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Effect of Concomitant Use of *Syzygium Cumini (L.) Skeels* Seed Powder and Metformin: An *In-vivo* Interaction Study

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ABSTRACT

Context: As the increasing herb-drug interactions have posed many questions about the safety and efficacy of concomitant use of many herbs and drugs. An attempt has been made to study the effect of Jamun (*Syzygium cumini* (L.) Skeels) seed powder (JSP) and metformin in diabetic rats.

Aim: Interaction between JSP and Metformin

Settings and Design: Dabur Research Foundation, Ghaziabad.

Methods and Material: Sprague Dawley rats were divided into five groups of six each and were fasted for 2 hr before the experiment with water ad libitum. All Group G1 & G2 received, citrate buffer, group G3 received 500 mg/kg body weight of JSP, G4 received metformin 200 mg/kg BW, and G5 received JSP + metformin 200 mg/kg body weight each respectively. Diabetes was induced by administering an i.p. injection of nicotinamide at the dose of 110mg/kg 15 min. before the 50 mg/kg STZ injection in an ice-cold 0.1 M citrate buffer (pH 4.5). Blood glucose levels were measured using a glucometer.

Statistical analysis: Body weight was analyzed by two-way ANOVA, rest data by one-way ANOVA. Data expressed as Mean ± SEM.

Results: The combined treatment showed a 25.37% and 43.0% reduction in BGL which is more than the individual effect of JSP and metformin on Day 1 and 8 respectively.

Conclusions: The results indicate that the combination of JSP and metformin is having an additive effect and can be used safely to obtain a prolonged and sustained antidiabetic effect.

Clinical significance: JSP along with Metformin can be given to manage very high blood glucose levels and also conditions like secondary failures to OHAs

Keywords: Additive effect, Herb-Drug Interaction, *Jamun*-metformin Interaction, Secondary failures, potential candidate, OHG's, *Jamun, syzygium cumini*.

INTRODUCTION

With increasing prevalence of diabetes[1]and resistance of conventional medicines, the patients are increasingly seeking herbal remedies to manage their blood glucose level and associated medical conditions to maintain their overall health[2] either on their own or as prescription. As less than 33% of the individuals inform their physicians about the polypharmacy[3] and is commonly noticed in chronic or incurable diseases. *Jamun* seed powder (JSP) and Metformin are widely used concomitantly

by the diabetics[4]. Though the respective antidiabetic effect of JSP[5] and Metformin[6]is proven but till date no data regarding their concomitant use is available. Thus, an in-vivo study is planned to provide significant database for their concomitant use.

MATERIAL AND METHODS

Drug and chemicals: Streptozotocin and Nicotinamide were purchased from Sigma-Aldrich CO (St. Louis, MO, USA). Fasting and Post parandial Glucose levels were determined using strip and glucometer (One Touch® Select Simple, Life Scan Europe, Switzerland). All other chemicals and solvents were of highest analytical grade.

Test item materials: Dried seeds of *Jamun* were collected from Pune (Maharashtra). The identification and authentication were done at FRLHT, Bangalore with voucher specimen no. 5261. The Seeds (1 kg) were mechanically crushed (mesh size 100). Metformin (Franco-Indian Pharmaceuticals pvt. ltd.) was procured from a local vendor with batch no. 18267.

Table no. 1: Preparation of test items

Test item (TI) No.	Test item name	Method of preparation	Final concentration
TI 1	JSP	1g JSP + 0.5% of (Na-CMC) sodium carboxy methyl cellulose	20ml. (500mg/10 ml/kg)
TI 2	Metformin	500 mg of metformin + 25mg of 0.5% Na-CMC	20 mg/ml (200 mg/10 ml/kg).
TI 3	JSP and metformin	500mg JSP + 500mg of metformin + 0.5% of Na-CMC	25ml (200 mg/10 ml/kg)

Animal care and ethical approval: Adult male *Sprague Dawley* rats of 6–8 weeks old age group, and body weight 200–300 g, were received from In-House breeding facility of Dabur Research Foundation and were used in the study. Rats were housed in polypropylene cages at an ambient temperature of 22 ± 3°C and 50±20% humidity with a 12 h each of dark and light cycle. All the rats were given a 7-day period of acclimatization before starting the experiment. Rats were fed with conventional feed purchased from a commercial supplier and filtered drinking water *ad libitum*. Animal housing, care and the conduct of experimental procedures were done in accordance with ethical norms approved by Ministry of Social Justices and Empowerment, Government of India. The study was initiated after getting ethics approval by Animal Ethics Committee Guidelines of Dabur Research Foundation (IAEC/49/798).

Induction of Diabetes Mellitus (DM): DM was induced by administering an i.p. injection of nicotinamide at the dose of

110mg/kg 15 min. prior to the STZ injection. After 15 min of nicotinamide administration, rats (n=34) were administered with freshly prepared STZ (i.p.) at the dose of 50 mg/kg in an ice-cold 0.1 M citrate buffer (pH 4.5). Rats (n=6) served as normal control group received citrate buffer (i.p.). Rats were kept from Day 1 to day 7 to induce diabetes. On Day-7 Blood glucose level (BGL) was measured using Glucometer.

Experimental design: Based on the BGL (200-400 mg/dL), rats per group (n=6) were selected and grouped as mentioned in Table 2

Table 2: Allocation of rats in groups for treatment

Group	Treatment	Dose and Route (Induction)	Dose and Route (Treatment)	Number of Animals
G1	Normal Control, 0.5%CMC	0.1M Citrate Buffer, i.p,	10 ml/kg, p.o. b.i.d.	6
G2	Diabetic Control, 0.5%CMC	Nicotinamide 110 mg/kg + STZ 50 mg/kg, i.p,	10 ml/kg, p.o. b.i.d.	6
G3	Diabetic + JSP	Nicotinamide 110 mg/kg + STZ 50 mg/kg, i.p,	500mg/kg, p.o. b.i.d.	6
G4	Diabetic + Metformin	Nicotinamide 110 mg/kg + STZ 50 mg/kg, i.p,	200 mg/kg, p.o. b.i.d.	6
G5	Diabetic +JSP+ Metformin	Nicotinamide 110 mg/kg + STZ 50 mg/kg, i.p,	200 mg/kg, p.o. b.i.d.	6

Assessment parameters

1) Body weight and Feed consumption: Body weight and feed consumption were measured daily.
2) Fasting and postprandial blood glucose

All rats were kept under fasting for 2 hrs, after 2 hrs all rats were treated with respective formulations as mentioned. After 1 hr Post-treatment BGL was measured using glucometer for all rats. Blood was drawn from tip of the tail with the help of disposable lancet. Blood glucose levels were estimated in each group before and after STZ administration and then on Day 1 and Day 8.

3) Liver and Kidney Function Test Estimation

At the end of experiment, animals were anesthetized, and blood was collected retro-orbitally to separate serum for the estimation of Kidney and Liver function tests. The mean value was tabulated for each group with S.E.M. The % change was

calculated with respect to disease control (G2) group.

4) Histopathological examination

At the end of the experiment, one rat from each group having the most prominent change in blood glucose levels was sacrificed for histopathological examination of pancreas. Mean of reduced number and size of islets of Langerhans, decreased cellularity in islets of Langerhans and degeneration of pancreatic cells were measured. Standard histopathological procedure was followed.

Statistical Analysis

Analysis was done with the help of standard statistical software, namely Microsoft Excel and Graph pad prism version 5.01. Data was expressed as Mean ± SEM. Body weight was analyzed by two-way ANOVA and rest data was analyzed by one-way ANOVA. Differences were considered significant at $p < 0.05$.

RESULTS

1) Body weight and feed consumption were carried out to study the any toxic effect of the test items and study is summarized in table 3 and figure 1,2 respectively.

Table 3: Body weight of rats for 8th days expressed as (MEAN ± SEM)

Groups	Body weight (MEAN± SEM)		Cumulative feed consumption for 7 days
	DAY 1	DAY 8	
G1	228.47±3.58	230.87±2.12	341.00
G2	232.33±3.17	223.10±3.16	374.00
G3	233.98±4.28	226.70±3.68	336.00
G4	234.28±5.57	226.48±2.83	317.00
G5	237.87 ±7.33	230.65±5.05	313.00

Figure 1: Body weight (MEAN ± SEM)

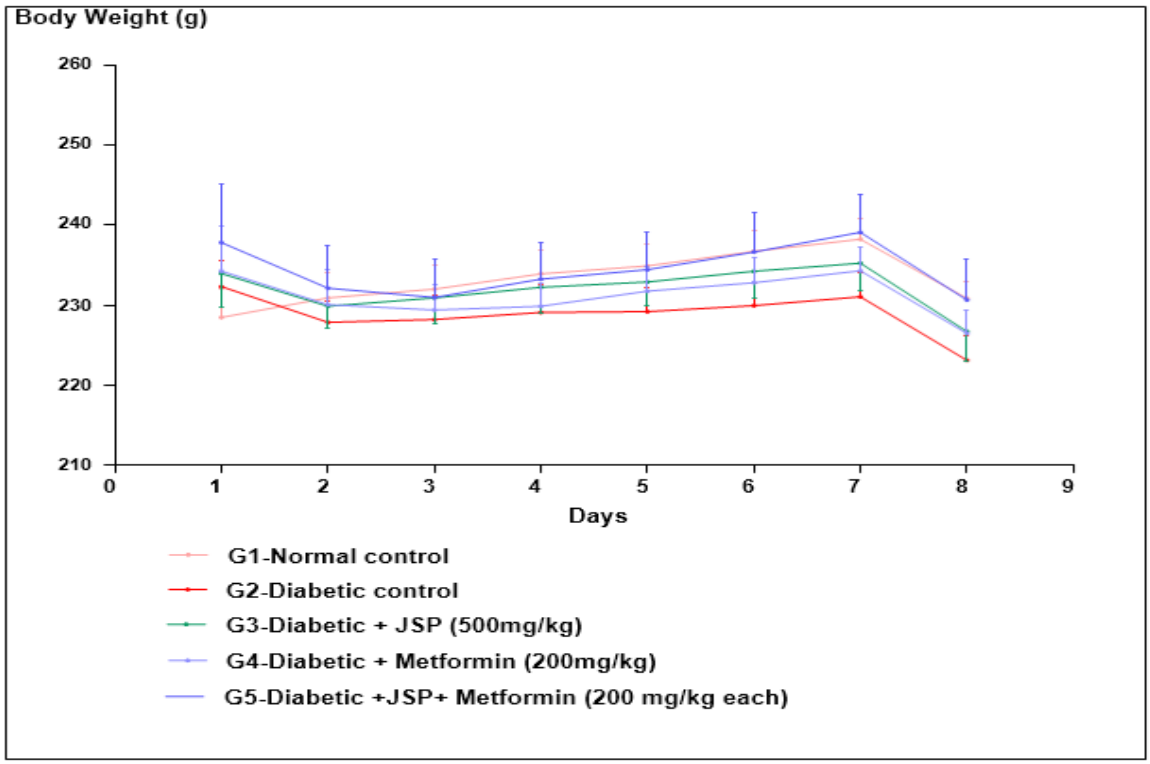


Figure 2: Cumulative Feed Consumption

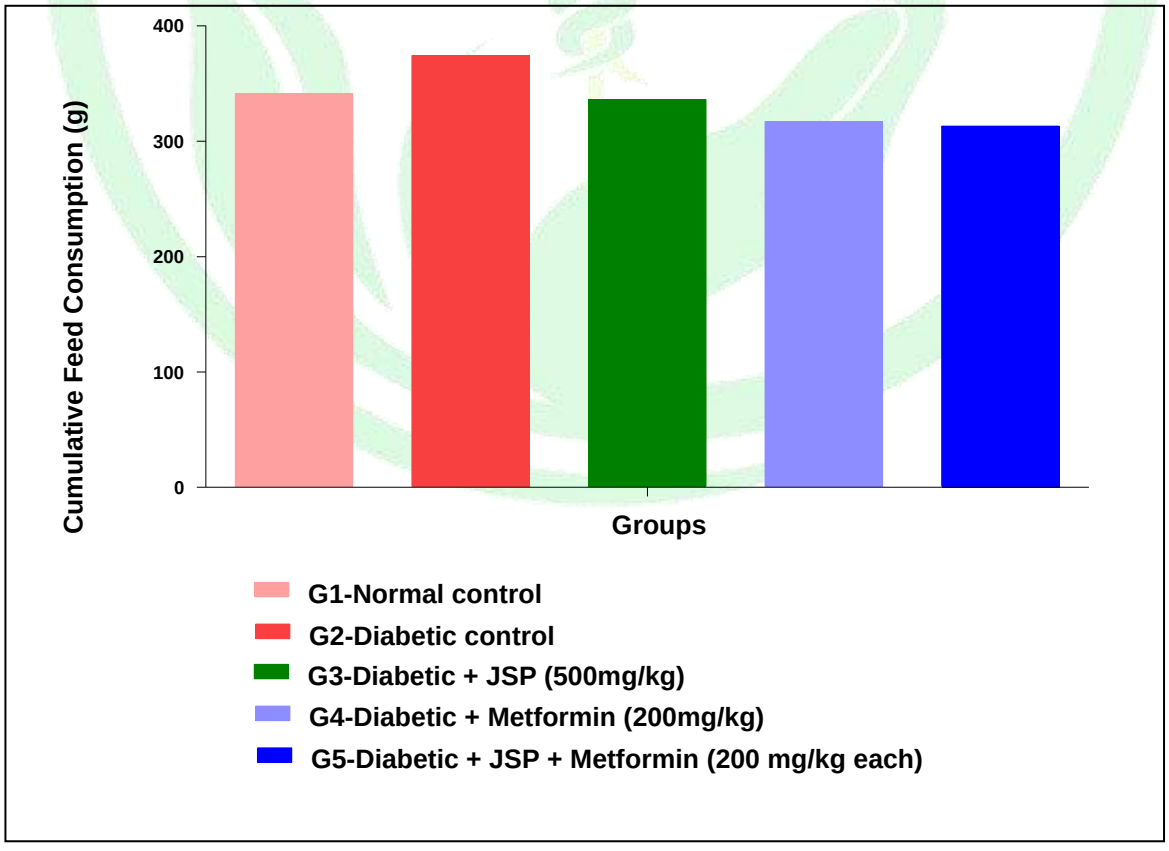


Table 4: Blood glucose level (fasting) - Day 1 and Day 8

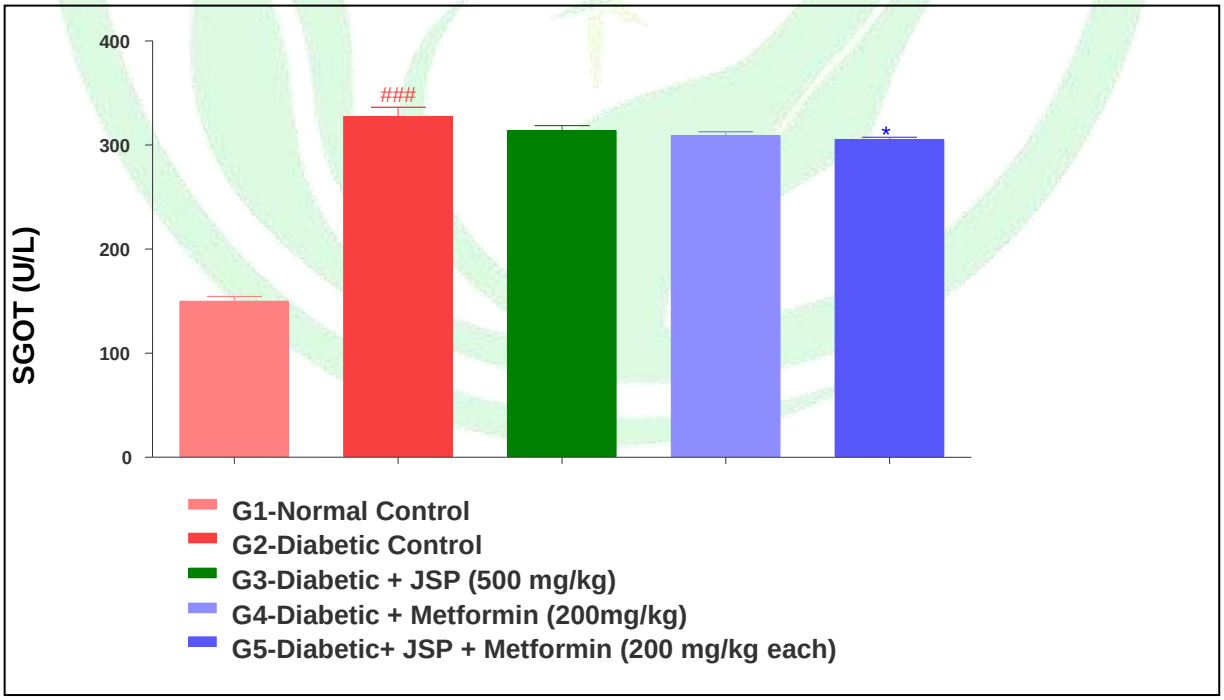
Group	Day 1 (BGL in mg/dl/) (MEAN ± SEM)		Day 8 (BGL in mg/dl/) (MEAN ± SEM)
	Before treatment	After treatment	
G1	116.00 ±2.85	100.50 ±2.92	122.50±2.54
G2	335.00 ± 3.21	323.17 ± 5.12	396.17± 6.69
G3	328.67 ± 9.68	301.50 ± 4.00	351.67 ± 8.38
G4	348.67 ± 2.63	263.33 ± 3.97	283.33 ± 4.77
G5	341.67 ± 2.64	241. 17 ± 1.96	244.83 ± 17.50

2) Biochemical parameters

Table 5: Liver function test and Kidney function test of rats on day 8

Group No.	SGOT(U/L) (MEAN ± SEM)	SGPT(U/L) (MEAN ± SEM)	Uric acid (mg/dl) (MEAN ± SEM)	Creatinine (mg/dl) (MEAN ± SEM)
G1	149.77± 4.63	27.05± 0.67	1.33 ± 0.07	0.31 ± 0.03
G2	327.20± 8.81	101.00±4.52	1.32 ± 0.06	0.37 ± 0.02
G	313.73 ± 4.73	95.43 ± 1.39	1.33 ± 0.04	0.33 ± 0.02
G4	309.08 ±3.52	94.42 ± 3.89	1.31 ± 0.05	0.32 ± 0.03
G5	305.10 ± 2.13	86.05 ± 1.58	1.33 ± 0.04	0.32 ± 0.02

- **SGOT**

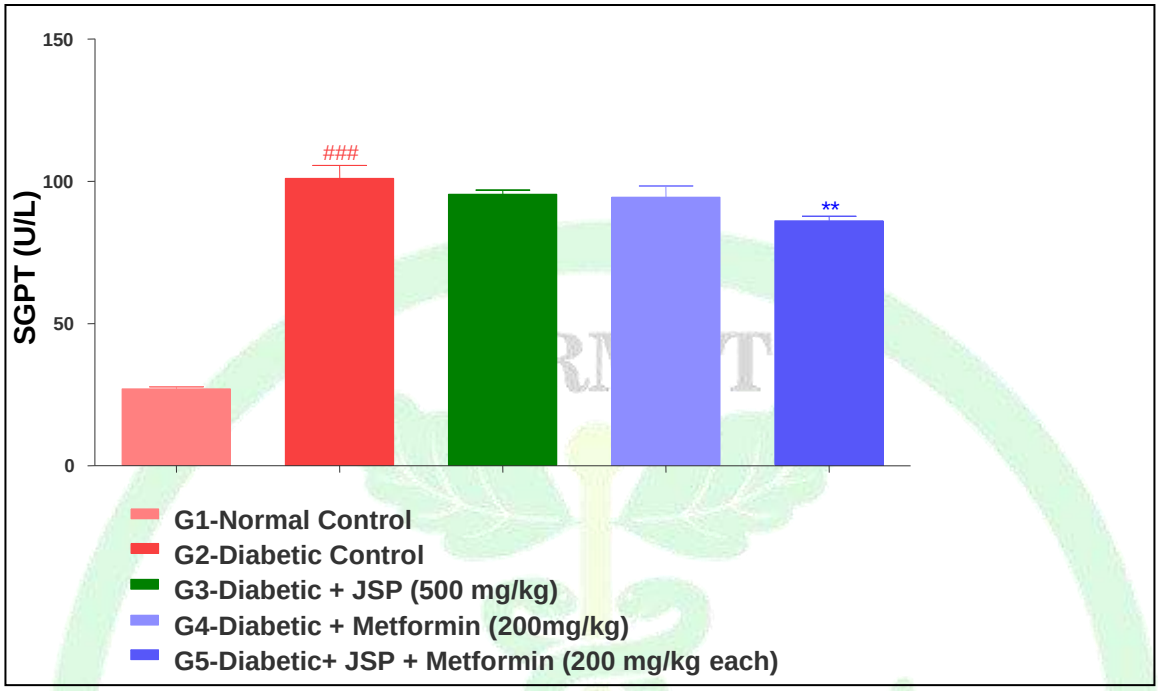


###p<0.001, values differ significantly from Normal Control (G1)

***p<0.01 & *p<0.05, values differ significantly from Diabetic Control (G2)*

Figure 3: SGOT level on Day-8

- **SGPT**



###p<0.001, values differ significantly from Normal Control (G1)

***p<0.01 & *p<0.05, values differ significantly from Diabetic Control (G2)*

Figure 4: SGPT level on Day-8

- **Creatinine**

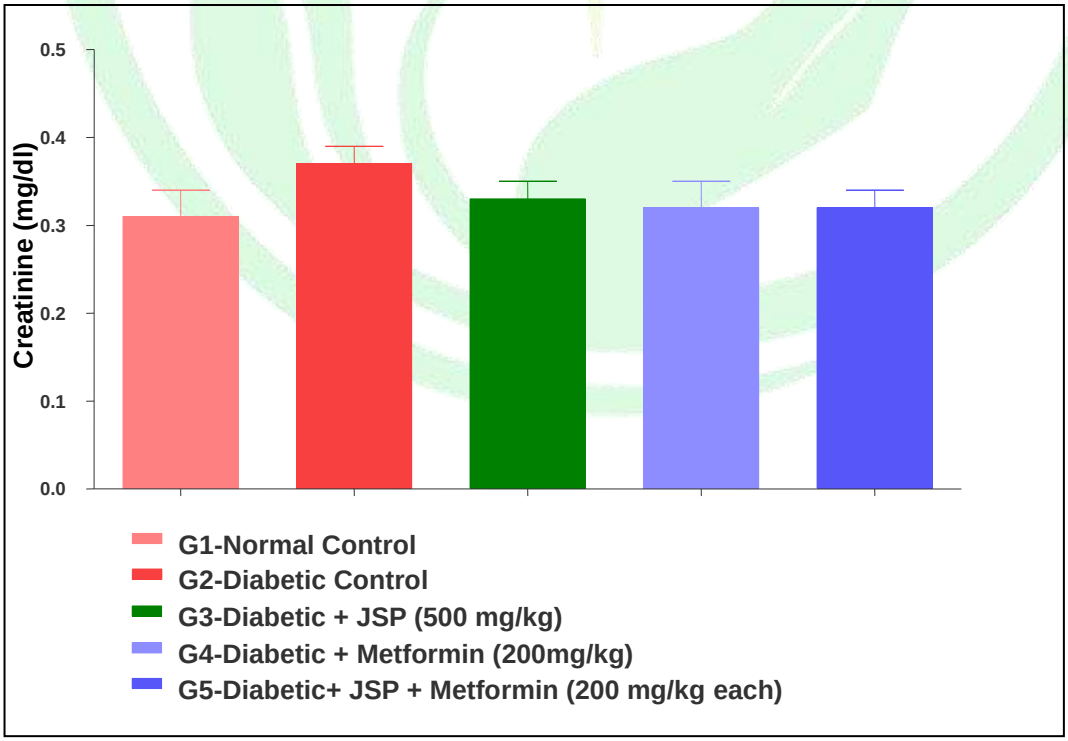


Figure 5: Creatinine level on Day - 8

- **Uric Acid**

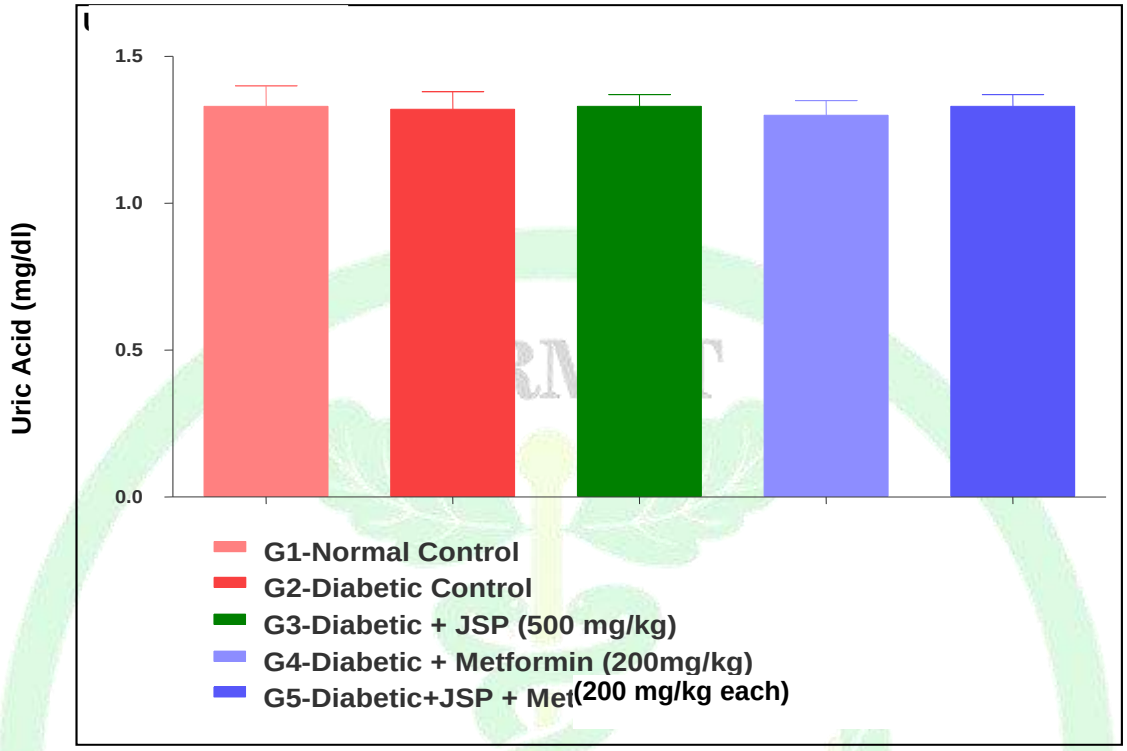


Figure 6: Creatinine level on Day - 8

3) Histopathology of pancreas

Rats from Diabetic control group shows a reduced mean β-cell number, size and markedly degenerated pancreatic islets as compared to normal control.

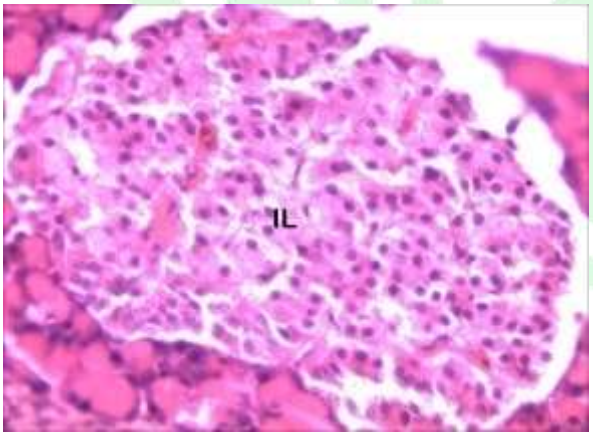


Figure 7: Histopathology of pancreas of G1

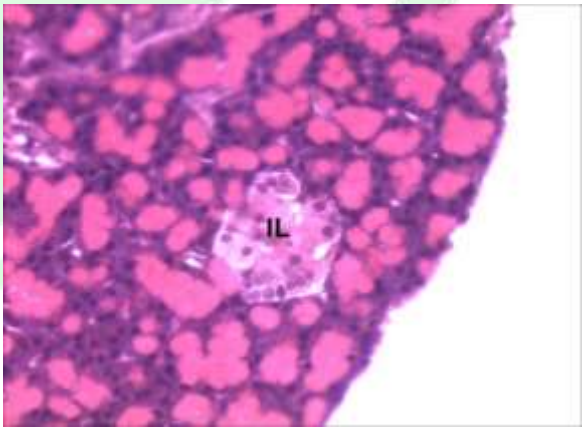


Figure 8: Histopathology of pancreas of G2

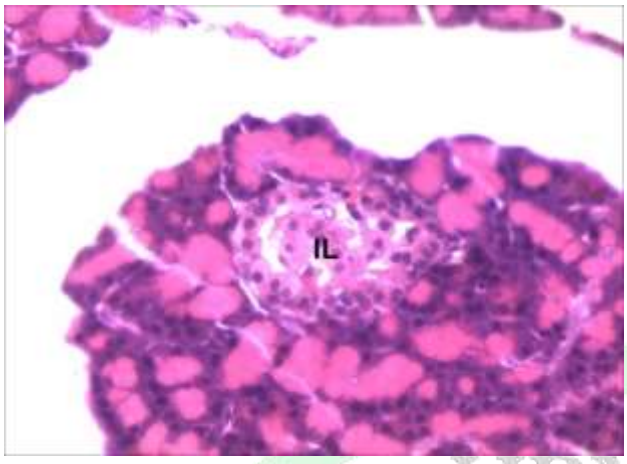


Figure 9: Histopathology of pancreas of G3

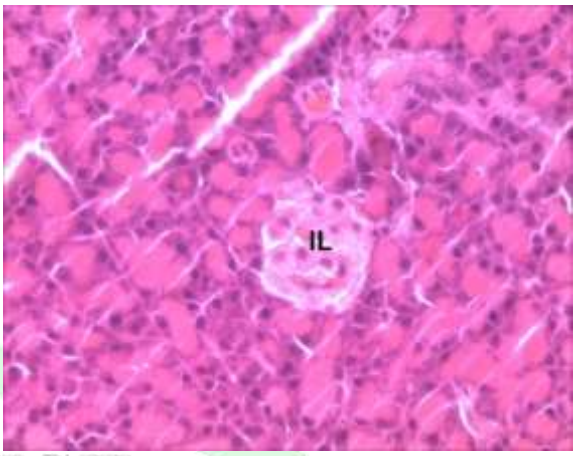


Figure 10: Histopathology of pancreas of G4

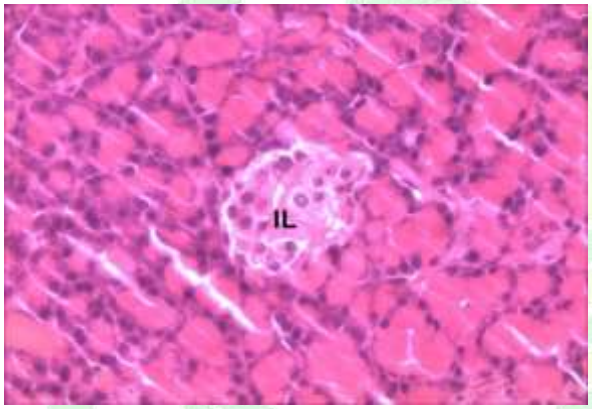


Figure 11: Histopathology of pancreas of G5

DISCUSSION

JSP is known to exhibit antihyperglycemic activity by insulin mimetic and insulinotropic effect. It may act as antidiabetic herb either by stimulation of insulin release from beta cells or by lowering glucose absorption of intestine, hepatic glucose production and boosting sensitivity of insulin by enhancement of peripheral glucose uptake and utilization, activation of nuclear receptor PPAR-γ[7].This activity may be due to the presence of flavonoids, Glycosides, tannins. Metformin is also a well-known antihyperglycemic which acts primarily at the liver by reducing glucose output and, secondarily, by augmenting glucose uptake in the peripheral tissues, decreasing gluconeogenesis, and decreasing absorption of glucose[8].Thus, a question was posed to know the effects of concomitant use of JSP with Metformin.

In-vivo herb-drug interaction study using JSP and Metformin indicated that the concomitant use of these two drugs does not have any significant effect on body weight and feed consumption of treated and control rats which also proves its safety (Table 3, Fig.1,2). The combined (JSP + Metformin) treatment showed

25.37% reduction in BGL which is more when compared with individual effect of JSP and Metformin on Day 1. On Day 8, the effect of combination (JSP + Metformin) treatment was 43.0% reduction in BGL which is highly significant (i.e. P<0.001) (Table 4). The results indicate that the combination of herb (JSP) and drug (Metformin) is having additive effect towards blood glucose lowering effect and dose of individual drug was reduced to 200 mg/Kg from 500 mg/Kg when used in combination as compared to the individual intake of herb or drug which states its efficacy.

The biochemical tests including LFT and KFT revealed no significant change in treated and controlled groups which indicates that the combination of JSP and metformin is safe and does not have any hepatotoxic and renotoxic effect (Table no.5, Fig. no.3-6).

The histopathology of the 4 groups, except the normal control group showed a reduced mean β-cell number, size and markedly degenerated pancreatic islets as compared to normal control. The treatment group TI - 1 (JSP) and TI – 3 (JSP + metformin), showed marginal effect; i.e. improvement in histopathological grading of various histological parameters as compared to diabetic control, which indicate tendency of protecting the islets from progressive damage due to NAD-STZ during the 7-day study period whereas TI - 2 (Metformin) treated rats showed improvement in severity of histopathological lesions (Fig. no.7-11).

Thus, this *in-vivo* interaction studies suggest that JSP and metformin if used concomitantly may have safe and additive effect for the management of ailments. The significant decrease in FBS and PPBS as noticed on Day 1 and Day 8 of the treatment (P<0.001) gives the confidence to use conventional medicines along with *Ayurvedic* medicines in the management of DM. Non-alteration of LFT and KFT parameters of the treated rats indicates towards the non-toxicity of the combination therapy. The histopathology study showed the tendency of JSP and combination of JSP and metformin for protecting the islets from progressive damage when compared to untreated rats.

The combination therapy shows additive effect of therapy when studied in experimental rats it may thus provide a lead in modulating treatment for patients experiencing secondary failures to conventional antidiabetic medicines. Also, the dose needs to be managed cautiously.

CONCLUSION

The concomitant use of JSP along with Metformin shows additive effect of the

therapy proving to be used as a potent therapeutic agent to manage blood glucose levels and also conditions like secondary failures to OHAs. However, further toxicological studies need to be carried out and the practical applicability of the combination needs to get explored.

CLINICAL SIGNIFICANCE

JSP along with Metformin can be given to manage very high blood glucose levels and also conditions like secondary failures to OHAs

Source of funding: Nil

Conflict of interest: None

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