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Structural Study of the Sciatic Nerve in Diabetic Rats Treated with Alium Sativum

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Abstract:-

The diabetes mellitus has been induced in Sprague Dawely rats, *by injection of alloxan 75 mg/ kg body weight,* once daily for three successive days. *Group of diabetic rats were treated with oral hyopgycemic (Glibenclamide) drug only and another group was treated with oral hyopgycemic (Glibenclamide) drug and Alium Sativum-garlic powder (250 mg / kg body weight, daily. Histological and histometric changes of sciatic nerve were investigated, by applying routine histological stains with specific application of osmium tetroxide for staining the nerve, detecting the structural changes and to measuring the improvement in the regeneration of the sciatic nerves. It has been found that the Alium Sativum treated group restored to certain degree the normal architectures of the sciatic nerve in diabetic rats and the improvement in thermal sensation as compared with non treated group or group treated with oral hypgycemic drug only.*

دراسه نسيجيه لعصب النسا للجرذان المصابه بالسكري والمعالجه بمسحوق الثوم

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المستخلص:

تم احداث مرض السكري في جرذان السبراغ-داولي المختبريه وذلك بحقن ماده الالوكسان 75 ملغ لكل كغم من وزن الجسم ولثلاثه ايام متتاليه. عولجت مجموعه من هذه الحيوانات بمثبطات الكلوكوز المعطى بالفم فقط ومجموعه اخرى عولجت بمثبطات الكلوكوز مع اضافه بودر الثوم بجرعه 250 ملغ لكل كغم من وزن الجسم يوميا. فحصت التغيرات النسيجيه لعصب النسا باستعمال الصبغات النسيجيه الروتينيه مع استخدام صبغه الاوزميم رباعي الاوكسجين الخاصه لصبغه العصب. وقد تبين نسيجيا بان المجموعه التي تجرعت ببودر الثوم قد استردت التركيبه النسيجيه القريبه من المجموعه السيطره الطبيعيه وكذلك التحسن في سرعه التحسس الحراري بالمقارنه مع المجموعه التي لم تعطى اي علاج لها أو المجموعه التي عولجت بمثبطات الكلوكوز فقط.

Introduction

The sciatic nerve is arised from 4th, 5th lumber, 1st, 2nd , and 3rd sacral segment, it leaves through the greater sciatic foramen below the piriformis muscle [1]. It is situated approximately midway between the ischial tuberosity and greater trochanter of the femur, under cover of the lower part of the gluteus maximum muscle [1-2]. It then passes distally, resting successfully upon the obturator internus tendon and lateral to the long head of biceps femoris, at the middle of the thigh [1] or at bellow the 2nd third of the thigh it splits to tibial and common peroneal nerves [3]. The terminal branches of the tibial nerve are the cutaneous medial planter nerve that supplies the skin in the sole of the foot, and the superficial lateral planter nerve that supplies the skin over the fourth and fifth toe [4, 5 and 6]. There were 135 million people worldwide were diagnosed diabetes in 1995 and this number is expected to rise at lest 300 million by 2025 [7]. Several effication have been used for the control of hyperglycemia, early detection and treatment of retinopathy, neuropathy, foot diseases; like the use of aspirin and ACE inhibitors (Angiotensin Converting Enzymes) inhibitor's, were used to reduce the burden of diabetes complications [8]. Approximately 8% of diabetic patients reveal clinical signs of peripheral nerve diseases at time of initial diagnosis of the diseases, this incidence increases to reach up to 50% after 25 years of the disease onset [9]. Recent studies have shown the complex composition of garlic, containing many compounds, that present potential positive effect in the filed of health. Garlic can decrease blood pressure, normalize plasma lipid activity, inhibit platelet aggregation, smooth the thickening in the wall of the artery which related to aging and atherosclerosis [10].The precise mechanisms linking hyperglycemia to the pathological changes underlying the clinical syndromes are not yet fully defined, it is thought that increased metabolism of glucose to Sorbitol via the Polyol pathway is of central importance in the pathogenesis (diabetic neuropathy) [11]. The histological changes accompanying diabetic neuropathy changes are; structural alterations of the micro vessel, as thickening of the basement membrane [12], hypertension [13] and hyperosmolarity [14]. These will further contribute to diabetes related deterioration of blood nerve barrier then reduce the delivery of nutrition of the nervous tissue [15]. Endoneurial arteries shows thickening and hyalinization, in their walls and intensive reduplication of the basement membrane [16-17]. The ensheathement and remyelination of regenerated axons is accompanied by nodal migration, myelin sheath breakdown, demyelination, elimination of redundant Shwann cells, and nodal fusion[18-19].

Materials and Methods

Laboratory animals

Ninety-six Sprague Dawely rats, weighing 250-275 gm at age of 5 to 6 months were used in this study. The rats were housed in a well-ventilated room at 20° C, had free access to food and water and they were under daily inspection. Seventy two rats acquired to be diabetic by injecting them alloxan (75 mg / kg /body weight) I.P once daily for three successive dayes [20] . A drop of blood was taken from injured tip of their tails before and after they have been diabetic. Blood and urine glucose was measured after three days of the last dose of alloxan by using the Glucometer Elite and reagent strips (Glucometer Elite, Bayer A/S, Noergardsvei-32 DKr-2800 Lynph, Denmark). Diabetic rats with blood glucose less than 17 mmol /ml was not considered as diabetic rats [21-22].High dose of chloroform inhalation was used for sacrificing the rats, inside a sealed glass box.The rats were sacrificed following the beginning of the experiment by; two weeks, one month, two months, three months, four months,

and five months, at a rate of four animals at each stage after measuring their blood sugar.Animals classified into four groups each of (24 rats) as follows:-

- **Group A:-** normal healthy female rats were used to study the morphological and histometrical features of the sciatic nerve fibers in normal rats.
- **Group B:-** To study the morphological and histometrical features of the sciatic nerve fibers in diabetic rats .
- **Group C:** - Diabetic rats were treated with Glibenclamide only (0.0714 mg / kg. B.W. orally. Twice / daily).
- **Group D:-** Diabetic rats were treated with Glibenclamide (0.0714 mg / kg. B.W.orally. Twice / daily) [20] + Garli (Bulbus Allii sativi)powder (250 Mg / Kg . B.W. Daily) [22].

Drug administration

Solid drug (garlic powder and glibencamide tablets) which given to the rats orally had been grained well and suspended in 0.2% Arabic Gum solution. The volume of each prepared dose of the drugs was not more than 0.2 ml. The Glibenclamide was used as a hypoglycemic drug in combination with garlic powder.

Animal dissection and removal of tissues

The sacrificed animal lay down on one side, an incision of 2 cm long was made to the skin along the opposite thigh. The thigh muscles were separated and the sciatic nerve (15 mm long) of both left and right thigh were removed.

Histological Technique

A part of the sciatic nerve was taken, fixed in aldehyde solution (1% formaldehyde + 2.5 % phosphate buffered gluterldehyde, pH 7.4) for 2 hours at 4° C, transmitted to osmium tetroxide (1%) for four hours at 20° C, then washed with phosphate buffer (pH 7.4), three times each of 15 minutes [23]. A segment of osmicated nerve was softened in 50% glycerin overnight, immersed in pure glycerin [20], then teased (separation of the nerve fibers from each other) by using very fine and pointed needles. A dissecting microscope specially equipped for microsurgery (Konan Camera R & INC, Japan) were used. The specimen was laid on clean slides mounted with a drop of glycerin, then the borders of the cover slides sealed by Canada balsam. Four different pieces of each specimen were teased and mounted on different slides. The slides were labeled and kept in refrigerator till the time of measurement.

Histological Measurement

The measurement of the teased nerve fibers used were included; mean internodal length (ML), mean internodal diameter (MD), percentage of short segment (%SS) less than (150 μm), and swollen paranodal area (%SPA) more than (15 μm) in diameter [24-25]. In this measurement a 10 X eyepiece ocular micrometer and a 40 X objective lens were used. The mean of 100 reading from four nerve bundle of each segment was calculated [26]. Corrections to microns were made, by multiplying the mean of the length and the diameter of each internodes by (2.5) as a correction factor [27-28].

Nerve conductivity

The responding time (sensation of the peripheral nerve) to cold application to the foot in this experiment adminstered as a simplified method for estimation of the conductive velocity and for the improvement in the sciatic nerve conductivity [29]. Electrically controlled stage (- 10 C°) have been used, the rats layed on the cooled satge under cover transperent non sealed glass container, the time measured once the rats starting to lifting their feet from the stage. As this indicating for the responding time to the cold stage.

Statistical Analysis

The ready computerized statistical program (SPSS) was used to analyze the data [30-31].

Results

Morphological and histometrical measurement

Group A:- Non diabetic rats

The sciatic nerve in the rats showed the same anatomical features as the location and relation to other structures as that of human sciatic nerve, but the length measured (2-3 cm) . Group A which represent normal non diabetic rats showed no (SS) nor (SPA) during the time of the experimentation. The osmicated nerve fibers of this group appeared thick with regular contour and thick myelin layer. The paranodal areas were smooth or slightly swollen (Figure 1 and table 1). The histometrical measurement of the individual nerve fiber revealed that the mean internodal (segment) is (1160 ± 5.5 μm). The mean diameter (13.36 ± 0.16 μm).

Group B:- non treated diabetic rats

Group C:- Diabetic rats treated with Glibenclamide

The results obtained from (Table 3, Figure 3), despite treated with glibenlamaide but still showed significantly increases in the percentage of (SS), and in the (SPA), but the (ML) and the (MD) were significantly less than that which measured from normal non diabetic rats.

Group D:-Diabetic Rats Treated With Glibenclamide + Allium sativum

The results showed minor deterurative changes in comparison with that results obtained from group B, diabetic rats without treatment and group C diabetic rats treated with glibenclamide only. These results can be indicated by the statistically significant decline in the percentage of (SS) and (SPA) at one month duration onward, however [2.3 %] of the (SS)], and low percentage [6.75 %]of the (SPA) were observed.At three-months slight increase in the ML, MD, and a proportional decrease in the percentage of (SS), and SPA. These changes continued to the next diabetic periods (DP), in that the specimen which were examined at the forth month and the fifth month of diabetic period showed statistically significant raising in the ML, MD, beside significant decline in the percentage of (SPA), and disappearance of (SS) were detected (Figur 4 and Table 4).

Nerve conductivity

The rats of group D responding to the cold application at a mean time of (174) second, that is to say more rapid in comparison to group Band C. this indicated that nerve conductivity of the sciatic nerve was better and improved in the diabetic rats that treated with Glibenclamide + Allium Sativum in comparison with that in group B, and group C (Table 5).

Discussion

The deteriorative changes of the sciatic nerve fibers in diabetic rats appeared through the morphological changes, which represented by the swelling of the paranodal areas (SPA) and shortening of internodal spaces (SS) of the nerve fibers which leads to decrease in the conductivity of the nerve. These findings were interpreted as following:

1- swelling of the paranodal area may ascribed to the followings

Depletion of myo-inositol, altered phosphoinositide metabolism, and decreases availability of NADPH, which is important in maintenance the reduced level of glutathione and prevention of tissue damage by the reactive oxygen species [32].The inter-cellular accumulation of Polyols, initiated by Aldose Reductase leads to an influx of the water that results in changes of the permeability, and eventually, leads to a loss of cellular integrity and so causing the cells to swell, specially the paranodal area of the peripheral nerve fibers; this area is considered as a site of high metabolic activity and concentration of mitochondria [33-34].

2- short internodal segment

The degenerative changes, resulted from metabolic disorders due to diabetes, may lead to the axonal and myelin degeneration. These changes may occur due to the accumulation of polyols which leads to changes in the permeability (retention of water), loss of cellular integrity, a decrease concentration of potassium, amine acids, inositol and ATP (Adenosine Try phosphate). Sodium and chlorine begins to build up, these lead to secondary osmotic changes; membrane become freely permeable to all substance other than large protein, increases of hydration and rupture [35]. Accordingly, these lead to degeneration, then followed by regeneration of different parts of the nerve fibers, a process by which result in the appearance of short inter nodal segments.

3- microvascular changes

It was detected by Ward [17], that diabetic peripheral neurological changes may be due to; abnormality of intraneural capillaries, as thickening of the basement membrane, and microthrombi formation. Such information was also suggested by Rechthand and Rapoport [26], they supposed that; insufficient nutrient and oxygen may reach the nerve fibers leading to atrophy and degeneration of them.The present study found that the administration of Allium sativum, may prevent or relief these abnormal changes (Histologically, and functionally). This is documented by the restoration of the sciatic nerve specimen to their morphological appearance microscopically, and histometrically, in addition to the improvement to their conductive velocity, this may be attributed to the following: -

1-The adequate amount of antioxidant enzymes and adenosine, a conistiuent of the Bulbus Allii sativi [10], this can inhibits the leakage of cytotoxic enzymes that indices the damage of cell membrane and another enzymes that result in the damage of mitochondria inside cells as a result of the diseases.

2-Garlic activates endogenous fibrinolytic activity in the serum that prevent microthrombi of the intraneural microvesseles resulted from deterioration in the endothelial lining of the blood vesseles [36-37] accordingly leads to rapid blood flow for the nerves , good oxygenation , and good nutritions, then regenertation of the affected peripheral nerves reducing the diabetic neuropathic changes, and consequently improving the nerve conduction velocity.

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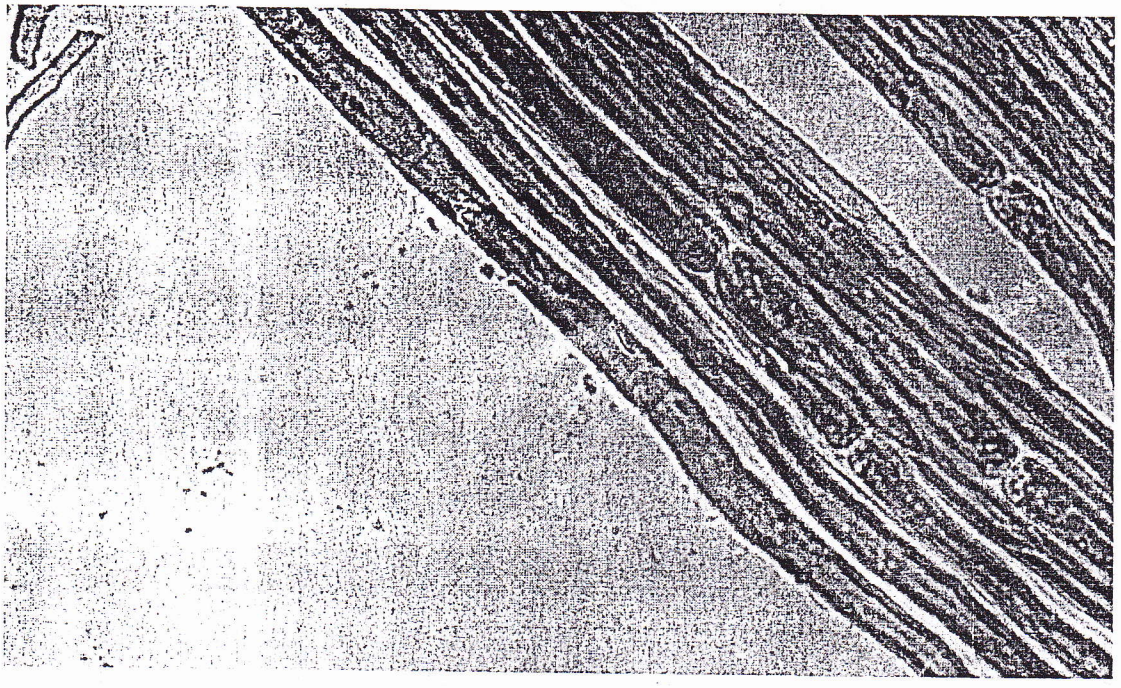

Figure (1): Osmicated nerve fibers from non diabetic animals; showing normal architecture and node of Ranvier. 160 X.

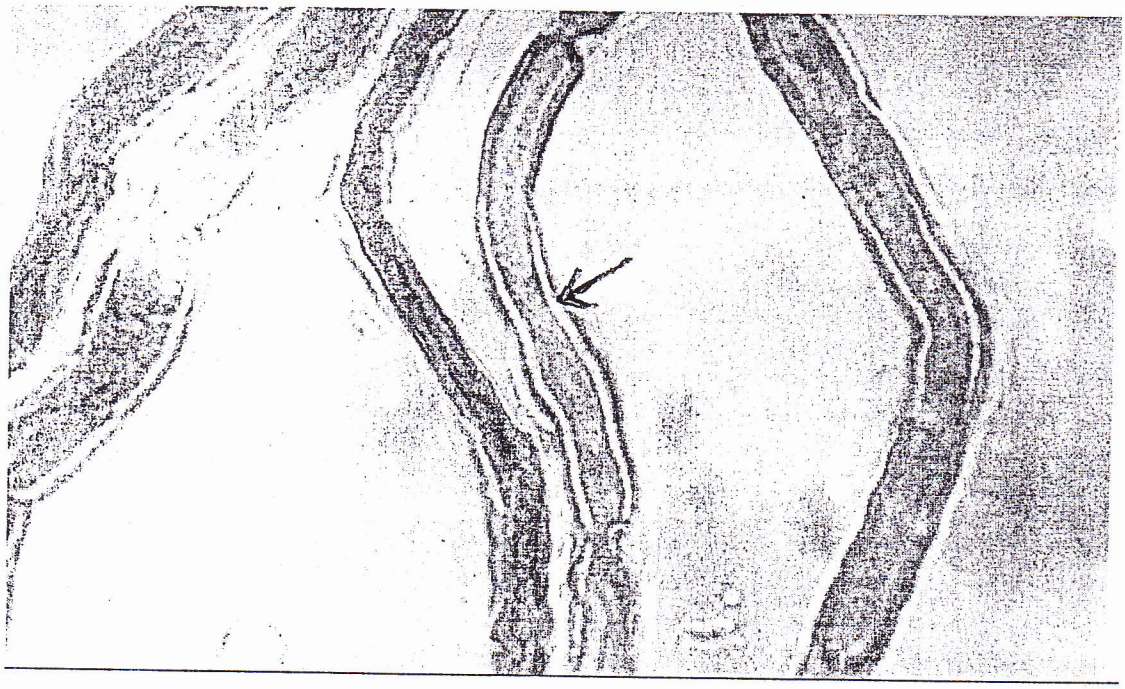

Figure (2): Osmicated nerve fibers from group (b) 2, showing (SS) (arrow). 160 X.

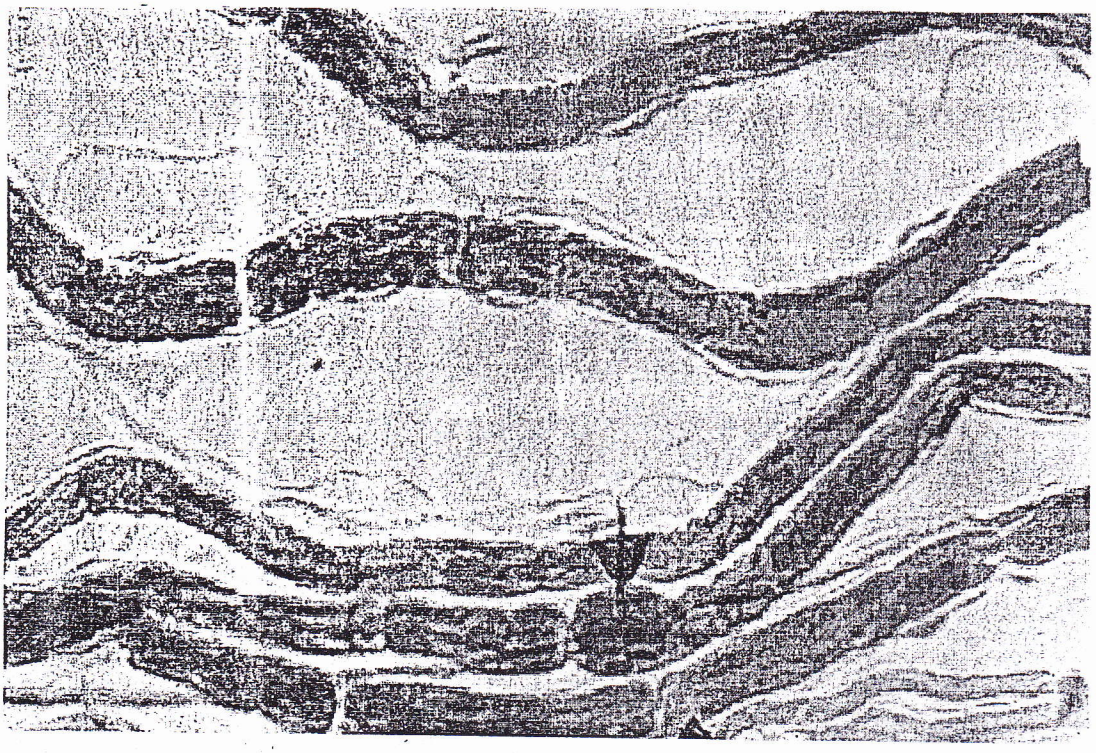

Figure (3):Osmicated nerve fibers from group (c) diabetic rats treated with glibenclamide; showing (SS) and (SPA) (arrowhead). 160X.

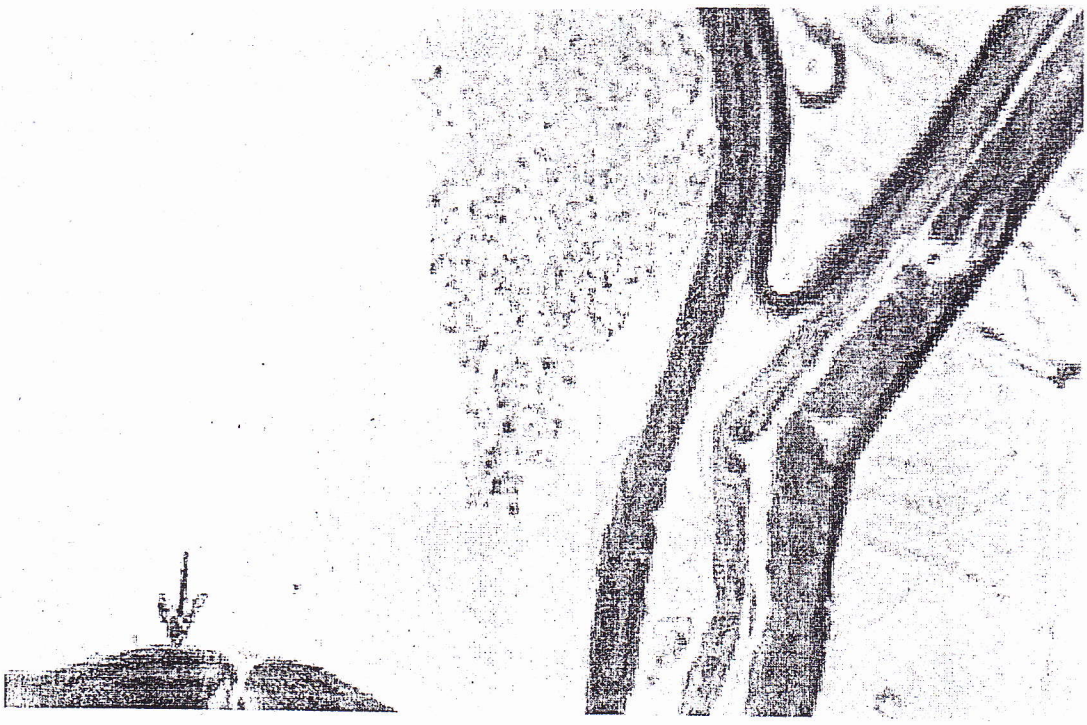

Figure (4):Osmicated nerve fibers from group (D) diabetic rats treated with garlic; Showing slight swollen para nodes (arrow). 160 X.

Table (1) :Control Group A/ The length, diameter (in μm), % of (SS) & % of (SPA)

Duration	Mean Length [ML]	Mean Diameter [MD]	% of Short Segment (SS)	Swollen Paranodal Areas (SPA)%
2 week	1358	13.11	0	0
1 Month	1355	13.52	0	0
2 Month	1342	13.48	0	0
3 Month	1344	13.34	0	0
4 Month	1342	13.36	0	0
5 Month	1340	13.32	0	0

Twenty-four rats [not diabetic] given .0.2 ml Arabic Gum [0.1 % orally twice daily. Each data = the mean of [4] reading each reading represent the mean of (100) internodal segment of one sciatic nerve specimen

Table (2):Group B/The length, diameter (in μm), % of (SS) & % of (SPA)

Diabetic period	Mean Length [ML]	Mean Diameter [MD]	% of Short Segment (SS)	Swollen Paranodal Areas (SPA)%
2 week	1351	13.49	0	0
1 Month	1161	11.26	10.31	12.14
2 Month	1125	10.98	12.53	13.36
3 Month	1101	10.65	12 .50	13.53
4 Month	1003	10.25	12.61	13.65
5 Month	1084	10.22	12:63	13.63

Twenty-four diabetic rat given only Arabic Gum (0.2 ml 0.1 %]) orally twice daily. Each data = the mean of [4] reading each reading represent the mean of (100) internodal segment of one sciatic nerve specimen.

Table (3) :Group C/The length, diameter (in μm), % of (SS) & % of (SPA)

Diabetic period	Mean Length [ML]	Mean Diameter [MD]	% of Short Segment (SS)	Swollen Paranodal Areas (SPA)%
2 week	1354	13.70	0	0
1 Month	1231	11.76	6.21	8.35
2 Month	1206	11.16	6.67	9.15
3 Month	1193	10.99	6.87	9.53
4 Month	1179	10.73	7.15	8.57
5 Month	1175	10.19	7.17	8.70

Twenty-four diabetic rats treated by Glibenclamide (0.014 Mg/ Kg. Twice Daily. S/C. twice daily). Each data = the mean of [4] reading , each reading represent the mean of (100)internodal segment of one sciatic nerve specimen.

Table (4):Group D/The length, diameter (in μm), % of (SS) & % of (SPA)

Diabetic period	Mean Length [ML]	Mean Diameter [MD]	% of Short Segment (SS)	% of Swollen Paranodal Areas (SPA)
2 Week	1369	13.82	0	0
1 month	1313	12.53	1.71	5.61
2 month	1257	12.34	2.39	6.75
3 month	1266	12.43	2.31	6.33
4 month	1316	12.59	0.1	5.64
5 month	1312	12.63	0	5.61

Twenty-four diabetic rats given Allium sativa L. 250 mg/ KG. + Glibenclamide Orally twice daily. Each data = the mean of [4] reading, each reading represent the Mean of (100) internodal segment of one sciatic nerve specimen.

Table (5):Nerve conductive velocity of the sciatic nerve (time in second)

Diabetic period	Animals No.	Not diabetic	Diabetic rats		
			Treatment		
			Not treated	Glibenclamide	Glibenclamide +Garlic
		A	B	C	D
4 Months	1	79	389	233	120
	2	67	341	218	100
	3	85	331	198	121
	4	71	371	293	133
	mean	75.5	358	235.5	118.8
5 Months	1	81	322	215	111
	2	89	321	210	123
	3	78	351	289	154
	4	82	368	288	200
	mean	81	340.5	250	174

The brisk withdraw of the hind foot as a test for estimation of the conductive velocity.