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ORIGINAL ARTICLE

Comparative Effects of Metformin, *Pleurotus ostreatus*, *Nigella Sativa*, and *Zingiber officinale* on the Streptozotocin-induced Diabetes Mellitus in Rats

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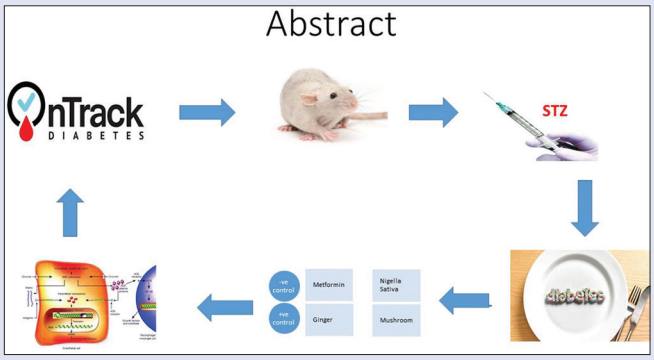
ABSTRACT

Background: Diabetes mellitus (DM) is a heterogeneous metabolic disorder occurring due to absolute or relative deficiency of insulin and causing chronic hyperglycemia that leads to crippling complications associated with morbidity and mortality. **Objectives:** The objective of the present work is to investigate the comparative antihyperglycemic effects of *Pleurotus ostreatus*, *Nigella sativa,* and *Zingiber officinale* with metformin. **Materials and Methods:** The study was conducted from June 2017 to February 2018. In this study, rats were divided into six groups according to the treatment regimen. Each group comprised five rats. Serum analysis of glucose was conducted during the treatment. After animals were sacrificed, histopathological examination of the pancreas was carried out. **Results:** Histopathological examination showed that streptozotocin caused damage to pancreatic beta-cells which secrete the insulin. Treatment with metformin, *N. sativa*, mushroom, and ginger produced a significant improvement in the function of these cells, regeneration of cells, and decrease in serum glucose levels. **Conclusion:** In two groups, *N. sativa* and ginger, regeneration of beta islets was observed, along with a significant reduction in the serum glucose levels, which was found in other groups as well.

Key words: Diabetes mellitus, metformin, *Nigella sativa, Pleurotus ostreatus, Zingiber officinale*

SUMMARY

- *Nigella sativa*, ginger, and mushroom have been shown to possess potent antidiabetic effect as compared to metformin
- *N. sativa* and ginger also caused the regeneration of beta islets of Langerhans in the pancreas
- *N. sativa* and ginger ameliorated the streptozotocin (STZ)-induced alterations
- *N. sativa*, ginger, and mushroom abrogated the STZ-induced insulin defect in the pancreas
- *N. sativa*, ginger, and mushroom attenuated the oxidative stress induced by STZ.



Abbreviations Used: DM: Diabetes mellitus; ROS: Reactive oxygen species; RNS: Reactive nitrogen species; AMPK: Activated protein kinase; STZ: Streptozotocin; RPM: Round per minute.

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INTRODUCTION

Diabetes mellitus (DM) is a dangerous metabolic disorder causing various complications, which significantly increases morbidity and mortality. DM characteristic features include increased serum glucose concentrations due to absolute or relative deficiency of insulin, causing biotransformation abnormalities in the glucose, fat, and proteins, characterized by hyperglycemia and dyslipidemia.[1] The glucose metabolism disorders can cause different complications, such as ketoacidosis, hyperosmolar coma, macro- and micro-angiopathy, nephropathy, neuropathy, and recurrent infections. These complications are the main reasons of illness and death in diabetic patients.[2,3]

In Type I DM, there is an absolute deficiency of insulin secretion due to the autoimmune destruction of beta pancreatic cells that lead to metabolic disturbances, especially affecting glucose homeostasis.[4]

In Type II DM, some mechanisms get damaged which regulate the cell sensitivity to insulin that ultimately leads to insufficient insulin secretion by the pancreatic beta cells, insulin dysfunction, and delayed insulin secretion through insulin resistance.[5] One of the known main culprits which damage the homeostatic mechanisms is oxidative stress.

Oxidative stress plays an important role in the development of complications in diabetes, particularly Type II diabetes.[6]

It has been found in several studies that DM is associated with oxidative stress, which causes increased production of free radicals, such as superoxide radical, hydrogen peroxide, and hydroxyl radical, or decreased antioxidant defense mechanism.[7,8] Role of oxidative stress in

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the pathophysiology of DM is not merely due to free radical production but also due to nonenzymatic protein glycation, damaged antioxidant enzyme system, and formation of peroxides.[9]

Free radicals are classified into reactive oxygen species, reactive nitrogen species (RNS),[10] and reactive chlorine species (RCS).[11]

In some conditions, oxygen may kill the cells when it produces reactive species which causes necrosis and ultimately the cell death. RNS and RCS also cause oxidation by the generation of some mechanisms which interfere with the normal physiological mechanisms in the cell.[12]

Several studies have found an effective role of polyphenols and antioxidants in reducing the oxidative stress by reducing the generation of free radicals.[13]

Metformin is a very well-known effective drug which reduces the serum glucose levels by targeting the insulin resistance and reduces gluconeogenesis in the liver. This effect is found to be due to the activation of activated protein kinase (AMPK).[14] AMPK is an enzyme that plays an important role in insulin signaling, whole-body energy balance, and metabolism of glucose.[15]

Mushrooms are an important component of the human meals due to their nutritional and medicinal value. They are a source of compounds responsible for antimicrobial, antioxidant, antitumor, and anti-inflammatory characteristics.[16-23] *Pleurotus ostreatus* revealed antitumor activity and hypoglycemic effects in experimentally induced diabetes.[24,25] *P. ostreatus* (oyster mushroom) is increasingly considered as an important food product with a significant role in human health and diet.[26] It is generally accepted that lowering high plasma cholesterol levels plays a significant role in preventing atherosclerosis. Oyster mushrooms are an ideal dietary substance for the prevention and treatment of hypercholesterolemia due to high content of dietary fiber, sterol, proteins, and microelements.[27] The fact that lovastatin is present in high proportions in this mushroom is makes it an important food supplement for patients suffering from hypercholesterolemia.[28] Mushrooms being neither plant nor animal have been placed in a kingdom called *Myceteae*. Among fungi, mushroom fungus is more prominent because it is a macrofungus with a distinctive fruiting body, which is large enough to be seen with the naked eyes and to be picked by hand.[29] The oyster mushroom is widespread in many temperate and subtropical forests throughout the world although it is absent from the Pacific Northwest of North America, being replaced by *Pleurotus pulmonarius* and *Pleurotus populinus*.[30]

Nigella sativa has conventionally been used as bronchodilator, antibacterial, diuretics, liver tonics, and analgesics.[31] The herb is also used in the treatment of several conditions including hypertension, cardiovascular diseases, diabetes, digestive ailments, hepatic and renal problems, diarrhea, cancer, skin disorders, infections, rheumatism, headaches, dysentery, asthma, and bronchitis.[32] A study conducted by Sangi *et al.* revealed that intraperitoneal administration of *N. sativa* (thymoquinone) significantly decreases hyperglycemia in streptozotocin (STZ)-induced DM in the rats.[33]

Ginger rhizomes are widely used in foods for their nutritional and medicinal benefits, especially in Asia. More recently, ginger juice was shown to have an antidiabetic effect in alloxan-induced diabetic rats.[34] In a previous study, ginger juice was reported to cause a significant reduction in the fasting glucose levels and an increase in the insulin levels in STZ-induced Type I diabetic rats.[35] Kazeem *et al*. found that oral administration of polyphenols from *Zingiber officinale* (especially free polyphenol) ameliorates these derangements and tends to restore the integrity of the pancreas and kidney.[36]

Ginger, *N. sativa*, and mushrooms, especially *P. ostreatus* species, have been found very rich in antioxidant substances which are mainly associated with the reduction in the production of free radicals and consequent oxidative stress and inflammation.[37-39]

MATERIALS AND METHODS

Preparation of fungal material and *Pleurotus ostreatus* liquid mycelial culture

Mycelium of *P. ostreatus* was isolated *in vitro* from the fruit bodies, and then liquid mycelium of *P. ostreatus* was obtained by inoculating 250 ml growth media consisting of malt extract and then incubated for 2 weeks at 25°C with gentle shaking.

Plant material

Seeds of *N. sativa* and rhizome of *Z. officinale* were purchased from the local market, and metformin was obtained from local pharmacies.

Preparation of ginger (*Zingiber officinale*) extract

After acquiring the plant material, aqueous ginger extract was prepared using the slightly amended method used by Alnaqeeb *et al*.[40] After peeling the ginger roots on crushed ice, small pieces of ginger root were made and homogenized in 0.9% NaCl in a high-speed blender for 2 min burst for a total of 12 min. Homogenized material was filtered thrice using filtering cloth, and centrifugation was done for the filtrate at 5000 round per minute. The concentration of the aqueous extract of ginger was made to be 500 mg/mL. Lower concentrations of extract were serially diluted using sterile 0.9% NaCl and stored in a Eppendorf's tube at −20°C.

Preparation of black seed (*Nigella sativa)* extract

Aqueous extract was prepared using the slightly changed method used by Bensiameur-Touati *et al*.[41] After acquiring the black seeds, these were cleaned, were dried at room temperature, and were powdered using a mechanical grinder. To prepare the aqueous extract, 100 g of powder was soaked in 1000 ml hot water. The mixture was boiled for 15 min, and filtration was done using a porous cloth. The filtrate was evaporated using a rotatory evaporator machine to get thick residue. The ruminant was suspended in 0.9% NaCl normal saline and stored in the Eppendorf's tube at −20°C.

Animals

Thirty healthy and active adult albino, male rats, aged 90–120 days, weighing 200–240 g, were selected. Rats were kept under an environmental condition with 24°C ± 3°C, 12-h light/dark cycle with good ventilation.

Induction of diabetes

The rats were made to fast overnight before the induction of diabetes by intraperitoneal administration of nicotinamide (110 mg/kg body weight) followed by a single intraperitoneal injection after 15 min of 60 mg/kg STZ freshly dissolved in distilled water.[42] Hyperglycemia was confirmed 4 days after injection by measuring the tail vein blood glucose level with an Accu-Chek Sensor Comfort Glucometer. Only the animals with fasting blood glucose levels ≥250 mg/dl were selected for the study.

Experimental design

Following acclimatization for 1 week before use, the animals were randomly divided into six groups each included five rats and labeled as A, B, C, D, E, and F according to the treatment. Group A animals were considered as control, Group B animals were made diabetic without treatment (control group), Group C was treated with ginger after induction of diabetes, Group D was given *N. sativa* after diabetes

induction, Group E animals were given *P. ostreatus* after STZ injection, and Group F animals were made diabetic and then treated with metformin.

The four diabetic groups were treated with 150 mg/kg/day of metformin, *P. ostreatus* (mushroom) (1000 mg/kg), *N. sativa* (80 mg/kg), and *Z. officinale* (500 mg/kg)[43] using oral stomach gavage (PO) (to make proper comparison with same route of administration as metformin is an oral hypoglycemic agent) in two divided doses (in 24 h). Serum glucose levels of all the study groups were assessed and compared with those of the metformin and control groups (positive and negative).

Histopathological examination

The treatment continued for 8 weeks; all groups were sacrificed in the 57th day; after that, pancreatic tissues were collected in 10% formalin and prepared for tissue processing. Histopathological examination was performed at the Department of Anatomy, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia.

Statistical analysis

The data analysis was carried out using the Statistical Package for the Social Science (SPSS Software Version 20, Chicago, IL, USA). All numeric variables were expressed as mean ± standard deviation. Statistical comparisons were performed using the one-way analysis of variance (ANOVA) test followed by *post hoc* least significant difference multigroup comparison. Homogeneity of variance was assessed using the one-way ANOVA test and Levene's statistic test. For all tests, $P < 0.05$ was considered statistically significant.

RESULTS

Serum analysis showed that in all groups, serum glucose levels increased after the induction of DM with STZ and came down when the rats were treated with metformin, mushroom, ginger, and *N. sativa*. Other groups showed almost equal results as were found with the use of the standard drug used in the study, i.e., metformin.

Statistical analysis

Serum levels of glucose in positive control, metformin, mushroom, *N. sativa*, and ginger groups were significantly higher than normal control (P = 0.0001 for all) and were significantly higher in positive control groups versus metformin, mushroom, *N. sativa*, and ginger groups (P = 0.0001 for all). In *N. sativa* group, blood glucose level was significantly higher than metformin, mushroom, and ginger groups (P = 0.009; P = 0.013, P = 0.001, respectively) [Table 1 and Figures 1, 2].

Histopathology of the pancreas

In sections from rat pancreas, studied under hematoxylin and eosin stain,after sacrifice of the animals,in the control Group A, the islets of Langerhans showed ,thin walls and non congested capillaries. The endocrine cell showed normal vesicular structure and homogeneously stained, acidophilic cytoplasm. The beta-cells were located periphery and with smaller nuclei [Figure 3].

In Group B, i.e., diabetic group, in the islets of Langerhans, there was marked disorganization of cellular components with aggregation of degenerated cells into clumped groups, and numerous vacuoles could also be seen denoting depleted cell population [Figure 4].

In Group C which was treated with ginger, after diabetic induction, histopathological examination of the pancreas showed the features of islets cell proliferation from intercalated ducts and small newly formed islets [Figure 5a]; on the other hand, there are normal Langerhans islets with cell population that has active rounded vesicular nuclei with dispersed chromatin granules. No vacuolation between the cells and capillaries among the cells looked normal [Figure 5b].

In Group D, sections from the pancreas after treatment with *N. sativa* showed a mild improvement of islets architecture; still, cell population showed clumped degenerated population. Other cells showed vacuolation seen in untreated diabetics [Figure 6].

In Group E that was treated with *P. ostreatus* (mushroom), sections showed that the active islets population cells are large and contain active vesicular nuclei [Figure 7].

In the group that was treated with metformin, i.e., Group F, islets of Langerhans revealed a marked protection from STZ-diabetic-induced changes, cell population more organized, no vacuolation between the cells, less vacuolation, and presence of new ductal cell proliferation with small islet formation [Figure 8].

DISCUSSION

The purpose of this research was to observe the effects of *P. ostreatus* (mushroom), *N. sativa*, and *Z. officinale*, in comparison with metformin on the serum glucose levels and pancreas, concentrating mainly on the pancreas of the diabetic rats. The results of the study showed that the herbals used in the study significantly reduced the serum glucose levels and the beta islets of Langerhans in the pancreas which were damaged by STZ and showed a reversal effect and started working; at the same time in the *N. sativa* and ginger groups, regeneration of beta islets of Langerhans was observed.

Therefore, as insulin resistance which is occurring secondarily to oxidative stress is blamed to be the main cause of Type II DM and inflammatory process[44] due to the activation of autoimmune mechanisms as the reason for Type I DM, the metformin is found to be effective in Type II

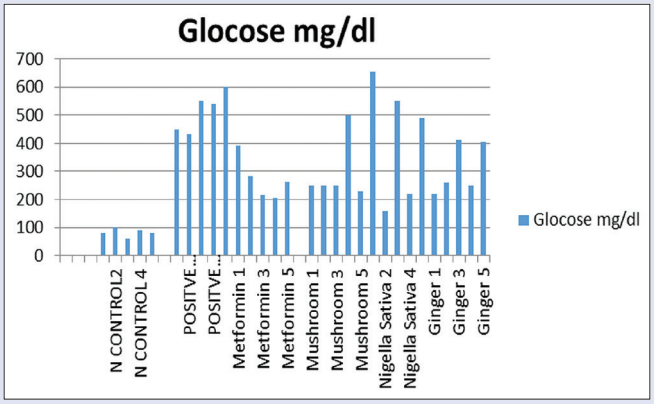

Figure 1: Glucose levels in different study groups

Table 1: Comparison of serum glucose measured in different studied groups

Parameter	Control	Positive control	Metformin	Mushroom	*Nigella sativa*	Ginger
Glucose (mg/dl)	82.20±14.46	514.00±71.62	251.20±38.94	255.70±25.69	328.20±53.59	229.20±24.41
P		0.0001[a]	0.0001[a]; 0.0001[b]	0.0001[a]; 0.0001[b]; 0.869[c]	0.0001[a]; 0.0001[b]; 0.009[c]; 0.013[d]	0.0001[a]; 0.0001[b]; 0.424[c]; 0.337[d]; 0.001[e]

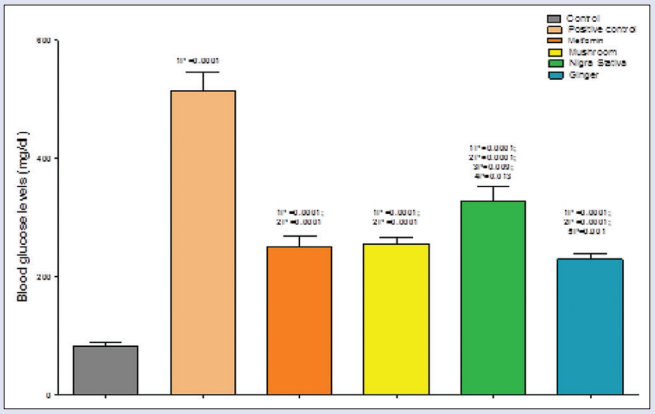

Figure 2: Comparison of blood glucose levels (mg/dl) in different studied groups. Data are expressed as mean ± standard deviation. 1P: Significance versus control; 2P: Significance versus positive control; 3P: Significance versus metformin; 4P: Significance versus mushroom; 5P: Significance versus ginger using one-way analysis of variance test (least significant difference)

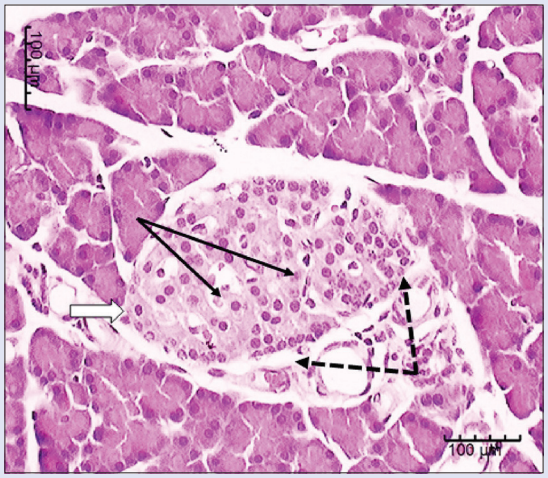

Figure 3: Section from Pancreas Group A showing islets of Langerhans (white arrow), capillaries (thin black arrow), endocrine cells (dotted arrows), and homogenously stained acidophilic cytoplasm. Beta cells (dotted arrows). Normal appearance of beta islets

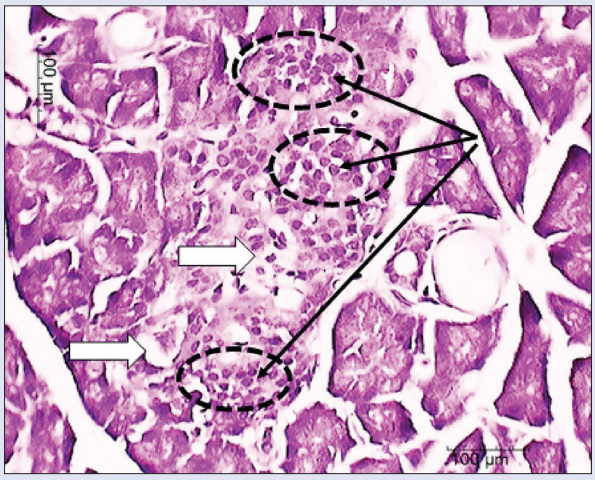

Figure 4: Pancreas sections from Group B showing islets of Langerhans (dark-stained nuclei), clumped groups (dotted circles and arrows), and numerous vacuoles (white arrows). Decreased number of beta islets is obvious

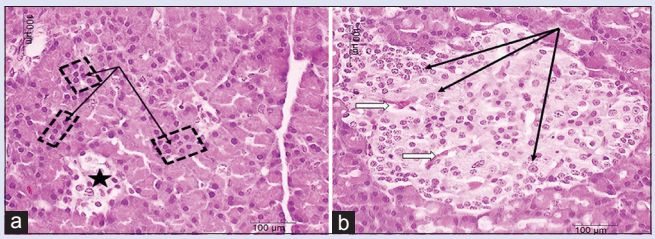

Figure 5: (a) Group C section showing region of pancreas cell proliferation (dotted squares and arrows) and newly formed islets (star). Regeneration of beta cells and normal function of the cell is clear. (b) Group C section showing normal Langerhans islets (black arrows) and cells and the capillaries (white arrows). Normal population and function of cells is indicated

DM because of its insulin-sensitizing effect and reduction of insulin resistance.[45] The insulin is required to be given exogenously in case of Type I DM.

The *N. sativa* and ginger have shown to affect the oxidative stress and reduction in the production of free radicals, and as these substances lead to regeneration of beta islets of Langerhans, both of these substances are useful for the treatment of both types of DM.[46,47] The anti-inflammatory effects of *N. sativa* and ginger are very well documented in previous studies, so this may be explaining the reason for regeneration of beta islets of Langerhans in the pancreas.

The mushroom used in the study *P. ostreatus* also showed the antihyperglycemic effects almost equal to metformin, but no regeneration of cells was observed in the pancreas, and it describes its usefulness in Type II DM only. Because of being rich in the antioxidants and anti-inflammatory substances, these effects can be correlated with their glucose-reducing effects.[48]

The results of this study are in conformity with the previous studies.

In the present study, we found that ginger has potential antihyperglycemic effect as have been found by Al-Amin *et al.*[49] that may also be due to its effects on serotonin receptors in addition to increase in the pancreatic secretion of insulin from beta cells or release of bound insulin.

In the present study, it was also observed that *N. sativa* produced beneficial effects on the serum glucose, by significantly reducing it, may be due to its antioxidant effects and may also be due to the regeneration of pancreatic beta cells, the results are in line with the study conducted by Abdelmeguid *et al.*[50]

A clinical trial conducted by Jayasuriya *et al.*[51] concluded that antihyperglycemic effect of *P. ostreatus* shows that mushrooms can be used as adjuvant therapy for the treatment of DM.

The histopathological results of this study regarding the destruction of beta islets of Langerhans in the pancreas by STZ and the regeneration of same cells with the use of ginger and *N. sativa* are due to reduction in the oxidative stress as also have been observed by Ali Sangi *et al.* and Abdelmeguid *et al.*[43,50]

Psilocin has been termed as the active ingredient of mushrooms, and its pharmacological actions on behavior are well documented and also it has been found to be rich in Vitamin E that is a well-known antioxidant agent.[52] Results of this study regarding antidiabetic effects of *P. ostreatus*

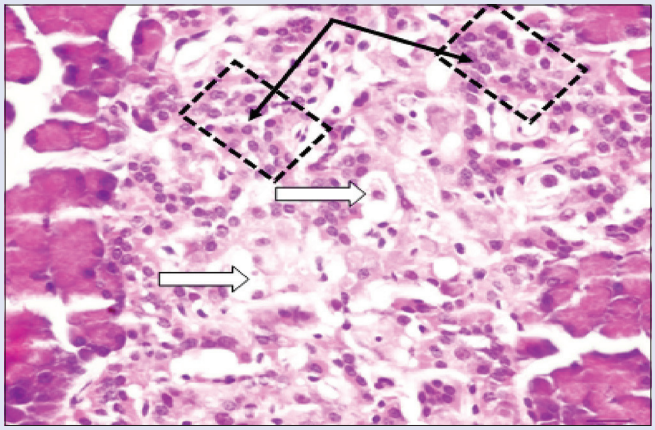

Figure 6: Group D section showing islets population (dotted squares and arrows) and vacuolation in nontreated diabetics (white arrows). Little improvement in cell structure still showing presentation of diabetic pancreatic beta islets

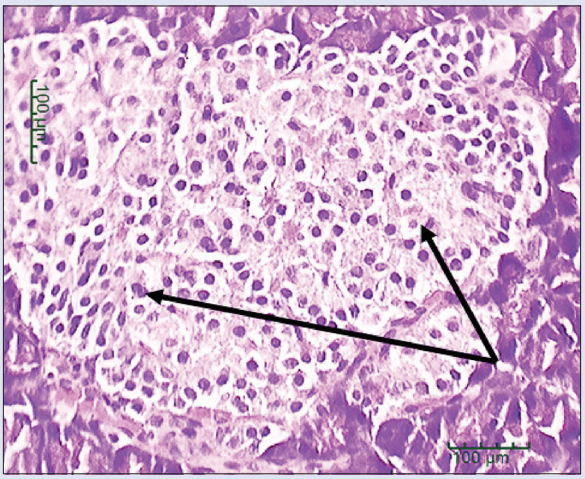

Figure 7: Group E section showing active islets population, cells are large and contain active vesicular nuclei (arrows). Reversal from the damage caused by streptozotocin to beta islets with no regeneration of the cells

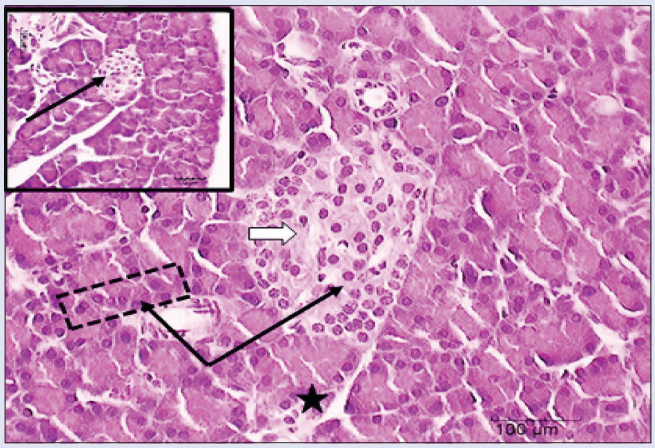

Figure 8: Group F section showing islets of Langerhans less vacuolation (white arrow), new ductal cell proliferation (dotted square and arrows similar to islets cells), and small islet formation (star). Organized structure of the cell, indicative of prevention from the damage

are consistent with the previous studies in which this substance has been found effective in Type I and Type II DM.[25,53] Regarding antihyperglycemic mechanism of action of P. ostreatus, it is yet to to be determined that which of its chemical constituents brings down serum glucose levels. Therefore, further phytochemical studies of the substance are needed.

Thymoquinone is a well-known active ingredient of *N. sativa*. In previous studies, it has been proved that this active ingredient is responsible for it antidiabetic effects.[33] Thymoquinone has found to increase the insulin receptor sensitivity and regeneration of beta islets of Langerhans in the pancreas.[54] However, *N. sativa* is very rich in the chemical constituents which are very rich in the antioxidant effects, and antioxidants also help reduce the oxidative stress with resultant decrease in insulin resistance.[55]

The chemical constituents of ginger (*Z. officinale*) aqueous extract include polyphenols, vitamin C, β carotene, flavonoids, and tannins.[39] In the current study, ginger has shown significant antihyperglycemic effects with the decrease in serum glucose levels and regeneration of beta cells in glucose along with improvement in beta islet cell function.

However, which of the abovementioned constituents is responsible for these actions will become clear when further immunohistochemical effects, effects on insulin receptors and glucose transporters, will be observed.

CONCLUSION

In metformin group, no regeneration of cells was observed, but the cells damaged by the effect of STZ showed improvement in function and cell morphology/structure, which indicates that decrease in insulin resistance also leads to improvement in islets function and structure. Serum analysis results were almost equal in all the groups with little variation. The herbals used in the study were found effective in decreasing the serum glucose levels. *N. sativa* and ginger caused regeneration of cells also. It is suggested that immunohistochemistry for cells secreting insulin to be done for verification of beta cells for functional improvement. *N. sativa* and ginger active ingredients should be used in the further studies and clinical trials. These substances possess potential to cure both types of DM.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Iso H, Date C, Wakai K, Fukui M, Tamakoshi A; JACC Study Group, *et al*. The relationship between green tea and total caffeine intake and risk for self-reported type 2 diabetes among Japanese adults. Ann Intern Med 2006;144:554-62.
2. Ahmed N, Babaei-Jadidi R, Howell SK, Thornalley PJ, Beisswenger PJ. Glycated and oxidized protein degradation products are indicators of fasting and postprandial hyperglycemia in diabetes. Diabetes Care 2005;28:2465-71.
3. Marles RJ, Farnsworth R. Economic and med plant research.Bokus 1994;6:149-54.
4. Ozougwu JC, Obimba KC, Belonwu CD, Unakalamba CB. The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. J Physiol Pathophysiol 2013;4:46-57.

5. Defronzo RA, Lily, Ferrannini E. Lecture 1987, the triumvirate: beta cell, muscle, liver. A collusion responsible for NIDDM. Diabetes 1988;37:667-87.
6. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Int J Biomed Sci 2008;4:89-96.
7. Rahimi R, Nikfar S, Larijani B, Abdollahi M. A review on the role of antioxidants in the management of diabetes and its complications. Biomed Pharmacother 2005;59:365-73.
8. Vincent AM, Russell JW, Low P, Feldman EL. Oxidative stress in the pathogenesis of diabetic neuropathy. Endocr Rev 2004;25:612-28.
9. Pari L, Latha M. Antidiabetic effect of *Scoparia dulcis*: Effect on lipid peroxidation in streptozotocin diabetes. Gen Physiol Biophys 2005;24:13-26.
10. Dröge W. Free radicals in the physiological control of cell function. Physiol Rev 2002;82:47-95.
11. Fridovich I. Fundamental aspects of reactive oxygen species, or what's the matter with oxygen? Ann N Y Acad Sci 1999;893:13-8.
12. Weseler AR, Bast A. Oxidative stress and vascular function: Implications for pharmacologic treatments. Curr Hypertens Rep 2010;12:154-61.
13. Asmat U, Abad K, Ismail K. Diabetes mellitus and oxidative stress – A concise review. Saudi Pharm J 2016;24:547-53.
14. Rena G, Pearson ER, Sakamoto K. Molecular mechanism of action of metformin: Old or new insights? Diabetologia 2013;56:1898-906.
15. Towler MC, Hardie DG. AMP-activated protein kinase in metabolic control and insulin signaling. Circ Res 2007;100:328-41.
16. Ajith TA, Janardhanan KK. Indian medicinal mushrooms as a source of antioxidant and antitumor agents. J Clin Biochem Nutr 2007;40:157-62.
17. Helano SA, Barros L, Martins A, Queiroz P, Santos-Buelga C; Ferreira ICFR. Fruiting body, spores and *in vitro* produced mycelium of *Ganoderma lucidum* from Northeast Portugal: A comparative study of the antioxidant potential of phenolic and polysaccharidic extracts. Food Res Int 2012;46:135-40.
18. Kwon AH, Qiu Z, Hashimoto M, Yamamoto K, Kimura T. Effects of medicinal mushroom (*Sparassis crispa*) on wound healing in streptozotocin-induced diabetic rats. Am J Surg 2009;197:503-9.
19. Ofodile LN, Uma NU, Kokubun T, Grayer RJ, Ogundipe OT, Simmonds MS, *et al.* Antimicrobial activity of some *Ganoderma* species from Nigeria. Phytother Res 2005;19:310-3.
20. Palacios I, Lozano M, Moro C, D'Arrigo M, Rostagno MA, Martínez JA, *et al.* Antioxidant properties of phenolic compounds occurring in edible mushrooms. Food Chem 2011;128:674-8.
21. Li H, Park S, Moon B, Yoo Y, Lee Y, Lee C. Targeted phenolic analysis in *Hericium erinaceum* and its antioxidant activities. Food Sci Biotechnol 2012;21:881-8.
22. Woldegiorgis AZ, Abate D, Haki GD, Ziegler GR. Antioxidant property of edible mushrooms collected from Ethiopia. Food Chem 2014;157:30-6.
23. Jose N, Ajith TA, Janardhanan KK. Antioxidant, antiinflammatory, and antitumor activities of culinary-medicinal mushroom *Pleurotus pufmonanus* (Fr.) Quel. (Agaricomycetidea). Int J Med Mushrooms 2002;4:329-35.
24. Yoshioka Y, Tabeta R, Saitô H, Uehara N, Fukuoka F. Antitumor polysaccharides from *P. ostreatus* (Fr.) quél.: Isolation and structure of a beta-glucan. Carbohydr Res 1985;140:93-100.
25. Chorváthová V, Bobek P, Ginter E, Klvanová J. Effect of the oyster fungus on glycaemia and cholesterolaemia in rats with insulin-dependent diabetes. Physiol Res 1993;42:175-9.
26. Alam N, Amin R, Khan A, Ara I, Shim MJ, Lee MW, *et al.* Nutritional analysis of cultivated mushrooms in Bangladesh – *Pleurotus ostreatus*, *Pleurotus sajor-caju*, *Pleurotus florida* and *Calocybe indica*. Mycobiology 2008;36:228-32.
27. Hossain S, Hashimoto M, Choudhury EK, Alam N, Hussain S, Hasan M, *et al.* Dietary mushroom (*Pleurotus ostreatus*) ameliorates atherogenic lipid in hypercholesterolaemic rats. Clin Exp Pharmacol Physiol 2003;30:470-5.
28. Alarcón J, Aguila S, Arancibia-Avila P, Fuentes O, Zamorano-Ponce E, Hernández M, *et al.* Production and purification of statins from *Pleurotus ostreatus* (Basidiomycetes) strains. Z Naturforsch C 2003;58:62-4.
29. Rathee S, Rathee D, Rathee D, Kumar V, Rathee P. Mushrooms as therapeutic agents. Rev Bras Farmacogn 2012;22:459-74.
30. Trudell S, Ammirati J. Mushrooms of the Pacific Northwest. Timber Press Field Guides. Portland, Oregon: Timber Press; 2009. p. 134.
31. Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, *et al.* A review on therapeutic potential of *Nigella sativa*: A miracle herb. Asian Pac J Trop Biomed 2013;3:337-52.
32. Gilani AH, Jabeen Q, Khan MA. A review of medicinal uses and pharmacological activities of *Nigella sativa*. Pak J Biol Sci 2004;7:441-5.
33. Sangi SM, Sulaiman MI, El-Wahab MF, Ahmedani EI, Ali SS. Antihyperglycemic effect of thymoquinone and oleuropein, on streptozotocin-induced diabetes mellitus in experimental animals. Pharmacogn Mag 2015;11:S251-7.
34. Ali BH, Blunden G, Tanira MO, Nemmar A. Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* roscoe): A review of recent research. Food Chem Toxicol 2008;46:409-20.
35. Asha B, Krishnamurthy KH, Devaru S. Evaluation of anti hyperglycaemic activity of *Zingiber officinale* (Ginger) in albino rats. J Chem Pharm Res 2011;3:452-6.
36. Kazeem MI, Akanji MA, Yakubu MT. Amelioration of pancreatic and renal derangements in streptozotocin-induced diabetic rats by polyphenol extracts of ginger (*Zingiber officinale*) rhizome. Pathophysiology 2015;22:203-9.
37. Reis FS, Barros L, Martins A, Ferreira IC. Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: An inter-species comparative study. Food Chem Toxicol 2012;50:191-7.
38. Forouzanfar F, Bazzaz BS, Hosseinzadeh H. Black cumin (*Nigella sativa*) and its constituent (thymoquinone): A review on antimicrobial effects. Iran J Basic Med Sci 2014;17:929-38.
39. Shirin Adel PR, Prakash J. Chemical composition and antioxidant properties of ginger root (*Zingiber officinale*). J Med Plants Res 2010;4:2674-9.
40. Alnaqeeb MA, Thomson M, Al-Qattan KK, Kamel F, Mustafa T, Ali M. Biochemical and histopathological toxicity of an aqueous extract of ginger in female rats. Kuwait J Sci Eng 2003;30:35-48.
41. Bensiameur-Touati K, Kacimi G, Haffaf EM, Berdja S, Aouichat-Bouguerra S. *In vivo* subacute toxicity and antidiabetic effect of aqueous extract of *Nigella sativa*. Evid Based Complement Alternat Med 2017;2017:8427034.
42. Alimohammadi S, Hobbenaghi R, Javanbakht J, Kheradmand D, Mortezaee R, Tavakoli M, *et al.* Protective and antidiabetic effects of extract from *Nigella sativa* on blood glucose concentrations against streptozotocin (STZ)-induced diabetic in rats: An experimental study with histopathological evaluation. Diagn Pathol 2013;8:137.
43. Ali Sangi SM, Abd Elwahab MF. Experimental evaluations of the nephroprotective properties of ginger (*Zingiber officinale*), *Cinnamomum verum* and *Nigella sativa* in STZ induced diabetic rats. Int J Biol Pharm Allied Sci 2017;6:1195-209.
44. Simmons RA. Developmental origins of diabetes: The role of oxidative stress. Free Radic Biol Med 2006;40:917-22.
45. Davies MJ, Tringham JR, Troughton J, Khunti KK. Prevention of type 2 diabetes mellitus. A review of the evidence and its application in a UK setting. Diabet Med 2004;21:403-14.
46. Al Wafai RJ. *Nigella sativa* and thymoquinone suppress cyclooxygenase-2 and oxidative stress in pancreatic tissue of streptozotocin-induced diabetic rats. Pancreas 2013;42:841-9.
47. Madkor HR, Mansour SW, Ramadan G. Modulatory effects of garlic, ginger, turmeric and their mixture on hyperglycaemia, dyslipidaemia and oxidative stress in streptozotocin-nicotinamide diabetic rats. Br J Nutr 2011;105:1210-7.
48. Ravi B, Renitta RE, Prabha ML, Issac R, Naidu S. Evaluation of antidiabetic potential of oyster mushroom (*Pleurotus ostreatus*) in alloxan-induced diabetic mice. Immunopharmacol Immunotoxicol 2013;35:101-9.
49. Al-Amin ZM, Thomson M, Al-Qattan KK, Peltonen-Shalaby R, Ali M. Anti-diabetic and hypolipidaemic properties of ginger (*Zingiber officinale*) in streptozotocin-induced diabetic rats. Br J Nutr 2006;96:660-6.
50. Abdelmeguid NE, Fakhoury R, Kamal SM, Al Wafai RJ. Effects of *Nigella sativa* and thymoquinone on biochemical and subcellular changes in pancreatic β-cells of streptozotocin-induced diabetic rats. J Diabetes 2010;2:256-66.
51. Jayasuriya WJ, Wanigatunge CA, Fernando GH, Abeytunga DT, Suresh TS. Hypoglycaemic activity of culinary *Pleurotus ostreatus* and *P. cystidiosus* mushrooms in healthy volunteers and type 2 diabetic patients on diet control and the possible mechanisms of action. Phytother Res 2015;29:303-9.
52. Khan MA, Tania M. Nutritional and medicinal importance of *Pleurotus* mushrooms: An overview. Food Rev Int 2012;28:313-29.
53. Krishna S, Usha PT. Hypoglycaemic effect of a combination of *Pleurotus ostreatus*, *Murraya koenigii* and *Aegle marmelos* in diabetic rats. Indian J Anim Sci 2009;79:986-7.
54. Balbaa M, El-Zeftawy M, Ghareeb D, Taha N, Mandour AW. *Nigella sativa* relieves the altered insulin receptor signaling in streptozotocin-induced diabetic rats fed with a high-fat diet. Oxid Med Cell Longev 2016;2016:2492107.
55. Dinagaran S, Sridhar S, Eganathan P. Chemical composition and antioxidant activities of black seed oil (*Nigella sativa* L). Int J Pharm Sci Res 2016;7:4473-9.