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Original Research

Effect of *Stereospermum personatum*, *Senna obtusifolia* and *Amomum subulatum* extract in Hypoglycemia on Swiss Albino mice model

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ABSTRACT

Stereospermum personatum, Senna obtusifolia and Amomum subulatum are common species throughout Bangladesh, India, Srilanka, Pakistan and other Asian countries. Combined formulation (polyharbal) of the methanol extract of *Stereospermum personatum* leaves, *Senna obtusifolia* leaves pod and *Amomum subulatum* seeds were examined for hypoglycemic activity. The potential of the combined formulation of the methanol extract of these leaves, leaves pod, and seeds to reduce blood glucose was examined in this study using a Swiss Albino mouse model. The polyherbal formulation's highest dose in this experiment, 400 mg/kg body weight, dramatically lowered the blood glucose level. The remaining dosages of this polyhedral formulation when taken orally likewise had notable hypoglycemia effects. When compared to the Control mean value, the combined formulation of the methanol extract of the leaves of *Stereospermum personatum*, the pod of *Senna obtusifolia*, and the seeds of *Amomum subulatum* exhibited significant oral hypoglycemic activity at various time intervals, indicating that all administered doses significantly decreased blood sugar levels.

1. Introduction

Chronic metabolic disease known as diabetes mellitus (DM) is a major global public health concern (IDF, 2021). Diabetes mellitus has routinely been listed among the top 10 causes of death worldwide over the last several decades (WHO, 2020). When this illness occurs, the body either produces too little insulin or the cells don't react to the insulin that is produced, which leads to high blood sugar levels. High blood sugar levels cause the characteristic symptoms of polyuria (frequent urine), polydipsia (increased thirst), and polyphagia (increased hunger), according to Alemzadeh et al. (2007). Type-1 and type-2 are the two main forms of diabetes mellitus. Juvenile onset diabetes mellitus and insulin dependent diabetes mellitus (IDDM) are other names for type 1 diabetes. The hallmark of type 1 diabetes is an autoimmune response by the patient's body to the

pancreas, which prevents the organ from producing insulin. Type-2 diabetes mellitus, also referred to as non-insulin dependent diabetes, is characterized by insufficient insulin production or inefficient insulin utilization by the pancreatic cells. Type 2 diabetes is a widespread chronic illness that can be inherited, accounting for more than 70% of cases of the condition (Wang, 2018; Kuddus et al., 2020; Kuddus et al., 2022). According to Deshpande et al. (2008), 90-95% of people have Type 2 diabetes, compared to about 5–10% who have Type 1 diabetes. There were 537 million diabetics worldwide as of 2021; by 2030, there were expected to be 643 million, and by 2045, there were expected to be 783 million (Sun et al., 2021). Ninety million people, or one in eleven, in Southeast Asia suffer from diabetes (IDF, 2021; Kuddus et al., 2021). According to Mohiuddin (2019), diabetes is the cause of 28976 deaths in Bangladesh overall, or 4.05% of all fatalities. Around the world, low- and middle-income nations are home to 80% of adult diabetics (IDF, 2019). Bangladesh has recently seen an epidemiological shift from communicable to noncommunicable diseases, according to Ahsan Karar et al. (2019). According to a recent metaanalysis study (Akhtar et al., 2020; Roy et al., 2019; Chakma et al., 2022), 8% of Bangladesh's population as a whole has diabetes mellitus. Bangladesh's fast urbanization, decreased physical activity, unhealthy eating habits, increased cigarette smoking rates, and cardiovascular issues are all contributing factors to the country's rising diabetes rate.

As anti-hyperglycemic medications, insulin, glimepiride+ pioglitazone, metformin, acorbose, nateglinide, pioglitazone, and sitagliptin are frequently used in Bangladesh (Ahmed et al., 2013; Orororo et al., 2018; Ekakitie et al., 2021). Skin rash or itching, weight gain, kidney complications, upset stomach, fatigue or dizziness, metallic taste, gas bloating, and diarrhoea are possible adverse effects of such oral anti-hyperglycemic medications (Ozkol et al., 2021). Large trees called Stereospermum Personatum can be seen growing in Bangladesh, Myanmar, and India. The majority of studies on stereospermum have concentrated on the bark and possible consequences of it (Alam et al., 2023a; Hossain et al., 2023). For the treatment of indigestion, hiccups, vomiting, diarrhea, pain, fever, diabetes, liver problems, and asthma, people take stereospermum orally. Stereospermum is applied topically to the skin to treat wounds. Stereospermum may lower fevers, lower blood sugar and cholesterol, lessen discomfort and swelling, and shield the liver from toxins. It may also shield the brain from disorders like Parkinson's disease or decreased blood flow. It appears that stereospermum has anti-oxidant properties.

Senna obtusifolia is a plant in the genus Senna, occasionally divided into the monotypic genus Diallobus. Common names for this species include Chinese senna, American sicklepod, sicklepod, etc (Bari et al., 2023). It is often regarded as a very dangerous weed that grows wild throughout Asia, Africa, Oceania, Central and South America, and Asia. In folk medicine, it is also used for its leaves, seeds, and root; its use is mostly in Asia. It is also thought to be good for the eyes and to have a laxative effect. The seeds are frequently roasted and then cooked in water to make tea as a traditional medicine. The Chinese Senna was formerly classified in the genus Cassia, so the name

"cassia gum," a thickening agent commonly used in food additives, comes from the plant's seeds. The ground and roasted seeds can also be used in place of coffee. Hairy roots from S. obtusifolia in vitro cultures have the potential to yield important secondary metabolites with therapeutic uses. Black cardamom, or Amomum subulatum, is a nutritional supplement with numerous health advantages. It is well known to support stronger immunity, lessen inflammation, and enhance digestion. Additionally, it has been shown to lower cholesterol, strengthen the heart, and lower the risk of developing several cancers (Sunny et al., 2021b). It can also lessen fatigue, enhance the quality of sleep, and lessen tension and worry. It can also lower the risk of diabetes, enhance cognitive function, and improve skin health. Several Indian traditional healers employ different parts of the plant to treat a variety of ailments, including piles, acne, diabetes, mouth blisters, cancer, colic, diarrhea, and digestive issues (Jain, 1991; Sunny et al., 2017; Sunny et al., 2021a). Pharmacological research examining the hypoglycemic effects of Senna obtusifolia, Amonum subulatum, and Stereospermum personatum have been carried out in Bangladesh and around the world since practitioners have mentioned these species as having hypoglycemic qualities (Irin et al., 2017). The present study set out to explore the possibility of developing novel, effective, and natural drugs for the management of diabetes mellitus by employing a formulation of methanol extracts from Stereospermum personatum, Senna obtusifolia, and Amomum subulatum.

2. Materials and Methods

2.1 Location and Study Period

The study was conducted from November 2017 to February 2018 at the University of Development Alternative (UODA), Lalmatia, Dhaka, at the Pharmaceutical Biotechnology Lab of the Department of Biotechnology and Genetic Engineering.



Figure 1: A. Stereospermum personatum (leaves) and B. Amomum subulatum (seeds) and C. Senna obtusifolia (leave pod)

2.2. Gathering vegetation and making a powder

Amomum subulatum seeds, Senna obtusifolia leaf pods, and Stereospermum personatum leaves were gathered from hills near Rangamati, Bandorban, and Gazipur and dried in the sun. Then, using a household blender, the dried leaves of Stereospermum personatum, Senna obtusifolia leaf pod, and Amomum subulatum seeds were ground into a powder and kept at room temperature in an airtight container until extraction.

2.3. Method of Extraction

The following describes how to extract leaves from *Stereospermum personatum*, pods from *Senna obtusifolia*, and seeds from *Amomum subulatum* using a non-polar (MeOH) solvent: The powder was created by blending dried Stereospermum personatum leaves, Senna obtusifolia leaf pods, and *Amomum subulatum* seeds. Then, using a digital balance, the dried powder of the seeds, pods, and leaves of *Senna obtusifolia*, *Stereospermum personatum*, and mixed (50+50+50=150 gm) of all three plants were measured. A beaker was filled to capacity (5:1) with methanol, and 100 g of mixed powder was added. The solvent and sample were mixed slowly with a glass rod or any other stainless steel rod to create a smooth liquid. Following many minutes of shaking, aluminum foil was placed over the beaker. For 48 hours, the mixers were left overnight. After filtering the solvent through a thin white cotton towel, we moved the extract into a water bath and waited to obtain dry extract (raw medication). After evaporating for seven days at a constant temperature of forty degrees Celsius, an extraction of seven grams was discovered. It was then scraped into a glass vial using a spatula. The extracted leftovers were stored in plastic jars, and the vial of extract was kept in a refrigerator between 4 and 8 degrees Celsius.

2.4. Animals used in experiments

A total of thirty Swiss albino mice were gathered from the Mohakhali, Dhaka, ICDDR'b Animal Resource Branch. The mice were fed maize and regular water while being kept in a steel cage with ten mice per cage. They were identified during the trial by having permanent markers in the colours red, black, and tube painted on their tails. The animals were chosen based on their body weight in order to maintain a somewhat constant average body weight across all groups.

2.5. Design experimentation

Initially, eight groups of forty mature mice (male: female = 4:1) were created, with five mice in each group. These groups were called Group-1 (Control), Group-2 (Standard), Group-3, Group-4, Group-5, Group-6, Group-7, and Group-8. Subsequently, 10 gm of glucose were dissolved in distilled water (net volume 10 ml) in a beaker, and 0.4 gm of the methanol extract from the seeds of *Stereospermum personatum*, *Senna obtusifolia*, and *Amomum subulat* was obtained and dissolved in DMSO (net volume 1 ml for each suspension). A 5-gram Glibenclamide tablet was swallowed in a vial and dissolved in 1 millilitre of DMSO in the meantime. Following an overnight fast, mice from the standard group were initially gavaged with a dose of 10 mg/kg body weight of Glibenclamide. Subsequently, mice belonging to Groups 3 and 4 were given a combination formulation containing a methanol extract of *Stereospermum personatum* leaves, *Senna obtusifolia* leaf pod, and *Amomum subulatum* seeds. The doses of the formulation were 100, 200, and 400

mg/kg body weight, respectively. Following an hour, glucose was injected intraperitoneal (IV) at a dose of 2 mg/kg body weight into every mouse, including the control group. Following a 120-minute glucose gavage, all of the mice were sacrificed and their blood was drawn by striking their tails. The glucometer was then used to measure the blood glucose level in milligrams per milliliter.



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

2.6. Determination of blood glucose levels

One method for figuring out how much glucose is in the central or peripheral blood is called glucosemetry. In cases of metabolic illnesses such as 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, these values, which can be expressed in mg/dl or mmol. are clinically important. A glucometer is the instrument used to measure blood glucose levels. Using a test strip, the glucometer will interact with a drop of mouse blood (Model: AccuSure Instant Digital Simple Glucometer Kit). After a chemical reaction, the metre displays the amount of glucose in mg/dl or mmol/L. This small, portable device quickly, easily, and affordably measures blood glucose levels with just a small bit of blood.

2.7. Data Analysis

All of the data was recorded using a pre-made data collection sheet. The study subject groups' continuous variables were compared using the unpaired Student's t test. The mean SD was used to describe continuous variables. Categorical variables were compared using the Chi-square test, and absolute frequencies and percentages were provided. The Spearman's rank correlation coefficient

(r) test was used to assess the link between different parameters and psoriasis. All p values were two-tailed, with significance established at p 0.05 or greater at the level of the 95% confidence interval. The analysis was performed using the Windows version of SPSS software, version 22.

3. Results and discussion

3.1. Test for saponins

A vigorous shaking was applied after mixing 0.5 g of the plant material's methanolic extract with water. A persistent foaming was created (which stays stable on heating). It suggests that the extract contains saponins.

3.2. Terpenoids

A second test tube was filled with approximately 0.5 g of plant extract and 2 ml of chloroform. Carefully, 5 ml of strong sulphuric acid was added to form a layer, which was then studied for the existence of a reddish-brown colour interface, which indicated the presence of terpenoid.

3.3. Test for alkaloids

In a test tube, 0.5 g of methanolic extract and 5 ml of 1% HCl were added. After a few minutes at 900 C in a water bath, the test tube was filtered using filter paper. Wagner reagent (Standard solution of I2 in KI) is used to treat the filtered solution. It revealed the formation of a brown precipitate, indicating the presence of alkaloid.

3.4. Test for flavonoids

A test tube containing a little amount of extract was filled with a few drops of concentrated hydrochloric acid. According to Bari et al. (2023), the rapid development of red colour signifies the presence of flavonoids.

3.5. Tannin examination

In a test tube, 0.5 g of methanolic extract was collected, diluted in 5–10 ml of distilled water, and filtered. The filtered solution was supplemented with 5% FeCl3. The rapid development of blue and blue-black colour suggests the presence of tannin (Bari, et al., 2023).

After 120 minutes of glucose gauging, the control sample's mean was 5.74 mmol/l and the Glibenclamide-treated sample's mean was 3.14 mmol/l, according to the current study's glucometric analysis from mice blood. However, the mean value of the MeOH extract treated with a modest dose of 100 mg/kg of Polyspermum personatum, Senna obtusifolia, and Amomum subulatum (polyherbal) was found to be 4.1 mmol/l. The mean value of the medium dose (200 mg/kg) treated was 3.82 mmol/l. The treated value at a high dose of 400 mg/kg was 3.48 mmol/l. Every dose considerably lowered blood sugar when compared to the control mean value.

Serial	Group 1 (Control)	Group 2 Standard	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
	Glucose	Gliben clamide	Stereospermu m personatum	Senna obtusifolia	Amomum subulatum	poly	poly	poly
	4 gm/kg	10 mg/kg	400 mg/kg	400 mg/kg	400 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
1	5.8	3.7	4.3	4.1	4.5	4.1	3.800	3.500
2	6.1	3.2	4.2	4.1	4.2	4	3.700	3.200
3	6.2	3.5	4.2	4.3	4.4	4.2	3.800	3.300
4	5.8	3.3	4.1	4.2	4.3	3.9	3.800	3.600
5	5.8	3.6	4.4	4.3	4.1	4.3	4.000	3.800
Sum	29.7	17.3	21.2	21	21.5	20.5	19.100	17.400
Mean	5.94	3.46	4.24	4.2	4.3	4.1	3.820	3.480
SD	0.194	0.207	0.114	0.1	0.158	0.158	0.110	0.239
SE	0.0871	0.092	0.050	0.044	0.070	0.070	0.049	0.107
t-value		19.484	16.832	17.758	14.610	16.392	21.200	17.847
p-value		0.0005	0.0005	0.0005	0.0005	0.0005	0.001	0.001
Significanc e level 5%		0.05%	0.05%	0.05%	0.05%	0.05%	0.001	0.001
significance level		Significant	Significant	Significant	Significant	Significant	Significant	Significant

Table 1: The hypoglycemic effect of a formulation that included MeOH extracts of *Stereospermum personatum*, *Senna obtusifolia*, and *Amomum subulatum*.

As we can see, our sample demonstrated a considerable reduction in blood glucose levels when compared to the standard greatest hypoglycemic activity; the treated mean value was 3.48 mmol/l, which is comparable to that of the standard medications (3.46 mmol/l). Given the plants' widespread distribution, particularly in Bangladesh's rural areas, their extract presents a feasible and potentially affordable option for obtaining raw materials for the identification of new compounds with antinociceptive properties (Irin et al., 2017; Islam et al., 2023; Alam et al., 2023b). Once more, specific chemical groups may be responsible for the antinociceptive effects shown in this study. To identify the specific bioactive component or components that are at fault, more investigation is necessary.

4. Conclusion

Our scientific research has shown that a formulation containing *Amomum subulatum*, *Senna obtusifolia*, and *Stereospermum personatum* possesses hypoglycemic properties at several dosages. We can draw the conclusion that the hypoglycemic activities of the *Stereospermum*

personatum, *Senna obtusifolia*, and *Amomum subulatum* species that are now found in Bangladesh are confirmed by certain required chemical entities. A wide range of novel medications and natural pharmaceutical raw materials will undoubtedly be produced by well planned, methodical cultivation of these plants and appropriate scientific research into their pharmacology. As a result, the nation's natural resources of *Senna obtusifolia*, *Amomum subulatum*, and *Stereospermum personatum* promise to be excellent sources of novel medications and pharmaceutical raw materials.

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Author Contribution

Every author participated in the conceptualization, fieldwork, data analysis, study design, and execution phases of the work. Upon reviewing the final publication, each author provided their approval.

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A statement of conflicting interests

The authors declare that none of the work reported in this study could have been impacted by any known competing financial interests or personal relationships.

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