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**Original Article**

# Effect of Different Concentrations of Jamun and Amla Extracts to Combat Diabetes Mellitus

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## ABSTRACT

Diabetes mellitus (DM) is a metabolic disorder affecting the health of people at large scale. It adversely affects the quality of life and can be managed by changes in life style including diet **Objective:** To compare the effect of different concentrations of jamun and amla on blood Glycemic Index of diabetes mellitus. **Methods:** A total 25 male Wister rats aged 10-12 weeks, weight above 150g were selected for the 2 months study. Male rats were purchased from IMBB research laboratory, The University of Lahore (UOL). During the efficacy study, streptozotocin (STZ) induced rats were divided into five homogenous groups with 5 rats in each group. To For control group and T1, T2, T3 and T4 includes normal diet with different concentration of Jamun and Amla extracts. The change in body weight (g) and fasting blood glucose (FBG)(mg/dL) for each group of rats were monitored on every 15 days to estimate any suppressing effect of seed formulation. **Results:** The mean age of diabetic rats selected for study were10.61±0.493 weeks. Weight of STZ induced rats were monitored but no significant difference was shown, however mean weight of 5% Amla and 5% Jamun supplemented diet groups show an increase in mean body weight pre- and post-treatment 18.8±15.95g and 8±19.85g respectively. The minimum post-treatment FBG level of rats was noted in 5% jamun and 5% amla supplemented group with mean FBG 111.4±24mg/dL and 131.2±57.1mg/dL respectively. **Conclusions:** The study concluded that jamun and amla both groups show a significant reduction in fasting blood glucose level but no significant mean difference was shown in body weight gain. At the end of the study 5% amla and 5% jamun composition groups had shown significant improvement in FBG levels among diabetic induced rats.

## INTRODUCTION

Diabetes mellitus (DM) is an ongoing, metabolic disorder described by raised blood glucose levels (BGL) [1,2], that leads over the long haul to cause severe damage heart, veins, eyes, nervous system and kidneys [2,3,4]. Worldwide, in 2017, there were above 450 million people with DM. These numbers were assessed to reeve above 690 million by 2045 [5,6]. A most recent survey in Pakistan [2nd NDSP 2016-2017] assessed that roughly 26.31% of local populace aged above 20 years is diabetic (known diabetic cases; 19.21% recently diagnosed diabetic cases; 714%) [7]. Uncontrolled diabetes is related with the advancement of difficulties that might compromise health-related quality of living and may cause increase in mortality risks. Diabetes mellitus complications such as peripheral retinopathy and neuropathy lead to possibly handicapping sequel [8,9]. Syzygium cumini (Jamun) of the family Myrtaceae has been broadly used to treat diabetes by customary experts over numerous hundreds of years [9,10]. Syzygium cumini is utilized for fixing different illnesses including DM, cough, loose bowels movement, irritation, and ringworm. Bark decoctions are taken for asthma and bronchitis, utilized as a mouth wash for the astringent impact on mouth ulcerations, and light gums and for stomatitis [11].The major bioactive mixtures

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present in the eatable part of the plant are oxalic acid, gallic acid, citronellol, myricetin, hotrienol, cyanidin diglucoside, flavonoids, phytosterols, polyphenols and carotenoids. The possible advantages of these bioactive components are to forestall/decrease metabolic abnormalities and different diseases [12-13]. Emblica Officinalis Gaertnor Phyllanthus Emblica Linn, Amla, also known as Indian gooseberry or Amla, is possibly the most important restorative herb in Ayurveda, the Indian traditional system of medicine [14]. Extracts from various parts of Emblica officinalis, particularly the fruit, contain a wide range of phytoconstituents, including a higher number of polyphenols such as ellagic acid and gallic acid, various tannins, micronutrients, minerals, amino acids, vitamins, essential oils, and flavonoids such as quercetin and rutin, as well as micronutrients, minerals, amino acids, vitamins, essential oils, and flavonoids such Cancer, inflammation, neurological problems, osteoporosis, hypertension, sedentary lifestyle diseases, infectious disease, and parasite infection have all been linked to the extract or plant [15]. Emblica officinalis notable for its supplement characteristics, contains an assortment of compound constituents including mucic acid, tannins, alkaloids, amino acids, flavone glycosides, carbohydrates, sesquiterpenoids and phenolic acid [16,17]. Emblica officinalis shows the most elevated total phenolic contents (TPC) about 3.62.44 ± 11.21 mg GAE/g [18].The prevalence of DM is increasing day by day, and its related complications have been increased in developing countries. In Pakistan its prevalence rate is also high and greatly affecting the economy of the country. Both Jamun and amla seeds are considered as highly acceptable with a high safety profile as a therapeutic agent. Therefore, the main purpose of the current research is to study the biochemical profile of Jamun seed and amla extract and their ability to reduce blood sugar levels.

## METHODS

This cross-sectional comparative study was performed in Food Science and Nutrition Laboratory (FAHS Lab 102), University Institute of Diet and Nutritional Sciences (UIDNS), Faculty of Allied Health Sciences, The University of Lahore, Lahore. This study was conducted to determine therapeutic potential. Study sample size was 25 rats, age ranges from 10-12 weeks and weight ranges from 150-200g, each group was estimated by using purposive (non-probability) sampling technique and 99% confidence level. Furthermore, materials to be used and protocols to be followed are described below:

**Procurement and handling of Raw Material:** Amla and Jamun fruits of fully ripened eating quality were acquired from a local fruit store in Lahore. To remove adherent dirt, dust, and other foreign particles, the fruits were carefully washed under running tap water. Fruits were deseeded and dried at room temperature for a few days after being washed. Using a small laboratory grinder, the dry components were crushed to a fine powder and then passed through a sieve for additional refinement. Following the production of the powder for each category, it was packaged separately in air-tight plastic jars and given to the rats as a supplement.

**Feed Plans for Experimental Rats:** During the efficacy study, rats were divided into five homogenous groups with5 rats (n=5) in each group. To For control group with normal diet and T1, T2, T3 and T4 includes normal diet with different concentration of Jamun and Amla extracts (Table 1).

To: Control (normal diet)
T1: Jamun Extract,(JE)(5% of normal diet)
T2: Jamun Extract,(JE)(10% of normal diet)
T3: Amla Extract,(AE)(5% of normal diet)
T4: Amla Extract,(AE)(10% of normal diet)

Administration of drugs:

Streptozotocin (70 mg/kg, BioShop, Canada) were injected intravenously. Dextrose 5% was given immediately after the injection of STZ to avoid any kind of hypoglycemic effect.

**Hypoglycemic Perspectives:** In each group glucose concentration was estimated by GOD-PAP method as described by Katz et al. [ ] whereas, glycemic level was estimated by following the instructions of Ahnet al.[ ].

**Data collection procedure:** Jamun and amla seeds supplementation/food products was provided to the study subjects. The selected subjects wereundergone a medical examination. Zero-day blood sampling was undertaken which was analyzed for glycemic effect. The feeding period of Jamun and amla extracts supplementation/food product was of 2-month duration and after every 15 days blood sugar levels (fasting blood sugar levels),and weight (in grams) of the study subjects was taken again till the end of the study. Feed was given to rats with the help of oral gavage needle.

**Statistical analysis:** The data obtained from entire study was subjected to statistically analyze by using SPSS version 24. Analysis of variance (ANOVA) was applied to calculate level of significance

| Diabetic Rats | | | | | |
|---|---|---|---|---|---|
| Diet | Normal diet | Jamun treated group | | Amla treated group | |
| Groups | Group I | Group II | Group III | Group IV | Group V |
| Diet | $T_o$ | $T_1$ | $T_2$ | $T_3$ | $T_4$ |

**Table 1:** Treatment groups

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## RESULTS

The mean age of diabetic rats was 10.60 weeks with standard deviation 0.492. The mean age of diabetic rat enrolled in study. The minimum pre-treatment weight(g) of rats was noted in group T0 with mean 151.08±0.8366 and maximum pre-treatment weight(g) of rats was noted in T1 group with mean of 163.2± 5.58. The minimum post-treatment weight(g) of rats was noted in group T0 with mean 127.2 ± 45.90 and maximum post-treatment weight(g) was observed in group T1 group with mean 182 ± 21.35. The mean body weight (in grams) of diabetic rats before and after treatment in group study are presented in the Table 2.

| Rats study group | Body weigh t (g) | Mean (g) ± SD | Minimum | Maximum |
|---|---|---|---|---|
| Diabetic control group (T0) | Pre | 151.08± 0.8366 | 151g | 153g |
| | Post | 127.2± 45.90 | 70g | 195g |
| 5% JE treatment group (T1) | Pre | 163.2 ± 5.58 | 158g | 170g |
| | Post | 182 ±21.35 | 164g | 209g |
| 10% JE treatment group (T2) | Pre | 161 ± 3.24 | 156g | 165g |
| | Post | 147 ± 31.91 | 107g | 180g |
| 5% AE treatment group (T3) | Pre | 162.6 ± 4.50 | 158g | 168g |
| | Post | 170.6 ± 24.35 | 137g | 195g |
| 10% AE treatment group (T4) | Pre | 158.8 ± 4.65 | 152g | 165g |
| | Post | 141.4 ± 31.31 | 94g | 176g |
| Grand Total | Pre | 159.48 ± 4.65 | 151g | 70g |
| | Post | 153.64 ± 31.31 | 170g | 209g |

**Table 2:** Average body weight pre and post Treatment among diabetic rats

| Rats study group | Blood glucose level (mg/dL) | Mean (mg/dL) ± SD | Minimum | Maximum |
|---|---|---|---|---|
| Diabetic control group (T0) | Pre | 326.4 ± 84.25 | 262 mg/dL | 468 mg/dL |
| | Post | 613.8 ± 39.8 | 575 mg/dL | 679 mg/dL |
| 5% JE treatment group (T1) | Pre | 361.4 ± 97.7 | 290 mg/dL | 532 mg/dL |
| | Post | 111.4 ± 24 | 71 mg/dL | 129 mg/dL |
| 10% JE treatment group (T2) | Pre | 412.2 ± 71.12 | 325 mg/dL | 522 mg/dL |
| | Post | 241.2 ± 106.3 | 146 mg/dL | 390 mg/dL |
| 5% AE treatment group (T3) | Pre | 492.8 ± 80.7 | 380 mg/dL | 600 mg/dL |
| | Post | 131.2 ± 57.1 | 81 mg/dL | 224 mg/dL |
| 10% AE treatment group (T4) | Pre | 351.2 ± 58.4 | 281 mg/dL | 420mg/dL |
| | Post | 155.4 ± 65 | 102 mg/dL | 268 mg/dL |
| Grand Total | Pre | 388.8 ± 94.35 | 262 mg/dL | 600 mg/dL |
| | Post | 250.6 ± 199.71 | 71 mg/dL | 679 mg/dL |

**Table 3:** Average level of blood glucose (mg/dL) pre and post Treatment among diabetic rats

The minimum pre-treatment fasting blood glucoselevel (FBG)(mg/dL)of rats was noted in group T0 with mean 326.4 ± 84.25 and maximum pre-treatment FBG level(mg/dL) of rats was noted in T3 group with mean of 492.8 ± 80.7. The minimum post-treatment FBG level of rats was noted in group T1 with mean 111.4 ± 24 and maximum post-treatment blood glucose level was observed in group T0 group with mean 613.8 ± 39.8. The mean blood glucose levels (md/dL) of diabetic rats before and after treatment in group study are presented in theTable 3. The follow up blood glucose level of diabetic rats were noted from day 0 till 60th day after intervals of fifteen days. The fasting blood glucose level of T0 increases by 88% and the FBG levels of T1, T2, T3 and T4 decreases by 69.17%, 40.48%, 72.97% and 55.75% respectively(Table 4).

| Parameter | | Blo od glucose level (mg/dL) | | | | |
|---|---|---|---|---|---|---|
| Treatments | | $T_o$ | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
| Study interval (Day) | 0 | 326.4±84.25 | 361.4±97.72 | 412.2±71.12 | 492.8±80.71 | 351.2±58.41 |
| | 15 | 342±83.06 | 254±57.63 | 373.6±28.37 | 268.6±44.41 | 439±157.8 |
| | 30 | 414±61.23 | 166.6±59.07 | 347.8±104.4 | 209.6±197.0 | 339±130.3 |
| | 45 | 505.6±73.77 | 140±51.21 | 276±69.85 | 176±59.66 | 240.4±74.73 |
| | 60 | 613.8±39.8 | 111.4±24.05 | 241.2±106.3 | 133.2±57.12 | 155.4±65 |
| Mean | | **404.36±68.42** | **206.6±57.93** | **330.1±76.01** | **256±87.78** | **305±97.24** |

**Table 4:** Effect of jamun and amla seed extract on blood glucose level in diabetic rats

| Parameter | | Body weight (g) | | | | |
|---|---|---|---|---|---|---|
| Treatments | | $T_o$ | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
| Study interval | 0 | 151.8±0.83 | 163.2±5.58 | 161.0±3.24 | 162.6±4.55 | 158.8±4.65 |
| | 15 | 151.0±4.00 | 160.2±6.76 | 163.8±8.04 | 147.8±9.70 | 157.2±8.07 |
| | 30 | 146.8±15.27 | 160.2±14.13 | 165.4±10.21 | 152.4±24.64 | 152.0± 16.79 |
| | 45 | 126.0±22.32 | 177.4±26.40 | 154.0±22.09 | 155.4±4.61 | 142.0±23.40 |
| | 60 | 127.2±45.90 | 182.0±21.35 | 147.0±31.91 | 170.6±24.35 | 141.4±31.31 |
| Mean | | **132.2±17.66** | **168.6±16.95** | **158.24±15.09** | **157.76±13.57** | **150.28±16.84** |

**Table 5:** Effect of jamun and amla seed extract on body weight in diabetic rats

The weight (in grams) of rats were calculated from day 0 and after every 15 days till to the end of the study at 60th day. The weight of T0, T2 and T4 group decreased by 16%, 8.57% and 10.9% respectively. The weight of T1 and T3 group increased 10.29% and 4.92% respectively (Table 5). The mean body weight of diabetic rats was 159.48 ± 5.64mg/dl before the treatment, whereas the mean body weight after treatment was 153.64 ± 35.67mg/dl. Findings showed statistically significant difference in pre and post treatment with p-values 0.012. The maximum mean body weight gain after treatment among diabetic rats is shown in group T1 with18.8 ± 15.95g and maximum mean weight lose post treatment is shown in T0 group with average of 23.8 ± 45.01g (Table 5). The statistical finding shows there is no significance difference between pre and post treatment and weight gain as all p-value are above 0.05. The mean blood glucose levels of diabetic rats were 159.48 ± 5.64mg/dl before the treatment, whereas the mean blood glucose levels after treatment was 153.64 ± 35.67mg/dl (Table 6). Findings showed statistically significant difference in pre and post treatment with p-values 0.003

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(Table 7,8).

| Body Weight | Mean (g) ± Standard Deviation | Paired sample t -test (p-value) |
|---|---|---|
| Pre | 159.48 ± 5.64 | 0.012 |
| Post | 153.64 ± 35.67 | |

**Table 6:** Comparison of average body weight pre and post treatment

| Fasting Blood Glucose Level | Mean (mg/dL) ± Standard Deviation | Median (IQR) | Friedman Test (p-value) |
|---|---|---|---|
| Pre | 388.8 ± 94.35 | 380(153) | 0.003 |
| Post | 250.68 ± 199.7 | 145(231) | |

**Table 7:** Comparison of average blood glucose levels pre and post treatment

| Paired Samples Test | | | | |
|---|---|---|---|---|
| Treatment group | Weight | Mean (g) ± SD | Change in body weight (g) | Sig. (2-tailed) |
| Diabetic control group (T0) | Pre | 151.08 ± 0.8366 | -23.8 ± 45.01 | **0.300** |
| | Post | 127.2 ± 45.90 | | |
| 5% JE group (T1) | Pre | 163.2 ± 5.58 | +18.8 ± 15.95 | **0.086** |
| | Post | 182 ± 21.35 | | |
| 10% JE group (T2) | Pre | 161 ± 3.24 | -14 ± 28.87 | **0.594** |
| | Post | 147 ± 31.91 | | |
| 5% AE group (T3) | Pre | 162.6 ± 4.50 | +8 ±19.85 | **0.232** |
| | Post | 170.6 ± 24.35 | | |
| 10% AE group (T4) | Pre | 158.8 ± 4.65 | -17.4 ± 26.66 | **0.656** |
| | Post | 141.4 ± 31.31 | | |

**Table 8:** Comparison of average weight pre and post treatment and change in body weight (g) post treatment

| Paired Samples Test | | |
|---|---|---|
| Treatment | FBG level, Mean difference (mg/dL) ± SD | Sig. (2-tailed) |
| Diabetic control group (T0) | -287.4 ± 99.33 | **.003** |
| 5% JE group (T1) | 250 ± 91.96 | **.004** |
| 10% JE group (T2) | 171 ± 68.88 | **.005** |
| 5% AE group (T3) | 361.6 ± 105.63 | **.002** |
| 10% AE group (T4) | 195.8 ± 52.96 | **.025** |

**Table 9:** Comparison of average fasting blood glucose level (mg/dL) pre and post treatment

| Fasting Blood Glucose Level 10% AE group (T4) | Mean (mg/dL) ± SD | Median (IQR) | Friedman Test (p-value) |
|---|---|---|---|
| Pre | 351.2 ± 58.4 | 340(113) | **0.025** |
| Post | 155.4 ± 65 | 137(93) | |

**Table 10:** Mean and SD fasting blood glucose levels

The mean difference of FBG level in pre and post treatment was -287.4 ± 99.33mg/dL, 250 ± 91.96mg/dL, 171 ± 68.88mg/dL, 361.6 ± 105.63mg/dL and 195.8 ± 52.96md/dL in T0, T1, T2, T3, and T4 group respectively (Table 9). Each group shows significant mean difference in change in FBG level among diabetic subjects of each group (p-values, 0.003, 0.004, 0.005, 0.002 and 0.025 of T0, T1, T2, T3 and T4 group respectively)(Table 9). Mean and SD pretest for fasting blood glucose was 351.2± 58.4 while post test value was 155.4 ± 65, p value 0.025 (Table 10).

## DISCUSSION

Syzygium cumini and Emblica officinali, are conventional therapeutic plants with different bioactive components dispersed in all pieces of the plant. The possible advantages of bioactive components present in the plant are to forestall/decrease metabolic abnormalities and cure different diseases [15,19]. Neha et al. in 2015 performed a study to determine the hypoglycemic impact of jamun seed on fasting blood glucose levels. The results of the experimental group were supplemented with 2 g of jamun seed powder per day for 60 days. The results showed a decrease in the blood glucose levels of experimental group[21].Same results are observed in current studies which show significant reduction in fasting blood glucose level of streptozotocin induced diabetic rats at both 5% jamun seed extract and 10% jamun seed extract supplementation. Mai et al in 2015 studied in streptozotocin-induced diabetic obese mice, the anti-hyperglycemic potential of the aqueous fruit extract of amla (E. officinalis). In the fifth and sixth weeks, blood glucose levels in the amla extract-treated groups were significantly lower (p 0.001)[22]. Similar findings were observed in current study which show the improvement in fasting blood glucose level in STZ induced diabetic rats as 5% amla extract 10% amla extract treated groups with a significant reduction in fasting blood glucose. Mahajan et al in 2018 investigated the antioxidant capabilities of amla extracts in rats, as well as their impact on oxidative stress in streptozotocin-induced diabetes. Oral treatment of amla extracts to diabetic rats reduced body weight gain somewhat and greatly reduced different oxidative stress markers in the diabetic rats' blood [23]. Similar results are observed in 5% amla extract supplementation group and 10% amla extract supplementation group in the present study.

## CONCLUSION

At the end of our study, it is concluded from the detail analysis and results that Syzygium cumini (Jamun) and Phyllanthus emblica (Amla) supplemented diet are effective in reducing fasting blood glucose (FBG) levels in Streptozotocin induced diabetic rats. Comparative study showed that best composition for reducing fasting blood glucose levels is a diet with 5% amla extract of normal diet with significant difference pre- and post-treatment (p-value is 0.002) after that diet with 5% Jamun extract showed a significant reduction in mean fasting blood glucose levels (p-value is 0.004). At the end, diet with 10% amla supplemented with p-value 0.025 showed effective reduction of FBG level but as compared to other composition it is listed at the end. Weight of STZ induced rats was monitored but no significant difference was shown however of 5% Amla and 5% Jamun supplemented diet groups shows an increase in mean body weight post-treatment by18.8±15.95g and 8±19.85g respectively.

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