# Antioxidative, Hepatoprotective, and Antidiabetic Properties of The Chitosan Nanoparticles Loaded with Hydroalcoholic Extracts of Aerial Part of the *Hypericum perforatum L.* and *Trigonella gracum* Seeds in Streptozotocin-Induced Diabetic Rats

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## Abstract

This study performed to compare the effects of metformin, *Hypericum perforatum L.*, and *Trigonella gracum* seeds alone and combined with chitosan nanoparticles on streptozotocin-induced diabetes in rats. 96 adult male Wistar were divided into 12 groups: Control, diabetic,

diabetic mellitus receiving buffer, pure chitosan nanoparticles, metformin at a dose of 250 mg/kg, hydroalcoholic extracts of *Hypericum perforatum L.* at a dose of 200 mg/kg and *Trigonella gracum* seeds at a dose of 100 mg/kg, and nano-extract of *Hypericum perforatum L.*, nano-extract of *Trigonella gracum* seed, alone and in combination. The healthy group received extracts of both plants at a dose of 300 mg/kg. The biochemical parameters of liver enzymes, blood glucose¸ rat weight, malondialdehyde (MDA), ferric reducing ability of plasma (FRAP) and superoxide dismutase (SOD), and histopathological changes in liver tissue were determined. The diabetic groups treated with metformin and nanoparticles containing two extracts alone and in combination significantly improved rats weight, alkaline phosphatase (ALP), MDA, and SOD (P-value $\leq$ 0.05). Chitosan nanoparticles containing the combined extracts showed more significant improvement of glucose levels than the diabetic groups treated with the extracts alone (P-value $\leq$ 0.05). The livers of diabetic rats treated with an alone extract and nano-extract of *Trigonella gracum* seeds and with a combination of the selected extracts of both plants alone and in combination with nanoparticles showed significant improvement in histopathological changes. It seems that chitosan nanoparticles containing combined extracts of *Hypericum perforatum L.* and *Trigonella gracum* seeds are good candidates for further evaluation as effective factors for control of diabetes.

**Keywords:** Metformin, Streptozocin, Rats, Chitosan, Nanoparticles, *Hypericum perforatum L.*, *Trigonella gracum*

# 1. Introduction

The number of people with diabetes mellitus worldwide has increased significantly in the last two decades. The International Diabetes Federation predicts there will be 578 million more adults with diabetes by 2030 and as many as 700 million by 2045. Based on World Health

Organization reported in 2020, the overall prevalence of diabetes in Iran is 10.3%. Individuals, societies, and economies are impacted by diabetes, which costs $760 billion annually in healthcare (1). Multiple pathogenic processes play a role in the development of diabetes. Insulin deficiency can be caused by autoimmune destruction of pancreatic β -cells or abnormalities leading to insulin resistance. The lack of insulin action in the target tissues is responsible for the abnormalities in carbohydrate, fat, and protein metabolism in diabetes (2). There is increasing evidence that oxidative stress plays a role in developing diabetes mellitus and its complications. The metabolic abnormalities of diabetes lead to mitochondrial superoxide overproduction (3). There are several treatment options for this disease, such as lifestyle changes and medications, the most well-known is using various drugs such as metformin and injectable insulin. Metformin or 1,1-dimethylbiguanide is the most widely prescribed as oral hypoglycaemic drug by improving insulin resistance (4). Medicinal plants are widely used to treat and control diseases because they are less expensive and have fewer side effects than synthetic drugs, including *Hypericum perforatum L.* (5) and *Trigonella gracum* (Fenugreek or Leguminosae) (6). The bioactive compounds of *Hypericum perforatum L.* include hypericin, pseudohypericin, hyperforin, adperforin, and phytoestrogens such as kaempferol, rutin, quercetin, luteolin, myristicin, and tannins. The most common use of this plant is for its antidepressant properties. It also has anti-inflammatory, antimicrobial, anticancer, antiviral activities, and obesity-associated complications such as Type II diabetes (5, 7, 8). The active components of *Trigonella gracum* include steroidal saponins such as diosgenin, gitogenin, alkaloids such as trigonelline, gentanin and carpaine choline, flavonoids such as quercetin, epigenin, orientin, isoorientin, kaempferol, vitexin, and tannic acid. The most common uses of this plant are for menstrual pain, relieving stomach problems, antioxidant, antibacterial, antifungal, anti-inflammatory, antihyperlipidemic, antihypertensive and antidiabetes. Its seeds is rich in fibre containing steroidal saponins and proteins comparable to those of soybean (6, 9-11).

## Objectives

Considering that the seeds of *Trigonella gracum* (6, 12) and *Hypericum perforatum L.* (7, 8, 12) are used alone in traditional medicine as antidiabetic agents and possess antioxidant properties, the main objective of this study was to evaluate the effect (to compare the impact) of metformin,

*Hypericum perforatum L.* (herbal number: Hyu325B107) and *Trigonella gracum* seeds (herbal number: Hju1142) alone and in combination with chitosan (low molecular weight) nanoparticles on streptozotocin (STZ)-induced diabetes in rats.

# 2. Material and methods

## 2.1. Collection of plants

*Hypericum perforatum L.* and *Trigonella gracum* were collected in April 2019 from the highlands of *Dena* in *Kohgiluyeh* and *Boyer-Ahmad* province-Iran. The samples were collected from Yasouj Agricultural and Natural Research Centre. After collection, the studied plants were cleaned and placed in the air protected from direct light for drying for several days, then crushed and prepared for extraction. This way, 100 grams of the dried plant was doused with 1000 mL of dissolvable (70% ethanol) which were obtained 8.5 grams extract of *Hypericum perforatum L.* and 2.4 grams of *Trigonella gracum*. The coming about blend was kept at 37 °C for 48 hours. The arrangement was sifted using the Whatman No. 1 philter paper. A revolving gadget concentrated the coming back blend as much as conceivable beneath vacuum conditions. At that point, the extricated was dried in an incubator at 50°C and put away in a cooler at -20 °C (١٣).

## 2.2. Animals and their classification

Male Wistar rats weighing 230–250 g and 84-91 days age were were obtained from Yasuj Animal Service Centre and maintained under standard conditions (12 light–dark cycle; 23±1 °C; 45-55% humidity) with free access to water and conventional rat chow. Diabetes mellitus was initiated in overnight fasting rats by the organization of intraperitoneal infusion of naturally arranged 55 mg/kg streptozotocin (STZ) in 0.01 M citrate buffer (pH 4.5) (CAS: 18883-66-4, Sigma-Aldrich, Germany) (14). After 24 h of STZ organization, rats have gotten glucose arrangement (2 mL/kg bw) to dodge hypoglycemic mortality. After 72, 120, 240, and 336 hours, a blood test was taken from the tail vein of fasting rats, and blood glucose was measured by glucometere to affirm diabetes mellitus. Rats with a fasting blood glucose of > 322 mg/dL were considered diabetic and were utilized for this study (15). After 14 days and diabetic

confirmation, the drugs (On the fifteenth day) were gavage to the animals for 14 consecutive days.

### 2.3. Experimental design

Animals were randomly divided into control (n=8) and diabetic (n=88) participants (four rats per cage). In this study, 96 adult male Wistar rats were used, divided into 12 healthy control groups (control), diabetes mellitus receiving 55 mg/kg STZ (DM), diabetes mellitus with buffer (DM +Bufer), pure chitosan (Molecular Weight of 50-190 kDa, deacitilation degree 75-85%, Sigma-Aldrich, Germany) nanoparticles (DM +Nano), metformin (CAS: 1115-70-4, Molecular Weight 165.62, Sigma-Aldrich) at a dose of 250 mg/kg (DM +Met) (4), *Hypericum perforatum L.* flower extract at a dose of 200 mg/kg (DM +HP) (7), *Trigonella gracum* seed extract at a dose of 100 mg/kg (DM +TG) (9), combined extracts of *Hypericum perforatum L.* and *Trigonella gracum* seeds at a dose of 300 mg/kg (DM +HP+ TG), nano extract of *Hypericum perforatum L.* at a dose of 200 mg/kg (DM +Nano HP), nano extract of Trigonella gracum seeds at a dose of 100 mg/kg (DM +Nano TG), combined nano extracts of *Hypericum perforatum L.* and *Trigonella gracum* seeds at a dose of 300 mg/kg (DM +Nano HP +TG), and healthy recipients of *Hypericum perforatum L.* and *Trigonella gracum* extracts at a dose of 300 mg/kg (Toxic). All prescriptions were administered by gavage at 8-10 am for 14 days. Gavage administration was performed in conscious animals using straight gavage needles (14 gauge, 7.6 cm length, 4 mm ball diameter). Body weight and blood glucose were measured between the first, seventh, and fourteenth days to study the changes.

### 2.4. Preparation method of chitosan nanoparticles

To prepare a clear chitosan solution, 10 mg of chitosan was dissolved in 5 mL of 2% acetic acid. 7 mg of tripolyphosphate was dissolved in 1 mL of distilled water. Then, depending on the dose of the extract used, for example, for *Trigonella gracum* seeds with a dose of 100 mg/kg, 100 mg of weighed extract powder was added to the clear chitosan solution, a magnet was put in it and placed on the stirrer at 900 rpm for 3 minutes. After 3 minutes, tripolyphosphate solution (7mg/1ml distilled water) were added drop by drop and placed on the stirrer for 30 minutes (16).

## 2.5. Biochemical assay

Animals were anesthetized with ether 24 hours after the last day of treatment, blood serum was isolated, and biochemical tests were performed. Serum liver enzymes, including alkaline phosphatase (ALP) (REF: 102400), alanine aminotransferase (ALT) (REF: 118400), and aspartate aminotransferase (AST) (REF: 118400), were determined by enzymatic colorimetric methods. All blood analyses were performed using Pars Azmoon kits (Pars Azmoon Inc., Tehran, Iran).

## 2.6. Measurement of oxidative stress indices

### 2.6.1. Lipid peroxidation assay

Eliza kits from Crystal Day Company-China (Cat. n: E0156Ra) were used to measure the serum level of MDA.

### 2.6.2. Superoxide dismutase (SOD) activity assay

Eliza kits manufactured by Crystal Day Company-China (Cat. n: E0168Ra) were used to measure serum levels of superoxide dismutase (SOD).

### 2.6.3. Measurement of Ferric Reducing Ability of Plasma (FRAP)

One mL of plasma test was put away at -70°C until utilized. The FRAP measure was performed agreeing to the strategy created by Benzie and Strain (17). Briefly, 10 mL of plasma was included in 1.8 mL of a naturally arranged FRAP arrangement, and the absorbance was measured at 593 nm.

## 2.7. Histological analysis

In the histopathologic examinations of rat liver by light microscopy, to evaluate the histological changes, the sample was kept in formalin, and then 5-micron sections were prepared from the tissues and stained with hematoxylin and eosin method (18). The dissected slides were

examined for portal vein inflammation, sinusoidal dilatation, focal inflammation in the liver parenchyma, fibrosis, and steatosis.

### 2.8. Statistical Analysis

Data are presented as mean ± SEM. Statistical differences between groups were analyzed by one-way ANOVA and Duncan test (post hoc). The significance level was set at a P value $\leq 0.05$. This work used Smirnov's Cumulogenov test to determine whether the variables studied had a normal distribution; if not, nonparametric tests were used.

## 3. Results

### 3.1. Functional Findings

The main functional parameters measured 15 days after treatment are summarized in Table 1. Compared with the control group, STZ-treated rats showed a significant increase in glucose levels. In addition, serum concentrations of glucose were significantly increased in all experimental groups compared with the control group. All diabetic rats showed a significant difference from the control group on the first day of the study. However, no significant difference was observed between the toxic and control groups. The control, toxic, DM +HP, and DM +TG groups showed a significant difference from the DM group on the first day of the study. The control, toxic, DM +Met, DM +TG, DM +HP+ TG, DM +Nano HP, DM +Nano TG, and DM +Nano HP +TG groups showed a significant difference from the DM group on the 15th day of the study. The combination of extracts alone and with chitosan nanoparticles caused a decrease in blood glucose levels compared to the control group. Metformin and the extracts improved blood glucose levels on day 15 compared with day 7 (Figure 1B).

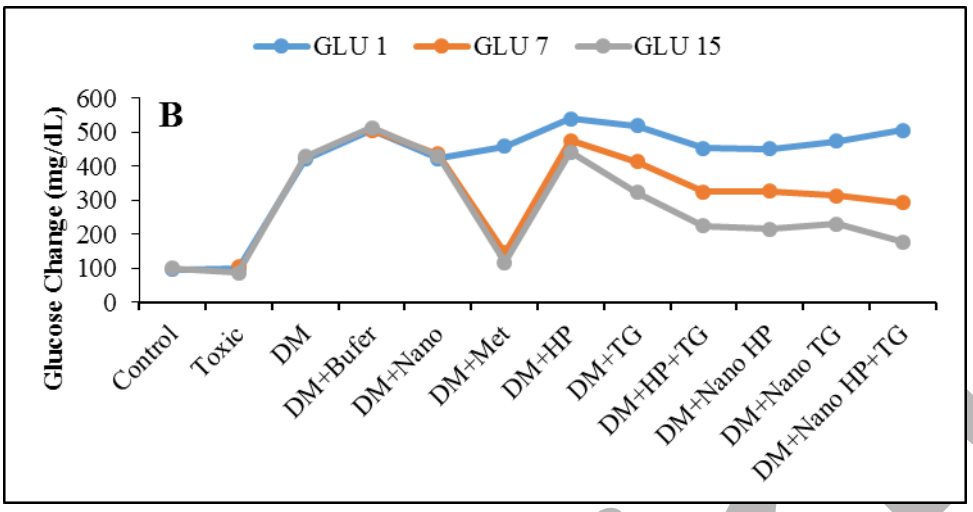
The result of the changes in body weight is shown in Figure 1C. Figure C shows that all diabetic rats differed significantly from the control group. In addition, no significant difference was found between the toxic and control groups. The control, toxic, DM +TG, DM +HP+ TG, DM +Nano HP, DM +Nano TG, and DM +Nano HP +TG groups showed a significant difference from the DM group on the 15th day of the study, but the DM +Met and DM +HP groups showed no significant difference. Figure 1C also shows that the changes in glucose levels on days 1 and 15

in all diabetic rats except the Toxic, DM +Nano, DM +HP, and DM +Buffer groups showed a significant difference compared with the DM and control groups.

Table 1: The effect of chitosan nanoparticles containing hydroalcoholic extracts from the aerial part of *Hypericum perforatum L.* and *Trigonella gracum* seeds on rats' blood glucose levels on day 1, day 7, and day 15.

| Groups | Glucose of 1st day (mg/dL) | Glucose of 7th day (mg/dL) | Glucose of 15th day (mg/dL) | Weight of 1st day (g) | Weight of 15th day (g) |
|---|---|---|---|---|---|
| **Control** | 97±4[b] | - | 101±4[b] | 237±5 | 261±7[b] |
| **Toxic** | 99±3[b] | 106±22 | 88±2[b] | 226±2 | 256±6[b] |
| **DM** | 420±7[a] | - | 429±12[a] | 237±5 | 190±4[a] |
| **DM+Bufer** | 508±27[a] | 506±29 | 514±23[a] | 244±5 | 219±6[ab] |
| **DM+Nano** | 424±14[a] | 435±25 | 430±21[a] | 235±5 | 195±6[a] |
| **DM+Met** | 458±19[a] | 146±14 | 118±7[b] | 232±3 | 203±4[a] |
| **DM+HP** | 540±14[ab] | 475±20 | 442±21[a] | 234±5 | 198±5[a] |
| **DM+TG** | 518±19[ab] | 414±19 | 324±26[ab] | 240±4 | 230±5[ab] |
| **DM+HP+TG** | 453±22[a] | 325±19 | 224±12[ab] | 241±3 | 221±4[ab] |
| **DM+Nano HP** | 451±7[a] | 326±21 | 216±15[ab] | 240±3 | 218±4[ab] |
| **DM+Nano TG** | 473±16[a] | 314±19 | 231±16[ab] | 243±3 | 223±3[ab] |
| **DM+Nano HP+TG** | 507±20[a] | 292±15 | 178±9[b] | 240±5 | 222±3[ab] |

**Abbreviation**: HP: *Hypericum perforatum*; TG: *Trigonella gracum*; Nano: Chitosan Nanoparticles; DM: Diabetic mellitus; Met: Metformin; mg/dL: milligrams per deciliter; g: gram. [a]Significant difference compared to the Control group (P-value $\leq$ 0.05). [b]Significant difference compared to the DM group (P-value $\leq$ 0.05).



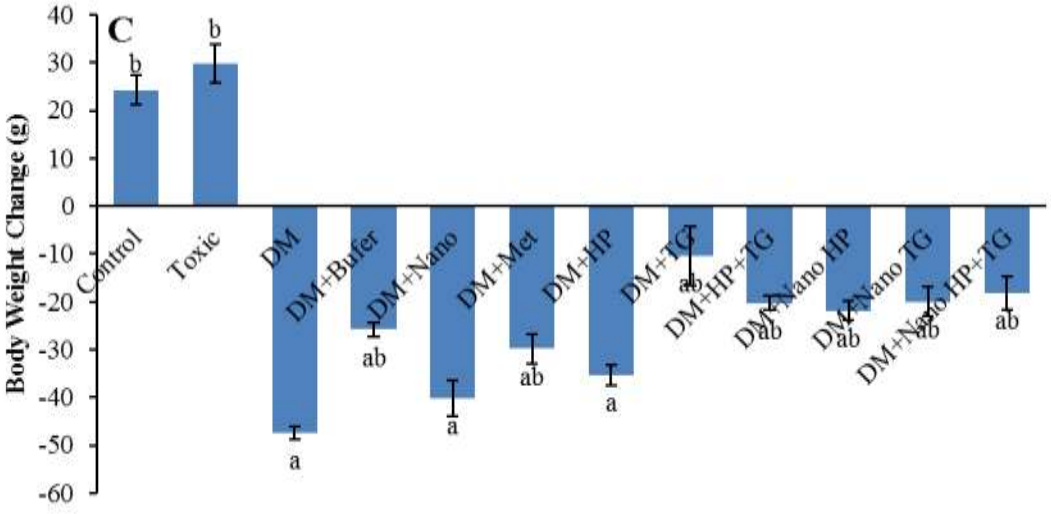
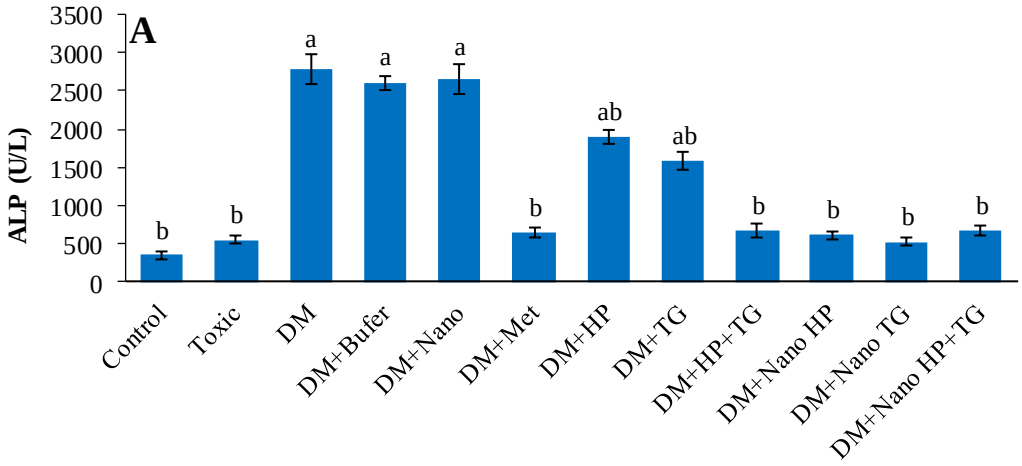




Figure 1. The effect of chitosan nanoparticles containing two hydroalcoholic extracts of *Hypericum perforatum* and *Trigonella gracum* seeds on blood glucose on the 15$^{th}$ day (A), changes in glucose during the study period (B), and comparison of the body weights in different groups based on the Duncan test during the study period (C). According to Duncan's test, the columns with at least one common letter differ significantly. **Abbreviation**: HP: *Hypericum perforatum*; TG: *Trigonella gracum*; Nano: Chitosan Nanoparticles; DM: Diabetic mellitus; Met: Metformin; mg/dL: milligrams per deciliter; g: gram. [a]Significant difference compared to the Control group (P-value ≤ 0.05). [b]Significant difference compared to the DM group (P-value ≤ 0.05). GLu1: Glucose of 1 day (mg/dL); GLu7: Glucose of 7 day (mg/dL); GLu15: Glucose of 15 day (mg/dL).

### 3.2. Evaluation of Oxidative/Antioxidant Status and biochemical parameters

٢٠٤ Liver injury was assessed by determining serum levels of liver enzymes. The average serum
٢٠٥ concentrations of biochemical parameters and oxidative stress markers in the studied groups
٢٠٦ were determined and compared in Table 2. As shown in Figure 2A, the serum level of the
٢٠٧ enzyme ALP significantly increased in the DM group (P value ≤ 0.05). However, the serum level
٢٠٨ increase was insignificant for the enzymes ALT and AST (Table 2). Injection of two
٢٠٩ hydroalcoholic extracts of *Hypericum perforatum L.* and *Trigonella gracum* alone and in
٢١٠ combination and with chitosan nanoparticles to diabetic rats resulted in a significant decrease in
٢١١ the serum level of ALP enzyme compared with the DM group (P value ≤ 0.05).

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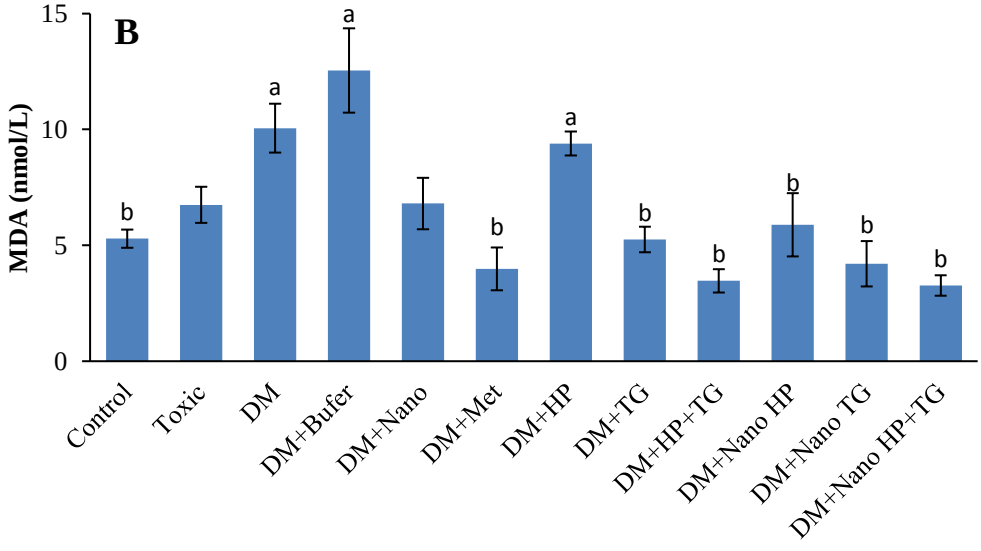


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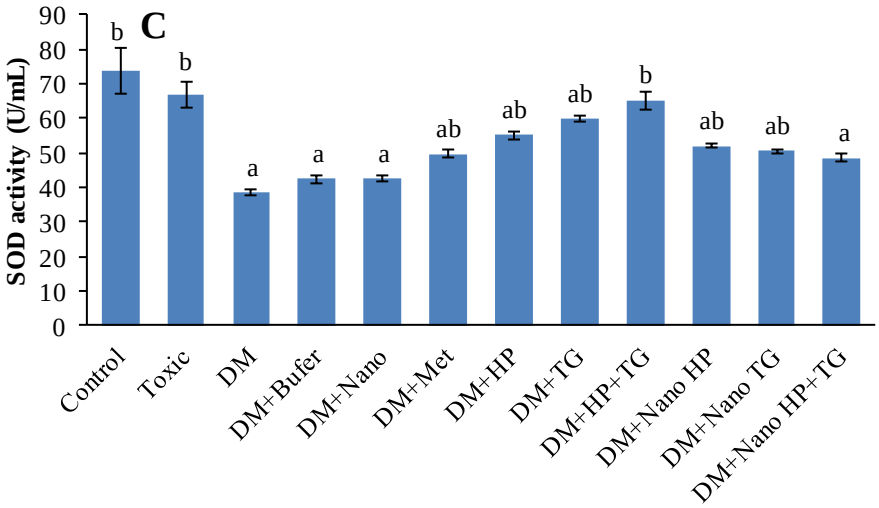


Figure 2: The effect of chitosan nanoparticles containing two hydroalcoholic extracts of *Hypericum perforatum* and *Trigonella gracum* seeds on liver enzymes (A) ALP, (B) serum MDA level, antioxidant enzyme (C) SOD in the studied groups. Comparison of the serum level of liver enzymes in different groups based on the Duncan test. According to Duncan's test, the columns with at least one common letter are not significantly different. **Abbreviation**: HP: *Hypericum perforatum*; TG: *Trigonella gracum*; Nano: Chitosan Nanoparticles; DM: Diabetic mellitus; Met: Metformin. ALP: Alkaline phosphatase; MDA: Malondialdehyde; SOD: Superoxide dismutase; U/mL: Units per litre; nmol/L: nanomoles per litre; U/mL: Units per millilitre. [a]: Significant difference compared to the Control group (P-value ≤ 0.05). [b]: Significant difference compared to the DM group (P-value ≤ 0.05).

As shown in Figure 2B, the MDA level in the DM group was significantly increased compared to the control group (P-value ≤ 0.05). It also appears from Figure 2B that administration of metformin and chitosan nanoparticles containing two hydroalcoholic extracts of *Hypericum perforatum L.* and *Trigonella gracum*, both alone and in combination, to diabetic rats significantly reduced the amount of MDA compared to the DM group (P-value ≤ 0.05). In addition, the amount of MDA in the DM+TG and DM+HP+TG groups showed a significant decrease compared to the DM group (P-value ≤ 0.05).

As shown in Table 2, the FRAP level in the DM group was significantly decreased compared to the control group (P-value ≤ 0.05). However, the level of FRAP non-significantly reduced with

the administration of chitosan nanoparticles containing combined extracts compared to the DM group.

As shown in Figure 2C, the SOD activity in the DM group was significantly decreased compared to the control group (P-value ≤ 0.05), while the administration of chitosan nanoparticles containing the combination of two hydroalcoholic extracts *Hypericum perforatum L.* and *Trigonella gracum* seeds to diabetic rats insignificantly reduced SOD enzyme activity in the DM+Nano HP+TG group compared to the DM group. However, the SOD activity in other groups (administration of metformin, extracts alone and together with chitosan nanoparticles, and also in combination) showed a significant increase compared to the DM group (P-value ≤ 0.05).

**Table 2**: The effect of chitosan nanoparticles containing hydroalcoholic extracts of aerial part of the *Hypericum perforatum L.* and *Trigonella gracum* seeds on biochemical parameters and serum oxidative stress markers in the studied groups.

| Groups | ALP (U/L) | ALT (U/L) | AST (U/L) | MDA (nmol/L) | FRAP (µmol/L) | SOD (U/mL) |
|---|---|---|---|---|---|---|
| **Control** | 346.60± 43.07$^{b}$ | 7.40±3.75 | 155.00±9.12 | 5.29±0.39$^{b}$ | 1468.37± 105.28$^{b}$ | 73.74±6.83$^{b}$ |
| **Toxic** | 554.60±42.73$^{b}$ | 65.00± 5.90 | 130.00± 8.40 | 6.74±0.78 | 1196.50± 187.54 | 66.71±3.76$^{b}$ |
| **DM** | 2781.60± 199.20$^{a}$ | 132.80± 88.86 | 278.40± 173.12 | 10.05±1.05$^{a}$ | 808.00± 19.02$^{a}$ | 38.49±1.07$^{a}$ |
| **DM+Bufer** | 2609.88± 88.28$^{a}$ | 73.13±7.36 | 114.88±10.91 | 25.55±3.82 | 1149.71± 125.64 | 42.41±1.11$^{a}$ |
| **DM+Nano** | 2655.60± 198.76$^{a}$ | 183.40± 48.09 | 455.40± 210.94 | 6.80±1.12 | 1103.50± 89.92 | 42.49±1.05$^{a}$ |
| **DM+Met** | 640.29± 64.22$^{b}$ | 178.80± 24.71 | 318.60± 38.37 | 3.98±0.93$^{b}$ | 1319.42± 119.19 | 49.63±1.30$^{ab}$ |
| **DM+HP** | 1897.00± 102.29$^{ab}$ | 171.75± 35.00 | 306.88± 95.83 | 9.39±0.52$^{a}$ | 979.31± 54.00 | 55.13±1.10$^{ab}$ |
| **DM+TG** | 1589.75± 113.87$^{ab}$ | 163.50± 47.75 | 348.88± 135.01 | 5.25±0.55$^{b}$ | 1056.19± 118.93 | 59.70±0.96$^{ab}$ |
| **DM+HP+TG** | 675.13±87.32$^{b}$ | 113.25± 9.54 | 187.13± 24.71 | 3.47±0.50$^{b}$ | 959.94± 102.64 | 65.04±2.35$^{b}$ |
| **DM+Nano HP** | 617.00±50.82$^{b}$ | 102.38± 17.26 | 196.38± 27.89 | 5.88±1.37$^{b}$ | 1070.88± 96.81 | 51.99±0.69$^{ab}$ |
| **DM+Nano TG** | 523.00±47.04$^{b}$ | 88.20± 18.88 | 208.80± 59.22 | 4.20±0.98$^{b}$ | 1327.21± 174.07 | 50.46±0.72$^{ab}$ |
| **DM+Nano HP+TG** | 671.00±76.67$^{b}$ | 107.00± 6.40 | 222.00± 11.41 | 3.26±0.44$^{b}$ | 739.83± 118.89$^{a}$ | 48.47±1.04$^{a}$ |

**Abbreviation**: HP: *Hypericum perforatum*; TG: *Trigonella gracum*; Nano: Chitosan Nanoparticles; DM: Diabetic mellitus; Met: Metformin; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: aspartate aminotransferase; MDA: malondialdehyde; FRAP: fluoride-resistant acid phosphatase; SOD: superoxide dismutase U/L: Units per litre; nmol/L: nanomoles per liter; µmol/L: micromoles per litre; U/mL: Units per millilitre. [a]: Significant difference compared with the control group (P value ≤ 0.05). [b]: Significant difference compared with DM group (P-value ≤ 0.05).

### 3.3. Histological examination

The dissected slides were examined for portal vein inflammation, sinusoidal dilatation, focal inflammation in the liver parenchyma, fibrosis, and steatosis, as shown in Figure 3. Histological examinations of the rats in the healthy group revealed no specific pathological findings (Figure 3 a-b). The liver sections of the diabetic rats showed sinusoidal enlargements around the portal tract (PT) and the central vein (CV) (Figure 3c). The livers of diabetic rats treated with the hydroalcoholic extract of *Hypericum perforatum L* alone and combined with chitosan nanoparticles showed no significant improvement in histopathological changes, and sinusoidal dilatation around CV was observed (Figures g, j). However, examination of liver tissue showed that the livers of diabetic rats treated with (DM +TG), (DM +Nano TG), (DM +HP+ TG), (DM +Nano HP +TG) showed significant improvement in histopathological changes (Figures h, i, k, l). No signs of steatosis and fibrosis and no specific pathology were observed in any of these studied groups. No specific pathology was observed in the healthy rats receiving the nanoparticle combination of two hydroalcoholic extracts of *Hypericum perforatum L*. and *Trigonella gracum* at a total dose of 300 mg/kg (Figure 3b). However, in the group receiving a mild buffer solution and chitosan nanoparticles, focal inflammation of the portal vein and focal sinusoidal dilatation

around the CV were observed (Figures 3 d,e).

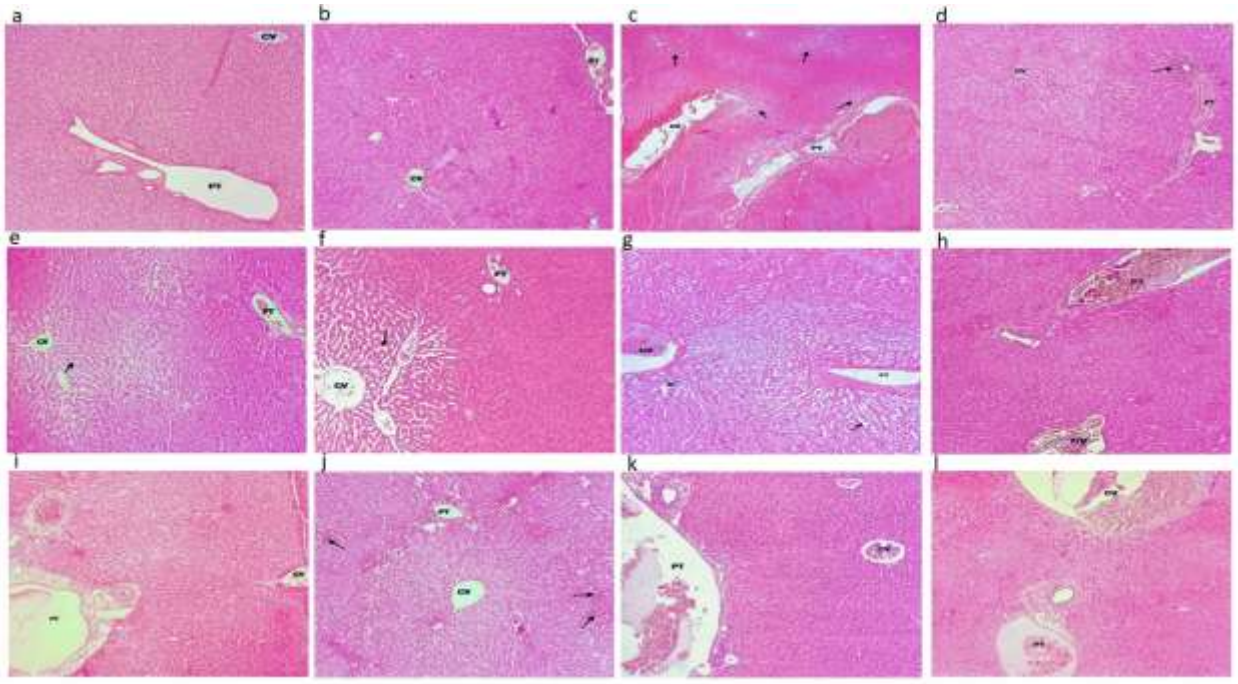

**Figure 3:** H and E tissue staining and scoring of hepatocytes. The animals were slaughtered at the end of the study, and the liver tissue was examined for the percentage of damaged cells. The study groups included a control group, a toxic group, and ten experimental groups. Group (a): Control; (b): Toxic; (c): Diabetic; (d): Diabetic rats received buffer; (e): DM+Nano; (f): DM+Met; (g): DM+HP; (h): DM+TG; (i): DM+HP+TG; (j): DM+Nano HP; (k): DM+Nano TG; (l): DM+Nano HP+TG. **Abbreviation**: HP: *Hypericum perforatum*; TG: *Trigonella gracum*; Nano: Chitosan Nanoparticles; DM: Diabetic mellitus; Met: Metformin.

## 4. Discussion

This study showed that STZ administration in adult male rats significantly increased blood glucose, MDA, and liver enzymes ALP and decreased body weight, FRAP, and serum SOD in the diabetic group compared to healthy rats (P-value $\leq 0.05$). Liver tissue in diabetic animals becomes necrotic, and the increase in enzyme activity is probably the result of its leakage from the liver cytosol into the bloodstream and injection of two hydroalcoholic extracts of *Hypericum perforatum L.* and *Trigonella gracum* seeds alone and in combination with nanoparticles. The results showed that metformin and extracts of *Hypericum perforatum L.* and *Trigonella gracum*

seeds individually and in combination, and chitosan nanoparticles containing a combination of hydroalcoholic extracts of *Hypericum perforatum L.* and *Trigonella gracum* seeds at a dose of 300 mg/kg, or alone significantly improved the above indicators compared with the diabetic group. Administration of chitosan to diabetic rats results in a significant decrease (P-value $\leq$ 0.05) in blood glucose and serum biochemical tests such as ALP, MDA, and the antioxidant enzyme SOD and liver tissue improve the condition in diabetic rats. The reduction in these activities is likely the result of the inhibition of induced liver damage.

In most patients with Type II diabetes, treatment with oral antidiabetic agents is the first-line treatment when lifestyle measures fail. Metformin, sulfonylureas, and thiazolidinediones, the most commonly prescribed antidiabetic agents, can temporarily improve glycemic control. However, despite the continuous introduction of blood glucose-lowering drugs, managing diabetes, and its associated complications remains a major global medical problem (19). Since ancient times, traditional medicine has always paid special attention to medicinal plants, and today, with the numerous researches conducted on medicinal plants, the practical and valuable effects of many plants have been achieved (6).

*Trigonella foenum-graecum* seeds are known for their carminative, tonic, and antidiabetic effects. Researchers have studied the hypoglycemic activities of the aqueous and methanolic extract of *Trigonella foenum-graecum* seeds in normal mice by oral administration (6). The current study and some previous reports indicate the therapeutic impact of *Trigonella graecum* against diabetes by ameliorating diabetic hyperglycemia and associated metabolic abnormalities and reducing oxidative stress (6, 9, 10, 20). Diosgenin saponin as the most bioactive substance of fenugreek has antioxidative effects and plays a pivotal role in improving the diabetic status by several mechanisms (9, 10).

Several plant-derived chemical compounds known as flavonoids and phytoestrogens have inhibitory effects on insulin secretion in humans and animals (9, 21). *Hypericum perforatum L.* and *Trigonella foenum-graecum* seeds contains a few phytochemical constituents, such as flavonoids counting rutin, kaempferol, quercetin and isoquercetin (5-10). For case, rutin has been detailed to advance insulin emission and lower blood glucose levels in diabetic creatures. In rats treated with an ethyl acetate extract of *Hypericum perforatum L.*, a significant decrease in blood

glucose levels and an increase in serum insulin levels were observed. The possible mechanism by which *Hypericum perforatum L.* exerts its hypoglycemic effect in diabetic rats may be that it potentiates plasma insulin action by increasing insulin secretion from existing pancreatic beta cells or its release from the bound form (8). It is also suggested that other than phytoestrogens from *Hypericum perforatum L.* and *Trigonella foenum-graecum* seeds such as quercetin (21, 22), fisetin (23), kaempferol (24), and myricetin (25) may be a potential means of glycemic control by increasing the activity of the insulin-dependent kinase receptor. Therefore, they induce insulin signaling and increase glucose transporters (GLUT4) and glucose uptake (12). Quercetin can stimulate glucose uptake in isolated cells without insulin, possibly due to the increased expression of GLUT4 in the plasma membrane. Quercetin influences flag transduction and utilizes glucose by controlling glucose transport and affront receptor signaling, which plays a comparable part to rosiglitazone as a PPARγ (peroxisome proliferator-activated receptor gamma) agonist and may also inhibit alpha-glucosidase activity. Insulin sensitivity-increasing factors lead to the improvement of diabetes (12, 21). In line with our study, the mentioned extracts may be useful and resulted in an antioxidant activity with an increase of SOD level and a decrease in MDA formation (7, 11). It seems that the hydroalcoholic extracts of *Hypericum perforatum L.* and *Trigonella gracum* seeds combined with chitosan nanoparticles, are good candidates for further evaluation as influential factors in controlling diabetes in the future.


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## Authors' Contributions

M.M, H.K.J, and M.G study concept and design. F.N, H.K.J, M.M, A.H.D, H.B, and M.A did experimental laboratory work, follow-ups, and medical analysis of statistical data. All authors drafting and reviewed the manuscript. All authors give their consent for publishing the article.

## Ethics

The Research Ethical Committee of Yasuj University of Medical Sciences approved this study. All experimental protocols, proposals, and methods followed relevant guidelines. They were approved by the Animal Ethics Committee at Yasuj University of Medical Sciences with the Code of Ethics IR.YUMS.REC.1397.167.

## Conflict of interest

All authors have no conflicts of interest relevant to this article.


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## Data Availability

There are no additional data. All data generated or analyzed during this study are included in this published article.

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