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Effects of *Nigella Sativa* and *Syzygium Cumini* Seed Extracts on Blood Glucose Levels in Swiss Albino Mice

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Abstract

A Swiss Albino mouse model was used to study the effects of two species that are common in Bangladesh: *Nigella Sativa L.* and *Syzygium Cumini L.* The trial looked at how a blend of methanol extracts from *Syzygium Cumini L.* and *Nigella Sativa L.* affected blood sugar levels using the oral glucose tolerance test (OGTT). 40 overnight starved mice were divided into eight groups with a 4:1 male to female ratio. After 120 minutes of glucose injection, blood glucose levels were measured using a glucometric method. However, the mean values of the samples that were only treated with high doses of *Syzygium Cumini L.* (Group 1) and *Nigella Sativa L.* (Group 2) were 3.92 mmol/l and 3.8 mmol/l, respectively. Group-5 low dose (50 mg/kg) treated mean value was 4.02 mmol/l, group-6 moderate dose (100 mg/kg) treated mean value was 3.7 mmol/l, group-7 poly medium dose (200 mg/kg) treated mean value was 3.38 mmol/l, and group-8 poly high dose (400 mg/kg) treated mean value was 3.12 mmol/l where combined extract formulations were used. The combined formulation of the methanol extract of *Nigella Sativa L.* (Seed) and *Syzygium Cumini L.* (Seed) possesses significant oral hypoglycemic activity at different time intervals in the cases when compared to Control mean value, showing that all doses given significantly reduced blood sugar levels.

Keywords: *Nigella Sativa*; *Syzygium Cumini*; Blood Glucose; Albino mice; Bangladesh

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Introduction

Diabetes mellitus (DM), a chronic metabolic condition, is a substantial global public health concern (IDF, 2021). Over the past few decades, diabetes mellitus has consistently been ranked among the top 10 global fatalities (WHO, 2020). A person with this disorder has high blood sugar levels, either as a result of the body producing insufficient insulin or as a result of the cells failing to respond to the insulin that is

generated. The typical symptoms of polyuria (frequent urination), polydipsia (increased thirst), and polyphagia (increased hunger), according to Alemzadeh et al. (2007), are brought on by high blood sugar levels. There are two basic types of diabetes mellitus: type-1 and type-2. Type 1 diabetes is also known as insulin dependent diabetes mellitus (IDDM) or juvenile onset diabetes mellitus. Type 1 diabetes is characterized by an autoimmune attack on the pancreas by the patient's own body, which stops the pancreas from generating insulin. The pancreatic cells of type-2 diabetes mellitus, commonly known as non-insulin dependent diabetes, are either unable to produce enough insulin or are unable to use it effectively. More than 70% of all instances of diabetes are type 2, which is a common chronic disease that can be inherited (Wang, 2018; Kuddus et al., 2020; Kuddus et al., 2022). Deshpande et al. (2008) found that while Type 1 diabetes affects only roughly 5–10% of persons, Type 2 diabetes affects 90–95% of the population. Globally, 537 million persons with diabetes as of 2021; this number is projected to rise to 643 million by 2030 and 783 million by 2045 (Sun et al., 2021). In Southeast Asia, 90 million persons (one in eleven) have diabetes (IDF, 2021). In Bangladesh, diabetes is the cause of 28976 deaths overall, or 4.05% of all fatalities (Mohiuddin, 2019). 80% of adults with diabetes live in low- and middle-income countries worldwide (IDF, 2019). According to Ahsan Karar et al. (2019), Bangladesh has recently experienced an epidemiological transition from communicable to non-communicable diseases. A recent meta-analysis study (Akhtar et al., 2020; Roy et al., 2019; Chakma et al., 2022) found that diabetes mellitus affects 8% of Bangladesh's population as a whole. Diabetes is on the rise in Bangladesh as a result of the country's rapid urbanization, decline in physical activity, poor eating habits, rising cigarette smoking, and cardiovascular problems.

Insulin affects metabolism in a substantial way by causing cells in the liver, muscles, and fat tissue to take glucose from the blood, storing it as glycogen in the muscles and liver, and limiting the use of fat as an energy source. When insulin is absent or low, the body begins to mobilize lipids from adipose tissue to the liver for use as a fuel source. This is one way the body uses fat as an energy source. Cells in the body cannot absorb glucose when insulin levels are low or nonexistent. Diabetes mellitus develops when the body is unable to regulate its insulin levels. Consequently, some types of diabetes mellitus are treated medically with insulin. In terms of accessibility and safety, medicinal herbs outperform conventional medication in the treatment of many disorders (Ahmed et al., 2013; Orororo et al., 2018; Ekakitie et al., 2021). By squelching free radicals, a variety of antioxidant-rich plants alleviate renal insufficiency (Ozkol et al., 2021).

Nigella sativa L. also known as black cumin or black seed, and *Syzygium Cumini L.* also known as Java Plum, respectively, are two of these plants that have a lot of extremely promising health benefits. Due to their effects in minimizing oxidative stress and the apoptotic cascade, as well as in reducing kidney damage biomarkers and histological features, they are widely recognized as a realistic option for combating nephron-toxicants (Hosseinian et al, 2018; Hossain et al, 2017; Egbune et al, 2023). Due to the significant amount of phytochemicals in these plants' seeds, researchers are attempting to support their alleged usefulness in treating renal failure.

The bioactive compounds included in black seed, also known as *Nigella sativa*, include tannins, fixed oil, alkaloids, protein, and essential oil (Ahmed et al., 2020). Antioxidant, hepatoprotective, nephroprotective, and anti-diabetic properties are just a few of *Nigella sativa* broad-spectrum biological effects (Abduallah et al., 2017; Okoye and Elvis, 2019; Akhtar et al., 2020; Hannan et al., 2021; Abd-Elkareem et al., 2022). Thymoquinone, the monoterpenes p-cymene and apinene1, nigellidine 2, nigellimine 3, and a saponin are only a few of the compounds found in black seed oil (Al-Seenia et al., 2018; Hannan et al., 2021; Abdelrazek et al., 2018). Other substances found in black seed that support its therapeutic effects include thymol, thymo-hydroquinone, dithymoquinone, nigellone, alpha-hederin, flavonoids, and fatty acids, according to a study by Daryabeygi-Khotbehsara et al. (2017). The interplay of these several

substances improves the medicinal benefits of black seed. Investigations on both humans and animals have demonstrated that black seed has no toxicological or adverse side effects (Yimer et al., 2019).

Syzygium cumini (L.) Skeels, popularly known as the Java plum, is rich in anthocyanins, glucoside, ellagic acid, isoquercetin, kaemferol, and myrecetin. It is claimed that the seeds contain the alkaloid jamboline and the glycoside jambolin or antimellin, which prevent the diastolic breakdown of starch into sugar. Additionally, it has been demonstrated that seed extract can lower blood pressure by 34.6%; this result is attributed to the presence of ellagic acid (Morton, 1987). The scavenging of free radicals and protective action on antioxidant enzymes are attributed to the seeds' reported high flavonoid content (Ravi et al., 2004 and Ravi et al., 2004; Bari et al., 2023). Additionally, they were discovered to be quite abundant in calcium and protein and to have a high total phenolic content with strong antioxidant activity (Bajpai et al., 2005; Hasan et al., 2023; Islam et al., 2018). According to the Council of Scientific and Industrial Research (1948), java plums are high in sugar, mineral salts, vitamin C, and PP, which strengthens the antioxidant, anthocyanin, and flavonoid properties of the fruit. Java plums have been used for a range of illnesses from all over the world, including cough, diabetes, diarrhea, inflammation, and ringworm (Reynertson et al., 2005; Sazzad et al., 2023; Sunny et al., 2021b). Different portions of the plant are used by different traditional healers in India to cure conditions like diabetes, mouth blisters, cancer, colic, diarrhea, digestive disorders, dysentery, piles, acne, and stomachaches (Jain, 1991; Sunny et al., 2017; Sunny et al., 2021a). Since practitioners have cited *Syzygium Cumini L.* and *Nigella Sativa L.* as having hypoglycemic properties, pharmacological studies investigating the hypoglycemic effects of these species have been conducted in Bangladesh as well as throughout the world. The goal of the current study was to investigate the potential for creating innovative, potent, and all-natural medications for the treatment of diabetes mellitus using a formulation that included both *Syzygium Cumini L.* and *Nigella Sativa L.* methanol extracts.

Materials and Methods

Experimental site and study period

The research was carried out at the University of Development Alternative (UODA), Dhanmondi, Dhaka, at the Pharmaceutical Biotechnology lab of the Department of Biotechnology & Genetic Engineering, between September 2015 and June 2016. Seed collection of *Nigella Sativa L.* and *Syzygium cumini L.* were obtained from a Dhaka's local market. Then they were cleaned and dried in the direct sunlight. Then, until extraction, the seeds of were powdered in a household blender and stored at room temperature in an airtight container.



Figure 1: A. *Syzygium cumini L.* (seed) and B. *Nigella Sativa L.* (seed).

Procedure of extraction

The seeds of *Syzygium Cumini L.* and *Nigella Sativa L.* were ground into a powder using a blender before being measured out to 100 gm using a digital balance for extraction with polar (methanol) solvent. A beaker was filled with methanol at a ratio of 5:1, or 500 ml for every 100 g of powder. The solvent and dry sample were then mixed together to create a soft solution by being carefully swirled with a glass rod or any stainless steel rod. After a few minutes, stirring was resumed and maintained for an hour while the beakers were covered with aluminum foil. The system was then abandoned for 48 hours over night. After 48 hours, the solvent was filtered through a thin cotton cloth (white in color) before the extract was placed in a water bath, where it was let it to dry until it had the consistency of a crude medicine. After 5 days of evaporation, 9.557gm of extraction was discovered with a constant temperature of 40°C, and it was finally collected by spatula in a designated glass vial. The vial holding the extract was kept in the refrigerator at a temperature of 4–8°C, and the extracted residues were stored in plastic jars.

Experimental animals

A total of 40 Swiss albino mice were collected from the ICDDR, B, Animal Resource Branch in Mohakhali, Dhaka. The mice were kept in steel cages @10 mice per cage and were fed corn and water. They were labeled for identification during the experiment using red, black, and blue permanent markers. The animals were selected based on their weight in order to maintain a reasonably constant average body weight across all groups.



Figure 2: Experimental Animal (Swiss albino mice) at animal laboratory of University of Development Alternative.

Experimental design

Firstly, 40 adult mice (male: female ratio = 2:3) were selected. They were then divided into 8 groups of 5 mice each, with the names Control, Standard, Group-1, Group-2, Group-5, Group-6, Group-7, and Group-8. Then, 0.4 gm of each of the *Nigella Sativa L.* and *Syzygium Cumini L.* methanol extracts were collected from a vial and dissolved in DMSO (net volume 1 ml for each suspension), while 10 gm of glucose was dissolved in distilled water (net volume 10 ml) in a beaker. In the meantime, a vial containing a 5 mg Glibenclamide tablet was filled with DMSO (net volume: 1 ml). Following an overnight fast, mice from the Standard group were gavaged with Glibenclamide at a dose of 10 mg/kg body weight, followed by mice from Groups 1 and 2 (high doses of 400 mg/kg and 400 mg/kg, respectively). (Low dosage) Group 5 At doses of 50 mg/kg, 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight, respectively, the combination

formulation of the methanol extract of *Syzygium Cumini L.* (seed) and *Nigella Sativa L.* (Seed) was given to Group-6 (moderate dose), Group-7 (medium dose), and Group 8 (high dose). Following an hour, 2 gm/kg of body weight of glucose was gavaged to all the mice, including the Control group. After 120 minutes of glucose gavage, all of the mice underwent blood collection by lancing their tails and glucose level analysis using a glucometer. The blood glucose levels were calculated using the glucometer reading, which was recorded in mmol/l.

Measurement of blood glucose level

Glucosemetry is a technique for determining the amount of glucose present in peripheral or central blood. These values, which can be expressed in mg/dl or mmol, are clinically important for metabolic illnesses like diabetes mellitus and malnutrition, as well as some of their side effects like hyperosmolar coma, malabsorption syndrome, and the most dangerous condition, hypoglycemia, or lower than normal blood glucose levels.

The measurement of blood glucose levels is done with a device called a glucometer. The glucometer will interact with a mouse's blood drop using a test strip (Model: AccuSure Instant Digital Simple Glucometer Kit). The meter shows the amount of glucose in mg/dl or mmol/L following a chemical reaction. With only a tiny amount of blood required, this tiny, portable device tests blood glucose levels swiftly, simply, and economically.



Figure 3: Glucometer used for the measurement of blood glucose level.

Data analysis

A prepared data collecting sheet was used to record all of the information. The unpaired Student's t test was used to compare continuous variables between research subject groups. Continuous variables were represented using mean SD. The Chi-square test was used to compare categorical variables, and absolute frequencies and percentages were given. To evaluate the relationship between various factors and psoriasis, the Spearman's rank correlation coefficient (r) test was applied. With significance set at p 0.05 or higher at the level of the 95% confidence interval, all p values were two-tailed. The SPSS version 22 program for Windows was used to conduct the analysis.

Results and Discussion

Oral glucose tolerance test (OGTT) was used in the present study to examine the effects of a combination formulation of methanol extracts from *Nigella Sativa L.* (Seed) and *Syzygium Cumini L.* (Seed) on lowering blood glucose levels. Eight groups of 40 overnight deprived mice (male:female ratio: 2:3) were created. They received 10 mg/kg of the conventional medication Glibenclamide (conventional group) and glucose by gavage (Control group). Only *Syzygium Cumini L.* (seed) extracts (400 mg/kg) were administered to group 1; in group 2, only *Nigella Sativa L.* (seed) extracts (400 mg/kg) were used. In groups 5, 6, 7, and 8, respectively, doses of 50, 100, 200, and 400 mg/kg body weight of a mixed formulation of the methanol extract of *Nigella sativa L.* (Seed) and *Syzygium Cumini L.* (seed) were gavaged. After 60 minutes, glucose (2 gm/kg body weight) was then administered. A glucometric approach was used to measure blood glucose levels 120 minutes after glucose injection.

Table 1. Results of hypoglycemic effect of formulation containing *Nigella Sativa L.* and *Syzygium Cumini L.* Seeds MeOH extract

Serial	Control	Standard	Group 1	Group 2	Group 5	Group 6	Group 7	Group 8
1	5.8	3.2	4	3.8	4	3.6	3.4	3
2	5.6	3.4	4.1	3.9	4.2	3.8	3.3	3.3
3	5.9	3.1	3.8	3.7	4.1	3.8	3.4	3.2
4	5.8	2.9	3.7	4	4	3.5	3.1	3
5	5.9	3.2	4	3.6	3.8	3.8	3.7	3.1
Sum	29	15.8	19.6	19	20.1	18.5	16.9	15.6
Mean	5.8	3.16	3.92	3.8	4.02	3.7	3.38	3.12
SD	0.12	0.18	0.16	0.16	0.15	0.14	0.22	0.13
SE	0.05	0.08	0.07	0.07	0.07	0.06	0.09	0.05
T-value		26.94	20.51	22.36	20.69	25.09	21.73	33.5
P-value		0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005
Significance level		0.05%	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%
5% Significance Level		Significant	Significant	Significant	Significant	Significant	Significant	Significant

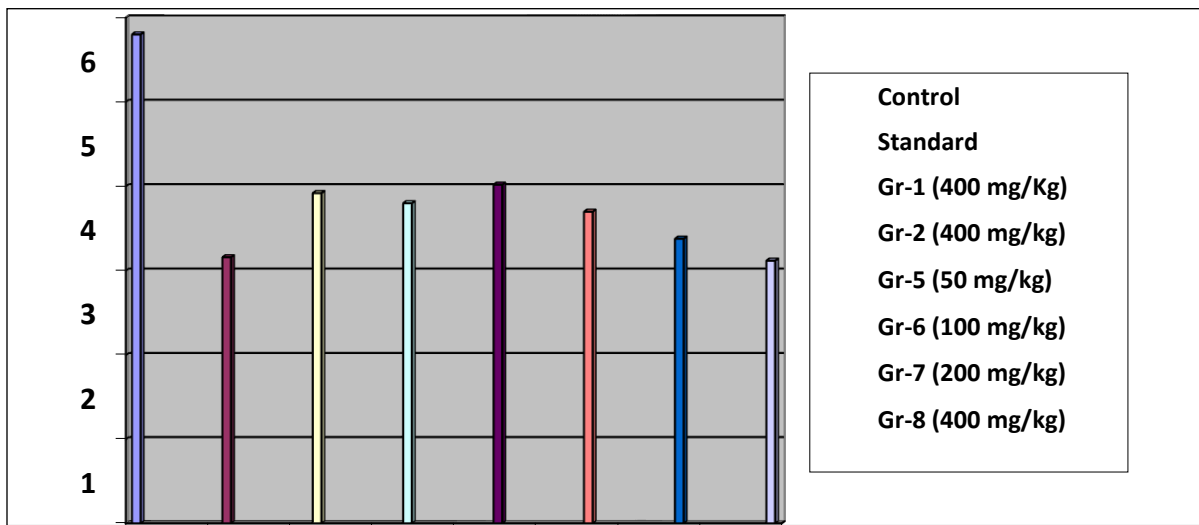


Figure 4: Measurement of blood glucose level of mice after 120 minutes of glucose gavaging with different treatments.

From the glucometric analysis of blood glucose levels after 120 minutes glucose gavaging, it was found that the Control mean value was 5.8 mmol/l and the Glibenclamide treated sample mean value was 3.16 mmol/l. On the other hand, only *Syzygium Cumini* L. MeOH extract high dose - 400 mg/kg (Group-1) treated mean value was 3.92 mmol/l, and only *Nigella Sativa* L. MeOH extract high dose-400 mg/kg (group-2) treated mean value was 3.8 mmol/l. group-5 low dose (50 mg/kg) treated mean value was 4.02 mmol/l, group-6 moderate dose (100 mg/kg) treated mean value was 3.7 mmol/l, group-7 poly medium dose (200 mg/kg) treated mean value was 3.38 mmol/l and group-8 poly high dose (400 mg/kg) treated mean value was 3.12 mmol/l where combined formulation of extract was used. Comparing with Control mean value all the given doses significantly decreased the blood sugar level and it demonstrates combined formulation of the methanol extract of *Nigella Sativa* L. (Seed) and *Syzygium Cumini* L. (seed) possesses significant oral hypoglycemic activity at different time interval in the cases.

Conclusion

From our scientific investigation we have observed that of formulation containing *Nigella Sativa* L. and *Syzygium Cumini* L. seeds has hypoglycemic activities at different doses. We can conclude that available *Nigella Sativa* L. and *Syzygium Cumini* L. species in Bangladesh, have some desired chemical entities that confirm its hypoglycemic activates. Planned and systematic cultivation and proper scientific investigation for pharmacological of these plants are sure to produce a large variety of new drugs and pharmaceutical raw materials of natural origin. Thus the indigenous *Nigella Sativa* L. and *Syzygium Cumini* L. promises to be a very good source of new drugs and pharmaceutical raw materials in the country.

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