Indian Journal of Experimental Biology
Vol. 45, October 2007, pp. 861-867

Effects of ethanolic extract of *Syzygium cumini* (Linn) seed powder on pancreatic islets of alloxan diabetic rats

N Singh & M Gupta

Environmental Endocrinology and Biomedical Research Unit
Department of Zoology, Meerut College, Meerut 250 003, India

Received 29 May 2006; revised 02 July 2007

The ethanolic extract of seeds of *S. cumini* increased body weight and decreased blood sugar level in alloxan diabetic albino rats. Level of significance for decrease in blood sugar after feeding alcoholic extract of *S. cumini* seeds in various doses was highly significant. The extract feeding showed definite improvement in the histopathology of islets. The most important finding is that the blood sugar level, which once dropped to normal levels after extract feeding was not elevated when extract feeding was discontinued for 15 days.

Keywords: Alloxan diabetes, Ethanolic extract, Pancreatic islets, *S. cumini*.

Seeds of *Syzygium cumini* (L), family Myrtaceae (commonly known as jamun in Hindi, black plum in English, neredam in Tamil and neereedu in Telegu) posses hypoglycaemic properties[1-8]. Bansal *et. al.*[9] compared the effect of *S. cumini* seed powder with chloropropamide. In the present study, an attempt has been made to find out the effect of jamun seed extract on beta cells of the islets of Langerhans and probable mode of hypoglycaemic action of alcoholic extract of *S. cumini* seeds.

Materials and Methods

Preparation of extract—Fresh *S.cumini* fruits were collected during July and August from the authenticated jamun trees. Plants were authenticated by Botany Department of Meerut College, Meerut. Flesh of the ripe fruits was removed and seeds were dried and powdered. Extract was prepared in absolute alcohol with the help of thermostatically controlled Soxhelet apparatus. The temperature was controlled at 78° C. The yield of the extract was 0.9 %.

Experimental animals—Male Wistar strain albino rats (7-8 weeks old) procured from Animal Division of IVRI, Izatnagar, were maintained in the animal facility of the Zoology Department of Meerut College, Meerut with standard food pellets and tap water *ad libitum*. All animals were cared for according to guidelines of the Institutional Animal Ethics Committee (IAEC) and experiments were also approved by IAEC.

Experimental design—Animals were acclimatized for laboratory conditions and kept on normal diet for two weeks. After 2 weeks, each rat received a dose of 6 mg/100 g body weight alloxan monohydrate in citrate buffer (*p*H 4.5) by intravenous route through caudal vein. Experimental animals were divided into following 5 groups of 18 animals each :

Group I: normal rats.

Group II: diabetic control group.

Group III: animals were given a dose of 25 mg/100 g body weight of alcoholic extract of *S. cumini* seeds, orally.

Group IV: animals were given a dose of 50 mg/100 g body weight alcoholic extract of *S. cumini* seeds orally.

Group V: animals were orally fed on 75 mg/100 g body weight dose of alcoholic extract of *S. cumini* seeds powder.

Out of 18 rats in each group, 6 animals were dissected after 15 days treatment; 6 after 30 days and remaining 6 were kept on normal diet for 15 more days after extract feeding for 30 days. The reversibility group was maintained to see whether the effect of the extract is palliative or curative.

During the experiment animals were kept under observation. The body weight was noted before

Phone: 0121 2641524, 09412205025
E-mail: n27singh@yahoo.com

starting the experiment and at the completion. The blood was collected for sugar estimation[10] from the caudal vein of normal control, diabetic control and treated rats before and after alloxan injection and at weekly intervals.

Histological studies—Pancreas was fixed in Bouin's fixative (without acetic acid) for histopathological studies. Sections of pancreas (6 µm thick) were stained with chromium haematoxylene-phloxine for differential staining of alpha and beta cells as per Gomori [11].

Liver was fixed in Bouin Hollande's fixative and stained in HE. For glycogen localization liver was fixed in cold absolute alcohol at 0-4° C. The sections were stained with PAS technique. For the confirmation of glycogen control slides were treated with 1% solution of diastase in phosphate buffer (*p*H 7).

Results

Body weight (Fig. 1)—There was no weight gain after 15 days of treatment with different doses of the extract. Individually the rats with high blood sugar lost 1.09-2.15% in body weight, whereas rats with moderately high blood sugar gained about 2.13 % in body weight. Feeding of extract for 30 days resulted in 1.53, 12.38 and 13.69% increase in body weight and after 30 days extract feeding and 15 days normal diet, there was 21.42, 24.18 and 18.15% increase in body weight with 25 mg, 50 mg and 75 mg doses of the extract respectively.

Blood glucose (Fig. 2)—Intravenous injection of alloxan-monohydrate raised the blood glucose upto 4 times of the normal range *i.e.* 90-120 mg/dl. However, the administration of alcoholic extract to diabetic groups decreased their blood glucose by 10-33% in 8 days, 33-52% in 15 days and 40-82% in 30 days. Level of significance for decrease in blood glucose after feeding alcoholic extract of *S. cumini* seeds in various doses was highly significant ($P<0.001$).

All three doses of the extract were able to lower the blood glucose level but 75 mg dose was most effective in lowering the blood glucose level.

Histopathological observations—Intravenous injection of alloxan monohydrate resulted in permanent damage of beta cells of islets, leading to elevation of blood glucose levels upto 4 times the normal range. The histopathological examination of islets of Langerhans from the pancreas of diabetic control group showed varying degree of damages. Regular arrangement of alpha and beta cells was disturbed, clumping and degranulation of beta cells was observed initially (Fig. 3b). In some islets hyperplasia took place with ballooning of cells and hydropic degeneration in some cells (Fig. 3c). After prolonged diabetes some islets were replaced by fibroblasts only (Fig. 3d).

Histopathologically acinar tissues were normal in all dose groups. The islets of Langerhans, in rats receiving 25 mg dose did not show any improvement after 15 days and were small in size or disfigured. After 30 days extract feeding, the remaining beta cells in the disfigured islets achieved granulation but presence of necrosed areas within islets indicated the damage caused by alloxan injection. Disfigurement still persisted after discontinuation of the extract

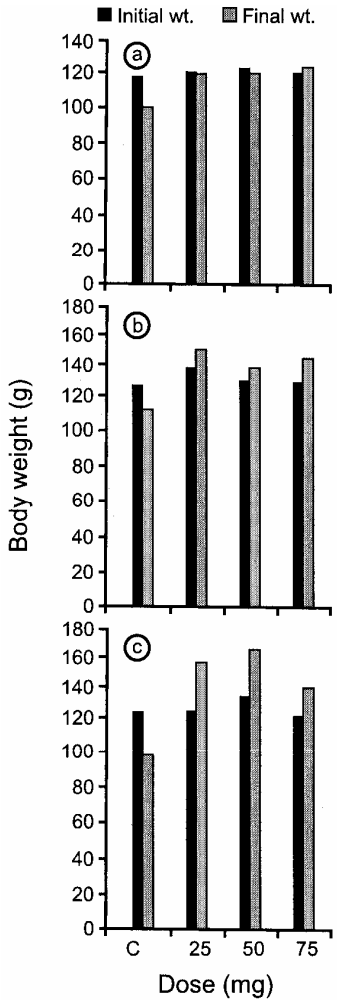


Fig. 1—Comparison of initial body weight and final body weight of alloxan diabetic rats and of experimental rats (n = 6) after feeding different doses of *S. cumini* ethanolic extract for 15 days (a), 30 days (b) and 30 days of extract feeding followed by 15 days normal diet (c). n = 6

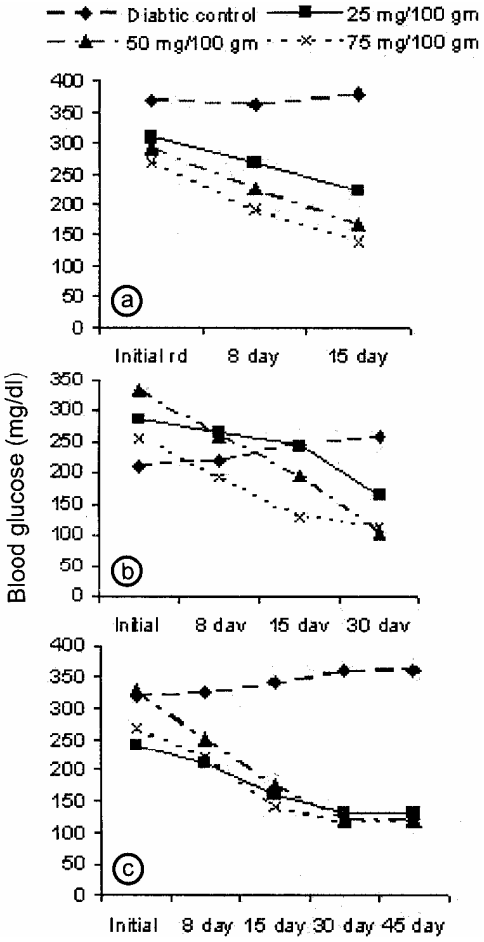


Fig. 2—Mean fall in blood sugar level after different doses of *S. cumini* ethanolic extract for 15 days (a), 30 days (b) and 30 days of extract feeding followed by 15 days normal diet (c). n = 6

feeding for 15 days, but the beta cells showed granulation.

In 50 mg dose group some islets showed signs of recovery in the form of regranulation of beta cells but otherwise islets with smaller size and hydropic degeneration were also present after 15 days. After 30 days extract feeding most of the islets showed recovery in the form of beta cells granulation except for some necrosed areas. The beta cells still preserved their granules when the extract was discontinued for 15 days.

In rats receiving 75 mg dose for 15 days, in small islets beta cells showed hydropic degeneration, and hypertrophy of certain islets was also observed. With the same dose after 30 days beta cells in most of the islets were able to achieve normal granulation except in the necrosed parts. The granulation of beta cells, which had appeared during the 30 days extract feeding period, did not show any change even when the drug was discontinued for 15 days. (Fig. 4).

In rats receiving 25 mg and 50 mg dose for 15 days, the liver showed hydropic degeneration, which after 30 days became normal and remained so even after discontinuation of the extract for 15 days. The rats receiving 75 mg dose showed signs of liver damage after 15 and 30 days extract feeding and hepatocytes did not show any recovery even after discontinuation of the extract feeding up to 15 days.

Despite the fact that blood sugar level was normal, the localization of glycogen in liver was very faint after 15 days in all three-dose groups. The intensity of glycogen localization increased after 30 days extract feeding of 25 and 50 mg doses and there was almost normal glycogen localization after discontinuation of the extract for 15 days. In the 75 mg dose group there was almost no glycogen localization after 30 days or after discontinuation of the extract for 15 days although blood sugar was normal.

Discussion

Although hypoglycaemic property of *Syzygium cumini* syn. *Eugenia jambolana* seeds, both on normal and alloxan diabetic animals has been reported, most of these studies were conducted only on hourly basis and only single study is available on the effect of *S. cumini* only histopathology of pancreas. Sepha and Bose[12] treated diabetic patients with *Eugenea* seed powder. Brahmchari and Augusti[13] concluded that seeds of *E. jambolana* contained some alcohol soluble orally effective hypoglycaemic principle. Shrotri *et al.*[3] tested the pulp of *S. cumini* fruit and its seed powder on normal rabbits, alloxan diabetic rabbits, normal dogs, alloxan diabetic dogs and on three pancreatectomized dogs and reported fall in blood sugar. Kedar and Chakrabarti[14] tested jambolana seed powder (1g/kg body weight) on streptozotocin diabetic albino rabbits and reported that the blood sugar level and glucose tolerance test became normal within 3 weeks. Sharma *et al.*[8] used ethanolic extract of *E. jambolana* (*S. cumini)* seed powder on alloxan diabetic rabbits and reported increase in body weight, reduced fasting blood glucose (FBG) and improved lipid profile. In severely diabetic group in blood sugar level a fall of 29% was recorded (P<0.001). Murthy *et al.*[15] observed that a compound purified from fenugreek (*Trigonella foenum graecum* Linn) seeds showed similar advantage of bringing down FBG levels of diabetic rats to normal level maintaining the FBG at normal level without treatment for 15 days.

During the present study, the alcoholic extract of *S. cumini* produced a fall in blood glucose level of 13, 23 and 28% after 8 days, 24, 46 and 50% after 15 days and 50, 69 and 58% after 30 days with 25, 50 and 75 mg doses, respectively. When these data were statistically analyzed and Student's '*t*' test was applied to calculate the level of significance, it indicated that the effect of alcoholic extract was dose dependent.

The results of diabetic control group of the present study, that alloxan produces permanent hyperglycaemia by selective destruction of the beta cells of the islets of Langerhans of pancreas are in agreement with those of Black[16], Bhaveja *et al.*[17]and Dunn *et al*[18].

With 15 days feeding of 25 mg dose of the extract, islets did not show any noticeable improvement over the changes produced by alloxan injection. But after 30 days of treatment, when blood sugar came down to normal level, islets of Langerhans also showed improvement in the beta cells granulation. In the 50 and 75 mg dose groups some islets showed recovery

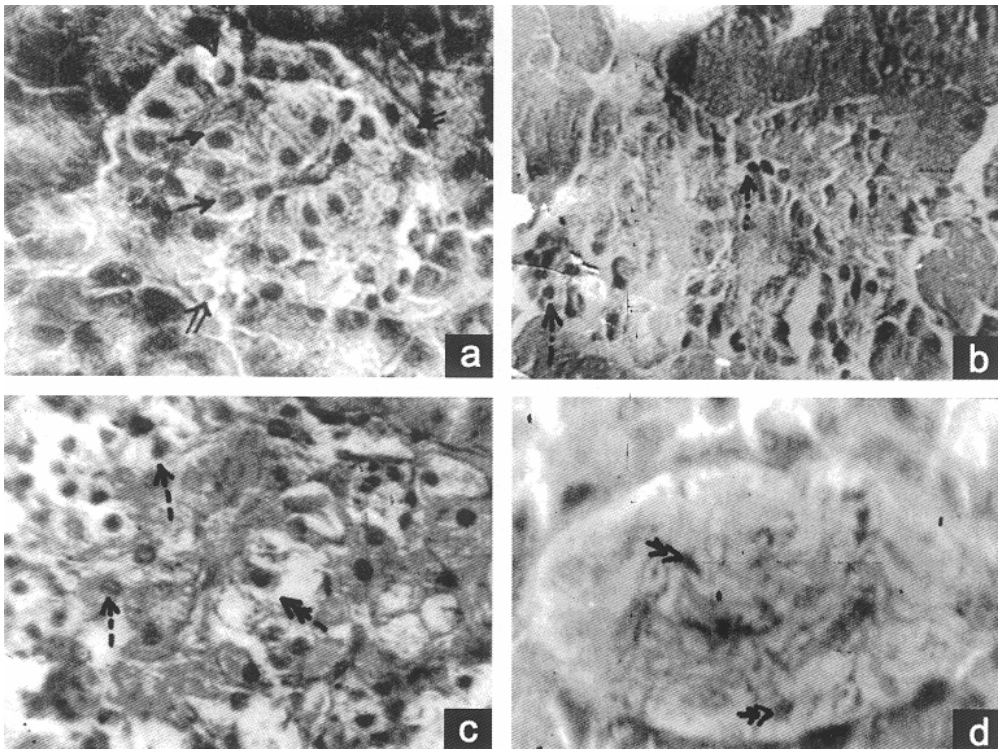
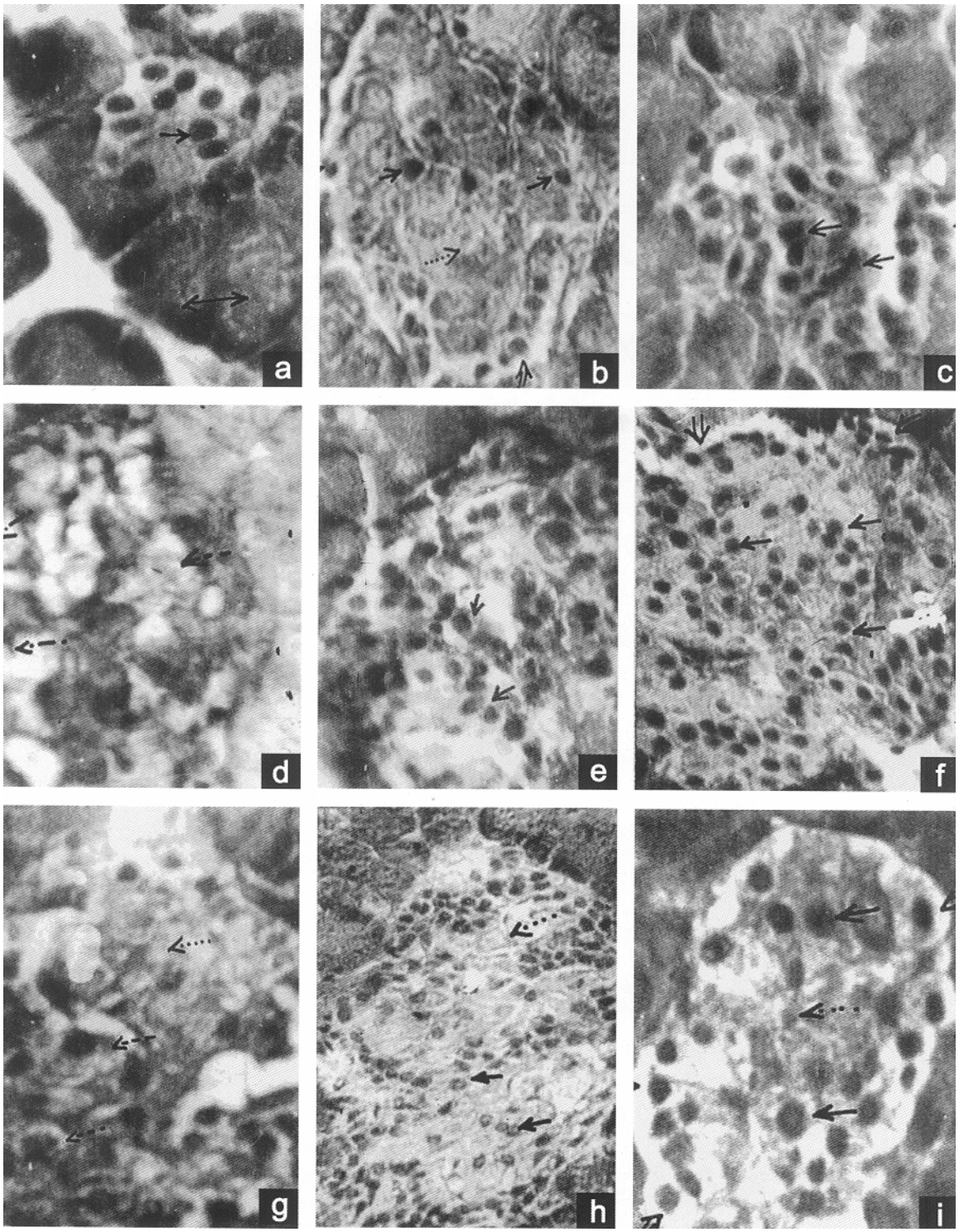


Fig.3—a: a normal islet of normal control group, showing alpha cells (⇒) at the periphery and beta cells (→) with granular cytoplasm in the centre (× 600); b: a totally disfigured islet of diabetic control group (15 days), showing clumping (⟝→) and degranulation (⋯>) of beta cells (× 475); c: hyperplasia of the islets with hydropic degeneration (– –→) and ballooning (–→→) of occasional beta cells (diabetic control group of 30 days) (× 600) ; d: complete fibrosis of the islets, showing fibroblasts (→›) in place of alpha and beta cells (diabetic control group of 45 days) (× 600)

Fig.4—a: a small islet (→) surrounded by normal acinar tissue (←→) after 15 days extract feeding. (25mg / 100g dose) (×600); b: a large islet with alpha cells on periphery (=>), necrosed areas in the centre (-- >) and remaining beta cells (→) with normal granulation after 30 days extract feeding. (25mg/100g dose) (×600); c: a medium sized islet with normal β-cells (→) and α cells (=>) after 30 days extract feeding followed by 15 days normal diet. (25mg/100 dose) (×600); d: a large islet showing excessive hydropic degeneration (--- >) and pyknosis of nuclei (.--.>)indicating no improvement after 15 days of extract feeding. (50mg / 100g dose) (×600); e: a large islet, with loss of regular arrangement of α and β cells granulation after 30 days extract feeding followed by 15 days normal diet. (50mg/100g dose) (×600); f: a large islet with α cell (=>) on periphery and β cells (→) in the centre with normal granulation after 30 days extract feeding followed by 15 days normal diet. (50mg / 100g dose) (×600); g: islets showing some hydropic degeneration (---->) of beta cells and few necrosed areas (…..>) after 15 days extract feeding. (75mg/100g dose) (×675); h: a large islet full of cells with some clumping (--.→) but most of the beta cells show granulation after 30 days extract feeding. (75mg/100g dose) (×400); i : an islet with alpha cells on the periphery (=>) and beta cells with normal granulation (→) in the centre. Some necrosed areas (…..>) indicating previous damage caused by alloxan, after 30 days extract feeding followed by 15 days normal diet (75mg /100g dose) (× 675)


a
b
c
d
e
f
g
h
i

even after 15 days and blood sugar level was also normal in those rats. After 30 days all the β-cells, which were partially damaged by alloxan injection showed recovery and blood sugar became normal in almost all the animals treated with 50 mg or 75 mg doses. Sharma *et al.*[8] have also reported improvement in islet picture after one months oral feeding of *E. jambolana* (*S. cumini)* seed powder extract. Regener-ation of islet cells by dietary components[19] and stimulation of insulin secretion by different plant extracts[20] have been reported. Most probably some alkaloid in the alcoholic extract of *S. cumini* acts in the same manner and causes improvement in beta cells.

The histopathology of liver is in agreement with histopathology of pancreas as well as blood biochemistry. Due to high level of blood sugar hepatocytes showed hydropic degeneration. As the normal granulation was achieved by β-cells of the islets, blood sugar level became normal and hepatocytes also reversed back to normal. The liver of rats, treated with 75 mg/100 g body weight dose of the extract showed hydropic degeneration and fatty infiltration, indicating some toxic effect of high dose.

Observations of liver histopathology, when subjected to comparison with the histochemical observations of liver, were in complete accordance with each other. There was moderate to normal glycogen localization in almost all hepatocytes after 30 days feeding of 25 and 50 mg doses of alcoholic extract, but 75 mg dose showed hepatotoxic results, so there was faint to normal glycogen localization in liver in this dose group. As blood sugar level did not increase even after 15 days discontinuation of the extract feeding, liver glycogen was also unaffected. Kedar and Chakrabarti[14] have also reported that liver glycogen is drastically reduced in diabetic group and jambolana seeds help in increasing the glycogen content but not equivalent to that observed in non-diabetic control animals. During the present study normal glycogen localization was observed after 30 days of extract feeding of 25 and 50 mg doses. Sharma *et al.*[8] have also reported decreased glycogen content in liver of diabetic rabbits and increase in liver glycogen content after treatment with alcoholic extract of *E. jambolana* seeds.

One important finding of the present study is that the blood sugar level which once dropped to normal level after extract feeding did not increase again after discontinuation of extract feeding up to 15 days. This finding is in accordance with the observations of Sepha and Bose[12] on diabetic patients.

It can be concluded that the effect of alcoholic extract of *S. cumini* appears to be curative rather than palliative. Improvement in the islet histopathology and glycogen localization in the liver also indicates towards same fact.

Acknowledgement

This work was supported by grant from ICMR, New Delhi.

References

1 Nadkarni K M, *Indian Materia Medica*, (Popular Book Depot, Bombay), (1954) 805.
2 Vaish S K, Therapeutic uses of Jamun seeds in alloxan diabetes, *Proceedings of Indian Sci Cong Assoc*; (1954) 230.
3 Shrotri D S, Investigation of hypoglycaemic properties of *Vinca rosea, Cassia auriculata* and *Eugenia jambolana, Indian J Med Res*, 51 (1963), 464.
4 Indira G, Seshagiri Rao A & Vimla Devi M, Studies on hypoglycaemic activity of aqueous extract of seeds of *Eugenia jambolana* in different formulations in rabbits, *Indian J Pharmacol*, 11(1) (1954) 65.
5 Lal B N & Chaudhary K D, Observations on *Momordica charantia* (Kavellaka) and Jamboo (*Eugenia jambolana*) as antidiabetic remedies, *J Res Ind Med*, 2 (2) (1968) 161.
6 Prince P S, Menon V P & Pari L, Hypoglycaemic activity of *Syzygium cumini* seeds: effect on lipid peroxidation in alloxan diabetic rats, *J Ethnopharmacol*, 61(1) (1998) 1.
7 Teixeira C C, Pinto L P, Kessler F H, Knijnik L, Pinto C P & Gastaldo F F D, The effect of *Syzygium cumini* seeds on post-prandial blood glucose levels in non-diabetic rats and rats with Streptozotocin induced diabetes mellitus, *J Ethnopharmacol*, 56(3) (1997) 209.
8 Sharma S B, Nasir A, Prabhu K M, Murthy P S & Dev G, Hypoglycaemic and hypolipidemic effect of ethanolic extract of seeds of *Eugenia jambolana* in alloxan induced diabetic rabbits, *J Ethanopharmacol*, 85 (2003) 201.
9 Bansal R, Ahmed N & Kidwai J, Effect of oral administration of *Eugenia jambolana* seeds and chloropropamide on blood glucose and pancreatic cathepsin B in rats, *Indian J Bioch Biophy*, 18(5) (1981) 377.
10 Asatoor A M & King E J, Simplified colorimetric blood sugar method, *Biochem J*, 56 (1954) 44.
11 Gomori G, Observations with different strains on human islets of Langarhans, *Am J Path*, 17 (1941) 395.
12 Sepha A C & Bose S N, Clinical observations on the anti diabetic properties of *Pterocarpus marsupium* and *Eugenia jambolana*, *J Indian Med Assoc* 27 (1956) 388.
13 Brahmchari H D & Augusti K T, Effects of orally effective hypoglycaemic agents from plants on alloxan diabetes, *J Pharm Pharmacol*, 14 (1962) 617.
14 Kedar P & Chakrabarti C H, Effects of jambolana seed treatment on blood sugar, lipids and urea in streptozotocin induced diabetes in rabbits, *Indian J Physiol Pharmac*, 27(2) (1983), 135.
15 Murthy P S, Moorthy Radha, Pugajhenthi S, Vankana Babu B, Prabhu K M, Ratnakar P, Shukla Rimi, Puri Dinesh, Dev G, Rusia U & Agarwal S, *Trends in Clinical Chemistry and*

Laboratory Medicine (Association of Clinical Biochemists of India, Delhi) 2003.

16 Black H E, Chemically induced (streptozotocin, alloxan) diabetes mellitus in dog : Biochemical & ultrastructural studies, *Am J Pathol*, 98 (2) (1980) 295.

17 Bhaveja U K, Aurora A L & Agarwal D S, Production & study of experimental diabetes in mice with alloxan, *Indian J Pathol Microbiol,* 25 (1982) 1-5.

18 Dunn J S, Sheehan H L & Maletchie N G, Necrosis of islets of Langerhans produced experimentally, *Lancet*, 1 (1983) 484.

19 Hellerstrom C, Andersson A & Gunnarsson R, Regeneration of islet cells, *Acta Endocrinol* (Suppl.), 83 (Suppl. 205) (1976) 145.

20 Shanmugasundaram K R, Panneerselvam C, Samudram P & Shanmugasundaram E R B, The insulinotropic activity of *Gymnema sylvestre*, R. Br. An Indian medical plant in controlling diabetes mellitus, *Pharmacological Res Communications*, 13 (5) 1981 475.