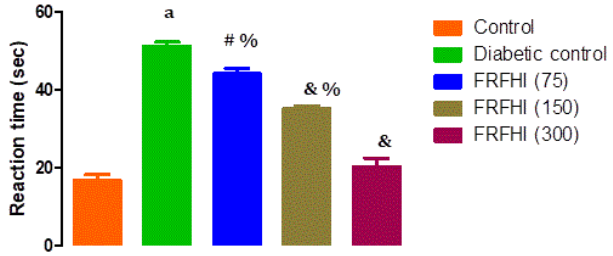

Figure 6: Effect of FRFHI on Walking function; [a]P<0.001 Vs Normal control, [&] P<0.001, [#]P<0.05 Vs Diabetic control, [%]P< 0.001 Vs FRFHI (300)

Locomotor activity

Locomotor count in actophotometer revealed a marked impairment of motor coordination in the diabetic animals when compared to normal control rats (P<0.001). Significant improvement in locomotor activity was observed in the animals treated with dose of 300 mg/kg of FRFHI as compared to FRFHI 75 and 150 mg/kg treated rats. (P<0.001 and P<0.01; respectively) (Figure 7).

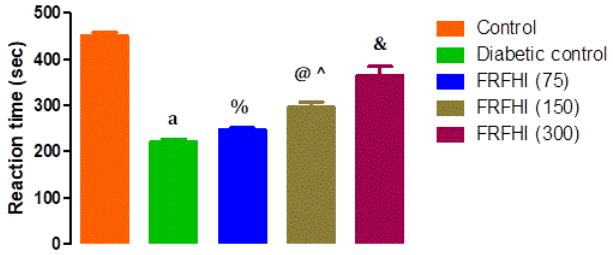

Figure 7: Effect of FRFHI on Locomotor activity; [a]P<0.001 Vs Normal control, [&] P<0.001, [@]P<0.01 Vs Diabetic control, [%]P<0.001, ^P<0.01 Vs FRFHI (300)

Effect on antioxidant parameters

A long standing hyperglycemia results in the production of oxidative stress and thus development of diabetic complications. In the present investigation we have observed the significant increase in the levels of MDA and NO concentration (P<0.001) and decreased SOD and GSH concentration (P<0.001) in the non-treated diabetic rats when compared with the normal rats. Significant decrease in MDA was observed in the animals treated with dose of 300 mg/kg of FRFHI as compared to FRFHI 75 and 150 mg/kg treated rats (P<0.001 and P<0.01; respectively). Also

increased levels of NO in diabetic rats was significantly decreased with FRFHI treated diabetic rats at a dose of 75, 150 and 300 mg/kg (P<0.05, P<0.001 and P<0.001 respectively). Further, significant decrease in NO was observed in the animals treated with dose of 300 mg/kg of FRFHI as compared to FRFHI 75 and 150 mg/kg treated rats (P<0.001 and P<0.001 respectively).

Further we have observed the rise in GSH and SOD concentration after the three weeks of FRFHI treatment at dose of 150 and 300 mg/kg (P<0.001 and P<0.001) in comparison with diabetic non-treated rats. Significant rise in GSH and SOD levels was observed with FRFHI (300 mg/kg) as compared to FRFHI (75 and 150 mg/kg) (P<0.001 and P<0.01 respectively) (Table 3).

Table 3: Effect of FRFHI on antioxidant parameters; [a]P<0.001 Vs Normal control, [&] P<0.001,[@]P<0.01, [#]P<0.05 Vs Diabetic control, [%]P<0.001, [^]P<0.01, [$]P<0.05 Vs FRFHI (300)

Groups	MDA (nmol/mg)	NO (ng/mg)	SOD (%activity)	GSH (ng/mg)
Control	1.367 ± 0.041	130.1 ± 1.129	5.860 ± 0.246	12.21 ± 0.362
Diabetic Control	3.279 ± 0.080[a]	229.4 ± 1.613 [a]	2.465 ± 0.620 [a]	3.833 ± 0.143[a]
FRFHI (75)	2.987 ± 0.106[%]	213.0 ± 5.132[#%]	2.775 ± 0.163[%]	4.408 ± 0.209[%]
FRFHI (150)	2.043 ± 0.065[&^]	185.5 ± 4.836[&%]	3.493 ± 0.167[@^]	7.762 ± 0.297[&%]
FRFHI (300)	1.574 ± 0.082[&]	144.2 ± 2.738[&]	4.584 ± 0.118[&]	9.835 ± 0.061[&]

Effect on histopathology of sciatic nerve

Histopathological examination of sciatic nerve of normal rat showed normal histology, nerve fibre (large arrow), schwann cell nuclei (small arrow) while diabetic control group showed disturbed structural organization (small arrow) and axonal swelling of neuron (large arrow). FRFHI at a dose of 75 mg/kg failed to restore the structural alterations of sciatic nerve. However, oral administration of FRFHI at doses 150 mg/kg and 300 mg/kg significantly restored the sciatic nerve morphology and minimal degeneration (small arrow) of nerve with reduced axonal swelling (large arrow) was observed (Figure 8).

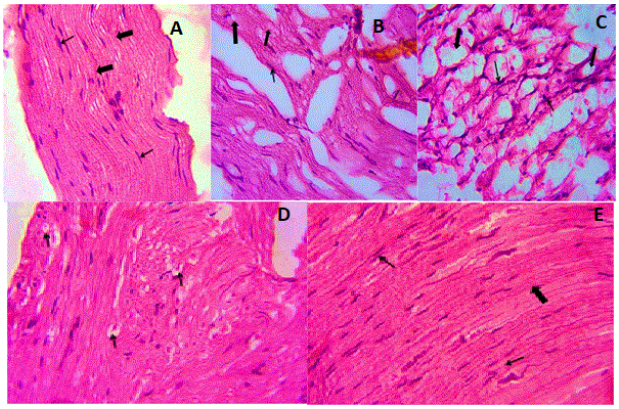

Figure 8: Histopathology of Sciatic nerve H and E stain (100X) (A) control rats (B) Diabetic non-treated rats, (C) FRFHI (75 mg /kg), (D): FRFHI (150 mg/kg) and (E): FRFHI (300 mg/kg)

DISCUSSION

Disorders of civilization are slow progressing, long lasting, largely preventable illnesses that result from numerous common modifiable risk factors [22]. Diabetes is a prime risk factor for the development of long-term microvascular and macrovascular complications and the pathophysiology of the diabetes associated complications is complex and multifactorial. Diabetic neuropathy affects all peripheral nerves including pain fibres, motor neurons and the autonomic nervous system [3]. Along with appropriate lifestyle changes, current pharmacotherapy for competent management of diabetic neuropathy mainly considers opioids, NSAIDs, antidepressants and anticonvulsants [6]. The limitations of these available therapeutic approaches force us to consider more effective and specific alternatives.

The human population is witnessing the overall limelight being gained by the herbal and polyherbal formulations owing to their effectiveness, reduced side-effects and cost effectiveness of the therapy. As reported in literature, different extracts of *Helicteres isora* (L) reported to display various pharmacological properties such as antimicrobial, antidiabetic, anti-cancer, anti-diarrheal, antioxidant and thus it is considered as a potent moiety for maintaining good health [9].

Increased levels of triacylglycerol are common characteristics of the dyslipidemia associated with type 2 diabetes mellitus [23,24]. In the present study, remarkable increase in serum triacylglycerol levels was observed thus suggesting dyslipidemia associated with insulin resistance.

Insulin deficiency or absolute absence of insulin in the body hampers the ability of cells to utilise glucose as major energy source. Thus, a considerable shift in substrate utilization for energy maintenance is observed with increased dependence on fat and protein sources. Significant weight loss as observed in diabetic condition is evident due to the breakdown of fats and proteins [25]. In accordance with reported literature, a significant increase in the body weight was observed with high fat diet regimen, which was significantly decreased after streptozotocin injection as a result of successful diabetes induction. Oral treatment with FRFHI (150 mg/kg and 300 mg/kg) significantly increased the body weight in comparison to non- treated diabetic control rats.

Prolonged hyperglycaemic condition owing to impairment in insulin-mediated glucose disposal (insulin resistance) and defective secretion of insulin by pancreatic b-cells is a hallmark of diabetes mellitus [26]. Diabetic rats treated with FRFHI at doses 75, 150 and 300 mg/kg daily for 3 weeks, significantly decreased the elevated fasting blood glucose levels when compared to control rats. Impaired fat metabolism on account of prolonged hyperglycaemia results into diabetes associated dyslipidemia [27]. A significant increase in serum HDL cholesterol and triacylglycerol while decrease in total protein levels was observed in non-treated diabetic control rats when compared to control rats. Diabetic rats treated with FRFHI at dose levels 75, 150 and 300 mg/kg produced a significant decrease in the levels of serum HDL cholesterol and triacylglycerol while increase in levels of total protein in comparison with the non-treated 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 [13]. Diabetic rats demonstrated reduced latencies in cold immersion and hot plate test. Since FRFHI at a dose of 150 and 300 mg/kg significantly increased tail flick latency and paw withdrawal latency, it strongly suggests the role of FRFHI in protecting unmyelinated C fibers and myelinated A-delta fibers. The use of walking track analysis provides a non-invasive method of assessing the functional status of the sciatic nerve. The beam-walk apparatus has been used to assess sensorimotor deficits [13]. Diabetic animals showed significant increase in latency time to travel 1 m distance in walking function test as compared to normal control. Three weeks repeated dose treatment of FRFHI (150 and 300 mg/kg) significantly reduced latency time as compared to diabetic control group. Locomotor count in actophotometer revealed a marked impairment of motor coordination in the diabetic animals when compared to normal control rats. Improvement in locomotor activity was achieved at the doses 150 and 300 mg/kg when compared to diabetic non-treated rats.

Increased oxidative stress is the major contributor towards the development of diabetes and associated complications [28]. Non-treated diabetic rats showed a significantly increased oxidative stress as elevated MDA and NO while decreased GSH and SOD activity. Significant effect on the restoration of antioxidant defence such as decreased MDA and NO as well as increased GSH and SOD was observed in the rats treated with FRFHI 150 and 300mg/kg, suggesting strong antioxidant property possessed by FRFHI.

Histopathological examination reveals the tissue toxicity and effect associated with the administered drug [29]. In the present investigation, degeneration and axonal swelling of neuron was observed in the stained sections of sciatic nerve of non-treated diabetic rats. Whereas, 3 weeks of oral administration of FRFHI at doses 150mg/kg and 300mg/kg significantly restored the nerve morphology. Thus FRFHI remarkably reduced the sciatic nerve damage in comparison with non-treated diabetic rats.

CONCLUSION

In conclusion, it can be said that flavonoid rich fraction of *Helicteres isora* fruits not only attenuated the diabetic condition but also reversed neuropathic pain through modulation of oxidative–nitrosative stress and restoration of

altered structural morphology of sciatic nerve Thus, FRFHI may find a clinical application to treat neuropathic pain in the diabetic patients.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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