Acta fytotechn zootechn, 26, 2023(2): 123–130
http://www.acta.fapz.uniag.sk

Original Paper

In vivo histopathologic and histomorphometric evaluation of the antidiabetic potential of *Syzygium cumini* and *Ficus racemosa* herb

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Article Details: Received: 2022-10-11 | Accepted: 2023-03-24 | Available online: 2023-06-30

https://doi.org/10.15414/afz.2023.26.02.123-130

Seeds of *Syzygium cumini* and fruits of *Ficus racemosa* individually are reputed to have hypoglycemic properties, but their consorted efficacy is yet to reveal, which may produce an improved antidiabetic effect because of synergistic phytochemical interaction. Hence, the antidiabetic potency of a combined extract of *S. cumini* seeds and *F. racemosa* fruits was investigated using glibenclamide as a reference drug in terms of physiologic, hematologic, histopathologic, and histomorphometric restoration. For a 30-day experiment, seventy-two Swiss albino mice were grouped as normal control, diabetic control (alloxan @ 150 mg.kg^{-1} b.wt), glibenclamide (@ 600 µg.kg^{-1} b.wt), and combined herbal extract (ethanolic extracts of *S. cumini* seeds @ 500 mg.kg^{-1} b.wt and *F. racemosa* fruits @ 200 mg.kg^{-1} b.wt). Diabetic control mice presented hyperglycemia and significantly lower body and pancreas weights. When the combined herbal extract was administered, the diabetic animals' body and organ weights increased, and glycemic levels fell; the exhibited hypoglycemic effect was better than the reported individual treatment and statistically similar (p <0.05) to the reference drug. Postprandial hypoglycemia found in the glucose tolerance test also indicates improved glucose usage ability of the combined extract by the cells and tissues. Diabetes-induced changes in the pancreas such as fibrosis, vascular congestion, decreased number, and diameter of the islets, were restored to near-normal after combined herbal extract and glibenclamide administration. Collectively, the combined herbal extract derived from *S. cumini* seeds and *F. racemosa* fruits was better than their individual dose and was nearly as effective as a standard hypoglycemic drug.

Keywords: *Syzygium cumini*, *Ficus racemosa*, antidiabetic, histomorphometry, pancreas

1 Introduction

Diabetes mellitus, marked by insistent hyperglycemia and organ dysfunction, has sparked widespread concern because of its complications. In Bangladesh, the prevalence of diabetes is 8% and it attributes 3% of total deaths, both of which are on a steady rise (Talukder and Hossain, 2020). Hypoglycemia in diabetic patients can be achieved through insulin treatment, oral antidiabetics, or dietetic means. However, synthetic antidiabetic products are expensive, have shocking results, and partial gestational period use (Rekha, 2010). In prolonged use, insulin therapy can cause insulin resistance, decreased appetite, fatty liver, and brain shrinkage (Piedrola et al., 2001). In addition, gastrointestinal disturbances, lactic acidosis, hypersensitivity, cardiovascular complications, nasopharyngitis, renal impairment, and decreased function of β-cells were observed in the long-term management of oral hypoglycemic drugs (Moller, 2001). In view of these, consumers are keen to utilize alternative health care such as herbal medicines, as drug safety with its economic viability is their chief concern. Local availability, lack of side effects, ease of administration, and low cost of herbal drugs have increased their popularity in developing and least developed countries, where the price of synthetic medicine leads many people to non-adherence to treatment in the long run. A clear message with scientific proof about the hypoglycemic effect of the herbal extract with the aforementioned

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phenomenal qualities may encourage the average citizens of developing and underdeveloped countries to use it as a free antidiabetic.

Before the revelation of insulin, *Syzygium cumini* or Jamun (Family: Myrtaceae) was a forefront antidiabetic drug in Europe, which had also been used in various integral and alternative medication frameworks of India (Rizvi and Mishra, 2013). A glibenclamide-like phytochemical, mycaminose, had been isolated from *S. cumini* seeds extract (Kumar et al., 2008). The other substances liable for the restraint of glucose are alkaloid- jambosine, glycoside- jambolin, saponins, flavonoids, and phenols (Rather et al., 2019). Jamun seeds have 31.62–41.4% carbohydrates, 1.97–8.5% crude protein, 2.3–16.9% total dietary fibers, 0.83–1.18% crude fat, and 2.18% ash (Kumar et al., 2022). *Ficus racemosa* of the Moraceae family is one of the spices referenced in all old sacred texts of Ayurveda, Unani, Homeopathy, and Siddha to have hypoglycemic, anti-inflammatory, gastro-defensive, and anti-filarial activity (Ahmed and Urooj, 2010). The nutrients contained in its fruits are 4.06 ±0.06% total protein, 16.65 ±0.10% crude fibers, 4.39 ±0.14% crude fat, 19.78 ±0.10% carbohydrates, 3.96 ±0.15% ash (Singh et al., 2013). *F. racemosa* are reported to contain flavonoids, alkaloids, α-amyrin acetate, and tannins, which may explain its hypoglycemic action (Zulfiker et al., 2011).

A handsome amount of published literatures have been well established the antidiabetic potency of *S. cumini* seeds and *F. racemosa* fruits alone, but their consorted efficacy is still unknown. Individual doses of *S. cumini* seeds @ 100, 250, 300, 500, 750, 1,250 mg.kg^{-1} b.wt, and *F. racemosa* fruits @ 100, 200, 250, 500 mg.kg^{-1} b.wt were used by the previous researchers (Zulfiker et al., 2011; Irfan et al., 2011; Nahar et al., 2010; Singh and Gupta, 2007). However, the therapeutic efficacy of combining *S. cumini* seeds and *F. racemosa* fruits may exceed that of either plant used alone. Polyherbalism, also known as an herb-herb combination, is a promising treatment option for many diseases because of the synergism and an immense source of phytoactive constituents which are unavailable in a single plant. When one herb facilitates the retention, circulation, metabolism, and exclusion of the other herbs or the active ingredients of both herbs having similar therapeutic activity are intended to be furnished by multiple mechanisms of action, synergism takes place. Polyherbal formulations enhance medicinal efficacy while lowering single herb concentrations, hence minimizing adverse effects and herbal overdosage. Therefore, long-term treatment of diabetes mellitus through combined herbal therapy is feasible as their reported antidiabetogenic activity is on par with other synthetic hypoglycemic agents (Heroor et al., 2013).

In addition, the published literature for histomorphometrical assay in diabetes is limited, as in general, many studies were conducted only to explore hypoglycemic properties. However, pancreatic β-cells release insulin when blood glucose concentration reaches ≥3 mmol.L^{-1}. Insulin decreases glycogenolysis and glucogenesis, induces glycogenesis to regulate glucose homeostasis (Szablewski, 2017). Therefore, both histomorphometric and hypoglycemic evaluations are critical for a better understanding of diabetes mellitus management.

Thus, this study investigates the antidiabetic efficacy of a herbal extract derived from *S. cumini* seeds and *F. racemosa* fruits using an alloxan-produced diabetic animal model in comparison to a long-established antidiabetic medicine.

2 Material and methods

2.1 Extract preparation

In order to collect seeds, *S. cumini* fruits were purchased from Kewatkhali market, Mymensingh. *F. racemosa* fruits were obtained from the Botanical garden of Bangladesh Agricultural University (BAU), Bangladesh. The Horticulture Department of BAU analyzed the ethnopharmacological data of both plants. After mincing, the experimental seeds and fruits were dried, ground into powder, extracted with 95% ethanol, and filtered. After concentrating with a rotary evaporator, the filtered solvent was dried in a lyophilizer. Seeds and fruits extract were stored at 4 °C. Dimethyl sulfoxide (DMSO, Sigma-Aldrich, Germany) was applied to dissolve the extracts, where 0.5 ml DMSO contained the experimented dose of *S. cumini* seeds and *F. racemosa* fruits for each animal (Nahar et al., 2010).

2.2 Experimental animal rearing

A total of seventy-seven (N = 77) young Swiss albino mice (3–4 weeks old; average weight: 26.5 g) were bought from icddr,b (International Centre for Diarrhoeal Disease and Research, Bangladesh). The mice were in good health and had no visible deformities. The animals were kept at 25.0 ±3.0 °C room temperature, 60 ±5% humidity, and an equal light and dark period in the anatomy laboratory of BAU. The animal had free access of drinking water and icddr,b prepared mice pellets. All laboratory animals maintained a week acclimatization period and uniform management practices. Five female mice were used for the acute oral toxicity study, while the remaining mice were used for the main experiment purpose. An Animal Welfare and Experimentation Ethics Committee of BAU approved the treatment and care of the mice involved (Protocol Number: AWEEC/BAU/2022-16).

2.3 Acute oral toxicity study

Randomly selected 05 (five) healthy adult female mice were used for this experiment following guideline 425 of the Organization for Economic Cooperation and Development (OECD). The combination of herbal extract (*S. cumini* seeds @ 1,500 mg.kg^{-1} b.wt and *F. racemosa* fruits @ 600 mg.kg^{-1} b.wt) was dissolved in DMSO and administered to the 3–4 hour fasted mice. At first, a test dose was given to just one animal. As the animal survived for 48 hours, test doses were administered to other animals sequentially.

After dosing, each animal was observed continuously during the first 30 minutes. Any physical signs of toxicity were closely monitored for the first four hours and periodically watched for the next twenty hours. Following that, the animals were surveilled daily for 14 days. All animals were killed humanely, and their organs were examined for macroscopic pathological changes after the test was completed (Boukhalfa et al., 2018).

2.4 Diabetic animal model preparation and experimental procedures

In accordance with the Completely Randomized Design, seventy-two mice were randomly assigned to four treatment groups (n = 6), and each group underwent three replications. Except for the normal control group, all other groups had diabetes, induced by a single intraperitoneal injection of alloxan monohydrate (@150 mg.kg^{-1} b.wt, Sigma-Aldrich, Germany). On day three, fasting blood glucose levels were monitored using a digital glucometer (Kare, Taiwan) to confirm the onset of diabetes. Animals with an FBG level of 11.1 mmol.L^{-1} or higher were eligible for this experiment (Zulfiker et al., 2011). FBG levels were evaluated at 15 (fifteen) days intervals. In addition, body and pancreas weights were recorded on the final day of the experiment.

Among all four groups, the first group of mice served as non-diabetic normal control (NC), while the second group was alloxan-induced diabetic control (DC). Glibenclamide (@ 600 µg.kg^{-1} b.wt) was gavaged to the third group (GL), whereas the fourth group (SF) received *S. cumini* seeds (@ 500 mg.kg^{-1} b.wt) and *F. racemosa* fruits (@ 200 mg.kg^{-1} b.wt) orally every day for 30 days. According to the literature survey, the alcoholic extract of *S. cumini* seeds and *F. racemosa* fruits exhibited their best antidiabetic effect at 500 mg.kg^{-1} b.wt and 200 mg.kg^{-1} b.wt dose, respectively (Zulfiker et al., 2011; Singh and Gupta, 2007). Hence the dose of the combined extract for the experiment was chosen.

After the experiment tenure, light anesthesia (single intraperitoneal pentobarbitone injection @ 35 mg.kg^{-1} b.wt) followed by cervical sub-laxation was done to ethically sacrifice the animals. For histomorphological analysis, the splenic lobe of the pancreas was collected.

2.5 Oral glucose tolerance test

After an overnight fast on the 15th day, all animals were tested for oral glucose tolerance. The extract was initially administered. An hour later, glucose solution (@ 2 g.kg^{-1} b.wt) was given. After glucose overload, a portable glucometer was used to assess glycemia at 0, 30, 60, 90, and 120 minutes (Rekha, 2010).

2.6 Histopathological observation

The eye estimation method was followed to measure the color and gross texture of the pancreas. Formalin-fixed samples were dehydrated by alcohol and implanted in paraffin. Finally, using a rotary microtome, the tissues were sliced into 6-micron thicknesses and stained with hematoxylin-eosin (H & E) (Rekha, 2010). The sections were examined, and image acquisition (40X) was performed under a Leica ICC50 E microscope (Leica microsystems, Wetzlar, Germany) with a digital camera attached. Automatic image analysis was performed using the 'ImageJ' software.

2.7 Histomorphometric analysis

The morphometric analysis of the pancreatic islets was carried out with the help of 'ImageJ' automated image analysis software. With a calibrated graticule and an ocular micrometer, ten (10) histological sections/group were examined to obtain quantitative information about the pancreatic islets. The total number of islets per 10 mm^2 of parenchyma was counted directly at 40X magnification. The diameter of the islet (*Di*) was estimated using the formula $Di = \sqrt{ab}$; where a = major axis, b = minor axis (Noor et al., 2017).

2.8 Statistical analysis

Graph Pad Prism (version 9.0) software was used for the graphical presentation of the findings. One-way analysis of variance and Tukey's multiple comparison test was followed to perform statistical analysis. The mean ±SEM (standard error of the mean) was used to summarize the data. It was considered significant when the difference between the two groups was greater than 0.05 (p <0.05).

3 Results and discussion

3.1 Acute oral toxicity study

The LD_{50} value of the combined herbal extract was confirmed to be more than 2,000 mg.kg^{-1} b.wt since the tested animals showed no indications of toxicity nor died during the 14-day monitoring period. Although

an individual toxicity study of *S. cumini* seeds and *F. racemosa* fruits has been done, no information is available on their combined extract toxicity. New chemical toxicity testing, however, is crucial because it shows the dose-, organ-, and species-specific adverse effects of an investigational product. An analysis of the available research indicates that 500 mg.kg^{-1} b.wt. of an alcoholic extract of *S. cumini* seeds and 200 mg.kg^{-1} b.wt. of *F. racemosa* fruits had the greatest antidiabetic effect (Zulfiker et al., 2011; Singh and Gupta, 2007). Hence, a combination of these two doses was selected as the experimental dose (700 mg.kg^{-1} b.wt combined herbal extract of *S. cumini* seeds and *F. racemosa* fruits) to investigate the antidiabetic effect.

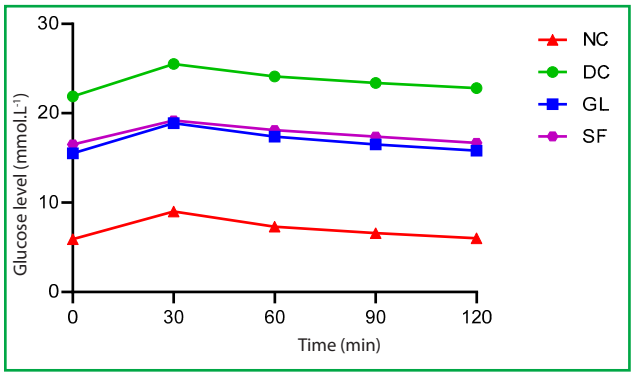

Figure 2 Results of oral glucose tolerance test in different experimental groups
NC – normal control; DC – diabetic control; GL – glibenclamide; SF – combined herbal extract

3.2 Physiological parameters

In the present investigation, the diabetic control group weighed considerably (p <0.05) less than the normal control in terms of body and pancreas weight (Figure 1a and b). The possible explanation for reduced body weight might be excessive muscle waste and glycogenolysis (Sophia and Manoharan, 2007). In insulin deficiency, muscle proteins are degraded to provide amino acids for gluconeogenesis as well as stored glycogen from the liver and muscle is depleted. Moreover, alloxan-induced death of the pancreatic beta cells decreases the width and number of pancreatic islets in the diabetic control group, leading to reduced pancreas weight (Lenzen, 2008).

However, the combined extract and glibenclamide treatment regenerate the pancreatic β-cells and increase the islet's number & diameter, resulting in increased pancreas weight and insulin production (Hasan et al., 2016). The produced insulin further stops tissue protein breakdown and glycogenolysis from the liver and muscle to prevent body weight loss. Statistically, these two groups had no significant differences in body and pancreas weight improvement. In accordance with our findings, previous studies also showed that *S. cumini* and *F. racemosa* could aid in weight gain (Sophia and Manoharan, 2007).

3.3 Oral glucose tolerance test

After 30 minutes of glucose administration, blood glucose levels peaked in all groups (Figure 2). As the test ended, the glucose level remained significantly higher in diabetic animals than those of the normal controls. However, the combined herb therapy significantly reduced blood glucose levels as much as glibenclamide (p <0.05).

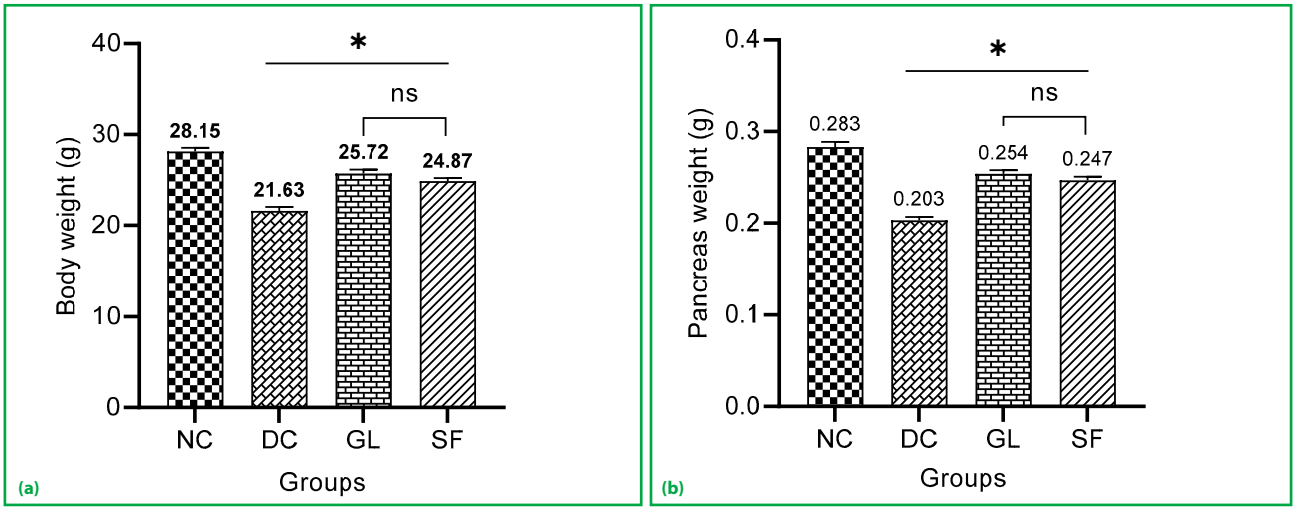

Figure 1 Changes in body weight (a) and pancreas weight (b) in different experimental groups. Values were given as mean ±SEM
Differences between groups were considered significant at p <0.05 level (*)
ns – non-significant; NC – normal control; DC – diabetic control; GL – glibenclamide; SF – combined herbal extract

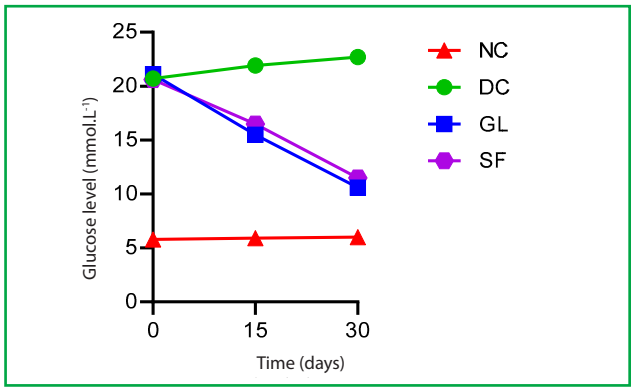

Figure 3 Changes in fasting blood glucose (FBG) levels in different experimental groups
NC – normal control; DC – diabetic control; GL – glibenclamide; SF – combined herbal extract

In diabetes mellitus, oral glucose tolerance test is conducted to determine the time required to eliminate glucose from the blood. Due to the absence of insulin, diabetic control mice failed to utilize glucose by cells and tissues, resulting in significantly high glucose levels in blood. However, the combined extract may lower the glucose absorption rate from the intestinal lumen through delayed carbohydrate digestion by inhibiting maltose, an α-glucosidase enzyme, and reduce postprandial blood glucose levels in a short term (Rekha, 2010). Heroor et al. (2013) and Rekha (2010) similarly reported a significant decrease in post-ingestion glycemia after combined extract treatment.

3.4 Hematological parameters

At the 0-, 15-, and 30-day follow-up evaluations, the diabetic control group had significantly higher (p <0.05) glycemic levels than the normal control (Figure 3). However, antidiabetic drugs and herbal extract significantly reduced alloxan-induced hyperglycemia (p <0.05). Glycemic levels dropped the most with glibenclamide, while the combination of *S. cumini* seeds and *F. racemosa* fruits showed statistically similar but slightly lower antihyperglycemic activity.

The death of insulin-producing pancreatic β-cells was caused by alloxan, a toxic glucose analog, resulting in hyperglycemia in diabetic animals (Lenzen, 2008). The presence of antidiabetic phytochemicals may contribute to the hypoglycemic activity of *S. cumini* and *F. racemosa* combined extract. Mycaminose, a phytochemical from *S. cumini*, promotes insulin secretion by acting on the pancreatic sulfonylurea receptor (Kumar et al., 2008). Moreover, alkaloid- jambosine and glycoside- jambolin present here end the diastatic transformation of starch into sugar (Swami et al., 2012). α-amyrin acetate, another phytochemical of *F. racemosa* fruits, improves glycemia possibly by its interaction with the cannabinoid system (Narender et al., 2009). Through the stimulation of pancreatic beta cells, saponins and tannins, present in both extracts, increase insulin release (Zulfiker et al., 2011). Although individual use of *S. cumini* seeds and *F. racemosa* fruits extract resulted in a 30.11% and 13.71% decrease in fasting blood glucose levels, respectively (Amin et al., 2021; Zulfiker et al., 2011), their combination was found to produce 44.17% post-prandial hypoglycemia in the present study. This increased therapeutic result is possible because of the presence of synergistically working phytochemicals with the co-administration of similar antidiabetic effects-producing herbs. When working with a composite extract, Heroor et al. (2013) and Rekha (2010) found better hypoglycemic results than the individual extracts, which is consistent with our research results.

3.5 Gross and histo-architectural changes of the pancreas

Grossly, the shape, size, and color of the pancreas were nearly identical among the experimental groups. Histologically, the endocrine islets of Langerhans with a regularly arranged α & β cells were randomly scattered among the exocrine pancreatic acini in the normal control group (Figure 4A). On the contrary, the diabetic pancreas showed fibrosis around the pancreatic duct, vascular congestion, and atrophied islets with considerable necrosis, resulting in a reduced number of insulin-producing cells (Figure 4B). In addition, there was hydropic degeneration, focal acinar damage, and inflammatory cells infiltration. Alloxan-induced pancreatic damage was dramatically reversed after the administration of combined plant extract and standard antidiabetic drug. Large islets with regularly distributed α & β cells in normal proportion were found in the group treated with the standard antidiabetic medication (Figure 4C). As well, the combined extract treatment repaired acinar damages and regenerated pancreatic β-cells, hereby restoring normal pancreatic architecture (Figure 4D).

In the diabetic control group, degeneration of islets of Langerhans with a disturbed arrangement of α & β cells confirms the diabetogenic action of alloxan monohydrate. The structural & cellular damages in the diabetic pancreas were reversed by the combined plant extract and standard antidiabetic drug treatment because of the existence of stable (quiescent) cells (Kumar et al., 1992). Stable cells are responsible for cell regeneration as they multiply to replace the missing cells. Furthermore, the phenolic constituents halted the destruction of the remaining β-cells, allowing other phytochemicals to have a chance to stimulate regenerative action (Eliakim-Ikechukwu and Obri, 2009). In addition, the alkaloids

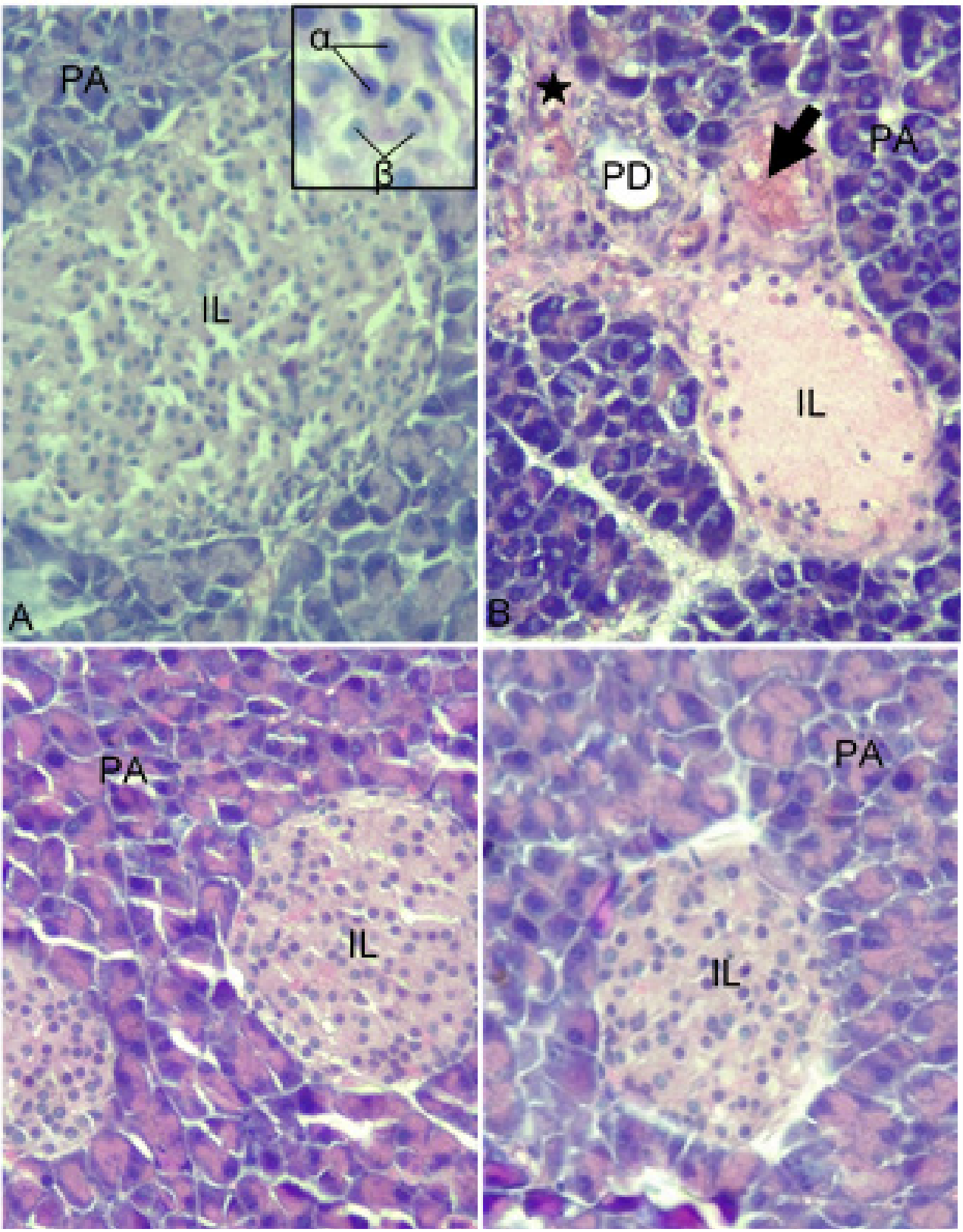

Figure 4 Photomicrograph of the pancreas stained with hematoxylin & eosin (H & E; 40X)
(A) The normal control group presented endocrine islets of Langerhans with a regularly arranged α & β cells among the exocrine acini. (B) The diabetic control group indicated fibrosis around the pancreatic duct (star), vascular congestion (short arrow) and shrunken islets with considerable necrosis. (C) Glibenclamide treated group showed large islets with apparently increased α & β cells. (D) The combined herbal extract-treated group exhibited normal pancreatic architecture where acinar damages were restored and pancreatic β-cells were regenerated. The scale bar stands for 100 µm. PA – pancreatic acini, PD – pancreatic duct, IL – islets of Langerhans

Table 1 Changes in histomorphometric parameters in different groups

Groups	Islet numbers (N.10 mm^{-2})	Islet diameter (μm.$islet^{-1}$)
Normal Control (NC)	11.20 ±1.80	125.24 ±7.12
Diabetic control (DC)	03.10 ±0.90[a]	61.87 ±4.82[a]
Glibenclamide (GL)	08.80 ±1.40[b]	114.76 ±6.06[b]
Combined herbal extract (SF)	07.90 ±1.90[b]	110.54 ±5.93[b]

results are mean ±SEM of 6 mice in each group; at p <0.05, the means with different letters differ significantly

and flavonoids in the extract play a role in restoring pancreatic β-cells (Chakravarthy et al., 1982). Previous researchers mentioned necrosed areas, vacuolation, and inflammation after single *S. cumini* or *F. racemosa* extract treatment (Adeyi et al., 2012; Singh and Gupta, 2007). However, the pancreas was almost devoid of diabetic alterations such as inflammatory cells, necrosis, fibrosis, degranulation, hydropic degeneration, vascular congestions, and hemorrhage with the combined extract administration; confirming the effectiveness of composite extract over individual extract in diabetes management. The present results agree with Rekha (2010), who worked with composite extracts of *S. cumini* and *Cinnamomum zeylanicum* and found histological improvement in the islets. Moreover, Masaenah et al. (2021) mentioned increased pancreatic β-cells numbers after dose-dependent combined extract treatment.

3.6 Histomorphometric study

As compared to the normal control, the diabetic control group had significantly (p <0.05) fewer pancreatic islets and a smaller islet diameter (Table 1). However, the combined extract and glibenclamide treatment exhibited significant (p <0.05) improvements in all parameters, with the highest recovery in the standard drug-treated group.

Death of pancreatic β-cells by the alloxan-generated reactive oxygen species and free radicals results in a decrease in both the number and width of pancreatic islets in the diabetic control group (Lenzen, 2008). However, the combined extract suppresses the histomorphometric alterations of the diabetic pancreas through antioxidant and anti-inflammatory phytochemicals. Flavonoids and α-amyrin acetate capture free radicals and inhibit lipid peroxidation, ultimately protecting the cell against alloxan-induced damage and increasing islets' number and width (Hasan et al., 2016). Masaenah et al. (2021) and Schossler et al. (2004) also reported insulin-positive cell development in the *S. cumini* – treated pancreas, which supports the present histomorphometric findings.

4 Conclusions

The herbal extract combination of *S. cumini* seeds and *F. racemosa* fruits produced near-normal physiological, hematological, histopathological, and histomorphometrical changes in diabetic mice after a 30-day administration. The synergistic phytochemical interaction between the two plant extracts made the combined therapy more effective than their individual use. Further research could determine the dose-dependent effects of the combined extract of *S. cumini* seeds and *F. racemosa* fruits.

Acknowledgments

This research was completed through the financial support of the Government of Bangladesh.

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