# In Vitro Hypoglycemic Effects Of Black Seed (Nigella Sativa)

**Fahad Saad Alhodieb[1]***

**Abstract**

Type-2 diabetes mellitus (T2DM) is one of the major noncommunicable disease worldwide wherein, inhibition of intestinal glucose absorption and transport from the luminal side to the circulation is considered a critical pathway for its prevention and treatment. The current study thus explored the antidiabetic potential of black seed using various *in vitro* methods, such glucose adsorption, glucose diffusion retardation, carbohydrate hydrolyzing enzyme inhibition, glucose transport enhancement across the yeast cells. Results indicated that black seed powder (BSP) effectively adsorbed significant amounts of glucose, inhibited its diffusion across dialysis membrane both in the presence and absence of α-amylase. BSP exhibited higher glucose dialysis retardation index (GDRI) compared to wheat bran. The aqueous extract of black seed inhibited α-glucosidase in a dose dependent manner and also promoted glucose transport across yeast cell membrane in a dose dependent manner. It is concluded that the observed hypoglycemic effect of black seed is due to the aforementioned mechanisms in addition to previously suggested mechanism of increasing insulin secretion by pancreatic cells.

**Keywords:** Antidiabetic, black seed, glucose transport, *in vitro*, starch digestion

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## INTRODUCTION

Type-2 diabetes mellitus (T2DM) characterized by defects in insulin secretion or action resulting in hyperglycemia accounts for nearly 90% of all diabetes mellitus cases worldwide irrespective of age, race, and gender. T2DM is a result of the interaction between genetic predisposition and environmental factors including dietary habits and lifestyle (Reimann et al., 2009; Hamdan et al., 2019). Consequently, medicinal plants and certain bioactive principles found in plants have been considered useful adjuvants in diabetes therapy (Hamdan et al., 2019). These natural substances can function in diverse mechanisms including inhibition of carbohydrate hydrolyzing enzymes, adsorption of liberated glucose in the intestinal lumen, promoting insulin secretion, action, sensitivity, and enhancing glucose transport across target tissues (Tiwari and Rao, 2002). Inhibition of intestinal glucose absorption and transport from the luminal side to the circulation is regarded as a critical pathway for the prevention and treatment of diabetes (Patel and Mishra, 2012). Thus, medicinal herbs have gained much scientific attention owing to their increased consumption some of which are considered to be safer than oral hypoglycemic drugs (Ahmed et al., 2013a; Hamdan et al., 2019).

*Nigella sativa* Linn (Rununculaceae) commonly referred to as “black seed” is one such plant product widely used as a spice in Middle Eastern countries and also valued as traditional medicine in a diverse array of diseases (Ahmad et al., 2021). Muslims see black seed as a medication that can heal all illnesses except death, whereas indigenous medical practitioners regard it as "the plant from heaven" (Ahmed et al., 2013b). As a consequence, the plant has received extensive scientific scrutiny confirming a range of



***Corresponding author:** Dr. Fahad Saad Alhodieb
**Address:** *[1]Department of Clinical Nutrition, College of Applied Health Sciences in Ar Rass, Qassim University, Ar Rass 51921, Saudi Arabia
Phone: +966 55 256 2571
E-mail: f.alhodieb@qu.edu.sa

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pharmacological effects; anti-diabetic, antitussive, anticancer, antioxidant, anti-inflammatory, organo-protective, and immunomodulatory effects (Ahmad et al., 2021). The seeds reportedly contain a range of secondary metabolites of which thymohydroquinone is widely researched (Kadam and Lele, 2017; Ahmad et al., 2021). The anti-diabetic effect of black seed is not only confirmed in animal studies (Abdelmeguid et al., 2010; Salama, 2011; Sultan et al., 2014) but has also been studied in human intervention trials (Ahmad et al., 2009; Bamosa et al., 2010; Hosseini et al., 2013; Bamosa et al., 2015; Heshmati and Namazi, 2015; Kaatabi et al., 2015). Animal studies indicated that black seed enhances β-cell regeneration thereby increasing insulin secretion and decreasing insulin resistance (Ahmad et al., 2021) while, clinical trials indicated that black seed significantly improves laboratory markers of hyperglycemia and diabetes management with a significant fall in fasting blood glucose, postprandial blood glucose, glycosylated hemoglobin, and insulin resistance, and an increase in serum insulin (Hamdan et al., 2019).

In light of the foregoing, the current study assessed the anti-diabetic potential of black seed using various *in vitro* methods, such as its effect on glucose diffusion, carbohydrate hydrolyzing enzymes, and glucose transport across the yeast cell membrane, because inhibition of intestinal glucose absorption and transport from the luminal side to the circulation is considered a critical pathway for the prevention and treatment of diabetes (Patel and Mishra, 2012).

## Materials and Methods

### Materials

*Nigella sativa* seeds, wheat bran, and baker's yeast were purchased from a local supermarket in Ar Rass, Saudi Arabia. Dialysis membrane (12 KD cut off), glucose oxidase peroxidase assay kit, α-amylase, α-glucosidase, 3,5-dinitrosalysilic acid, and p-nitrophenyl-α-D-glucopyranoside were purchased from Sigma Aldrich, Saudi Arabia. All the other chemicals and reagents used in the study were of extra pure quality.

### Processing of the sample

*Nigella sativa* seeds were dried in a hot air oven at 50ºC overnight to remove moisture. Dried seeds were then ground in a laboratory blender to a fine powder. The resultant black seed powder (BSP) was stored in an air-tight container in a refrigerator and used subsequently for the determination of glucose adsorption, glucose diffusion retardation, and amylolysis kinetics *in vitro*. Black seed extract (BSE) was prepared for enzyme inhibition and glucose transport experiments by extracting the seed powder with hot water (70ºC) in a mechanical shaker for 24 hours, followed by filtering and freeze drying.

### Determination of glucose absorption capacity

Glucose absorption capacity of BSP was determined *in vitro* wherein 250 mg of sample powder was added to 25 mL glucose solution with increasing concentration (5, 10, 20, 50 and 100 mM) in 50 mL centrifuge tubes and vortexed for 10 sec. The tubes were then incubated in a mechanical shaker water bath at 37°C. After 6 hours, the tubes were centrifuged at 4000 ×g for 20 min and glucose content in the supernatant was determined using glucose oxidase peroxidase assay kit. The glucose bound was calculated using the following formula and expressed in mM. Wheat bran was used as a reference (Ahmed, 2021).



$$Glucose\ bound\ (mM) = \frac{Glucose\ concentration\ of\ original\ solution - glucose\ concentration\ after\ 6h}{Weight\ of\ the\ sample} \times Vol\ of\ solution$$

### Determination of glucose diffusion retardation capacity

The ability of BSP to retard the diffusion of glucose across dialysis membrane (12 KD) was studied using 25 mL of glucose solution (20 mM) with 250 mg of sample powder taken in the dialysis bag. The dialysis bag was then placed into beakers containing 200 mL of distilled water. The beakers were then placed in a shaking water bath (30 strokes per minute) at 37°C. The amount of glucose diffusing across the dialysis membrane was determined at intervals of 60 minutes for 4 hours using glucose oxidase peroxidase assay kit. A control test without BSP and a standard reference test with acarbose (50 mg) were also carried out. Glucose dialysis retardation index (GDRI) which indicates the effectiveness of the sample to retard diffusion of glucose was calculated by using the following formula (Ahmed, 2021).

$$GDRI = 100 - \frac{Glucose\ content\ with\ addition\ of\ sample}{Glucose\ content\ of\ control} \times 100$$

## Determination of amylolysis kinetics

Amylolysis kinetics of BSP to study its effect on starch degradation was studied using a closed system containing 25 mL of starch solution, 0.4% α-amylase (100 mg), and 1% sample (250 mg) in a dialysis bag. The sealed dialysis bags were placed into a beaker with 200 mL of distilled water. The beakers were then placed in a shaking water bath (30 strokes per minute) at 37°C. The amount of glucose diffusing across the dialysis membrane was determined at intervals of 60 minutes for 4 hours using glucose oxidase peroxidase assay kit. A control test without BSP and a standard reference test with acarbose (50 mg) were also carried out. Glucose dialysis retardation index (GDRI) was calculated as mentioned earlier (Ahmed, 2021).

## Effect of black seed extract on α-glucosidase activity

To measure the α-glucosidase inhibitory effect of BSE, 10 μL of α-glucosidase solution (1 U/mL) was incubated with varying concentrations of BSE (10–50 μL) for 10 min at 37°C. The volume was made up to 210 μL with maleate buffer, pH 6.0, and the enzyme reaction was started by addition of 200 μL of 2 mM p-nitrophenyl-α-d-glucopyranoside solution. The mixture was further incubated at 37°C for 30 min and the reaction was terminated by treating the mixture in a boiling water bath for 5 min. Absorption of the released p-nitrophenol was measured at 400 nm after the addition of 1.0 mL of 0.1 M disodium hydrogen phosphate solution. A control test without BSE was carried out and the following formula was used to compute the percentage α-glucosidase inhibitory activity (Ahmed and Urooj, 2010a):

$$Inhibition\ (\%) = \frac{Absorbance\ of\ control - absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

## Effect of black seed extract on glucose uptake by yeast cells

A 10% (v/v) yeast suspension was prepared using yeast cells obtained by repeated washing dried baker's yeast with distilled water until the supernatant fluids became clear followed by centrifugation at 3000 ×g for 5 min. Various amounts of BSE (1-5 mg) were added to 1 mL of glucose solution (5-25 mmol/L) and incubated for 10 minutes at 37 degrees Celsius. The reaction was initiated by adding 100 L of yeast suspension, vortexed, and incubated at 37°C for 60 minutes. After 60 minutes, the tubes were centrifuged (2500 g, 5min) and the glucose concentration in the supernatant was determined. A control test without BSE was carried out and the following formula was used to compute the percentage increase in glucose absorption by yeast cells (Ahmed and Urooj, 2010b):

$$Gulcose\ uptake\ increase\ (\%) = \frac{Absorbance\ of\ control - absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

## Statistical analysis

The data presented were the Mean ± SD of triplicate determinations. Data were analyzed by ANOVA followed by Tukey's post-hoc test for significant differences using SPSS 20.0 software. Values were considered significant at $p \leq 0.05$. Graphs were plotted using OriginPro 2018 software.

## Results and discussion

Diabetes mellitus is a chronic condition with diverse etiologies that is distinguished by persistent hyperglycemia and disruption in carbohydrate, lipid, and protein metabolism as a result of abnormalities in insulin production, insulin action, or both. In the management of T2DM, controlling postprandial hyperglycemia, which is crucial can be achieved by slowing starch digestion via carbohydrate hydrolyzing enzyme inhibition. Thus, the current study assessed the anti-diabetic potential of black seed using various *in vitro* methods, such as its effect on glucose diffusion, carbohydrate hydrolyzing enzymes, glucose transport across the yeast cell membrane, and isolated rat hemidiaphragm.



### *Glucose adsorption capacity of black seed powder*

The glucose adsorption ability of BSP at different molar concentrations of glucose is presented in Figure 1. It was observed that BSP adsorbed significant amounts of glucose at all molar concentrations of glucose and glucose adsorption capacity of BSP was directly proportional to glucose concentration, wherein BSP adsorbed more glucose with increasing concentration of glucose. Similar trends were seen with wheat bran. Glucose adsorption capacity of foods indicates that the fibers, both soluble and insoluble fraction bind glucose liberated as a result of starch digestion in the intestinal lumen and thereby prevent its absorption into the blood stream (Bhutkar et al.,

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2017). Furthermore, higher adsorption of glucose at higher concentration of both sample and glucose offers effective control of postprandial hyperglycemia.

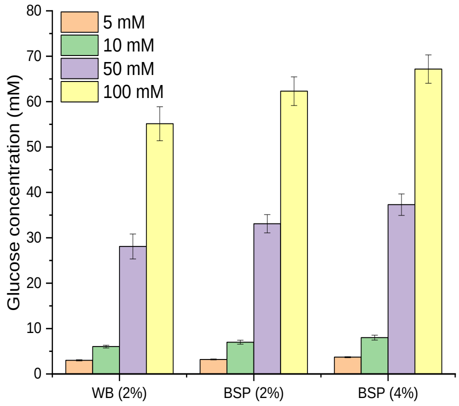


**Figure 1.** Glucose adsorption capacity of BSP and wheat bran. WB; Wheat Bran, BSP; Black seed powder.

## *Effect of black seed powder glucose diffusion*

The effectiveness of BSP in retarding diffusion of glucose across dialysis membrane is shown in Figure 2. The glucose that diffused into the dialysate in the presence of BSP was significantly lower ($p \leq 0.05$) than that of control and wheat bran at all times, consequently the glucose dialysis retardation index (GDRI) was lower than that of wheat bran and control indicating a strong trapping of glucose by BSP primarily by the dietary fibers present in it as different dietary fibers have been shown to effectively entrap varying amounts of glucose in different *in vitro* experiments (Chau et al., 2003; Ahmed et al., 2011; Liu et al., 2017). The GDRI is a useful index for predicting the in vitro effects of dietary fiber on the retardation of glucose absorption in the gastrointestinal tract (Chau et al., 2003). The higher GDRI observed for BSP compared to wheat bran infers that BBSP is able to bind more glucose resulting in lower diffusion which could be beneficial in blunting postprandial blood glucose raise (Chau et al., 2003). A number studies have indicated that polysaccharides that increase viscosity of intestinal contents are effective in delaying glucose from the GI tract wherein the dietary fibers entrap glucose in the fissures and crevices' present in the fiber meshwork thereby reducing its absorption in to the blood stream (Elsenhans et al., 1980; Lopez et al., 1996).

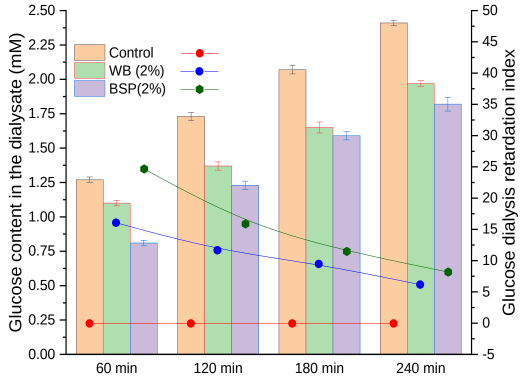


**Figure 2.** Effect of black seed powder glucose diffusion

## *Effect of black seed powder amylolysis kinetics*

Starch digestibility characteristics in the presence of BSP, wheat bran and acarbose are presented in Figure 3. It was found that acarbose completely inhibited α-amylase and showed no diffusion of glucose in to dialysate at all time intervals. Acarbose is know inhibitor of α-amylase which is in clinical use (Dalli et al., 2021). On the other hand, BSP also showed complete inhibition of α-amylase at 60- and 120-min time intervals and later there was diffusion of glucose which was significantly lower ($p \leq 0.05$) than that of wheat bran. Wheat bran also effectively reduced diffusion of glucose by inhibiting amylolysis kinetics. The retardation glucose diffusion in this system happens due two process wherein, the fibers and the phytochemicals present in BSP may reduce accessibility of α-amylase to starch molecules thereby preventing starch digestion and also by entrapping liberated glucose from diffusing across the dialysis membrane (Chau et al., 2003; Srichamroen and Chavasit, 2011; Liu et al., 2017). In case of BSP earlier studies have shown that different extracts of black seed inhibit α-amylase to varying degrees which is attributed to the presence of gallic acid, ferulic acid, quercetin, kaempferol, p-coumaric acid, naringenin, and trans-chalcone (Divya et al., 2020; Dalli et al., 2021). In amylolysis kinetics the effect of BSP on starch digestion inhibition is even more profound as indicated by GDRI of 100 found at 60- and 120-min time intervals is because BBSP is a strong inhibitor of α-amylase due to the presence of different phytochemicals and also due to the physical entrapment of



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liberated glucose by the fiber meshwork (Chau et al., 2003; Divya et al., 2020; Dalli et al., 2021).

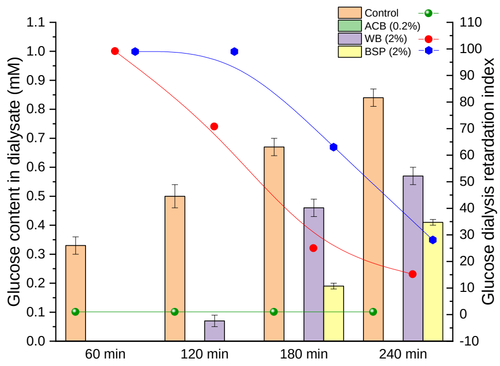


**Figure 3.** Effect of black seed powder amylolysis kinetics

## *α-glucosidase inhibitory activity of Effect of black extract*

α-glucosidase hydrolyze carbohydrates into glucose and therefore synthetic drugs that inhibit α-glucosidase are used in clinical practice to manage diabetes. However, because drugs are frequently associated gastrointestinal disturbances, utilization of α-glucosidase inhibitors from dietary sources has gained prominence (Assefa et al., 2019). In our study, BSE was found to inhibit α-glucosidase in a dose dependent manner (25 – 73%) which can be attributed to the presence of ferulic acid, rutin, naringenin, gallic acid, catechin, palmitic acid, linoleic acid and carvacrol, all of which have been shown to inhibit carbohydrate hydrolyzing enzymes to varying degrees (Dalli et al., 2021).

## *Glucose uptake by yeast cells*

Figure 4 depicts the dynamics of glucose transport across isolated yeast cells *in vitro*. It was found that BSE exhibited dose dependent increase in glucose uptake by yeast cells, wherein glucose uptake increased significantly with increasing sample concentration. However, an inverse relationship was found between the glucose concentration and uptake, implying that higher glucose concentration may result in lowered glucose uptake by yeast cells. These finding are consistent with earlier studies wherein, different plant extracts proportional increase in glucose transport with sample concentration and inversely proportional increase in glucose transport with increasing sample concentration (Harish et al., 2011; Bhutkar and Bhise, 2013; Bhutkar et al., 2017). Furthermore, yeast cell glucose transport may differ from that of other eukaryotic or human body cells. Glucose transport across the yeast membrane may entail enhanced diffusion rather than the mediation of a phosphotransferase enzyme system or any other undiscovered mechanism. Several factors, such as glucose levels inside the cells and subsequent glucose metabolism, can influence glucose uptake by yeast cells. If most of the internal sugar is rapidly metabolized, the internal glucose concentration falls and high glucose transport into the cell is promoted. Similarly, it is possible that the increased glucose absorption by yeast cells in the presence of BSE is due to both easier diffusion and increased glucose metabolism. Undoubtedly, it will be intriguing to investigate the *in vivo* activity of natural products, which may aid in increased glucose transport by muscle cells and adipose tissues of the body. The extract was able to bind glucose and transfer it across the cell membrane for further metabolism (Rehman et al., 2018).

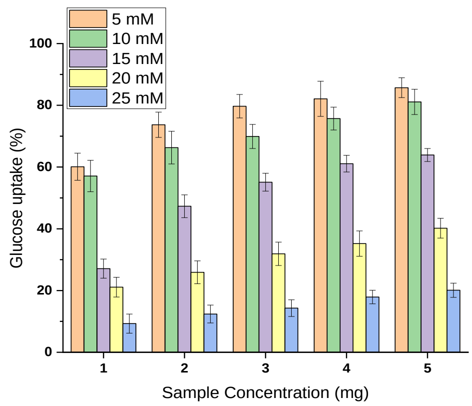


**Figure 4.** Effect of black seed extract on glucose transport in yeast cells.

## Conclusion

The findings of the study demonstrate that, black seed possesses significant hypoglycemic effect mediated by physical adsorption of glucose, inhibiting glucose diffusion, carbohydrate hydrolyzing enzymes and enhancing glucose in target tissues in addition to previously suggested mechanism of increasing insulin secretion by pancreatic cells.

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## Conflict of interest

There are no conflicts of interest.

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