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Impact of Nanoparticle Ociumum Basilicum on Lipid Profile in Diabetes Mellitus Albino Rats

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder that significantly impacts global health. The objective of the present study was to evaluate the therapeutic effects of chitosan nanoparticles loaded alcoholic basil extract against diabetes mellitus in rats. Forty-two adult male rats were divided into six groups, with seven rats in each group. Group one (Control negative). The other five groups were induced with diabetic mellitus using alloxan at a dose of 100 mg/kg. Group two (Control positive). Group three (empagliflozin) at a dose of 25 mg/kg, group four (Nano chitosan) at a dose of 250 mg/kg. While group five (basil seed alcoholic extract) at a dose of 250 mg/kg. Finally, group six (Nanoparticle-basil seed extract) at a dose of 250 mg/kg. Treatments were given orally for 21 days. Blood was collected via cardiac puncture to obtain serum for measuring the parameters of the present study. The induction of diabetes mellitus in groups 2, 3, 4, 5, and 6 was evidenced by increased blood glucose levels. Post-treatment, groups 3, 4, 5, and 6 showed a decrease in glucose concentration compared to the control positive, with all treated groups exhibiting reduced cholesterol levels. The study concluded that the use of basil seed extract combined with Nano-chitosan positively impacts blood sugar reduction and decreases low-density lipoprotein, triglycerides, and total cholesterol levels while increasing HDL and improving lipid profile levels. The use of Nano-chitosan or basil seed extract alone was less effective compared to the nanoparticle basil seed extract.

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INTRODUCTION

Nowadays, a lot of research and studies have been directed towards using plant extracts as an alternative to chemical treatments; such as Moringa oleifera [1], Phoenix dactylifera [2], Zingiber officinale [3], Nigella sativa [4], Alchornea cordifolia [5], and Ociumum Basilicum [6]. Basil seeds

(Ociumum Basilicum) are traditionally used for therapeutic purposes to improve digestive health, regulate blood sugar, aid in weight loss, cool the body, relieve stress, lower blood pressure, improve vision, lower cholesterol, and reduce inflammation [7]. Recent studies have indicated the hypoglycemic and hypolipidemic effects of extracts from various parts of Ociumum sanctum and Ociumum basilicum when tested on rats [8]. It could be believed that the therapeutic effect of Nano-chitosan and its components on modulating lipid synthesis and sterol excretion is probably due to its unique properties. Nano-chitosan decreases the absorption of cholesterol and inhibits the activity of enzymes involved in cholesterol synthesis. Nano-chitosan contains various compounds that likely have therapeutic effects on serum levels of glucose, lipid profile, and insulin. [9]. Diabetes, a major health concern, affects the heart and blood vessels. Researchers are investigating alternative treatments using natural substances like plants, with a promising focus on nanomaterials to enhance the delivery of herbal remedies.

MATERIAL AND METHODS

Basil Seed Extraction

The seeds purchased from the market were ground into a fine powder. 100 grams of this powder were macerated with 1000 ml of 70% methanol, and magnetically stirred for 24 hours at 45°C. The resulting extract was first filtered through gauze, then through No. 1 filter paper. It was concentrated using a rotary evaporator at 40°C and 90 rpm under reduced pressure. The concentrated extracts were then stored in a refrigerated sample container [10].

Treatment Preparation

Deacetylate chitosan, also known as poly (D-glucosamine), is a natural polymer derived from chitin from EPTUI (80-100) nm, Chitosan nanoparticles are prepared using a modulating method. An amount of 4mg/ml chitosan solution was created by dissolving 200 mg of chitosan powder in 50 ml of deionized distilled water with 1% acetic acid. This solution was left to stand at room temperature for 24 hours. It is then stirred at 900 rpm with a magnetic bar on a hotplate stirrer for 30 minutes, resulting in a semi-colloidal solution. The pH was adjusted to 4.6. The optimum level of chitosan adsorption on the surface was determined by adding NaOH (0.1N) and using a pH meter [11]. This process resulted in the formation of chitosan nanoparticles, which were suitable for drug loading for various applications. The samples were then centrifuged at 900 rpm for 15 minutes to separate the solution [12].

Loading Basil Seed Extract with Chitosan Nanoparticles

The extract and biosynthesize chitosan nanoparticles (CNPs), 10 ml of Ocimum basilicum seed extract was combined with 10 ml of a chitosan solution. The mixture was magnetically stirred at 60 °C and 110 rpm, resulting in an opalescent solution [13].

Animals:

This study was conducted according to instructions and regulations of animal care and use committee, Faculty of Veterinary Medicine / University of Kufa. Forty-two adult male rats, weighing between 225 and 275 grams, were utilized. The experimental procedures commenced following a two-week acclimatization period. The animals were randomly allocated into different cages, with each cage containing seven animals.

Induction of Diabetes:

Thirty-five experimental rats were induced with diabetes mellitus. They underwent a fasting period of 24 hours, followed by the administration of a 100 mg/kg body weight dose of alloxan. Six hours later, the animals received a 5% sucrose solution. Blood sugar levels of each rat were measured after three days using a glucometer from tail puncture.

Experimental design:

The control negative group consisted of 7 rats, while the 35 rats induced with diabetes mellitus were divided into five groups for the following treatments:

Group (1) Control negative: Received distilled water.

Group (2) Control positive: Rats received a single dose of alloxan at dose100 mg/kg intraperitoneal.

Group (3) Basil alcohol extract: Rats received a single dose of alloxan at dose100 mg/kg intraperitoneal plus Ociumum basilicum seed alcoholic extract at dose 250 mg/kg orally once every day.

Group (4) Nano chitosan: Rats received a single dose of alloxan at dose100 mg/kg intraperitoneal plus nano-chitosan at dose 250 mg/kg orally once every day.

Group (5) Nano particles basil seed extract: Rats received a single dose of alloxan at dose100 mg/kg intraperitoneal plus Nano-chitosan basil at dose 250 mg/kg orally once every day.

Group (6) Empagliflozin: Rats received a single dose of alloxan at dose100 mg/kg intraperitoneal plus empagliflozin (Behringer Ingelheim) at dose 25 mg/kg orally once every day.

The groups (3,4,5,6) underwent treatment for a 21-day period. At the experiment's end, all animals were prepared for blood sample collection via heart puncture using a 23-gauge needle syringe for further assessment tests and analysis.

At the end of the experiment, blood was collected from all animals, allowed to rest for 15 minutes, and then centrifuged to obtain serum. The animals were anesthetized using intramuscular Ketamine (90 mg/kg body weight) and Xylazine (40 mg/kg body weight).

Parameters of serum tests

Glucose concentration

Over the course of three weeks was tested blood glucose level once a week, blood sugar levels are monitored constantly using a glucose-meter (on call plus) by make a small puncture in the tail is used to collect a blood sample.

Lipid profile

Wet chemistry analyzers operate on the photometric principle, utilizing a liquid reagent system. These analyzers typically employ one or more optically clear cuvettes to take photometric readings of the reaction mixture. They may use a halogen or Xenon lamp for illumination. Depending on their size and capabilities, analyzers might utilize filters, prisms, or diffraction gratings as monochromators. Spectrophotometric measurements are commonly conducted in the range of 304 to 670 nm. The tests rely on forming a colored complex of the analytic with specific reactive reagents. Refer to appendix number 6 for the procedural steps.

RESULTS

The result of nanoparticles basil seed extract was positive nano-particle according the tests

Glucose concentration

Groups that were injected singly intraperitoneal alloxan of at dose 100 mg/kg of were showed a very high significant increase at ($P<0.05$) in blood glucose concentration at three weeks.

The first week after treatment the groups 3,4,5, and 6 were showed significant difference reducing glucose concentration of all treatment groups comparative with control positive while appearances normal glucose concertation in negative control group at ($p<0.05$). fig. 1.

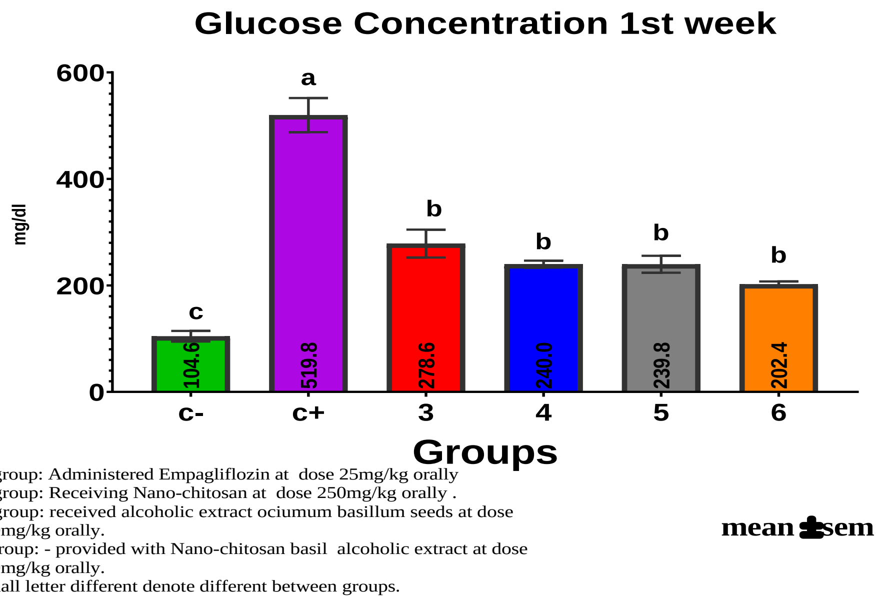

Fig. 1. Measurement glucose concentration at first week in all groups

The second week, it was appeared glucose concentration results, indicate that the control positive group was increase a significantly difference at ($p<0.05$) compared to treated and negative groups, fig. 2.

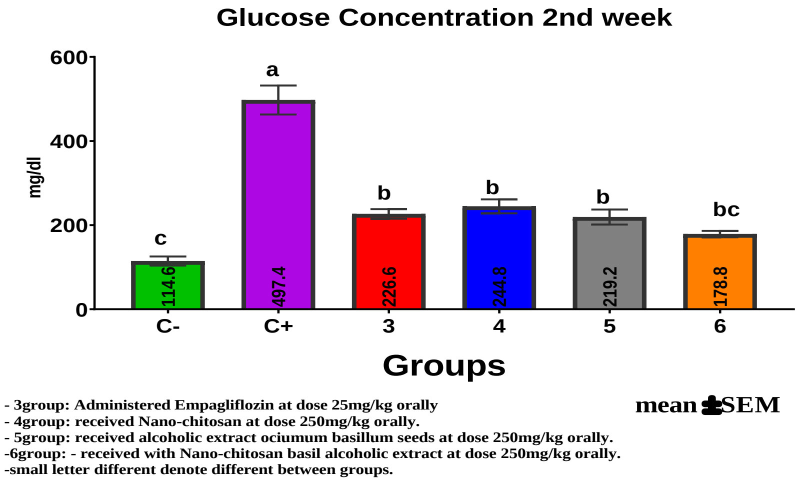

Fig. 2. Measurement glucose concentration at second week in all groups

The results for the third week, as shown in fig. 4-6, indicated that the control positive group was showed a significantly high increase in blood glucose concentration compared to all other treated groups at ($P<0.05$). However, there was no significant differences observed among 3, 4, 5 and 6 groups comparative with control negative group, fig. 3.

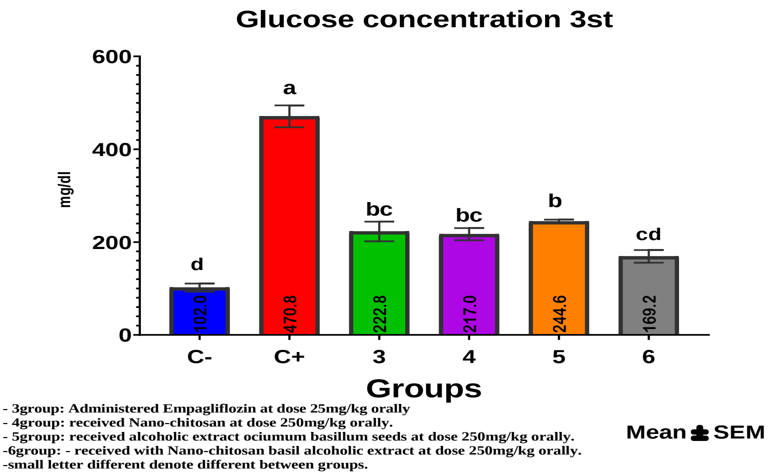

Fig. 3. Measurement glucose concentration at third week in all groups

Lipid profile

Cholesterol levels

As depicted in fig. 4, the cholesterol levels results were showed that the control positive group had a remarkably high statistically significant at ($p<0.05$) when compared to all other treatment groups that reduced cholesterol levels and control negative group showed normal cholesterol levels.

Triglyceride levels

Fig. 5, indicated that the control positive group exhibited increase significant difference in triglyceride levels from all other treatment groups at the same significance lower level while control negative group demonstrated a significant high from all treated groups at ($p<0.05$). Interestingly, the group six displayed a slight increase but no significant compared to the 3, 4 and 5 groups.

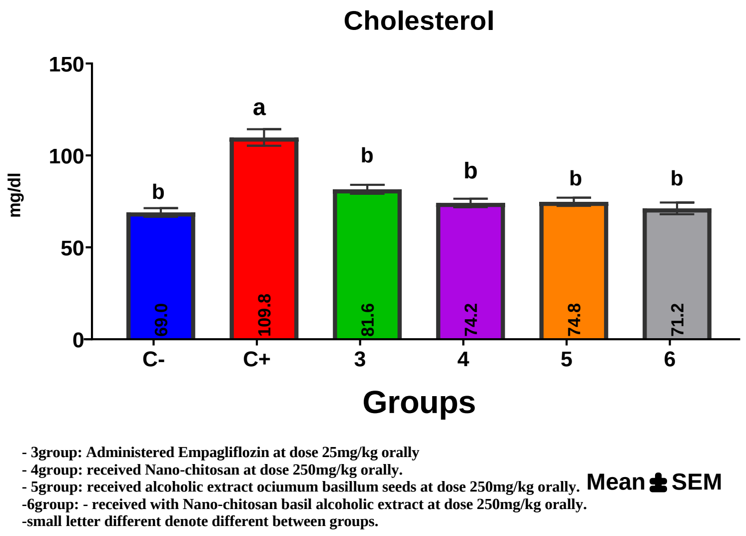

Fig. 4. Measurement serum total cholesterol level in all groups

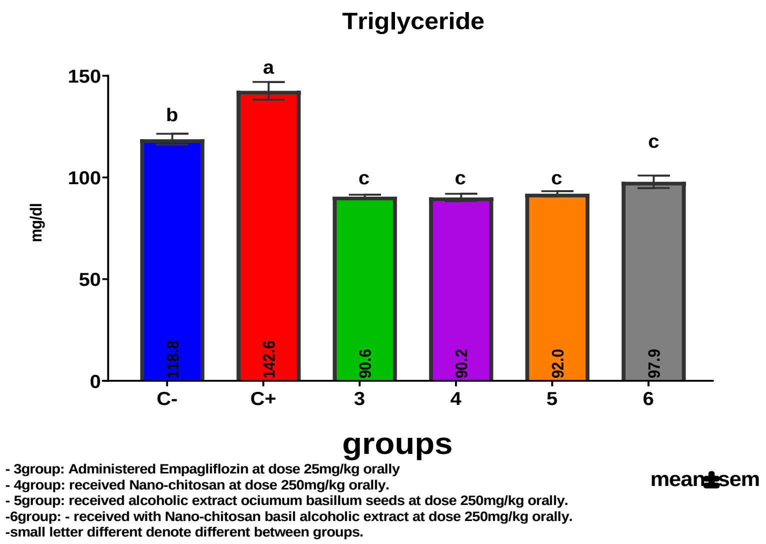

Fig. 5. Measurement serum triglyceride level in all groups High density lipoprotein

The fig. 6 presents the results of the High-Density Lipoprotein (HDL) levels were showed group six the same level with Control negative group but exhibited significantly higher than 3,4 and 5 groups at ($p<0.05$), that showed slightly different results. These differences were not statistically significant, but showed high a significant compared to the control positive group ($p<0.05\%$).

Low-density lipoprotein levels

In Fig. 7, the outcomes of the low-density lipoprotein assessment (LDL) were presented. The control positive group showed a significantly high results when compared to all other treated groups (p<0.05). While the sixth group exhibited a low statistical significance when compared with the fourth and fifth groups. However, there was no significant difference was observed when compared with the third and control negative groups (p <0.05).

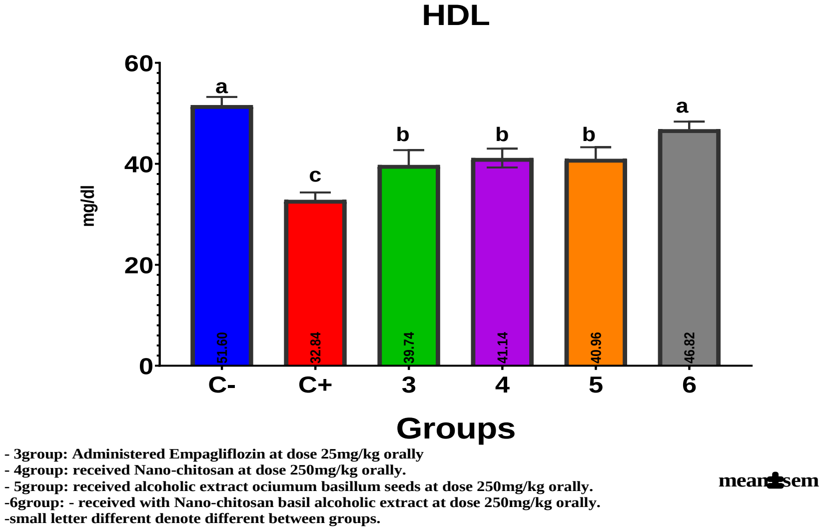

Fig. 6. Measurement serum high-density lipoprotein level in all groups

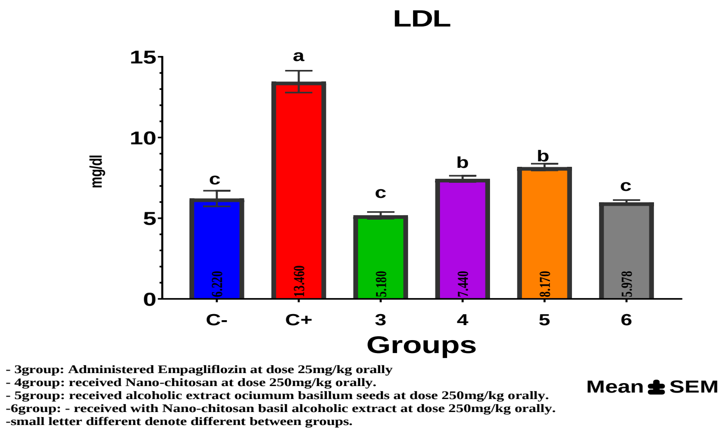

Fig. 7. Measurement serum low-density lipoprotein levels in all groups

DISCUSSION

During a three-week observation of blood glucose levels in rats, suffering from diabetes induced by alloxan, a noticeable sugar levels was seen in the positive Control. Alloxan triggers diabetes by damaging the insulin-producing cells in the pancreas, leading to a state of low insulin (hypoinsulinemia) and high blood sugar (hyperglycemia). Alloxan specifically induces hyperglycemia through its toxic effects on pancreatic beta cells. One of the mechanisms for its toxicity is the generation of free radicals, which has been demonstrated in both living organisms according to report [14]. Simultaneously, and a significant decline an observed in the remaining groups.

The results appeared the effect of empagloflazin by decreasing glucose level. The study by Forycka et al. [29] supported similar findings. Empagliflozin is an inhibitor of the sodium–glucose cotransporter 2 (SGLT2), which causes increased urinary glucose excretion. Thus, it contributes to improve glycemic control, better glucose metabolism and reduced gluco-toxicity. Chitosan can play a hypoglycemic effect without decreasing the appetite in diabetes rats [15]. Our study confirmed the effect of nanoparticle-chitosan in lowering blood sugar levels and increasing insulin levels, and the study by [16, 17] supported similar results. Notably, chitosan oligosaccharides have been shown to promote the proliferation of β-cells and the recovery of damaged β-cell functions, which can produce insulin and thus increases insulin level.

The group of alcoholic basil seeds extract have been found to have anti-hyperglycemic potential, which means they can help lower blood sugar levels. The explain the lower glucose concentration agreement with [18]. The group of basil nano-chitosan appear best lower glucose concentration than other groups due to combination between the nano-chitosan particles with alcoholic basil seed extract effects of these two substances could be responsible for lowering of the glucose concentration this result observed in study by [19].

Lipid profile (LDL, HDL, Triglycerides and Cholesterol) concentration: -

Diabetes mellitus is often linked with hyperlipidemia, which results in significant changes in lipid levels and composition. The alterations in lipid concentrations were associated with diabetes mellitus that could play a role in the onset of vascular disease. Fatty acids, which are crucial components of cell membranes and precursors to eicosanoids. That are essential for the structure and functionality of all cells in the body. Alloxan causes a notable increase in TC, Triglycerides (TG), LDL levels and decrease in HDL in positive control group. Rajagopal and Sasikala. [20] founded high concentration of serum lipids in diabetes mellitus. It was primarily due to an increase in the mobilization of free fatty acids from peripheral fat depots, as insulin inhibits hormone-sensitive lipase. Therefore, the pronounced hyperlipidemia that characterizes diabetes can be seen as a result of the unchecked actions of lipolytic hormones on fat depots. Alloxan induced excessive fatty acids in the plasma that can stimulate the liver to convert some of these acids into phospholipids and cholesterol. These substances, combined with an excess of triglycerides which were produced by the liver, may then be released into the bloodstream in the form of lipoproteins.

Consequently, serum basil extract has been shown to reduce glucose concentration in alloxan-induced diabetic rats. It has also proven highly effective in managing diabetes mellitus complications, such as managing hyperlipidemia, and it prevents defects in lipid metabolism [21]. The results of lipid profile in treated groups were shown the nano-chitosan basil particles group. It appearances the lower total cholesterol level at all treated groups and control negative. Empagliflozin was recorded similar results by Han et al. [22]. However, the substantial glucose waste caused by empagliflozin treatment led to an energy deficit in these mice. To compensate, empagliflozin increased reliance on lipid metabolism. It promoted intestinal stem cell proliferation, lipid absorption, and stimulated lipid metabolism in the kidney and liver. Unfortunately, in this case, empagliflozin exacerbated lipid metabolism disorders, worsening blood lipid abnormalities, hepatic lipid profile alterations, and kidney and liver steatosis in diabetic mice [23].

The treatment of rats with basil extract reduced the high lipid levels. This trend in lipid profile has been observed in previous studies. Which reported that basil decreases cholesterol, triglyceride, LDL and VLDL levels and increases HDL level. These effects could be attributed to the hypolipidemic effect of Ocimum basilicum, which may inhibit the key enzymes in triglycerides and cholesterol synthesis or increase cholesterol excretion through bile acid formation [24, 25, 26]. Chitosan nanoparticles have a superior cholesterol-binding capacity compared to commercial chitosan due to their smaller particle size and significantly larger surface area, which is ideal for adsorbing organic compounds such as lipids and fatty acids. Additionally, they can effectively reduce lipid levels in both plasma and liver in rats. As the particle size decreases, the surface area per unit mass increases found enhancing the potential for biological interaction [27, 28]. Nano-Chitosan lowers the lipid profile by binding to negatively charged molecules like lipids and proteins at pH levels above their isoelectric point. Furthermore, it acts as a fiber in the gastrointestinal tract, increasing the elimination of fat in the stool.

CONCLUSION

The data of the present study indicated that the potential of nanoparticle of Ociumum Basilicum as a therapeutic agent could enhance diabetes in male rats. In contrast, the individual use of nano-chitosan or basil seed extract alone was less effective in reducing blood sugar, LDL, TG, and TC levels, while increasing HDL levels, underscoring the superior delivery capabilities of nano-chitosan for herbal substances.

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