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Research Article

Role of Polysaccharides isolated from *Nepeata septemcrenata* in diabetic albino rats

Sawsan Ahmed Nasr[1], Amel Abd El-Moneim Saad[2], Mai Selmy khadar[3]

[1]Physiology Department, [2]Biochemistry Department, and [3] Botany Department

National Organization for Drug Control and Research (NODCAR)

ABSTRACT

The present study was designed to investigate the antidiabetic activity of polysaccharide extracted from *Nepeta septemcrenata* (*N. septemcrenata*). Diabetes was induced by intraperitoneal injection of streptozotocin (STZ) (30 mg/kg b.w.). Twenty four adult male albino rats were divided into control group, diabetic group, diabetic group treated with gliclazide as a reference drug (10mg/kgb.w.) and diabetic group treated with polysaccharide extracted from *N. septemcernata* (50mg/kg b.w). The experiment was lasted for 21 days. STZ was significantly increased blood glucose, Alanin aminotransferase (ALT), Asparate aminotransferase (AST), alkaline phosphatase (ALP), total cholesterol, triglyceride and amylase associated with significantly decrease of total protein and albumin levels. Administration of polysaccharide extracted from *N. septemcrenata* significantly attenuated glucose, α-amylase, ALT, AST, ALP, total protein, albumin, total cholesterol and triglyceride. In Conclusion: The polysaccharide extracted from *N. septemcrenata* display a significant antidiabetic properties in comparison with gliclazide.

Keywords: Diabetes mellitus, Streptozotocin, *Nepeta septemcrenata*, Gliclazide, liver function.

INTRODUCTION

Diabetes mellitus (DM) is a serious chronic metabolic disease and be divided into two major types by etiology, namely type1 diabetes mellitus (T1DM) or type2 diabetes mellitus (T2DM). It is a global disease that a thorny problem in the world medicine [1]. Diabetes needs long-term treatments in order to have their conditions brought under control and to prevent complications. Exacerbate symptoms associated with hyperglycemia is primarily attributed to microvascular and macrovascular changes which can cause atherosclerosis, retinopathy, renal failure, and peripheral artery diseases. Once complications are allowed to creep in, the outcome is in danger [2].

There is an increasing demand by patients for the use of medicinal plant. Hypoglycemic plants are still prevalent in developing countries, where they have been used to treat diabetes for many centuries More than 1200 species of plants have been used empirically for their alleged hypoglycemic activity. This fact is attributed to the high cost and the lack of availability of current therapies for the majority of patients in developing countries. Nevertheless, many medicinal plants claimed effective by folk medicine require scientific investigation to ascertain their effectiveness, toxicity and then provide alternative drugs and therapeutic strategies [3]. World Health Organization (WHO) approves the use of plant drugs for different diseases, including diabetes mellitus. Traditional plant medicines are used throughout the world for treatment of diabetes mellitus [4].

Nepeta is one of the most important genera of lamiaceae family with regard to the number of its species. Some species of this genus are important medicinal plants and their extracts have been used for medicinal purposes [5]. It was reported that *Nepeta* plants were prepared as tea and used in traditional medicine as anthelmintic, febrifuges, expectorants, to treat bronchitis, bites, stings of scorpions, skin diseases (*Nepeta hindostana*) and anticatarrhal (*Neptea hederacea*) [6].

Nepeta septemcrenata (*N. septemcrenata*) is an erect slender plant with branches at base leaves are oppositely alternated, ovate with crenate or slightly dentate margins. This plant is found in Saint Catherine, Sinai, Egypt [7]. *N. septemcrenata* known to be used by the native Bedouins in folk medicine as antipyretic, sedative, cardiotonic, eye wash and as a gargle in sore throat [8]. *N. septemcrenata* had antipyretic effect, significantly decrease the thickness of the inflamed rats paw and induced protection against writhing response, finally it had much medicinal prosperity with high margin of safety [9]. An isopimarance type diterpence and 7-omethyl apigenin were isolated from the ethanolic extract of *N. septemcernata* herb [8, 10]. *N. septemcrenata* extract contains a highly concentration of volatile oils (ester, alcohol, keton, hydrocarbons and phenol), carbohydrate,

*Corresponding author: Sawsan Ahmed Nasr | E-mail: sawsannasr@hotmail.com

flavonoides (apigenin-7-methyl etherand flavone glycoside) and unsaturated sterols. In the same time the other constituents (cardenolides, tannins, saponins, alkaloids and coumarins) were found in trace amount [11].

Aim of the work:

Therefore, this study was designed to investigate the antidiabetic effect of polysaccharide extracted from *N. septemcrenata*. Gliclazide was used as a reference drug for diabetic treatment.

Materials and Methods

Plant material preparation:

Roots and aerial parts of *N. septemcrenata* (family, Labiatae) were collected from south Sinai desert, Egypt. The plant portions of aerial and root were air dried in the absence of direct sunlight, grounded and kept in dark.

Preparation of the Polysaccharides:

Dried powdered root (100g) and aerial parts (100g) previously defatted with petroleum ether and then exhausted with 95% ethanol, were separately extracted with boiling distilled water by placing on boiling water bath with occasional stirring for 3hr, then filtered while hot. The process was repeated twice and the combined filtrates from each part powder were concentrated under vacuum at a temperature not exceeding 70^{o}C. The polysaccharide were precipitated from the concentrated aqueous extracts by adding slowly while stirring 4 volume of absolute ethanol, the precipitates collected by centrifugation were washed several times with ethanol, then vigorously stirred in absolute acetone, filtered and then dried in vacuum desiccator over anhydrous calcium chloride. Percentage of polysaccharides obtained from the organs was calculated on dry weight bases.

Analysis of the precipitated polysaccharides:

For the determination of sugar composition, the monosaccharide residues released by acid hydrolysis (100 mg of the prepared polysaccharide was hydrolyzed with 10ml 0.5N HCL on a boiling water bath for 8hr, $Ba(OH)_2$ was added. Mixture was centrifuged and precipitate was water washed twice. Evaporation was carried out till the volume reached 2ml). Gas Chromatography (GC) and thin layer chromatography (TLC) were used for analysis [12]. TLC of polysaccharides monomers in hydrolysates was carried out using solvent system; ethyl acetate: methanol: acetic acid: water (60:15:15:10).The chromatogram was dried and visualized using thymol sulfuric acid reagent [13].

Materials for analysis:

Standard materials for GC analysis of the sugars were kindly supplied by Misr University for Science and Technology, TLC was carried out on silica gel plates (Merck 60F254, 20x20 cm).

GC and MS analysis were conducted using Finnigan SSQ 7000 under the following: conditions Column: DB5 capillary column, I.D. 0.25mm; ionization mode: EI (70-ve); Temperature program: 50-250 (4^{o}C/min) Detector: FID. Sample volume was 2µl, Mass range: 50-750 mass units. GC for sugar analysis was done using Hewlett-Packard 6890.

Chemicals:

Streptozotocin (STZ) and all other chemicals used in this study were obtained from the Sigma Chemical Company (USA).

Animals:

Male albino adult rats (170 ± 20 g) were obtained from animal house, National Organization for Drug Control and Research, NODCAR, Giza, Egypt. They were housed in wire cages with natural ventilation, illumination and allowed free water and standard diet for acclimatization in two weeks before being used for the experiment. All rats were maintained under standard laboratory conditions of light/dark cycle (12h /12h) and temperature (25 ± 2^{0}C). Rats were allowed free access to standard diet and tap water. The local ethics committee of NODCAR approved study protocols.

Induction of Experimental Diabetes:

Diabetes was induced by injection of a double intraperitoneal dose of STZ (30 mg/dL) (freshly prepared in 0.01 M citrate buffer, pH 4.5) [14]. After injection, animals had free access to food and water and were given 5% glucose solution to drink overnight to counter hypoglycemic shock [15]. After 3 days of STZ injection, diabetes was identified by polydipsia, polyuria (visual observations) and measuring blood glucose concentration [16]. Rats with a fasting blood glucose level above 200 mg/dl were considered diabetic and were used in this study [17].Control rats were only injected intraperitoneally with citrate buffer.

Experimental design:
A total number of 24 male albino rats were randomly divided into following four groups; each group consists of six rats as follows:

Group 1: Rats served as normal control.
Group 2: Diabetic group (STZ induced diabetic rats), Rats served as diabetic group (200-300 mg/dL), injected by STZ (30 mg/kg b.w.).

Group 3: Diabetic rats treated with polysaccharides extracted from aerial parts of *N. septemcrenata* were orally supplemented to rats (50 mg/kg b.w. /day for 15 days) for STZ diabetic induced rat groups) [18]. This dose is previously used by Nasr, [19] after toxicity study of the ethanol extract of *N. septemcrenata*.

Group 4: Diabetic rats treated with a daily oral dose of gliclazide as a reference drug (10 mg /kg b.w.) for 15 days [20].

Blood sampling:
Blood samples were collected from all the groups under fasting condition between 8.00 and 9.00 am, for every three days, in clean and dry test tube, left 10 minutes to clot and centrifuged at 3000 r.p.m for serum separation. Individual blood samples were obtained after 15 days from rats of each group, animals were sacrificed and autopsy performed immediately sera were separated and kept at-20 ^{0}C for biochemical analysis.

Biochemical analysis:
Glucose was determined in serum by colorimetric assay according to the method of Caraway and Watts [21]. Liver function enzyme (Alanin aminotransferase (ALT) and Asparate aminotransferase (AST) were estimated according to the method of Reitman and Frankel [22]. Alkaline phosphate (ALP) activity was determined by the method [23]. Albumin was determined with bromocresol green as described by Doumas *et al*., [24] and Tietz [25]. Total protein was estimated according to Biuret reaction as described by Gornall *et al*., [26] and Tietz [27]. Cholesterol activity was estimated according to methods of Richmond [28] & Ellefson and Caraway [29], Triglyceride [30]. α-Amylase activity was determined according to Winn-Deen *et al*. [31] and Tietz [32]. All the above mentioned parameters were determined using the corresponding diagnostic kits of Spectrum Company.

Statistical Analysis:
The results were expressed as mean value ± standard error (Mean values $\pm$ SE) for 6 rats in each group. Variable between groups was analyzed using one-way analysis of variance (one-way ANOVA test). Subsequent multiple comparisons between the different groups were analyzed by Duncan's multiple comparison tests. Data were statistically analyzed using the statistical package for social science (SPSS 11.0 software, Chicago, USA) values at $P < 0.05$ were considered significant [33].

Results
The extracted polysaccharides of the aerial parts and the roots were detected in 10% and 1 % respectively. They were precipitated as a yellowish brown amorphous powder. According to the chromatographic peak of polysaccharides reference substance and samples, the composition of the polysaccharides in *N. septemcrenata* was identified. Thin layer chromatography and GC analysis showed that there were diversity and heterogeneous nature, the hydrolysate polysaccharides (monosaccharide) obtained from the aerial parts composed mainly of arabinose in relatively high percentage 25.15 %,then xylose 11.10% and variable amounts of ribos, rhamnose , mannitol ,sorbitol, galactose- mannose , glucose and glucoronic acid as 2.7%, 8.3 %,0.97%,1.34% ,18.48%, 22.74% and 9.1% respectively. While, the root showed different structure as it composes of arabinose 13.5 %, xylose 3.05 %, ribose 0.45%, rhamnose 2.08 %, sorbitol 1.14%, fructose 0.71% galactose and mannose 36.89%, glucose 42.1% (table 1). Arabinose here is not the major consistent but in a lower percentage than that of galactose and mannose which are present in higher amount than that of the aerial part. Uronic acid is present in slightly lower percentage in the aerial parts, while is absent in the root as show in table (1). The plant contains a relatively high percentage of polysaccharides chromatograms of the polysaccharides are shown in figures (1 and 2).

Table (1): Polysaccharides component of aerial parts and roots of *N. septemcrenata*

Polysaccharide Component (%)	Aerial parts	Root
Arabinose	25.15	13.5
Xylose	11.10	3.05
Ribos	2.7	0.45
Rhamnose	8.3	2.08
Mannitol	0.97	-----
Sorbitol	1.34	1.14
Galactose- mannose	18.48	36.89
Glucose	22.74	42.1
Glucoronic acid	9.1	-----
Fructose	-----	0.71

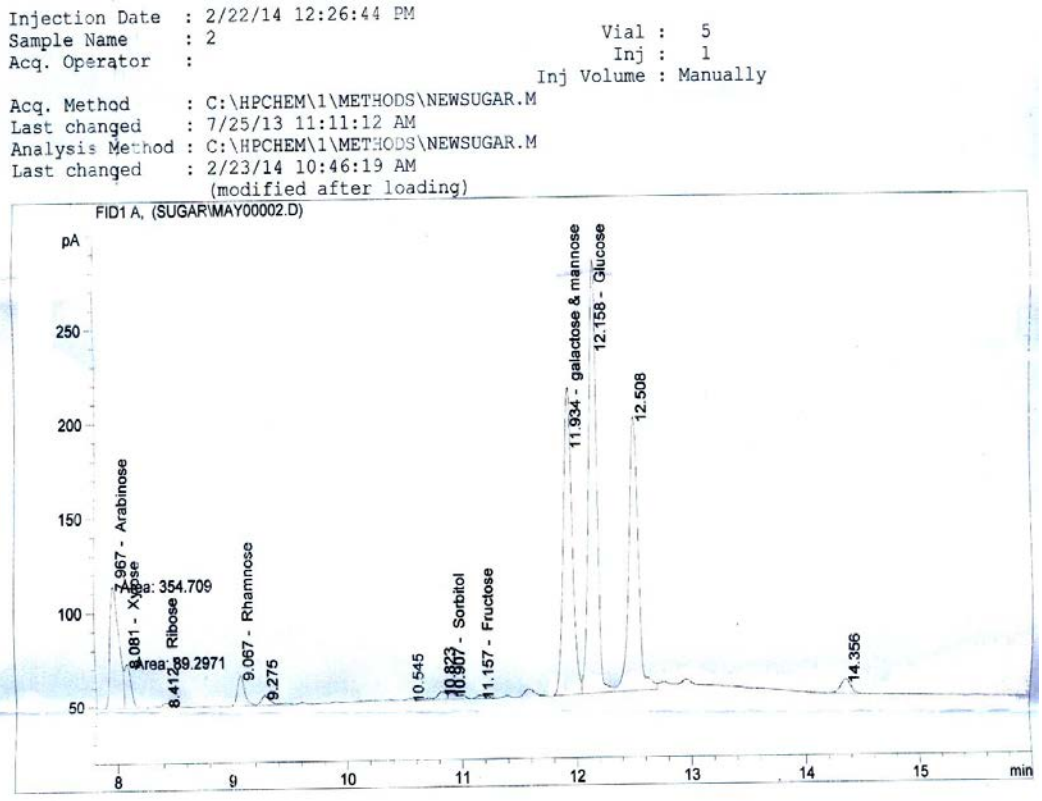

Figure (1): G.C. Spectrum of hydrolysate polysaccharides and polysaccharides from the aerial parts of *N. septemcrenata*.

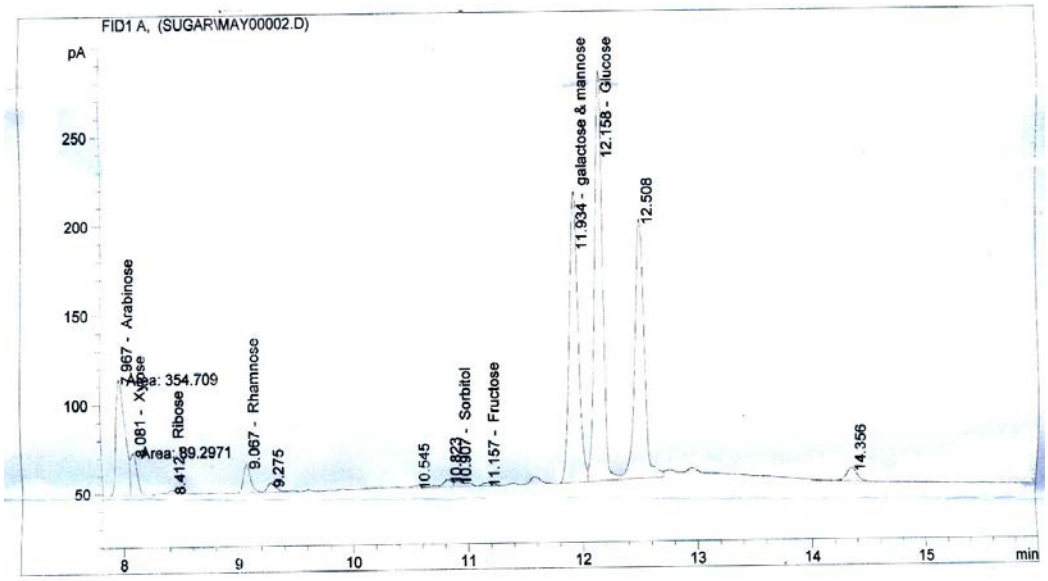

Figure (2): G.C. Spectrum of hydrolysate polysaccharides and polysaccharides from the aerial parts of *N. septemcrenata.*

Table (2) shows blood glucose of normal control, diabetic, diabetic + polysaccharide and diabetic + gliclazide. Concerning diabetic group, significant increase is noticed in blood glucose level post injection of STZ record 258.85±6.96 mg/dl ($p<0.5$) with percentage increase (+171.70%) as compared to control group. Significant amelioration is noticed in blood glucose levels in all diabetic treated groups, the most pronounced effect for the polysaccharide (4.10%) followed by gliclazide as a reference drug (11.49%) when compared with diabetic group. In addition, there is a significant decrease in polysaccharide treated group when compared with drug treated group in curative effect.

Table (2): Effect of *N. septemcrenata* polysaccharide against STZ induced diabetic rats on glucose level (mg/dl).

Group	Glucose
Control	95.28±2.42
Diabetic	258.85± 6.96*
Diabetic + polysaccharide	99.19±0.07□∞
Diabetic + gliclazide drug	106.23± 0.50*□

Data are reported as mean± S.E.
* Significant difference versus control (P < .05)
□ Significant difference versus diabetic (P < .05)
∞ Significant difference versus gliclazide drug (P < .05)

Table (3) demonstrates the level of liver function enzymes ALT, AST and ALP activities in normal control, diabetic, diabetic+ polysaccharide and diabetic+ gliclazide groups. With regard to diabetic condition, significant increase in all enzyme activities post STZ injection reached to 87.84±4.27, 128.73±1.92 and 444.43±2.27 with percent of elevation (+207.88%, +92.65% and 92.86 %) for ALT, AST and ALP respectively. ALT enzyme still record insignificant reduction and elevation post polysaccharide and gliclazide drug treatment amounted 26.26±0.31and 32.46±1.11 with percentage of decrease -7.95% and increase +13.77% respectively. There is a significant decrease in ALT of polysaccharide treated

group when compared with drug treated group in curative effect. The level of AST activity is improved as a result of polysaccharide and gliclazide drug treatment which recorded (64.54±1.08) and (62.38±1.21) with percentage of change (-3.41% and - 6.64%) respectively. While, the effect of polysaccharide and gliclazide drug on diabetic rats can easily be noticed through the normalization of enzyme of ALP tested return to the level of normal control, where an insignificant increase is observed with polysaccharide (+ 0.72%) there is a significant reduction with drug (- 8.96 %).

Table (3): Effect of polysaccharide of *N. septemcrenata* against STZ induced diabetic rats on ALT, AST and ALP activities (U/ml)

Group	ALT	AST	ALP
Control	28.53±1.06	66.82±1.9	230.43±4.43
Diabetic	87.84±4.27*	128.73±1.92*	444.43±2.27*
Diabetic + polysaccharide	26.26±0.31□ ∞	64.54±1.08□	232.10±1.2□
Diabetic + gliclazide drug	32.46±1.11□	62.38±1.21□	209.78±.63*□

Data are reported as mean± S.E.
* Significant difference versus control (P < .05)
□ Significant difference versus diabetic (P < .05)
∞ significant difference versus gliclazide drug (P < .05)

Table (4) shows the levels of total protein and albumin in control, polysaccharide and drug treated groups. With regard to diabetic groups significant reduction in total protein and albumin observed post STZ injection as compared to normal control group record (5.49±0.09 mg/dl) with percent of reduction (-14.35%) for total protein. On the other hand nearly simultaneously decreased level of albumin is recorded post injection of STZ with percentage of decrease (- 18.47%). All diabetic treated groups showed a non-significant enhancement in total protein levels as compared to control group. In both polysaccharide and drug treated groups, total protein reach to 6.54± 0.02 and 6.48±0.01 mg/ dl respectively with percent of + 2.02% and +1.09%. Remarkable an insignificant enhancement is noticed in albumin level post different type of treatment (polysaccharide, drug) as compared to normal control with percent (- 0.88% and -3.52%).

Table (4): Effect of *N. septemcrenata* polysaccharide against STZ induced diabetic rats on total protein and albumin levels (mg/dl).

Group	Total protein	Albumin
Control	6.41±0.07	3.41±0.03
Diabetic	5.49±0.09*	2.78± 0.09*
Diabetic + polysaccharide	6.54±0.02□	3.38 ±0.11□
Diabetic + gliclazide drug	6.48±0.01□	3.29 ± 0.10□

Data are reported as mean± S.E.

* Significant difference versus control (P < .05)
□ Significant difference versus diabetic (P < .05)

Table (5) shows the levels of total cholesterol and triglyceride in control, polysaccharide and drug treated groups. With regard to diabetic groups, a significant elevation is observed for total cholesterol and triglyceride post STZ injection as compared to normal control group, in treated groups reached to (143.37±0.74 mg/dl) with percentage of increase amounted (+81.5%) for cholesterol and record (208.35±4.87 mg/dl) with percent of elevation (+119.20%) for triglyceride. On the other hand, insignificant improvement is noticed in cholesterol level post STZ injection as compared to normal control group amounted, 82.87±3.08 and 83.10±4.33 mg /dl with percent of elevation + 4.91 and +5.20% respectively. All diabetic treated groups show significant enhancement in triglyceride level as compared to the normal control. In polysaccharide and gliclazide treated groups, triglyceride reach (154.94±4.27and172.03 ±0.029 mg /dl) respectively with percent of + 63% and + 80.98%respectively. There is a significant decrease in triglyceride of polysaccharide treated group when compared with drug treated group in curative effect.

Table (5): Effect of *N. septemcrenata* polysaccharide against STZ induced diabetic rats on total cholesterol and triglyceride levels (mg/dl).

Group	Total Cholesterol (mg /dl)	Triglyceride (mg / dl)
Control	78.99± 5.83	95.05±1.64
Diabetic	143.374±0.74*	208.35±4.87*
Diabetic + polysaccharide	82.87 ±3.08□	154.94±4.27*□ ∞
Diabetic + gliclazide drug	83.10± 4.33□	172.03 ± 0.029*□

Data are reported as mean± S.E.
* Significant difference versus control (P < .05)
□ Significant difference versus diabetic (P < .05)
∞ Significant difference versus gliclazide drug (P < .05)

Table (6) records the activity of α-amylase of the different studied groups. It can be concluded that amylase enzyme is affected with diabetic condition, shows a significant elevation reached to 363.53±4.15 with percent + 20.3%. Treatment of the diabetic rats with *N. septemcrenata* polysaccharide and gliclazide drug produce obvious enhancement in amylase enzyme. This can be easily seen through amylase activity which show significant change as a result of treatment diabetic rats with polysaccharide and drug as compared to normal control reached to (327.75±0.36 and 326.33± 3.169) with percent of decrease (- 8.47% and -7.99%).

Table (6): Effect of *N. septemcrenata* polysaccharide against STZ induced diabetic rats on α-amylase activity (U/L)

Group	Amylase
Control	302.17±5.58
Diabetic	363.53±4.15*
Diabetic + polysaccharide	327.75±2.17*□
Diabetic + gliclazide drug	326.33±3.17*□

Data are reported as mean± S.E.
* Significant difference versus control (P < .05)
□ Significant difference versus diabetic (P < .05)

Discussion

Streptozotocin (STZ) is one of the most commonly used substances to induce diabetes in the rat. This toxin causes the death of pancreatic cells by alkylation of DNA resulting in reduced synthesis and release of insulin [34]. STZ is believed to partially destroy the pancreatic β-cell of islet of langerhans of the pancreas [35].

The potential role of the medicinal plants as hypoglycemic agents has been reviewed by several authors, supported by the ethnobotanical surveys and traditional medicines of different cultures [36- 38]. There are more than 200 compounds from plant sources that have been reported to show blood glucose lowering effect. The wide variety of chemicals classes indicates that a variety of mechanisms of action are likely to be involved in lowering blood glucose levels [20].

A double intraperitonial injection of STZ given to rats produced hyperglycemia in 7 days with blood glucose level of 200–300 mg/dL. It was observed that treatment of polysaccharide of *N. septemcrenata* for 15 days reduced the fasting glucose to significantly normal level. The daily administration of polysaccharide (50 mg/kg) to STZ-induced diabetic rats caused a significant reduction in blood glucose level when compared with the diabetic control group. There is a significant difference between polysaccharide and gliclazide drug treated groups. The changes in blood glucose concentration reflect abnormalities in β-cell functions. The fluctuation in blood sugar might also be attributed to the sensitivity of STZ that varies with species, strain and nutritional state [39]. The obtained results in the current study run parallel with Wu *et al*. [40], Akbarzadeh *et al.* [41] and Aly *et al.* [20] who used STZ to induce diabetic mellitus in rats.

The present results revealed a significant amelioration in blood glucose level post treatment of diabetic rats with *N. septemcrenata* polysaccharide (50 mg/kg for 15 days). These results run parallel with Wu *et al*. [40] who found that *Astragalus* polysaccharide in a dose 400 mg/kg for 5 weeks give anti -diabetic effect with rat. Also, Zheng *et al.* [42] who used a dose of *Ganoderma lucidum* polysaccharides (200 mg/kg for 8 weeks) and Huang *et al.* [43] used 20 mg/kg b.w. for 8 weeks by oral gavage dose of *Pleurotus tuber*-regium polysaccharides give the same result. In line with the present study Vats *et al* .[44] found that the crude ethanol extract of *Ocimum sanctum* (Labiatae) leaves significantly improve β-cell function and enhances insulin secretion leading to lowering blood glucose level. Furthermore, the previous authors reported that the antihyperglycemic effect of *Ocimum sanctum* is at least partially dependent upon insulin release from the pancreas and significantly increased the activity of three key enzymes involved in carbohydrate metabolism, namely phosphofructokinase, glucokinase and hexokinase towards normal levels.

The effects on blood glucose and the effects on pancreatic cells could all be the indirect result of polysaccharide of *Ophiopogon japonicus* as reported by Chena *et al*. [45] who attributed the reduction of blood glucose to the polysaccharide extracted from *Ophiopogon japonicus*. Pancreatic cells are highly specialized cells which are responsible for producing all of the insulin required by an organism to maintain glucose homeostasis. Defects in development, maintenance, or expansion of β-cell mass can result in impaired glucose metabolism and diabetes [46]. In the present study, we observed that *N. septemcrenata* contains a relatively high percentage of polysaccharides varying in their nature which in turn might lead to a wide range of therapeutic effects like antidiabetic effect and our pervious study on *N. septemcrenata* found antoxidant action which lead to decrease free radical generation resulted from STZ injection that improvement the β- cell destruction and consequently decrease blood glucose [19]. So the effect of polysaccharide of *N. septemcrenata* on blood glucose may be due indirect action also. Another explanation from Zheng *et al.*[42] who reported that *Ganoderma lucidum* polysaccharides showed an anti-hyperglycemic effect in STZ-induced diabetic rats possibly due to its protective effect on pancreas cells through preventing apoptosis and enhancing cells regeneration.

The present results demonstrated that there is a significant increase in ALT, AST and ALP enzyme activities in all treated groups post STZ induction rats. On the other hand, there is an improvement in ALT, AST and ALP enzyme activities when treated with polysaccharide and gliclazide drug. High serum levels of (ALT, AST and ALP enzymes) post STZ treatment are associated with inflammation and /or injury to liver cells, a condition known as hepatocellular liver injury and apopotosis [47]. Significant improvement in liver function enzyme markers was noticed in treatment of diabetic rats with polysaccharide and gliclazide drug when compared with diabetic groups. This result is agreement with Fu *et al*. [48] who reported that *Acanthopanax senticosus* polysaccharide plus metformin could significantly reverse the pathophysiologic parameters of diabetic rats to normal level than only metformin administration. Also, Bhateja and Singh [49] reported that *Acacia tortilis* polysaccharide lowered serum ALT and AST levels which showed the protective effect and normal functioning of liver in reversing the organ damage due to diabetes which was clearly observed by high levels of ALT and AST

in diabetic control. Dahech *et al*. [50] demonstrated that levan polysaccharide is efficient in inhibiting hyperglycemia and oxidative stress.

Our results are consistent with previous studies that administration of some antioxidants (as zinc, selenium vitamin C and E) to diabetic rats normalized the elevated activities of liver function enzymes AlT, AST, ALP induced in response to diabetes mellitus [51]. The possible mechanism of action of polysaccharide could be correlated with promoting insulin secretion by closure of K^+- ATP channels, membrane depolarization, and stimulation of Ca^{2+} influx, an initial key step in insulin secretion [52]. The mechanism of hepatoprotective ability of extracts may be attributed to numerous bioactive compounds such as terpenoids, flavonoids, sterols, essential oil, alkaloids and polysaccharides. Most of them (especially falvonoids, triterpenoids such as ursolic acid) showed a mechanism to improve the function of liver and pancreas cells and hence normalization of liver enzymes [38], [53-55].

The present results revealed a significant reduction in serum total protein content post STZ injection. This finding runs parallel with Gupta *et al*. [56] and Sivajothia *et al*. (2008) [57]. The reduction in serum total protein content in the present results may be related to reduction in albumin which is the most abundant blood plasma protein (70%) produced in liver. Non enzymatic glycation of albumin was found the potential to alter its biological structure and function [58]. STZ induced animals showed serum albumin level significant decline and this result in agreement with Sivajothia *et al*. (2008)[57] who reported that hypoalbuminemia is a common problem in diabetic animals and is generally attributed in the presence of nephropathy. While, Jiang *et al.* [59] used *Rhizoma dioscoreae* polysaccharide improve significantly the level of albumin in diabetic rats.

Hyperlipidaemia is a common complication of lipidaemia mellitus in experimental animals. The effect of polysaccharide on serum total cholesterol (TC) and triglyceride (TG) in STZ-induced diabetic rats is shown in table (5). When compared to diabetic controls, TC and TG decreased remarkably. This suggests that polysaccharide may have a hypolipidaemic effect in diabetic rats. This result is in agreement with Liu *et al*. [60] and Huang *et al*. [43].The viscous structure of polysaccharides has been suggested to be important by interfering with the absorption in the digestive tract, and thereby increasing the excretion of cholesterol. On the other hand, the hypotriglyceridaemic effect might be due to a delayed absorption of triglyceride in the small intestine caused by the high viscosity of the intestinal contents [61]. Furthermore, fibers of polysaccharides may influence the gastrointestinal functions, including nutrients (fat) absorption, slow gastric emptying and bacterial fermentation in the colon [62]. These phenomena may result in reduced calorie uptake. Reduced energy uptake may initiate the mobilization of stored energy from body's fat depots and transport *via* circulation to site of high metabolic demand [63].

Concerning α-amylase significant elevation was noticed in diabetic rats post STZ injection. This result is an agreement with Najafian [64].While, there is a significant decrease in polysaccharide and gliclazide treated groups. This result was in parallel with Wei *et al*. [65]. Suppression of the activities of digestive enzymes may delay the degradation of carbohydrate, starch, and oligosaccharides, causes a reduction in the rate of glucose adsorption and postprandial blood glucose level. The tea extract can inhibit several kinds of digestive enzyme, which includes α-glucosidase, α-amylase [66]. Wei *et al.* [65] found that green tea flower polysaccharides (TFPS) had an inhibitory effect on α-amylase and α-glucosidase, which indicates that TFPS could inhibit starch or glycogen degrading to oligosaccharides and glucose. Thus blood glucose will not ascend fast and the glycemic index reduces. The inhibition of *N. septemcrenata* polysaccharide on starch digestion may occur in this way. Aly *et al*. [20] reported that liver and serum amylases are immunological identity and both are very similar to parotid gland and their differences from pancreatic amylase strengthens previous suggestion that liver is the main source of serum amylase and, further, eliminates the possibility of the pancreas being a source. The fall in amylase content either in pancreas or in liver is may be due to increased secretion or intracellular degradation in vivo and a decreased rate of synthesis. In addition the reduction in amylase content is paralleled by a change in specific messenger RNA content suggesting that insulin regulates the synthesis of amylase at the level of transcription [67]. Inhibition of α-amylase leads to inhibition of starch breaking and results in lower levels of blood glucose [68]).

In this study, the following compounds (arabinose, xylose, ribos, rhamnose, mannitol, sorbitol, galactose- mannose, glucose and glucoronic acid) which are isolated by thin layer chromatography and GC analysis of aerial parts of *N. septemcrenata* polysaccharide. These results were parallel with Hu *et al*. [69] who reported that *Pseudostellaria heterophylla* polysaccharide consists of galacturonic acid, glucose, galactose and arabinose by HPLC analysis. Also, Chena *et al*. [45] revealed that monosaccharides isolated from the root of *Ophiopogon*

japonicus is composed of arabinose, glucose and galactose which analyzed by high performance gel permeation chromatography (HPGPC).

Conclusion

This study revealed first time the antidiabetic activity of *N. septemcrenata* polysaccharide and its use in treatment of diabetes, hyperlipidemia and liver toxicity. However, the precise mechanism by which *N. septemcrenata* polysaccharide reduced fasting blood glucose level in diabetic rats will require further detailed study. Therefore, future research and clinical trials in this area may lead to the use *N. septemcrenata* polysaccharide as a new type of therapeutic agent in treatment of diabetes.

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