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Original Article

ANTI-DIABETIC EFFECT OF A NOVEL NANO POLYMER OF THYMOL IN STREPTOZOTOCIN-INDUCED DIABETIC WISTAR RATS

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ABSTRACT

Objective: The aim of this study was to evaluate the effect of thymol and thymol nano polymer on the blood biochemical parameters and anti-diabetic activity in Streptozotocin (STZ)-induced diabetic rats.

Methods: The synthesized nano polymer (NP) was characterized by using different spectroscopy methods, such as IR, HNMR and CNMR. Loading and releasing of thymol were investigated by HPLC. Eleven groups of the Streptozotocin-induced diabetic and normal rats (overall 110 males) were tested through various biochemical factors such as: serum glucose, insulin, liver function-related enzymes including ALT, AST, ALP and bilirubin by ELISA kit methods.

Results: It has shown that thymol nano polymer is desirable for transferring drug. The amount of thymol loaded on NP estimated at 43±2.5 %. Then, 65% of the loaded drug was released. LD_{50} for thymol and thymol nano polymer were 435 and 583 mg/kg, respectively. thymol nano polymer at doses of 30, 60 and 90 mg/kg, in a dose-dependent manner, reduced blood glucose, increased insulin levels, and controlled liver enzymes ALT, AST, ALP and bilirubin in the STZ-induced diabetic rats.

Conclusion: The use of thymol nano polymer appears to be a new aspect concerning to protect diabetes-induced damage in the animal model.

Keywords: Thymol, Diabetes, Nano polymer, Streptozotocin, Drug delivery

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INTRODUCTION

One of the most common metabolic diseases is diabetes mellitus (DM), which is defined by hyperglycemia due to the weakness of body cells to use glucose properly [1]. It is likely that by the year 2030 about 500 million people will get diabetes [2]. Hyperglycemia is the common symptom of these diseases that alterative of glucose and changes in liver enzyme levels [3]. These changes include abnormal liver enzyme levels, necrosis, inflammation, cirrhosis, hepatocellular carcinoma, hepatitis, nonalcoholic fatty liver disease and acute liver failure [4]. Fluctuating levels of alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are mostly the result of the leakage of these enzymes from the cytosol of hepatocytes in the bloodstream [5].

Treatment for this pathology is multifaceted, which includes physical activity, dietary changes, and decrease in absorption of glucose in the gastrointestinal system and drug therapy [6]. Despite the use of common drugs, the development of new methods with fewer side effects to prevent DM is growing in the field of ethnobotanical treatments [7]. So, the hypoglycemic activity of a number of medicinal plant extracts has been evaluated and confirmed in animal models [8]. Previously, extracts of herbs such as *Syzygium cumini* and *Psidium guajava* have been identified as effective compounds for diabetes mellitus [9]. It has also been shown that the root extract of *ashwagandha* has improved diabetic disorders by reducing oxidative stress [10].

An important part of the essential oil of plants such as Thymus vulgaris is thymol, which is widely used as a flavoring and preservation capacity in the food industry. It has also been used in folk medicine since the ancient Greeks, Egyptians and Romans [11]. Studies have shown that thymol has antioxidant and anti-inflammatory properties [12, 13]. The antioxidant effects maybe return to its phenolic structure, as phenolic compounds often exhibit redox properties, which may adsorb and neutralize free radicals [14, 15]. Also, the ant diabetic effects of thymol on high fat diet-induced diabetic mice have been reported previously [16]. On the other hand, it has been shown that thymol is bonded to a variety of nanoparticles for various diseases [17, 18].

Nanoscale drug carriers are generally synthesized from natural and polymeric materials for slow and targeted release in sizes of 10 to 1,000 nm. Therefore, because the structure of drug carriers has important benefits such as biocompatibility and biodegradability, it is widely used to develop pharmaceutical industries. Nanoparticles, either encapsulated or absorbed, carry the drug more effectively to be used in the target tissue [19].

Thus, this is the first study that was initiated with the aim of evaluating the effect of thymol and thymol nano polymer on the blood biochemical parameters and anti-diabetic activity in Streptozotocin-induced diabetic rats. The efficacy was compared with glibenclamide, a standard hypoglycemic drug.

MATERIALS AND METHODS

Preparation and characterization of nano polymer

By using Citric acid (CA) (Carlo Erba) as AB3 monomer and glycerol (G) (Carlo Erba) as A3 monomer at different CA/G molar feed ratios, nano branching polymers were synthesized through the melting polycondensation. Glycerol (0.5 ml, 6.6 mmol) and CA monohydrate7 g (33 mmol), 11.09 g (52.8 mmol), and 16.64 g (79.2 mmol), corresponding to the CA/G molar feed ratios of 5, 8, and 12 respectively, were mixed in a polymerization ampule equipped with gas inlet, vacuum inlet, and magnetic stirrer at 90 °C, and heated to 110 °C for 20 min under constant stirring. The temperature of the polymerization ampoule was increased and adjusted to 120 °C for 30 min, 130 °C for 40 min, 140 °C for 40 min, 145 °C for 50 min and 150

for 60 min, respectively under vacuum to remove the water formed during the reaction. The mixture was cooled at room temperature. Viscose compound was dissolved in Tetrahydrofuran (THF) (Merck) and filtered to obtain a clear solution. The resulting solution was then concentrated under reduced pressure and was precipitated several times in cyclohexane (Merck). Precipitated compound (1 g) was dissolved in THF (10 ml) and then placed in a dialysis bag (Mn cutoff 2000, sigma-Aldrich) immersed in THF (100 ml) for 4 h and THF in the outside medium of dialysis bag was replaced with the fresh solvent. Finally, to obtain pure product as colorless and viscose compound, THF was evaporated under the reduced pressure. In the Second step, nano polymer modified by oleic acid (NP) was synthesized. Frist 3 g initial nano polymer was poured in a polymerization capsule and then 192 µl oleic acid was added. The resulting mixture was then heated at 90, 100, 120, 140 and 160 °C respectively, and stirrer at 1,000 rpm magnetic stirrer for 5 h under the vacuum condition. To remove residue oleic acid, the mixture was washed several times with n-hexane. Then, it was washed in 20 ml ethanol. The produced polymer and ethanol solvent were put into a dialysis bag up at room temperature for 4 h [20].

Fourier transforms infrared (FTIR) spectroscopy

The IR spectra of the NP were performed with a Nicolet 320 spectrophotometer FT-IR spectrometer which was prepared by mixing the fine powder with KBr and pressing. The spectra were obtained at a resolution of 4 cm^{-1}in the range 4,000–500 cm^{-1}[21].

Nuclear magnetic resonance (NMR)

All NMR experiments were conducted on a Bruker DRX 400 (400 MHz, USA) apparatus in D_2O as solvents. Identical spectra were obtained by dissolving samples in D_2O and the spectra were recorded at 500 MHz (in ^1H and ^{13}C NMR spectra for all temperatures and concentrations). The resulting data were processed and analyzed using ACDLABS/1D NMR software [22].

Encapsulation of thymol and extract by NP

One-tenth gram (1.67×10^{-2} mmol) of NP was dissolved in 5 ml distilled water and stirred for one h. Then thymol (Sigma Aldrich) dissolved in Dimethyl sulfoxide (DMSO)(Merck) as stock solutions (0.1 mmol) was added dropwise to NP mixture and various concentrations of thymol were obtained (25, 50, 100, 150 µM). These solutions were sonicated at room temperature for obtaining the final product [23].

Loading and releasing capacity by HPLC method

HPLC method was used to determine the loading capacity, according to previous studies [24]. To estimate the amount of pure thymol loaded on NP, a reversed-phase HPLC with a Knauer liquid chromatography (Smart line; Knauer, Berlin, Germany) equipped with an ultraviolet detector (Well chrome, K-2600; Knauer) and a reverse-phase C18 column (Nucleosil H. P.; 25 cm _ 0.46 cm internal diameter, pore size mm; Knauer) using isocratic elution with UV absorbance detection was used. The mobile phase was made up of 40% methanol and 60% aqueous solution of formic acid (0.1%). The test equipment was calibrated at a wavelength of 284 nm with a column temperature of 25 ° C, with a volume of injection of 1 µl, and a mobile phase flow rate of 1 ml/min. After 1 h sonication for encapsulation, a water solution of NP-pure thymol was prepared. To remove the non-encapsulated pure thymol residue, the solution was centrifuged at 10,000 rpm and, after precipitation, the supernatant was filtered [25]. An aliquot of the solution after filtration was injected into the HPLC to determine the concentration of encapsulation. *In vitro* release of pure thymol from NP was carried out by dissolving 5 mg of pure thymol loaded NP in 3 ml of PBS (0.1 M, pH 7.4). The NP solutions containing the pure thymol (1 ml) were manipulated according to the previous section [26].

Experimental animals

In the present study, one hundred ten Wistar rats (Pasteur Institute of Iran), weighing 240±30 g were used. The animals were easily accessible to standard water and pellet diet and were kept in standard plastic cages in 12 h of darkness and 12 h of light at an ambient temperature of 25±2. To acclimatize them to the laboratory environment, it was given ten days before the time of the study. The ethical approval for this study was obtained from the Animal Care and Ethics Committee (ACEC) of the Ilam University of medical science (IR. MEDILAM. REC.1396.84). According to ACEC recommendations, we tried our best to minimize research animal pain and suffering. To minimize effects of transportation-induced physiological changes on subsequent biomedical research, it is advisable to consider two factors According to the first factor, in the present study, it was noted that the animal transfer according to the physiological conditions in accordance with the international protocols with the least stress of the animal is carried out and according to the second factor, in general, mediators of stress response to reach the desired conditions (for example, for 24 h) Was considered.

Acute toxicity test (LD50)

The toxicity test of the thymol and thymol nano polymer were carried out using the Acute Toxic Method [38]. Each dose of thymol and thymol nano polymer were administered to three male rats (240–250 g; total). The initial dose of 10 mg/kg was gradually increased up to 1500 mg/kg, according to the mortality rate during the 24 h exposure period. The animals were kept under observation for 14 d [27].

Induction of diabetes

Diabetes was induced in overnight fasted Wistar rats by single dose intraperitoneal injection of freshly prepared Streptozotocin (STZ) (Sigma-Aldrich) at 60 mg/kg body weight. Positive control for diabetic rats, glibenclamide (2.5 mg/kg, Merck) has been used. The non-diabetic rats (negative control) also received normal saline. After 72 h STZ administration, tail blood samples of the overnight fasted rats were collected to measure blood glucose levels [28].

Experimental design

To evaluate the anti-hyperglycemic effect of thymol in normal and STZ-induced diabetic rats, the animals were divided into 11 equal groups (n=10), as follows:

Group 1: Untreated diabetic rats;

Group 2: Untreated normal rats;

Group 3: Normal rats treated with thymol (60 mg/kg).

Group 4: Normal rats treated with thymol nano polymer (60 mg/kg)

Group 5: Diabetic rats treated with thymol (30 mg/kg)

Group 6: Diabetic rats treated with thymol (60 mg/kg)

Group 7: Diabetic rats treated with thymol (90 mg/kg)

Group 8: Diabetic rats treated with thymol nano polymer (30 mg/kg)

Group 9: Diabetic rats treated with thymol nano polymer (60 mg/kg)

Group 10: Diabetic rats treated with thymol nano polymer (90 mg/kg)

Group 11: Diabetic rats treated with glibenclamide (2.5 mg/kg)

Ten days after induction of diabetes, the diabetic animals received the thymol, thymol nano polymer and glibenclamide, respectively. Non-diabetic animals received (60 mg/kg) thymol and thymol nano polymer by gastric gavage needle (NG tube). The untreated normal and diabetic rats were given normal saline [28].

Collection of blood and determination of biochemical parameters

Blood samples were collected from tail vein rat in 2 w after the administration of thymol and thymol nano polymer and blood glucose levels were determined. At the completion of the treatments, the animals were fasted overnight and then blood samples were drawn from their retro-orbital plexus. Immediately after blood

samples collection, serum was isolated by centrifugation at 3,000 rpm for 10 min and then analyzed for various biochemical parameters. The serum samples were stored at −80 °C in a freezer until they were analyzed. For biochemical analysis were used standard commercial kits according to the manufacturer's instructions. Fasting serum glucose level was determined on day 14 by glucose oxidase-peroxidase method using the kit. Alanine and aspartate aminotransferase (ALT and AST) and alkaline phosphatase (ALP) were measured using kits (Nanjing Jiangcheng Bioengineering Institute, Nanjing, China). Serum bilirubin was determined using a commercial kit (RANDOX Laboratories Ltd., UK) [28].

Statistical analysis

The results were expressed as mean±SD. Data analysis was performed by the SPSS version 21 using one-way analysis of variance (ANOVA) tests. To assess the individual variations between the control and treatment groups, P<0.05 was considered the significance level. Terms half-maximal lethal dose (LD_{50}) refers to the concentration of a chemical, drug or toxic substance that produces a response halfway between the baseline and maximum after a specified exposure time.

RESULTS

Evaluation of hyperbranched polyester

By using spectroscopic methods such as IR spectra, ^{1}H, and ^{13}C NMR spectra, the structure of the synthesized nano polymer modified by oleic acid (NP) were evaluated. The IR spectrum of the NP compound is shown in fig. 1. The peak of the carbonyl group appears at 1738 ppm. This displacement in this spectrum is due to the formation of a new carbon derived from the carbonyl group, which is derived from the combination of hydroxyl groups of the NP compound with the oleic acid. As shown in the fig., after the addition of the oleic acid group (due to the presence of a long chain of carbon) to the NP compound, the peak strength of the hydroxyl group in the NP composition decreased to the NP compound, with a peak of 3485 ppm.

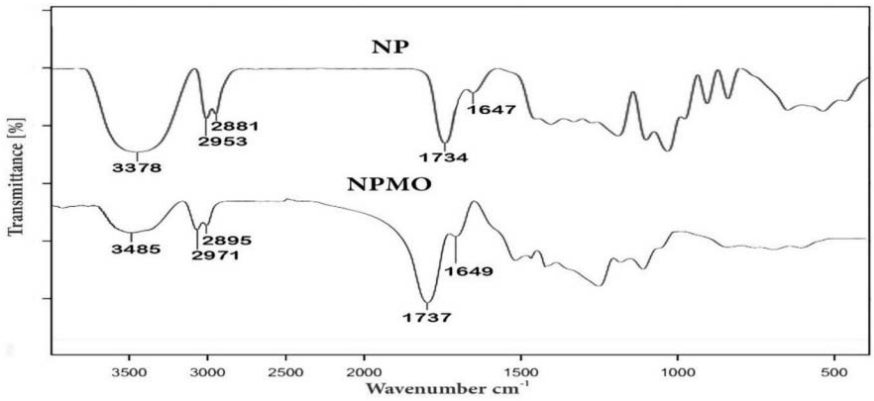

Fig. 1: FT-IR spectrum of nano polymer modified by oleic acid (NP)

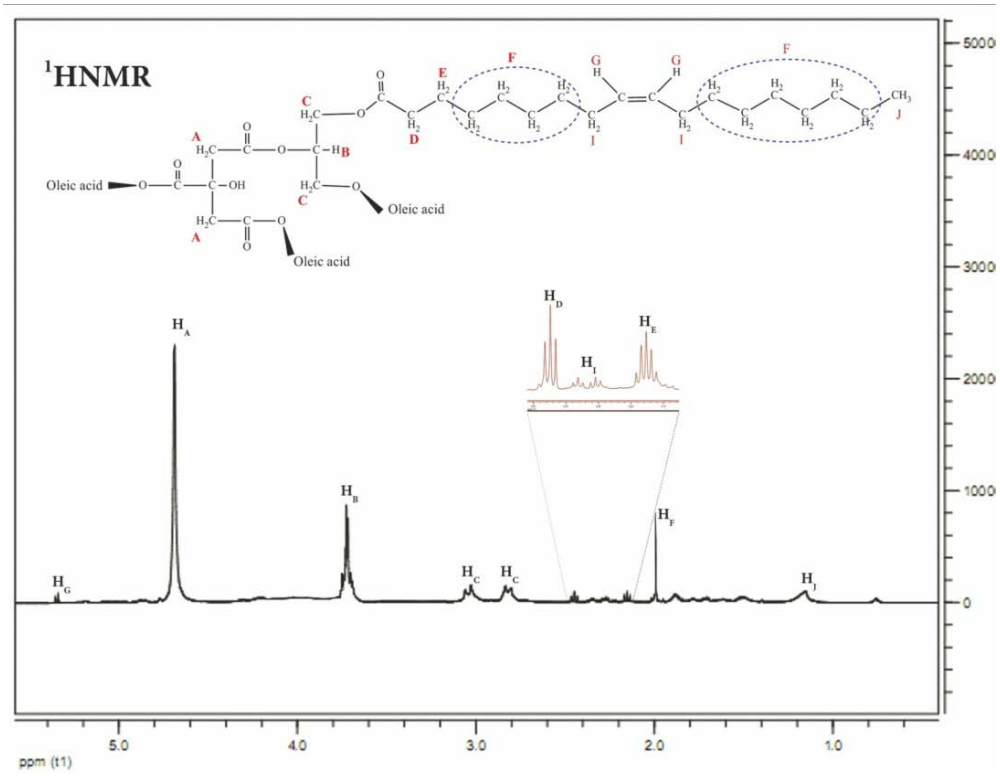

Fig. 2: ^{1}H NMR spectrum of nano polymer modified by oleic acid (NP) in D2O

The HNMR spectrum of the NP compound is shown in fig. 2. For a more straightforward interpretation, these combinations of hydrogens with identical positions are named with the same letters. Hydrogen A is the hydrocarbon content of the monomeric citric acid present in the compound, a single peak at the highest intensity at 4.37 ppm. Hydrogen B is actually single hydrogen-related monomer glycerol, which appears at 3.87 ppm. Hydrogen C is methylated glycerol, which has two separate couriers due to the presence of neighbor hydrogen. Hydrogen D is related to the methane adjacent to the carbonyl group in the oleic acid monomer, split into a triplet peak at 2.45 ppm due to the presence of two hydrogen neighbors. Hydrogen E is a methyl group adjacent to hydrogen D, which has been split into a five-pixel peak due to the presence of four hydrogen neighbors. Hydrogen, named after the letter "F", appears in a single-pixel at 2 ppm because of the same space-spatial position. Because of the dual-bonded proximity to hydrogen, I hydrogen has a different position with neighboring hydrogen and appears as a peak in the range of 2.2 to 2.3 ppm. The G-hydrides appear to be in the form of a double peak at 5.26 ppm due to the presence of the double bond, and eventually, the J-hydrides belong to the end-methyl group of the oleic acid chain, which, with minimal displacement, is a multiplicative peak at 1.15 ppm appear.

The CNMR spectrum of the NP compound is presented in fig. 3. Regarding the fact that there are 12 types of carbon in the synthetic composition, in the CNMR spectrum, this combination has a 12-peak index of the carbon compounds of this composition, which, according to the form, is named all the carbons with the letters A to M, each of which Are on the spectrum. Equivalent carbon is indicated by a letter as described below. Carbon A is the middle carbonic acid group of citric acid, which appears at the end of the spectrum with a displacement of 177 ppm. Carbon B is the fourth type of citric acid that appears at 42 ppm. Methionine citric acid carbonated with the letter C appears at 28 ppm. The two carboniferous carbon represented by the letter D appears at 172 ppm. The middle carbon of glycerol is marked with the letter E, which is given at 73 ppm. Methyl glycerol carbonates have been identified with the letter F, which appear as a peak at the highest intensity of 68 ppm. Carbon is the carbonyl oleic acid group with the letter G, which has the largest displacement and peak at 183 ppm. Carbonyl adjacent carbonyl oleic acid group has a specific displacement due to its direct connection with the carbonyl group, with a different displacement than its other neighboring carbon, with the letter H marked with a peak of 38 ppm. Carbon labeled with the letter "I" has a roughly identical position and appears as a peak of more intensity than the other oleic acid carbon at 26 ppm. The adjacent dual-carbon carbons are positioned differently from other neighboring carbon and are marked with the letter J and have a peak at 21PPM. The carbons that are connected by a double bond are named with the letter K and appear as a peak at 130 ppm. At the end of the carbon, the methyl group has appeared with a minimum displacement of 9 ppm.

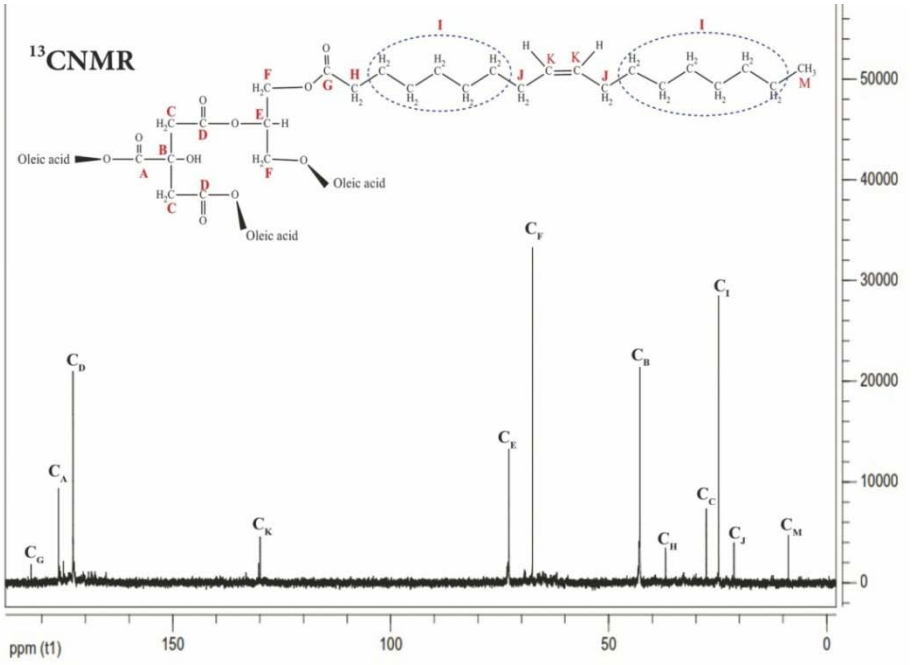

Fig. 3: ^{13}C NMR spectrum of nano polymer modified by oleic acid (NP) in D2O

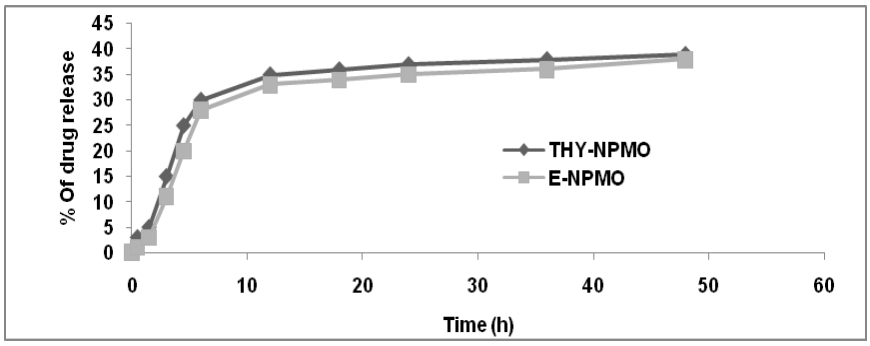

Fig. 4: *In vitro* release of thymol loaded NP (0.1 g, 1.67×10^{-2} mmol) in distilled water (HPLC grade) at 37 °C. Data represented as mean±SEM, (n=3)

Evaluation of the loading and releasing

By HPLC, loading capacity and release of thymol were evaluated from the NP. Thymol standard curve was used to calculate thymol loaded into the NP. After three repetitions and injection into HPLC, the loading rate of thymol was estimated at 43±2.5 %.

Thymol was released in two fast and slow phases of the NP. In the first twelve hours, approximately 65% of thymol released from a fast and steep gradient of thymol nano Polymer, and the remaining 80-85 % to 48 h was released at a lower rate and less slope. NP showed slow biphasic release kinetics with a faster release phase during the initial period as shown in fig. 4.

Toxicity test (LD50)

The result of the acute toxicity test showed that the LD_{50} for thymol and thymol nano polymer were 435 and 583 mg/kg, respectively. So doses of 30, 60 and 90 mg/kg were selected to continue the experiment.

Effect of thymol and thymol nano polymer on body weights

Bodyweight of different tested groups was recorded in table 1. There was no significant difference in body weight between groups before induction of diabetes. By the end of the second week after induction of diabetes, diabetes symptoms appeared such as weight loss when compared with the non-diabetic rats. A significant (P<0:05) decrease (14.6%) in the body weights of diabetic rats was observed after induction of STZ in the animals. Weight loss, in the group treated with thymol nano-polymer 90 mg/kg, similar to diabetic group.

Effect of thymol and thymol nano polymer on hyperglycemia of diabetic and normal rats

The results show that after induction diabetes, in untreated diabetic rats (Group 1), serum glucose levels increased compared to those of non-diabetic rat (Group 2) in weeks 2. Treatment with thymol and thymol nano polymer at 30, 60 and 90 mg/kg for 2 w reduced the glucose concentration in diabetic rats by dose-dependent manner, respectively. In non-diabetic groups (group 3 and 4), 60 mg of thymol and thymol nano-polymer were used, which was not significantly different from glucose level in group 2. (table 2, P<0.05).

Effect of thymol and thymol nano polymer on an insulin level of diabetic and normal rats

The results show that in the groups treated with thymol and thymol nano polymer (30,60 and 90 mg/kg, respectively), as with the diabetic group treated with glibenclamide 2.5 mg/kg, serum insulin levels significantly increased compared to diabetic group. In this study, the highest insulin secretion into serum was observed in the diabetic group treated with thymol nano polymer 90 mg/kg (group 10). However, this effect did not have a significant difference with the group treated with glibenclamide 2.5 mg/kg (table 3, *P*<0.05).

Table 1: Effects of thymol and thymol nano polymer on body weight (g) in STZ-induced diabetic rats and normal rat

Number	Treatment groups	Initial body weight (g)	Secondary body weight (g)
1	Diabetic rat+normal Saline	240.5±1.5	205.2±1.3*
2	Non Diabetic rat+normal Saline	245.2±1.6	293.3±1.9#
3	Non Diabetic rat+thymol 60 mg/kg	247.6±2.1	281.8±2.6*#
4	Non Diabetic rat+thymol nano polymer 60 mg/kg	251.3±0.8	287.6±1.4*
5	Diabetic rat+thymol 30 mg/kg	248.4±1.2	231.1±0.9
6	Diabetic rat+thymol 60 mg/kg	250.8±1.7	210.2±1.1*
7	Diabetic rat+thymol 90 mg/kg	252.1±2.1	201.4±2.7*
8	Diabetic rat+thymol nano polymer 30 mg/kg	249.5±1.7	218.9±2.3*
9	Diabetic rat+thymol nano polymer 60 mg/kg	247.7±1.5	205.5±0.7*
10	Diabetic rat+thymol nano polymer 90 mg/kg	251.6±2.2	199.4±1.3*
11	Diabetic rat+glibenclamide 2.5 mg/kg	245.9±1.8	203.7±1.6*

Notes,±: Standard Error of the Mean; Significant differences each group versus diabetic rats are indicated: *p<0:05; Significant differences each group versus non diabetic rats are indicated: #p<0:05.

Table 2: Effect of thymol and thymol nano polymer on blood glucose in STZ-induced diabetic rats and normal rats

Number of groups	Treatment groups	BG mg/dl
1	Diabetic rat+normal saline	510.11±5.21*
2	Non Diabetic rat+normal saline	91.31±3.24
3	Non Diabetic rat+thymol 60 mg/kg	82.44±2.9
4	Non Diabetic rat+thymol nano polymer 60 mg/kg	87.35±2.3
5	Diabetic rat+thymol 30 mg/kg	410.71±4.43*
6	Diabetic rat+thymol 60 mg/kg	380.63±4.9*
7	Diabetic rat+thymol 90 mg/kg	320.12±4.1*
8	Diabetic rat+thymol nano polymer30 mg/kg	303.67±2.9*
9	Diabetic rat+thymol nano polymer60 mg/kg	300.45±3.38*
10	Diabetic rat+thymol nano polymer90 mg/kg	180.40±2.91
11	Diabetic rat+glibenclamide 2.5 mg/kg	160.35±2.31

Notes,±: Standard Error of the Mean; Significant differences each group versus diabetic rats are indicated: *p<0:05; Significant differences each group versus non-diabetic rats are indicated: #p<0:05.

Table 3: Effect of thymol and thymol nano polymer on serum insulin in STZ-induced diabetic rats and normal rats

Number	Treatment groups	INSULIN mIU/ml
1	Diabetic rat+normal saline	6.21±0.5*
2	Non Diabetic rat+normal saline	15.12±1.6#
3	Non Diabetic rat+thymol 60 mg/kg	14.39±2.2
4	Non Diabetic rat+thymol nano polymer 60 mg/kg	14.81±0.9
5	Diabetic rat+thymol 30 mg/kg	8.37±0.8*
6	Diabetic rat+thymol 60 mg/kg	10.61±1.3
7	Diabetic rat+thymol 90 mg/kg	11.54±1.2
8	Diabetic rat+thymol nano polymer 30 mg/kg	16.19±0.71
9	Diabetic rat+thymol nano polymer 60 mg/kg	17.32±0.8
10	Diabetic rat+thymol nano polymer 90 mg/kg	18.71±1.0
11	Diabetic rat+glibenclamide 2.5 mg/kg	16.32±1.2

Notes,±: Standard Error of the Mean; Significant differences each group versus diabetic rats are indicated: *p<0:05; Significant differences each group versus non-diabetic rats are indicated: #p<0:05.

Evaluation of the effect of thymol and thymol nano polymer on hepatic markers

Our results ascertained the liver function by evaluating the major biochemical indicators of liver functions, including ALT, AST, ALP and bilirubin. As expected, the inductions of diabetes by STZ lead to a significant elevation of the indicators when compared to the control animals (fig. 5-fig. 8). Thymol and thymol nano polymer could significantly lower the levels of the elevated liver parameters.

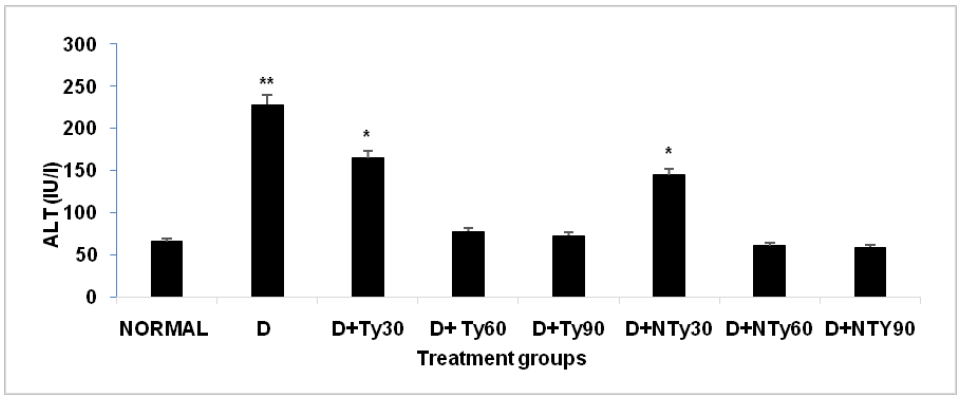

Fig. 5: Effect of thymol and thymol nano polymer on the liver marker enzyme alanine amino transferase (ALT) in STZ-induced diabetic rats and normal rat. Normal: non diabetic rat+normal saline, D: Diabetic rat+normal saline, D+TY 30: Diabetic rat+thymol 30 mg/kg, D+TY 60: Diabetic rat+thymol 60 mg/kg, D+TY 90: Diabetic rat+thymol 90 mg/kg, D+NTY 30: Diabetic rat+thymol nano polymer 30 mg/kg, D+NTY 60: Diabetic rat+thymol nano polymer 60 mg/kg, D+NTY 90: Diabetic rat+thymol nano polymer 90 mg/kg. Significant differences each group versus diabetic rats are indicated; *p<0:05

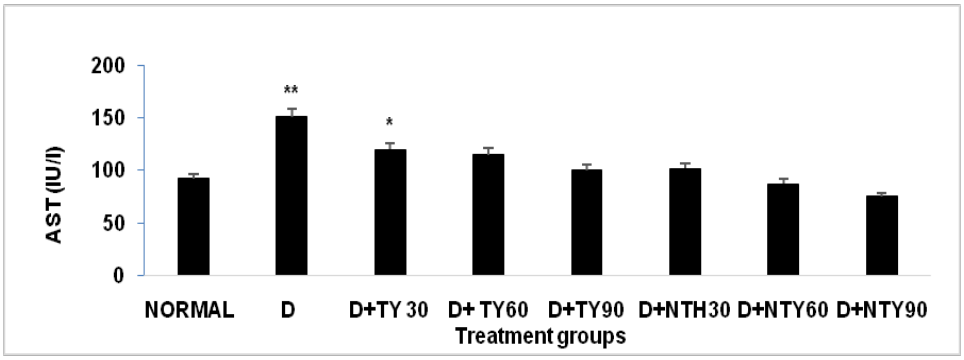

Fig. 6: Effect of thymol and thymol nano polymer on the liver marker enzyme aspartate aminotransferase (AST) in STZ-induced diabetic rats and normal rat. Normal: non diabetic rat+normal saline, D: Diabetic rat+normal Saline, D+TY 30: Diabetic rat+thymol 30 mg/kg, D+TY 60: Diabetic rat+thymol 60 mg/kg, D+TY 90: Diabetic rat+thymol 90 mg/kg, D+NTY 30: Diabetic rat+thymol Nano polymer 30 mg/kg, D+NTY 60: Diabetic rat+thymol nano polymer 60 mg/kg, D+NTY 90: Diabetic rat+thymol nano polymer 90 mg/kg. Significant differences each group versus diabetic rats are indicated; *p<0:05

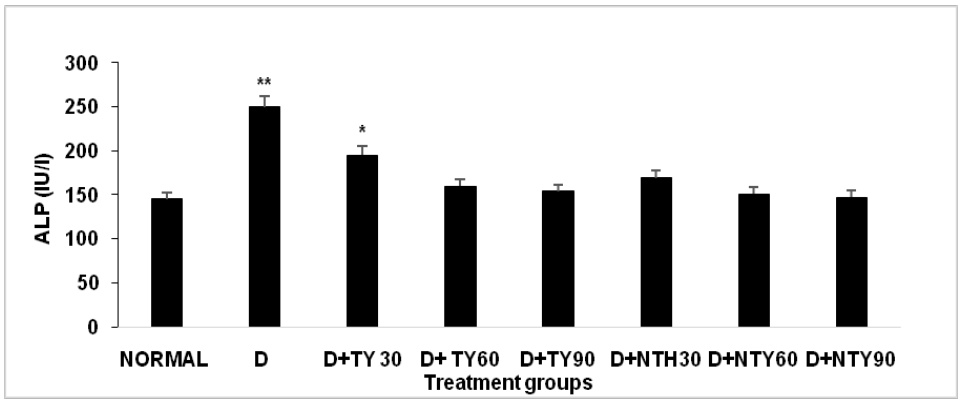

Fig. 7: Effect of thymol and thymol nano polymer on the liver marker enzyme alkaline phosphatase (ALP) in STZ-induced diabetic rats and normal rat. Normal: non diabetic rat+normal saline, D: Diabetic rat+normal saline, D+TY 30: Diabetic rat+thymol 30 mg/kg, D+TY 60: Diabetic rat+thymol 60 mg/kg, D+TY 90: Diabetic rat+thymol 90 mg/kg, D+NTY 30: Diabetic rat+thymol nano polymer30 mg/kg, D+NTY 60: Diabetic rat+thymol nano polymer 60 mg/kg, D+NTY 90: Diabetic rat+thymol nano polymer 90 mg/kg. Significant differences each group versus diabetic rats are indicated; *p<0:05

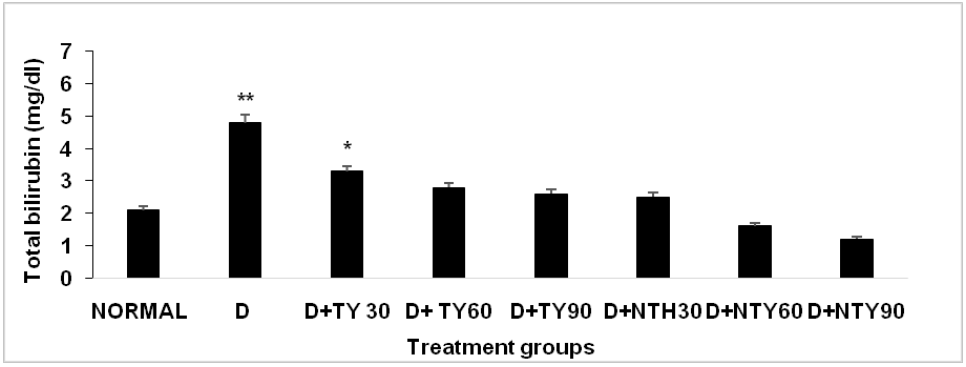

Fig. 8: Effect of thymol and thymol nano polymer on the liver marker enzyme total bilirubin in STZ-induced diabetic rats and normal rat. Normal: non diabetic rat+normal saline, D: Diabetic rat+normal saline, D+TY 30: Diabetic rat+thymol 30 mg/kg, D+TY 60: Diabetic rat+thymol 60 mg/kg, D+TY 90: Diabetic rat+thymol 90 mg/kg, D+NTY 30: Diabetic rat+thymol nano polymer 30 mg/kg, D+NTY 60: Diabetic rat+thymol nano polymer 60 mg/kg, D+NTY 90: Diabetic rat+thymol nano polymer 90 mg/kg. Significant differences each group versus diabetic rats is indicated; *p<0:05

DISCUSSION

To reduce the side-effects and dosing intervals, researchers are now focusing on drug delivery systems. In this regard, some components of medicinal plants such as thymol, with a terpenoids structure, are used for a variety of disorders and diseases [29]. Thus, this is the first study that was initiated with the aim of evaluating the effect of thymol and thymol nano polymer on the blood biochemical parameters and anti-diabetic activity in Streptozotocin-induced diabetic rats. The efficacy was compared with glibenclamide, a standard hypoglycemic drug. Recent research has focused on the possibility using hyperbranched and functionalized polymers to enhance the efficiency of drug delivery systems due to their specific characteristics such as low viscosity, high solubility, plenty of terminal groups and lack of chain entanglements [30, 31]. Due to factors such as small size and excellent biocompatibility, nano polymers modified by oleic acid(NP) are easily fluid in the bloodstream and will reach target tissues as well as increase binding to cell receptors [32]. This polymer has some cavities for loading drugs and used for loading thymol as a diabetes drug release system. It seems that all interactions between thymol and NP were non-covalent [33, 34]. In an investigation of nanoparticles, citric glycerol hyperbranched polyester was synthesized in different concentrations that were monitored cisplatin as an anticancer drug for loading in polyester [20].

In this study, different analytical techniques have been used to get NP structure, including Fourier transforms infrared **(**FTIR), ^{1}H and ^{13}C nuclear magnetic resonance (NMR). The FTIR spectrum demonstrated in nano polymer (NP) carbon derived from the carbonyl group at 1738 cm^{-1} and the hydroxyl group at 3485 cm^{-1}. The ^{1}HNMRspectrum showed that there are 7 types of hydrogens. Hydrogen A is at 4.37 ppm. Hydrogen B is at 3.87 ppm. Hydrogen C is methylated glycerol, which has two separate couriers due to the presence of neighbor hydrogen. Hydrogen D is at 2.45 ppm due to the presence of two hydrogen neighbors. Hydrogen E is a methyl group adjacent to hydrogen D. Hydrogen F appears in a single-pixel at 2 ppm because of the same space-spatial position. Hydrogen I has a peak in the range of 2.2 to 2.3 ppm. The G-hydrides appear to be in the form of a double peak at 5.26 ppm. Hydrogen J is a multiplicative peak at 1.15 ppm appear.

The ^{13}CNMR spectrum of the NPMO compound displays 12 types of carbon in the synthetic composition. Carbon A is displacement of 177 ppm and carbon B is at 42 ppm. Methionine citric acid carbonated with the letter C appears at 28 ppm and carbon D appears at 172 ppm. Carbon E is given at 73 ppm and F, which appear as a peak at the highest intensity of 68 ppm. Carbon G, has the largest displacement and peak at 183 ppm. Carbon H marked with a peak of 38 ppm and "I" has a roughly identical position at 26 ppm. The adjacent dual-carbon carbons J have a peak at 21 ppm and K appears as a peak at 130 ppm. At the end of the carbon, the methyl group has appeared with a minimum displacement of 9 ppm.

By HPLC method, it is possible to evaluate the amount of NP loading capacity. In this analysis, subsequent to drawing standard curve amount of the loading rate of thymol was estimated at 43±2.5%. In one study, chitosan nanoparticle of thymol with anti-bacterial effects had a loading capacity of about 2.5% [35]. However, in another study, thymol nanospheres as an effective anti-bacterial agent, similar to that of our study, was reported to be about 43 % [36].

In our study, approximately 65% of thymol from the NP in the first 12 h (fast phase) and the remaining 80-85% in the next 48 h was released with slower kinetics and less slope, which indicated two phases the release of thymol from NP. The initial and rapid release of the drug from the polymer is more closely related to the particles of the drug that are surface-coated with nano polymers, and thus the release of the drug increases [37]. In a study, it has been shown that thymol has released about 60% of nanoparticles from natural lipids for up to 18 h [38]. In another study, it has been shown that thymol loaded with hierarchically-structured biogenic silica in the first 48 h has been released about 50% of thymol [39].

Our result of the acute toxicity test showed that the LD50 for thymol and thymol nano polymer was 435 and 583 mg/kg, respectively. In one study, *Tephrosia calophylla* extract has improved the symptoms of diabetics caused by alloxan-induced with a significantly IC50value 400 mg/kg [40].

Previous investigations showed that thymol has various anti-diabetic effects, especially type II diabetes [41, 42]. Decreased body weight in STZ diabetic animals is associated with increased blood glucose, inhibition of insulin level, and enhancement of muscle wasting [43, 44].

Our results indicated that there was no significant difference in body weight between groups before induction of diabetes. Diabetes symptoms appeared such as weight loss when compared with the untreated diabetic rats. Weight loss, in the group treated with thymol nano-polymer 90 mg/kg, similar to diabetic group. In a study similar to this study, it has been shown that the weight of diabetic rats has significantly decreased. However, the use of different concentrations of *Zataria multiflora* extract**,** which contains thymol and carvacrol [45], has not been able to reduce the weight of the diabetic control group in diabetic rats [46]. Also, according to the previous study, *Thymus praecox* subsp. *skorpilii*,which contains thymol, significantly decreased enhanced oral glucose tolerance, insulin tolerance and weight loss [47].

Our results showed that after induction diabetes, in untreated diabetic rats, serum glucose levels increased compared to non-diabetic rats. Treatment with thymol and thymol nano polymer reduced the glucose concentration in diabetic rats by dose-dependent manner. Also, the results from this study showed that in the groups treated with thymol and thymol nano polymer, as with the diabetic group treated with glibenclamide, serum insulin levels significantly increased compared to the diabetic group. Previous

studies, in confirmation of our study, have shown that increase blood glucose levels and decrease insulin levels in STZ diabetic rats, confirmed that hyperglycemia is a main cause for disruption of insulin in the optimal rate [16, 41, 42] Moreover, in one research treatment by thymol led to a significant reduces in the blood glucose level and increase in serum insulin level after 28 d in the STZ-induced diabetic rats [48]. However, the anti-diabetic mechanism of thymol, which is responsible for increasing the rate of serum insulin secretion and decreasing of blood glucose, is still unclear [42].

Our results demonstrated the liver function by evaluating the major biochemical indicators of liver functions, such as ALT, AST, ALP and bilirubin. As expected, the inductions of diabetes by STZ lead to a significant elevation of the indicators when compared to the non-diabetic animals. In all the tested parameters, the liver functions of the diabetes-induced rats could be significantly improved by thymol and thymol nano polymer. ALT and AST are the important hepatic enzymes due to their levels in serum as the specific and reliable biomarker for liver damages [42, 49, 50]. In confirmation of our results, it was shown in a paper that thymol 40 mg/kg has been able to significantly reduce ALT and AST in diabetic rats by STZ and help animal health [48]. It has been documented that the *Zataria multiflora* essential oil has been able to protect the increase of ALP produced by STZ in diabetic rats [51]. It has already been documented that the ethanolic extract of the *Artocarpus heterophyllus*, which contains terpenoids, reduces the amount of bilirubin in diabetic rats in a dose-dependent manner [52]. Also, the aqueous extract of the *Canarium odontophyllum,* containing terpenoid and flavonoid, reduced the amount of total bilirubin in streptozotocin-induced diabetic rats [53].

CONCLUSION

Thymol and thymol nano polymer affected by increasing insulin secretion, lowering blood glucose, and improving the function of liver enzymes in STZ-diabetic rats. The effects of thymol nano polymer on improving the above mentioned were greater than thymol. The use of thymol nano polymer seems to be of interest to diabetics as alternative medicine.

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ABBREVIATION

Diabetes mellitus (DM), Nano polymer (NP), glycerol (G), Citric acid (CA), Tetrahydrofuran (THF), Fourier transforms infrared (FTIR), Nuclear magnetic resonance (NMR), Streptozotocin (STZ), alkaline phosphatase (ALP), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT).

AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICT OF INTERESTS

The authors state no conflicts of interest in the manuscript

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