Original Research Article

Mapping of Potential Variation of Diabetes Amelioration Activity of *Spondias mombin* on Alloxan-induced Diabetic Wister Rat Concerning the Availability of Phytochemical Constituents based on Seasonal Contrast Phenomenon

Abstract

Diabetes mellitus is the seventh leading cause of death globally, and it is a disease that is progressing with deadly implications on a global scale. Furthermore, the treatment regimen is not affordable to all socioeconomic groups. As a result, scientists are working to develop cheaper, more effective phytochemical-based medications. *Spondias mombin* is a tree and flowering plant in the Anacardiaceae family that is commonly known as the "tapereba" or "caja" tree in Brazil. It is found in both the Atlantic Forest and the Amazon in upland and floodplain forest environments, and it is also present in inhabited areas, albeit in a sub-spontaneous state. The purpose of this study was to determine the anti-diabetic properties of crude ethanol extracts of *Spondias mombin* obtained in three seasons of the year: following winter, summer, and the rainy season. The goal of this study is to determine which season of the year the plant under consideration has the most anti-diabetic action, change in body weight, and serum lipid profile. The anti-diabetic effects were assessed using a one-touch glucometer after administration of 500 mg/kg doses of the extracts from each seasonal extract at the following time intervals: 1h, 2h, 4h, 6h, 12h, 24h, and 72h. The extract following the rainy season showed optimum anti-diabetic efficacy with a serum lipid profile at a dose of 500 mg/kg. However, the 500mg/kg extract following winter and summer did not show any significant benefits in diabetes or serum lipid profiles. This study discovered that the anti-diabetic properties of *Spondias mombin* vary seasonally.

Key words: *Spondias mombin*, Diabetes mellitus, anti-diabetic, rainy season

Introduction

Diabetes and its consequences constitute a significant healthcare burden globally, posing significant problems to individuals, health services, and national economies. According to WHO, the global population will grow by 37% between 2000 and 2030, while the number of diabetics will grow by 114% [1]. Diabetes is a disorder characterized by abnormally high blood glucose (or blood sugar) levels. In this scenario, the body does not produce enough insulin or respond to insulin correctly. Diabetes is classified into two categories: they are classified as type 1 and type 2. Diabetes mellitus is another name for type 2 diabetes. Type 2 diabetes is a rapidly expanding worldwide health problem that is intrinsically linked to the obesity pandemic. Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia caused by changes in carbohydrate, lipid, and protein metabolism, leading to abnormalities in insulin synthesis, insulin action, or both [2]. Type 2 diabetes is caused by insulin resistance and pancreatic beta cell loss [3]. Sulphonylureas, meglitinides, biguanides, thiazolidinedione, alpha-glucosidase, acarbose, and other oral medicines are used to treat diabetes [4].

However, these medications can cause hypoglycemia, congestive heart failure, gastrointestinal side effects, bone fractures, and liver damage [5]. In addition, the expensive cost of these oral drugs makes it difficult for patients to adhere to the treatment regimen. As a result, physicians and scientists are looking for herbal medicines with anti-diabetic efficacy, fewer unwanted effects, and a fair price. The number of people seeking alternative and herbal therapies is increasing at an alarming rate.

Herbal remedies are the result of hundreds of years of therapeutic expertise by generations of traditional medicine practitioners. Herbal medicine is still the major source of primary health care for around 75-80% of the world's population, primarily in underdeveloped nations [6]. This is largely due to the widespread belief that herbal medications have no negative effects and are inexpensive and locally available [7]. According to the World Health Organization (WHO), the use of herbal treatments outnumbers conventional medications by two to three times [8]. Plant-derived chemicals have played a crucial role in the treatment of a variety of diseases as well as drug discovery operations for thousands of years. Plant-based drugs have been shown to be beneficial in treating TB, skin disorders, cancer, diabetes, jaundice, AIDS, hypertension, mental health issues, and a range of other infectious diseases [9].

Spondias mombin, often known as hog plum or yellow mombin, is a tree and flowering plant in the Anacardiaceae family. It occurs in the rainforest and along the shore. It may grow to a height of 15 to 22 meters. A medium-sized to giant tree with long compound leaves with an odd number of leaflets ranging from 9 to 19. Typically, the leaves are alternate but clustered towards the ends of the branches, radiating from the branch in all directions like the spokes of a wheel [10]. Tannins, saponins, phenol, alkaloids, flavonoids, phytate, oxalate, and cyanogenic glycosides are found in the leaves of this plant [11-12]. It has anxiolytic, antibacterial, anti-diabetic, antioxidant, abortifacient, antipsychotic, oxytocic, antidepressant, anti-inflammatory, and other activities [13-21].

In our study, we evaluated the anti-diabetic benefits of this plant's leaves gathered in three distinct seasons: following winter, summer, and the rainy season (January to December). Seasonal fluctuations may affect metabolite concentrations or the concentrations of various metabolites in different seasons. This might be related to changes in environmental variables that occur during the harvesting season of plant resources. The activity of an enzyme that aids in the creation of various chemical substances is frequently governed by environmental factors. The purpose of this research is to discover the season in which the plant under consideration possesses the greatest anti-diabetic activity. Thus, this study sought to investigate, from an ecophysiological standpoint, the effect of climatic environmental factors on the synthesis of secondary metabolites as well as whether the change in these metabolites alters the plant's antidiabetic action.

Method and Materials

Plant Collection and Extraction

At the end of the winter, summer, and rainy seasons, leaves of the *Spondias mombin* were collected from the same location. Collected leaves were thoroughly cleaned with distilled water and sun-dried for 7 days. The leaves were then put into the oven for 7 days at 45°C, followed by the winter, summer, and rainy seasons. All the leaves were stored in a cool, dry place. At the same time, leaves from seasonal times were processed into a fine powder. The maceration process was individually repeated thrice for 3 different preparation in same manner. 500 gm of fine powder was added in 20ml of 95% ethanol and occasional shaking was ensured. Next the solvents was dried in a rotary evaporator and filtered through filter paper. All extracts are kept at 4°C.

Chemicals and drugs

Alloxan, which was purchased from Sigma Aldrich, was used to induce diabetes in healthy male rats. Metformin, a common anti-diabetic medicine, was received as a gift sample from Incepta Pharmaceutical Ltd.

Animal

The experiment involved 100 male rats with an initial weight between 105.4 and 160.4 g. Each of them was kept in all-around ventilated cages for 15 days prior to the experiment in the animal houses of the Institute of Nutrition and Food Science University of Dhaka. 100 rats were divided into 10 groups.

Group 1: considering as negative control group /administered respective solvent only (10ml/kg).

Group 2: Considering as alloxan-controlled, no medicament was given in this group.

Group 3: Treated with alloxan, moderate dose of metformin (250 mg/kg) was given to this group.

Group 4, 5 and 6: Alloxan induced disease model, treated with different seasonal extracts after winter, after summer and after rainy extracts respectively at same dose (750 mg/kg).

Group 7: Only treated moderate dose of metformin (250 mg/kg) respectively

Group 8, 9, 10: Only treated with different seasonal extracts after winter, after summer and after rainy extracts respectively at same dose (750 mg/kg).

Investigation of Antidiabetic activity and hematology test

Extracts were administered by dissolving in propylene glycol (100mg/500µL). Alloxan was given in 150 mg/kg body weight of rat via intraperitoneal root. Blood samples from the rat's were taken once a week from the tip of its tail. On the first, seventh, fasting blood glucose was estimated. Body weight was measured just before starting the experiment and before sacrificing animals. X…….X. Serum was separated, examined for triglycerides, cholesterol, HDL, LDL, creatinine, urea, and serum SGOT and SGPT were estimated.

Result and Discussion

In this study, we investigated the anti-diabetic effects of *Spondias mombin* extracts from three distinct seasons (after winter, after summer, and after rainy seasons). With these extracts, we also measured total lipid profile alteration as well as urea and creatinine levels in mice. This data suggests that seasonal fluctuation influences *S. mombins'* anti-diabetic activity and total lipid profile.

Body weight

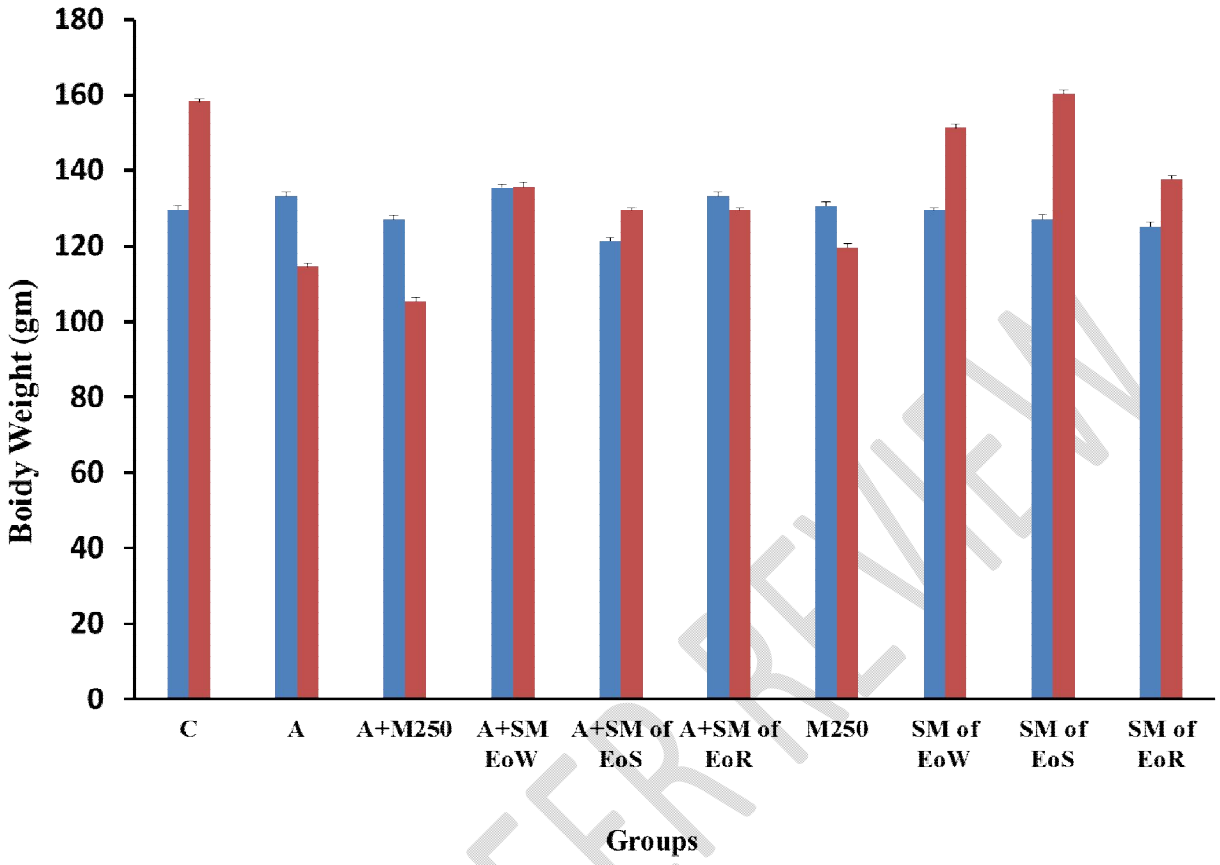

Figure 1: Variation in bodyweight before and after administration of drugs. Here, C=Control, A=Alloxan, M=Metformin, SM= *Spondias mombin* , EoW= Extract of Winter , EoS= Extract of Summer , EoR= Extract of Rainy Season

The body weight was lower in the groups that were given alloxan, metformin, or alloxan plus metformin (250 mg). However, groups 4 and 5 that were alloxan induced and then given the extract dosage of 500 mg following winter and summer observed loss of weight. **Group 6** treated with the extract following the rainy season at a dosage of 500 mg showed no reduction or change in body weight (**Figure 1**). This suggests that the extract following the rainy season had a significant influence on the body weight. But the group 8, 9 and 10 treated with the only extract 500 mg of each seasons didn't show any variation in with the control group.

Blood glucose level

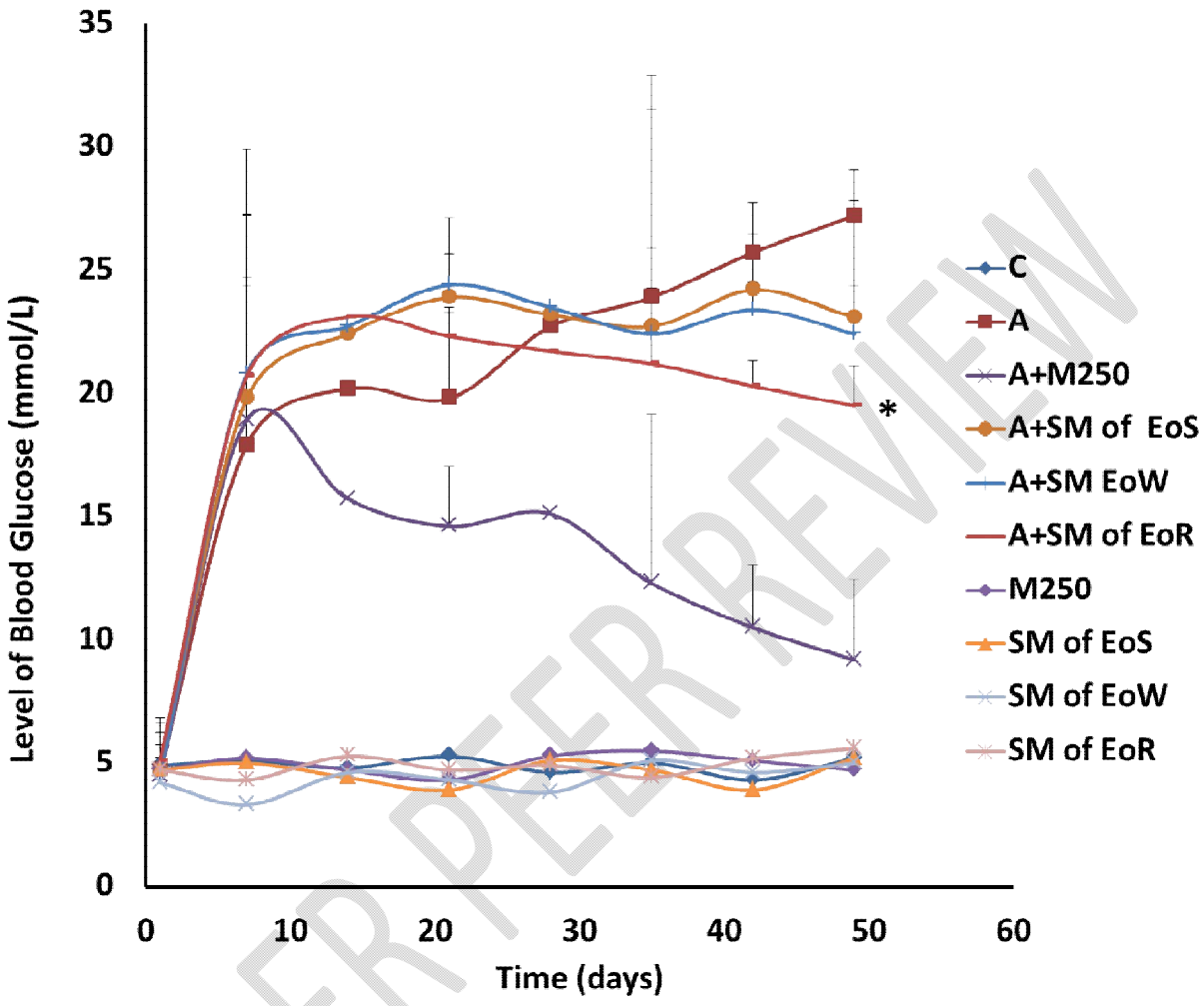

Figure 2: Variation in Blood Glucose levels of rats belonged to different groups. Here, C=Control, A=Alloxan, M=Metformin, SM= *Spondias mombin* , EoW= Extract of Winter , EoS= Extract of Summer , EoR= Extract of Rainy Season

Depicts a comparison of the anti-diabetic actions of extracts from three different seasons (winter, summer, and rainy) at a dosage of 500 mg for each extract. Distinct results were seen in the treatment group, which was given alloxan and three different seasonal extract doses. The blood glucose levels in the two groups (4, 5) treated with alloxan and 500 mg extract in the following winter and summer decreased, although not statistically significantly. However, group 6 was treated with alloxan and 500 mg extract following the rainy season, and the results were

statistically significant ($p<0.05$)*. Only this group's results were comparable to those of the control group. The groups 8, 9, and 10 treated with the solitary extract of 500 mg of each season, on the other hand, did not defer from the control group.

This data clearly suggests that the extracts from the post-rainy season have hypoglycemic properties. This might be related to the high flavonoid content of the extract treated with the after-rainy-season extract. Similar results were obtained in *Zizyphus spina-christi (L.), Loranthus micranthus,* and *Eucalyptus globulus* L. plants [22-24].

Lipid profile, liver function and kidney function test

Table 1: Lipid profile, liver function and kidney function test of *Spondias mombin*.

Parameter	Control(G1)	A(G2)	A+M250(G3)	A+SM EoW(G4)	A+SM EoS(G5)	A+CR EoR(G6)	M250(G7)	CR of EoW(G8)	CR of EoS(G9)	CR of
Serum lipid profiles (mg/dl) Mean ± SD										
Total Cholesterol	103.86 ± 02.01	184.34±04.33	133.22±05.09	182.08±15.58	180.62±03.94	176.66±06.02	104.24±03.35	100.54±03.04	103.48±03.03	101.82 ± 01.76
Triglyceride	52.71 ± 6.23	106.576± 4.84	84.64±6.72	110.38± 6.86	71.16± 2.41	106.26 ± 2.5	52.94±4.92	55.448± 5.51	53.322±4.82	53.984 ± 5.6
HDL	78.932 ± 4.76	40.26± 1.22	57.062± 1.58	41.484± 2.98	46.024±5.42	43.18 ± 2.2	77.166 ± 4.78	76.052± 4.94	75.004± 3.48	74.18 ± 4.43
LDL	41.956±3.48	80.104±3.67	58.878± 7.92	76.104± 9.66	78.696±3.25	77.932± 3.46	41.62 ± 3.78	43.79 ± 4.71	45.526 ± 3.4	43.244 ± 2.48
Liver function test (U/L) Mean ± SD										
SGOT	44.774±4.13	82.202±5.35	67.86±3.66	79.198± 5.67	77.964±2.84	76.494±3.19	42.158±5.15	41.888±4.37	43.07±4.01	40.49±3.96
SGPT	34.62 ± 4.56	82.46±4.95	50.72± 7.33	80.12±5.9	76.52± 2.53	77.51 ± 2.14	37.45±3.24	30.47±3.95	27.81± 2.81	33.41±2.85
kidney Function test (mg/dl) Mean ± SD										
Creatinine	0.63 ± 0.11	2.93±0.21	1.19±0.23	2.27±0.21	2.17±0.14	2.4±0.13	0.83±0.11	0.57 ± 0.13	0.52 ± 0.11	0.71 ± 0.14
Urea	32.418±2.35	68.148± 5.07	48.024±4.93	70.054± 5.62	65.372±3.38	63.87±4.75	30.25± 3.11	33.022 ± 4.5	28.15± 4.29	30.058 ± 1.99

Here, n=5; C= Negative control; A= Positive control; A+M250= Alloxan + metformin 250 mg; A+SM EoW= Alloxan + metformin 250 mg + Plant extract (Winter season); A+SM EoS= Alloxan + metformin 250 mg + Plant extract (Summer season); A+CR EoR= Alloxan + metformin

250 mg + Plant extract (Rainy season); M250= Metformin 250 mg; CR of EoW= metformin 250 mg + Plant extract (Winter season); CR of EoS= metformin 250 mg + Plant extract (Summer season); CR of EoR= metformin 250 mg + Plant extract (Rainy season), G=group.

Table 1 displays the total cholesterol, triglyceride, HDL, LDL, SGOT, SGPT, creatinine, and urea levels. The total cholesterol, triglycerides, HDL, LDL, SGOT, SGPT, and urea levels in groups 4 and 5 that received alloxan and a 500 mg extract of *S. mombin* after summer and winter did not drop appreciably. However, in group 6 treated with alloxan and 500mg of extract of the following rainy season, these parameters were dramatically reduced ($p< 0.05$) *. But in the case of creatinine, the levels fell in all groups treated with alloxan and extract of following winter, summer and rainy seasons (4, 5, and 6). The outcome is statistically significant ($p< 0.05$) *. This is due to the narrow range of creatinine levels.
There was no significant difference between groups 8, 9, and 10, which were only given extracts from three different seasons.

All of the foregoing data suggest that due to the high rainfall in the months of July and September, certain phytoconstituents may have been leached away. Again, during the peak of the dry season (January-March), most trees drop their leaves, would almost certainly result in a decrease in the number of phytoconstituents in the trees, as well as a decrease in plant phytoconstituents, and therapeutic activity may be reduced.

More research is needed to determine its exact chemical ingredients, antidiabetic efficacy, and molecular biology.

Conclusion

Seasonal fluctuation is related to the vegetative and reproductive stages of the plant, and it has a direct impact on the variation of the plant's chemical contents. This study demonstrates that the antidiabetic effect of the plant under investigation varies seasonally. This activity is at its peak following the rainy season. Future research may focus on the isolation, purification, and characterization of the bioactive chemicals found in these plants, as well as the development of a powerful antidiabetic dosage form. The findings of such research may serve as a starting point for selecting a certain season for raw material collection in order to create possible anti-diabetic medications.

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