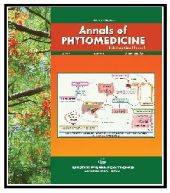
Annals of Phytomedicine 13(1): 548-557, 2024

DOI: http://dx.doi.org/10.54085/ap.2024.13.1.57

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Role of spices in mitigating diabetic complications: A study on streptozotocin-nicotinamide-induced type 2 diabetes in rats

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Article Info

Article history
Received 2 May 2024
Revised 18 June 2024
Accepted 19 June 2024
Published Online 30 June 2024

Keywords
Streptozotocin
Diabetes
Enzymes
Black cumin
Liver
Kidney

Abstract

Type 2 diabetes mellitus (T2DM) is a very common metabolic disorder characterized by complications which affect multiple organs, including the liver and kidneys. The present investigation aimed to examine how extracts from black cumin, fenugreek, garlic, and their combination, regulate blood glucose levels, liver and kidney enzymes, and tissue alterations in rats with type 2 diabetes induced by streptozotocin-nicotinamide. Male Wistar rats were split into six different groups: A normal control group, a diabetic control group, and four different groups treated with extracts from black cumin, fenugreek, garlic, and a combination thereof. Rats with diabetes showed noticeable decreases in body weight, polyphagia, polydipsia, increased blood glucose levels along with raised levels of liver and kidney-specific enzymes, and noticeable histopathological lesions. Administration of spice extracts, notably black cumin, reduced elevated glucose levels, lowered liver and kidney enzyme concentrations, and improved microscopic lesions compared to other spices in treated rats. These findings suggest potential therapeutic benefits of these spices in managing type 2 diabetes mellitus (T2DM) and associated complications involving the liver and kidneys.

1. Introduction

Diabetes mellitus is a serious metabolic disorder. It is characterized by insufficient insulin secretion from beta cell islets of the pancreas and/or tissues becoming resistant to insulin's effects. These results in hyperglycemia and subsequently cellular changes in various tissues. In recent decades, the prevalence of diabetes has skyrocketed worldwide, with the vast majority of cases being type 2 diabetes. It now accounts for around 90% of all diabetes diagnoses globally (Putta and Malarkodi, 2023). Experts predict that, by the year 2040, approximately 642 million people will be living with type 2 diabetes globally (Guariguata *et al*., 2014; IDF, 2019). India has emerged as a particularly severe diabetes hotspot. With over 77 million diagnosed cases, India now trails only China as the country with the second-highest number of diabetics in its population (Pradeepa and Mohan, 2021). Streptozotocin (STZ) is a widely used chemical in experimental animal models to induce insulin-dependent diabetes mellitus. This compound acts by inducing necrotic effects in beta cells of the islets of the pancreas (Fadillioglu *et al*., 2008; Punithavathi *et al*., 2008). Numerous studies conducted on both humans and animals have shown that sustained hyperglycemia in diabetes is linked to morphological alterations in the liver (Smith and Adam, 2011; Bilal *et al*., 2016; El-Sayed *et al*., 2020). The liver plays a significant role in controlling glucose metabolism through various hormones, including insulin and glucagon.

Creating precise animal models that closely resemble type 2 diabetes is crucial for discovering treatment approaches. Affordable access to treatment is vital for people living with diabetes. Current medications often have significant side effects and are costly. Traditional Indian medicine, has documented over 100 medicinal plants for managing diabetes. Indeed, these plants have shown effectiveness either individually or in combination (Akolade *et al*., 2019; Sanjeev and Divya, 2021). The objectives of this study were to evaluate the impact of three different Indian spices, *viz*., black cumin, fenugreek, garlic, and their combination, on various parameters in a streptozotocin-nicotinamide-induced type 2 diabetes mellitus (T2DM) rat model. Specifically, to evaluate the effects of these extracts on blood glucose control, liver and kidney functions, as well as histopathological changes.

2. Materials and Methods

2.1 Animal handling and diets

Male Wistar rats weighing between 150 and 200 g, including both sexes, were obtained from the experimental animal facility at Cadila Pharmaceutical Limited in Dholka, Gujarat, India. These rats were provided with pellet feed and had unrestricted access to water. Throughout the experiment, the rats were maintained on a standard chow diet, as detailed in Table 1. To ensure optimal conditions, the animals were acclimated to a temperature-controlled environment set at 25°C, with a 12 h light:dark cycle and a humidity level of 60 ±

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5%. All experiments adhered to the regulations outlined by the institutional animal ethics committee and were approved by the concerned governing body (No: VET COLL-13-2011).

2.2 Physical parameter monitoring

The body weight of each rat was recorded one day before treatment initiation (Day 0) and subsequently at weekly intervals throughout the study period. The final body weight was documented one day before blood collection, precisely preceding an overnight fasting period on Day 90. Daily food intake records were maintained, and body weights were systematically monitored weekly throughout the experimental timeline. After the 13-week duration, the animals were euthanized following a 12 h fasting period to facilitate blood and tissue collection. The euthanasia procedures strictly followed the standard guidelines. Additionally, a predetermined volume of water was provided to each experimental group, and the remaining quantity in the bottles was measured using a graduated cylinder on subsequent days to accurately assess water consumption.

2.3 Experimental group assignment

Prior to the experiment, the rats underwent a 7-day acclimatization period. The study involved 60 animals, which were distributed into six groups, each consisting of an equal number of rats (10 per group). The first group, comprising non-diabetic rats, received an intraperitoneal injection of normal saline in a volume determined by their body weight. The second group to sixth group were induced to have diabetes through an intraperitoneal injection of 45 mg/kg body weight of streptozotocin (STZ), followed by an injection of nicotinamide (NA) at 110 mg/kg body weight, 15 min after the STZ injection. Rats in Groups III, IV, V, and VI were administered oral aqueous extracts of spices as treatments, specifically black cumin, fenugreek, garlic, and a mixture thereof, over a period of 90 days.

2.4 Diabetes induction

Non-insulin-dependent diabetes mellitus (NIDDM) was induced in adult male Wistar albino rats following an overnight fast. This was achieved through a single intraperitoneal injection of 45 mg/kg streptozotocin, administered 15 min after an intraperitoneal dose of 110 mg/kg nicotinamide, as described by Pellegrino *et al*. (1998). Streptozotocin (STZ) and nicotinamide were dissolved in normal saline before injection. The presence of hyperglycemia was confirmed by elevated blood glucose levels, measured using a glucometer at 72 h post-injection and again on day 7. A fasting plasma glucose level exceeding 126 mg/dl was considered the diagnostic threshold for diabetes (American Diabetes Association, 1998). Only rats demonstrating persistent NIDDM were included in the subsequent antidiabetic study.

2.5 Aqueous extract preparation

2.5.1 *Nigella sativa* (black cumin) seed aqueous extract

The *N. sativa* seeds were sourced from a local market and meticulously washed with water. Following this, they were left to air-dry in a controlled environment at 25°C for one week, with periodic rotation to ensure consistent drying. Subsequently, the dried seeds were finely ground into a powder using a grinder. An aqueous extract of the seeds was then prepared by combining 100 g of the powdered seeds with 200 ml of distilled water, and thorough mixing was achieved using a magnetic stirrer. The resulting mixture underwent filtration to remove any solid particles, followed by lyophilization to obtain a dry extract. A stock solution was subsequently prepared by dissolving 600 mg of the lyophilized powder in 10 ml of distilled water. From this stock solution, concentrations of 12 and 25 mg/ml were prepared for utilization in the in vivo study (Kasim *et al*., 2012).

2.5.2 *Trigonella foenum-graecum* (Fenugreek) seed extract

Seeds were harvested from the local vicinity and subjected to a 24 h drying process at 37°C in an oven. Exposure to direct sunlight was strictly avoided to preserve the integrity of active constituents. Following drying, the seeds underwent grinding in a mechanical grinder. Subsequently, 50 g of the finely ground seeds were placed into a non-metallic container, and one liter of freshly boiled distilled water was poured over them. This mixture was then left to steep at room temperature for a period ranging from 5 to 8 h, resulting in the preparation of an infusion concentrated at 5% (W/V) fenugreek (Farman *et al*., 2009).

2.5.3 *Allium sativum* (garlic) aqueous extract

The process began by measuring 0.6 g of garlic powder, which was then dissolved and thoroughly mixed with 6 ml of distilled water for 20 min. Following this, the solution underwent centrifugation at 20,124 × g for 5 min at 4°C. The resulting supernatant was carefully retrieved and utilized for further experimentation (José *et al*., 2004).

2.6 Glucose-loaded animal study (oral glucose tolerance test)

Following an overnight fasting period, blood samples were obtained from both the diabetic control rat group and the diabetic groups treated with spice extracts at the 0 min mark. Subsequently, all rat groups were administered glucose orally at a dose of 2 g/kg, 30 min after receiving either the drugs or vehicle (for control). Blood glucose levels were then monitored at 30, 60, 120, and 180 min post-glucose administration to evaluate the impact of various extract doses on the animals' blood glucose levels.

2.7 Biochemical assays

Rats from all experimental groups were rendered unconscious using ether anesthesia. Blood samples were then collected from each rat by puncturing the retro-orbital plexus. The serum was collected from the blood, once it coagulated at room temperature. The serum was subjected to analysis of biochemical parameters, *viz.,* alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), cholesterol, creatinine, blood urea nitrogen, uric acid, and creatinine, by using specific spectrophotometric diagnostic kits procured from Merck Specialties Private Ltd., Mumbai, India.

2.8 Histological examination

On the 91st day of the experiment, all rats were sacrificed. For histopathological examination, collected tissues were immersed in a neutral buffer formaldehyde (10%) solution (pH 6.8) for fixation. The formaldehyde-fixed samples were processed in alcohol and xylene, followed by embedding in paraffin wax; blocks were prepared and sectioned at 5-6 microns through a microtome machine (Leica RM 2255). The sectioned tissues were subsequently stained by the hematoxylin and eosin (H&E) procedure for the identification of pathological lesions, observed through light microscopy.

2.9 Statistical analysis

The data are presented as mean values ± standard error (SE). Statistical analyses were carried out using SPSS version 20 software. One-way analysis of variance (ANOVA) was performed, followed by Duncan's multiple range tests for multiple comparisons among groups. Statistical significance was established at the 5% probability level.

3. Results

3.1 Body weight

The study investigates the influence of diabetes and the application of spice treatments on various physiological parameters in rats, as elaborated in the corresponding table. The final body weight displayed a notable decrease of approximately 10% in streptozotocin-nicotinamide (STZ-NA)-induced diabetic rats when compared to both the normal control and spice-treated rats (Figure 1). Moreover, among the spice-treated rats, those administered black cumin exhibited the least (1%) reduction in body weight in comparison to the normal untreated rats. This decline in body weight commenced in the 6th week of the experiment and endured until its completion.

3.2 Water consumption

The insulinopenic state of STZ-NA-induced diabetic rats produced significant changes in food and water consumption parameters, as mentioned in Figures 2 and 3. Water consumption increased (approximately three fold) in STZ-NA-induced rats compared to control rats. Among the treatment groups, the rats receiving black cumin and fenugreek exhibited elevated water consumption compared to the remaining groups. Food consumption in diabetic rats notably rose in comparison to control rats. Rats undergoing treatment also exhibited elevated food consumption compared to normal control rats; nonetheless, it was lower than that observed in the control STZ-NA-induced rats, suggesting that the extract treatments significantly curtailed food intake. Furthermore, in line with the diabetic condition, water intake surged (approximately six fold) in STZ rats.

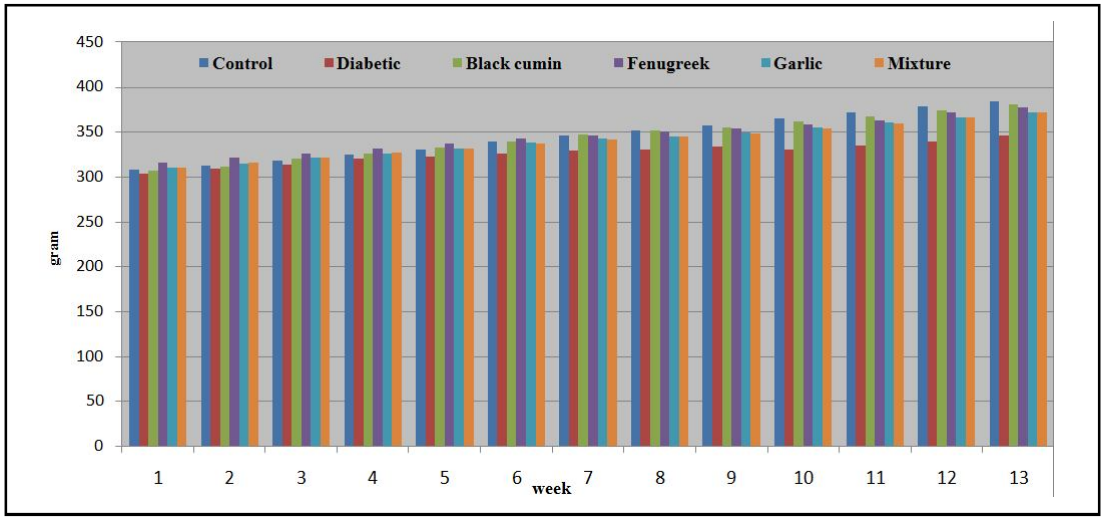


Figure 1: Body weight comparison in different experimental group during 90 days study.

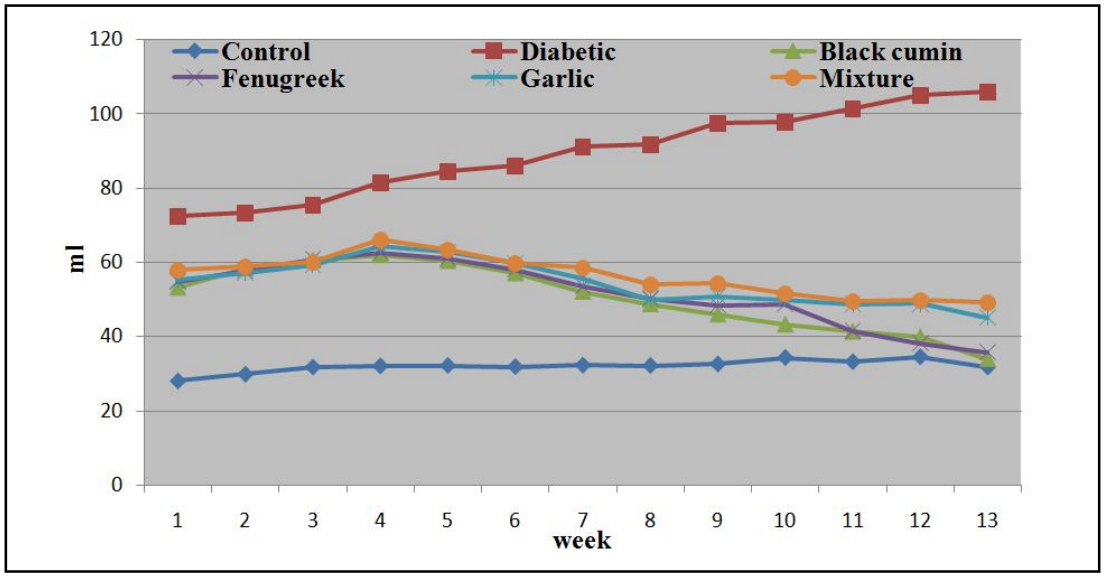


Figure 2: Water consumption in different experimental group during 90 days study.

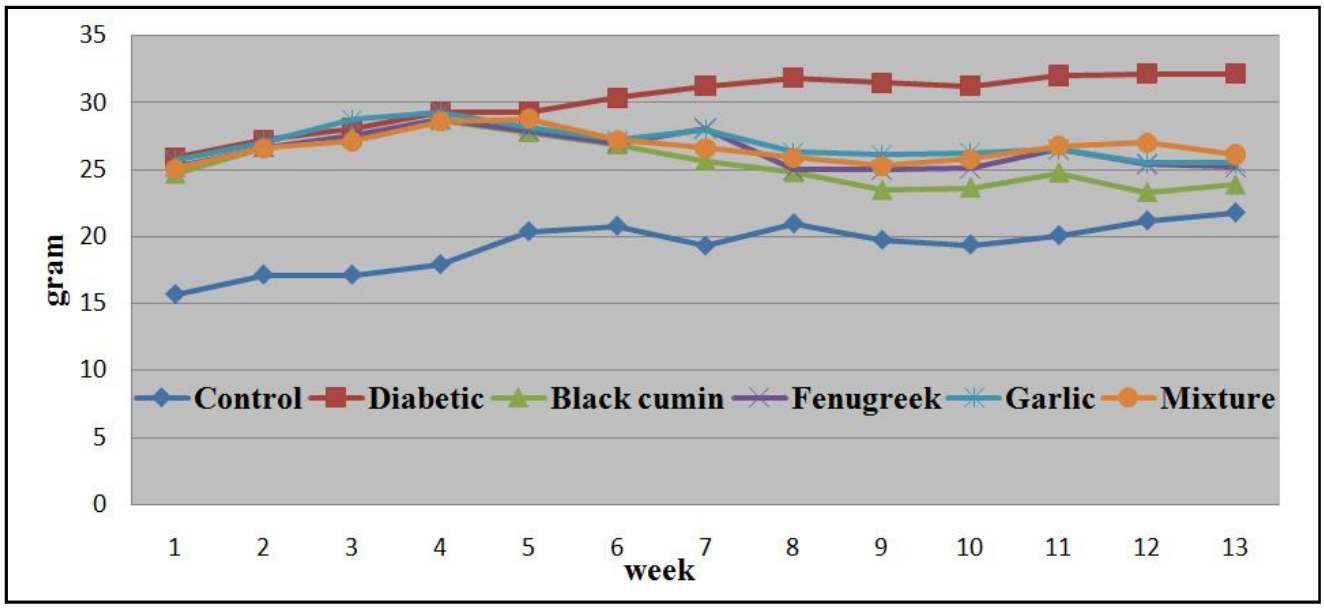


Figure 3: Food consumption in different experimental group during 90 days study.

3.3 Food consumption

By the study's conclusion, rats subjected to various extract treatments exhibited diminished food consumption compared to STZ-NA-induced diabetic rats. Nevertheless, no significant alteration in food consumption was noted in STZ diabetic rats, irrespective of treatment.

Diabetic rats treated with aqueous spice extracts displayed a statistically significant increase in liver and kidney weight compared to positive control diabetic rats (Table 1). However, regardless of treatment, no notable difference in organ weight was detected among groups for other collected tissues.

Table 1: Absolute organ weight (g) in different experimental groups on day 91st of study

Organ	Treatment groups					
	Group I	Group II	Group III	Group IV	Group V	Group VI
Liver	10.17 ± 0.35^{b}	12.67 ± 0.40^{a}	10.06 ± 0.28^{b}	10.20 ± 0.24^{b}	10.09 ± 0.23^{b}	10.13 ± 0.21^{b}
Kidney (both)	2.66 ± 0.07^{b}	3.24 ± 0.09^{a}	2.37 ± 0.08^{c}	2.39 ± 0.05^{c}	2.35 ± 0.06^{c}	2.34 ± 0.06^{c}
Lung	2.02 ± 0.05	1.92 ± 0.12	1.98 ± 0.08	1.91 ± 0.08	1.94 ± 0.06	1.98 ± 0.04
Brain	1.87 ± 0.05	1.85 ± 0.05	1.88 ± 0.07	1.83 ± 0.07	1.91 ± 0.09	1.81 ± 0.09
Spleen	0.63 ± 0.02	0.63 ± 0.03	0.63 ± 0.07	0.63 ± 0.02	0.67 ±0.04	0.67 ± 0.04
Heart	1.07 ± 0.04	0.99 ± 0.03	1.04 ± 0.09	1.00 ± 0.04	0.99 ± 0.03	1.01 ± 0.04
Testes (both)	3.41 ± 0.08	3.36 ± 0.07	3.18 ± 0.12	3.22 ± 0.21	3.13 ± 0.19	3.46 ± 0.11
Adrenal (both)	0.13 ± 0.01	0.15 ± 0.01	0.12 ± 0.02	0.15 ± 0.01	0.13 ± 0.0	0.14 ± 0.01

Compare data raw wise, different in superscript refer significant (p<0.05) variation between values.

3.4 Oral glucose tolerance test

The diabetic control group consistently showed dramatically higher blood glucose levels (ranging from 79% to 196% higher) compared to the control group at all time points, indicating severe and persistent hyperglycemia (Table 2). Among the treatment groups, black cumin showed the smallest increase in blood glucose levels compared to control (ranging from 16% to 41% higher), suggesting better glycemic control. The other treatment groups (fenugreek, garlic, and mixture) exhibited higher blood glucose elevations compared to the control (ranging from 21% to 81% higher), with garlic and mixture showing generally higher levels than fenugreek. These percentage changes further highlight the ability of the aqueous extracts, especially black cumin, to improve glycemic control in diabetic rats, although the extent of improvement varied among the different extracts.

3.5 Biochemical analysis

The impact of dietary aqueous extracts on liver and kidney-specific enzymes is elucidated in Table 3. Diabetic rats in Group II exhibited significantly elevated activities of ALT and AST enzymes, demonstrating an 82% and 59% increase, respectively, compared to their normal counterparts in Group I. However, administration of fenugreek, black cumin, garlic, and their combination in Groups III, IV, V, and VI, respectively, resulted in significant decreases in ALT (39%, 35%, 19%, 27% reduction) and AST (27%, 32%, 21%, 18% reduction) levels compared to Group II diabetic rats.

Furthermore, as depicted in Table 3, diabetic rats showed a notable increase in plasma total cholesterol (42% increase) and alkaline phosphatase (38% increase) concentrations compared to non-diabetic rats. Treatment with fenugreek, black cumin, garlic, and their combination led to significant reductions in cholesterol (39%, 33%, 23%, 31% reduction) and alkaline phosphatase (21%, 20%, 12%, 20% reduction) concentrations compared to untreated diabetic rats. Fenugreek extracts demonstrated the most substantial decrease in cholesterol levels, followed by black cumin, although significant reductions were observed in other treatment groups as well when compared with diabetic-positive control rats.

Table 2: Oral glucose tolerance test in different experimental group on day 91st of study

Minute interval	Treatment groups					
	Group I mg/dl	Group II mg/dl	Group III mg/dl	Group IV mg/dl	Group V mg/dl	Group VI mg/dl
0 min	118.3 ± 2.4^{d}	315.0 ± 7.7^{a}	167.4 ± 11.8^{c}	202.1 ± 6.7^{b}	214.2 ± 6.4^{b}	204.3 ± 3.5^{b}
30 min	189.5 ± 3.6^{e}	339.1 ± 6.5^{a}	219.4 ± 3.8^{d}	228.3 ± 3.3^{cd}	246.7 ± 5.4^{b}	240.0 ± 7.6^{bc}
60 min	200.5 ± 3.0^{d}	405.5 ± 5.1^{a}	272.6 ± 5.7^{c}	285.5 ± 3.5^{bc}	295.1 ± 4.6^{b}	271.5 ± 7.2^{c}
90 min	151.3 ± 7.8^{d}	376.8 ± 4.4^{a}	224.6 ± 4.9^{c}	234.1 ± 6.8^{bc}	250.0 ± 4.1^{b}	246.5 ± 8.2^{b}
120 min	119.1 ± 5.1^{d}	351.5 ± 7.2^{a}	157.2 ± 3.1^{c}	176.3 ± 5.4^{b}	188.7 ± 5.0^{b}	177.8 ± 5.3^{b}

Compare data raw wise, different in superscript refer significant (p<0.05) variation between values.

Table 3: Various biochemical parameters on day 91 of experiment for different treatment groups

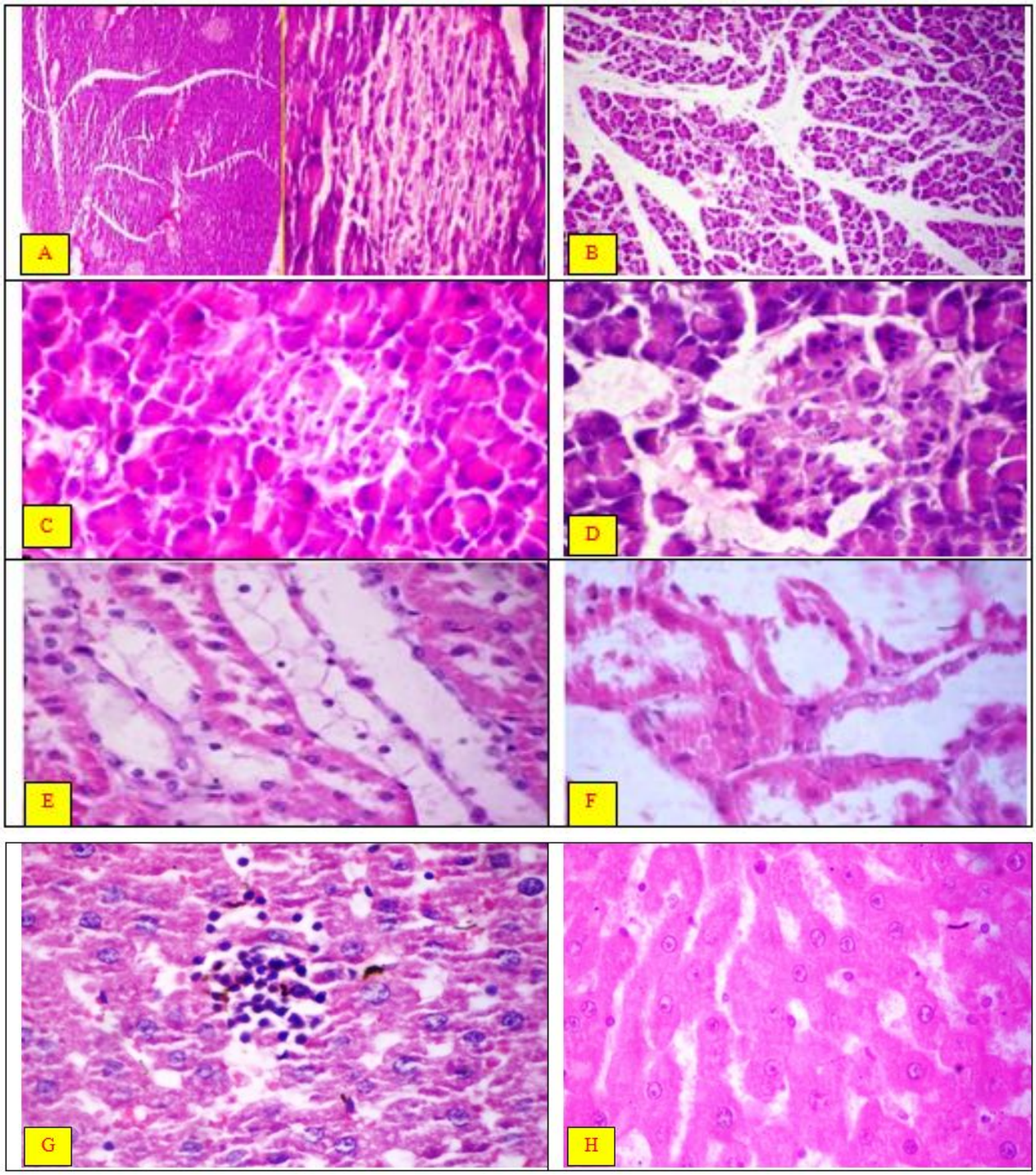
Parameters	Treatment groups					
	Group I mg/dl	Group II mg/dl	Group III mg/dl	Group IV mg/dl	Group V mg/dl	Group VI mg/dl
ALT (IU/l)	51.6 ± 2.3^{d}	93.2 ± 4.5^{a}	60.8 ± 3.9^{cd}	56.2 ± 3.9^{d}	75.0 ± 4.4^{b}	68.3 ± 3.0^{bc}
AST (IU/l)	80.2 ± 4.0^{d}	127.2 ± 6.0 ^{a}	86.8 ± 4.1^{cd}	92.6 ± 4.1^{bcd}	100.0 ± 4.5^{bc}	103.6 ± 8.2^{b}
ALP (IU/l)	107.3 ± 4.0^{c}	148.8 ± 9.3^{a}	118.1 ± 4.5^{bc}	116.8 ± 5.5^{bc}	129.6 ± 3.6^{b}	118.1 ± 6.0^{bc}
Chol (mg/dl)	62.7 ± 4.8^{c}	107.1 ± 9.8^{a}	72.2 ± 3.7^{bc}	65.7 ± 2.9^{c}	82.3 ± 5.0^{b}	74.0 ± 5.6^{bc}
UA (mg/dl)	0.5 ± 0.1^{c}	3.1 ± 0.2^{a}	0.7 ± 0.1^{bc}	0.9 ± 0.2^{bc}	1.0 ± 0.1^{b}	0.9 ± 0.1^{b}
Creat (mg/dl)	0.4 ± 0.1^{c}	1.1 ± 0.1^{a}	0.4 ± 0.1^{c}	0.5 ± 0.1^{c}	0.7 ± 0.0^{b}	0.5 ± 0.1^{c}
BUN (mg/dl)	21.7 ± 2.4^{c}	34.8 ± 1.3^{a}	23.9 ± 1.2^{bc}	23.7 ± 2.0^{bc}	27.2 ± 1.6^{b}	27.6 ± 1.4^{b}

Compare data raw wise, different in superscript refer significant (p<0.05) variation between values.

A similar pattern, albeit with smaller changes, was observed in blood urea nitrogen (BUN), creatinine, and uric acid measurements. Diabetic rats exhibited markedly increased levels of BUN (38%), creatinine (63%), and uric acid (83%), compared to normal rats. However, treatment with extracts reversed the elevation of these markers in Group III (BUN - 32%; creatinine - 63%; uric acid - 77% reduction), Group IV (BUN - 32%; creatinine - 54%; uric acid - 70% reduction), Group V (BUN - 20%; creatinine - 36%; uric acid - 67%), and Group VI (BUN - 20%; creatinine - 54%; uric acid - 70%) compared to untreated diabetic rats. Notably, black cumin exhibited the most sustained reversal of these increased enzyme activities compared to other extract-treated groups.

Microscopic examination of the pancreas revealed lesions predominantly in the endocrine component, particularly the islets of Langerhans, in streptozotocin-nicotinamide-induced diabetic rats (Group II). Untreated diabetic rats exhibited notable reductions in islet size (atrophy), deterioration, and depletion of islets, along with complete depletion of the islets of Langerhans, diminished cellularity, and the presence of pyknotic nuclei in pancreatic islet cells, as well as dilation of the pancreatic duct (Figures B and C), in comparison to normal control animals (Figure A). Conversely, rats administered black cumin, fenugreek, garlic, and a mixture (Groups III to VI) showed relatively milder to moderate lesions, with the restoration of architectural detail (Figure D). In the kidney sections, progressive damage was observed in both the cortex and medulla of streptozotocin-nicotinamide-induced type II diabetic rats (Group II). Lesions in untreated diabetic rats included loss of cytoplasm in tubular linings, the presence of pyknotic nuclei and loss of nuclei (Figures E and F), dilated tubules in the medulla with cellular transformation, and the presence of proteinaceous casts. However, rats treated with the aqueous solution were protected from nephropathy.

Histological analysis of liver sections from streptozotocin-nicotinamide-induced diabetic rats after 90 days revealed feathery degeneration, micro and macrocellular fatty changes, inflammatory cell infiltration around the portal tract (Figure G), and hepatocyte hypertrophy (Figure H) with mononuclear cell infiltrate, leading to distortion of the usual concentric arrangement of hepatocytes.



A. Photomicrograph of pancreatic islets of Group I rats containing plenty of endocirne cells (100X left, 400X right, H&E)
B. Pancreas of Group II diabetic rats with severe depletion of islets of Langerhans (100X H&E)
C. Pancreas of Group II diabetic rats with severe depletion of islets of Langerhans cells with distortion (400X H&E)
D. Similar depletion of cells in isltes of pancrease in Group VI diabetic rats tretated with combination of spices (400X H&E)
E. Kideny section showing necrotic and clear tubular cells in Group II diabetic rats (400X H&E)
F. Cortex of kindly showing dilated tubules with pyknotic nuclei and degenerated cell (400X H&E)
G. Section of liver showing focal mononuclear cell infiltration (400XH&E)
H. Section of liver showing hypertrophied and granular cells hepatocytes (400X H&E)

4. Discussion

Dehydration (polyuria), lipid catabolism, and loss of body weight are frequently linked with diabetes mellitus (Nath *et al*., 2017). In diabetic rats, heightened food consumption and diminished body weight were noted, suggesting a state of polyphagia and weight loss attributable to excessive breakdown of tissue proteins (Kamalak kannan and Prince, 2006). A deficiency in insulin leads to a reduction in protein content in muscle tissue due to increased protein breakdown (proteolysis) (Vats et al., 2004). However, after 8 weeks of consuming a standard diet, all diabetic groups regained their body weight, achieving similar values as the healthy control group on the standard diet. This recovery can be attributed to the restoration of glucose homeostasis (glycemic control), as dietary fiber intake is associated with an increase in the production or duration of action of a peptide similar to glucagon-like peptide-1 (GLP-1), thereby enhancing insulin secretion in a glucose-dependent manner. Evaluating the impact of *N. sativa* oil on gluconeogenesis and liver glucose production aids in elucidating its hypoglycemic mechanism, given that hepatic glucose production *via* gluconeogenesis plays a pivotal role in diabetic patients' hyperglycemia (Ishikawa *et al*., 1998). A study indicates that treatment with *N. sativa* oil markedly reduces glucose output from gluconeogenic precursors in isolated hepatocytes, implying that its antidiabetic effect is partly mediated by diminishing hepatic gluconeogenesis (Al-Awadi *et al*., 1991). Fenugreek seeds possess the ability to reduce blood glucose levels and partially restore the activities of crucial enzymes involved in carbohydrate and lipid metabolism in various animal models (Vats *et al*., 2003). The compounds responsible and the mechanisms are not fully understood but may involve steroid saponins, alkaloids, and trigonelline inhibiting intestinal glucose uptake (Petit *et al*., 1995; Yoshikawa *et al*., 1997; Al-Habori *et al*., 2001), and the amino acid 4-hydroxyisoleucine stimulating insulin secretion and improving glucose tolerance (Sauvaire *et al*., 1998, Visuvanathan *et al.,* 2022). Other components, like trace elements (Mohamad *et al*., 2004) and flavonoids (Shang *et al*., 1998), may also contribute to the antidiabetic effects.

The blood glucose-lowering effects of garlic have been attributed to sulfur-containing compounds such as (di-2-propenyl) disulfide and 2-propenyl propyl disulfide, which are believed to directly or indirectly stimulate insulin secretion (Carson, 1987). These disulfide compounds may also preserve insulin from inactivation by interacting with thiol-containing molecules (Augusti, 1996). Moreover, garlic extract could potentially improve glucose utilization by either reinstating a delayed insulin response or impeding intestinal glucose absorption.

This study unveiled significant increases ($p<0.05$) in AST, ALT, and ALP levels in the livers of streptozotocin-nicotinamide (STZ-NA)-treated rats, indicating liver cell damage induced by STZ-induced diabetes. The elevation in these enzymes suggests subtle changes in membrane permeability that allows their release into the bloodstream (Garella, 1997). Prior investigations likewise documented elevated AST, ALT, and ALP levels in hyperglycemic STZ-treated animals (Voss *et al*., 1988) and hepatic function alterations in STZ-induced diabetic rats (Barneo *et al*., 1990), linked to increased arginase activity (Salimuddin *et al*., 2008). This study confirms that STZ-NA induced diabetes impairs hepatic function and structure (Zafar *et al*., 2009).

Treatment with *N. sativa* oil reduced AST, ALT, and ALP activities, alleviating liver damage. Its active ingredient, thymoquinone, protects liver enzymes (Daba and Abdel-Rahman, 1998; Sheikh *et al.,* 2021; Prairna *et al.,* 2022). Fenugreek treatment decreased plasma AST, ALT, and ALP levels, attributed to compounds like 4-hydroxyisoleucine promoting insulin secretion and glucose utilization (Eidi *et al*., 2006; Saravanan *et al*., 2009; Khaled *et al*., 2010; Ramesh *et al*., 2010). Garlic extract administration lowered serum cholesterol in diabetic rats by potentially inhibiting hydroxymethylglutaryl coenzyme A reductase involved in cholesterol synthesis (Mariee *et al*., 2009; Ugwuja *et al*., 2010). STZ-NA induced diabetes increased blood urea, uric acid, and creatinine levels, indicating impaired kidney function. Possible causes include serum protein depletion, increased circulating amino acids, and deamination leading to ammonia and urea formation, as well as amino acid breakdown during gluconeogenesis (Ganong, 2003). Other reasons for elevated uric acid levels might be due to metabolic disturbances like increased xanthine oxidase activity, lipid peroxidation, and elevated triglycerides and cholesterol (Madianov *et al*., 2000), as well as protein glycation contributing to muscle wasting and purine release (Anwar and Meki, 2003). Black cumin oil treatment reduced creatinine, blood urea, and uric acid levels, potentially by increasing insulin secretion (Al-Logmani and Zari, 2009) and preventing renal failure through decreased oxidative stress and preserved antioxidant activity (Sayed-Ahmed and Nagi, 2007, Prairna and Ahmad, 2022). Fenugreek extract decreased cholesterol, plasma uric acid, urea, and creatinine levels (Khaled *et al*., 2010; Ramesh *et al*., 2010; Singh *et al.,* 2023), suggesting improved renal function in diabetic hyperglycemia (Almadal and Vilstrup, 1988). Garlic administration reduced altered blood urea levels, indicating a protective role in protein metabolism. It also restored elevated uric acid levels to near-normal, likely through free radical scavenging activity, and reduced creatinine levels, indicating improved kidney function (Kemmak *et al*., 2009; Eidi *et al*., 2006).

We did not find any gross lesions in any of the concerned tissues. The histopathological findings in the pancreas, kidney, and liver of diabetic rats were attributed to the cytotoxic effects of streptozotocin (STZ), which has been implicated in pancreatic β-cell damage via nitric oxide liberation, leading to the development of diabetes (Klaidman *et al*., 2001). For the kidney, severe hyperglycemia induced by STZ has been implicated as a causative factor in renal damage, ultimately leading to the development of diabetic nephropathy (Zafar *et al*., 2009).

Notably, the administration of aqueous extracts of spices exhibited protective effects on the pancreatic islet architecture. These observations corroborate previous studies that documented the beneficial effects of these plant extracts on pancreatic function (Abdelmeguid *et al*., 2011; Elaziz, 2011). The hypoglycemic and antioxidant properties of black cumin, in particular, confer protective effects on pancreatic β-cells (Mansi, 2005).

These hepatic lesions were correlated with elevated blood glucose levels and disruptions in liver function, which have been linked to diabetic complications (Pari and Sankaranarayanan, 2009). Moreover, heightened serum aminotransferase activities, commonly seen in liver diseases, are more prevalent among individuals with diabetes (Arkkila *et al*., 2001).

In short, the histopathological findings of this study in streptozotocin-nicotinamide-induced diabetic rats are consistent with previous reports, underscoring the deleterious effects of hyperglycemia on various organs, including the pancreas, kidney, and liver.

The protective effects of natural plant extracts, such as black cumin, fenugreek, and garlic, observed in this study emphasize their potential therapeutic efficacy in alleviating diabetic complications through maintaining blood glucose levels.

5. Conclusion

The study examined the impact of aqueous extracts of spices on diabetic rats over 90 days. Diabetes induction caused significant physiological changes, including decreased body weight, increased food and water consumption, persistent hyperglycemia, elevated liver and kidney markers, and organ lesions. However, treatment with these extracts effectively improved glucose control, reduced liver and kidney enzyme levels, and protected against organ damage. Black cumin extract showed the most potent therapeutic effects, maintaining body weight, lowering blood glucose levels, and reversing liver and kidney marker activities more effectively than other treatments. These findings underscore the beneficial antidiabetic and organ-protective properties of these spice extracts, particularly black cumin, suggesting their potential as alternative or complementary therapies for managing diabetes and its complications.

Acknowledgements

Authors are highly thankful to Department of Veterinary Pathology, S. D. Agricultural University, Gujarat for financial support.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Citation **T.J. Sheikh, D.V. Joshi, S.H. Rawal, Ranjan Rajeev, C. M. Modi and Amit Kumar Jha (2024). Role of spices in mitigating diabetic complications: A study on streptozotocin-nicotinamide-induced type 2 diabetes in rats. Ann. Phytomed., 13(1):548-557. http://dx.doi.org/10.54085/ap.2024.13.1.57.**