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Attenuation of streptozotocin mediated oxidative stress, hyperglycemia and toxicity in rats by treatment with B-20 drops - a homoeopathic preparation

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SUMMARY

The present study is aimed at finding the effect of B-20 drops, a homoeopathic formulation, in streptozotocin (STZ) induced diabetic rats. B-20 drops comprises of the constituents derived from plants and other natural sources, and are generally prescribed by the homoeopathic physician, in cases of hyperglycemia and diabetes. The elevated levels of fasting blood glucose and pancreatic lipid peroxides observed in rats treated with STZ were significantly reduced by the treatment of B-20 drops. The reduced liver glycogen contents were also brought back to near normal level by B-20 drops treatment in STZ diabetic rats. STZ induced histopathological changes in pancreas and liver was also partially reversed by B-20 drops. The findings indicate that B-20 drops help in improving the glycogen stores in the liver and prevents STZ induced damage through free radicals by decreasing the pancreatic lipid peroxides levels.

Key words: B-20 drops; Homoeopathic formulations; Hypoglycemia; Liver glycogen; Lipid peroxides; STZ

INTRODUCTION

Homoeopathy today is a rapidly growing system and is being practiced almost all over the world. The rationale of homoeopathic treatment of diabetes mellitus with constitutional predisposition is reported in literature (Mamchevko and Kolesova, 1992). In India, it has become a household name due to the safety of its pills and gentleness of its cure. A study indicates that about 10% of the Indian population solely depends on homoeopathy for their health care needs. It is more than a century and half now that homoeopathy is being practiced in India. It has blended so well into the roots and tradition of the country that it has been recognized as one of the Natural System of Medicine and plays an important role in providing health care to a large number of people.

In recent years there has been an upsurge in the clinical use of indigenous drugs. Indian medicinal plants and their derivatives have been an invaluable source of therapeutic agents to treat various disorders including diabetes (Koehn and Carter, 2005). Polyherbal preparations, originally used in the traditional systems of medicine, are now being investigated and effectively tried in a variety of pathophysiological states (Shah *et al.*, 1997). Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes

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(Ajgaonkar, 1979; Alarcon-Aguilara *et al.*, 1998). The hypoglycemic activity of a large number of these plants been evaluated and confirmed in different animal models (Karaway *et al.*, 1984; Bopanna *et al.*, 1997; Bhandari *et al.*, 1998; Jouad *et al.*, 2000; Sugihara *et al.*, 2000; Takako *et al.*, 2005). Side effects and expenses associated with allopathic drugs have provoked the need for research into drugs, which are without the side effects, especially those belonging to the traditional systems of medicine like Ayurveda, Homoeopathy, and Unani etc. B-20 sugar drops is a homoeopathic formulation comprising of the constituents derived from plants and other natural sources. Although B-20 drops are generally prescribed by the homoeopathic physician, in cases of hyperglycemia and diabetes in India, yet no scientific reports are available in literature. It is manufactured by M/s Bakson Drugs and Pharmaceutical Private Limited, Himachal Pradesh, India and is a herbomineral formulation, which contains herbal extracts and minerals of known antidiabetic activity. Its main constituents are *Acidum phosphoricum, Arsenium album, Lycopodium, Natrum sulphuricum, Phaseolus nanus, Secale corrutum and Syzyium jamb.*

The present study aims at investigating the effect of B-20 drops on the blood-glucose level of rats with a view to elucidate the rationale behind its use in the management of diabetes by homoeopathic physician and to evaluate the efficacy of the same in streptozotocin (STZ) induced diabetes in rats. The effect of B-20 drops on β cells, lipid peroxidation (LPO) and liver glycogen level was compared with gliclazide - a standard oral hypoglycemic agent.

MATERIALS AND METHODS

Drugs and chemicals

B-20 drops were provided by M/s Bakson Drugs and Pharmaceutical Pvt. Ltd., Himachal Pradesh, India. STZ was purchased from Sigma, U.S.A. Gliclazide was purchased from Serdia, Mumbai, India. All the biochemicals and reagents used were of analytical grade.

Animals

Experiments were carried out using Wistar albino rats, weighing 100 - 150 g, housed in groups of 6 animals per cage and maintained on standard pellet diet (Amrut Labs, Chakan Oil Mills Ltd., Pune) and water *ad libitum*, according to the Animal Ethics Committee guidelines established by the University.

Induction of diabetes

STZ (Sigma, U.S.A.) was dissolved in 0.05 M citrate buffer (pH 4.5) and was administered to 40 neonatal Wistar rats at a dose (100 mg/g, i.p.) (Barbera *et al.*, 1997). To another group of 10 neonatal rats, vehicle alone (0.05 M citrate buffer and 0.9% saline in equal volumes) was injected. Animals of this group served as group I (vehicle control). Blood glucose was determined for all animals after 2 months to confirm hyperglycemia. Rats showing fasting blood glucose levels more than 250 mg/dl were selected and randomly divided into four groups II, III, IV and V each containing 10 rats.

Group I (vehicle control) served as normal control and received normal saline twice daily. Animals of group II (STZ-treated) served as diabetic control and received normal saline 1 ml/kg p.o. twice daily. Rats of groups III (STZ-treated) received B-20 drops[1] (2 drops diluted with 1 ml of water, p.o.) twice daily. Rats of group IV (STZ-treated) received B-20 drops[2] (3 drops diluted with 1 ml of water, p.o.) twice daily. Diabetic rats of group V received gliclazide (25 mg/kg, p.o.). Treatment was continued for a period of 15 days.

Biochemical estimations

Assessment of pancreatic LPO: Animals were sacrificed at the end of 15 days after respective assigned treatment. Pancreas was removed for the determination of the level of lipid peroxides. Samples were homogenized in 20 volumes of 50 mM Tris

HCl (pH 7.5) using a glass homogenizer with a Teflon pestle (Ohkawa *et al.*, 1979) and then incubated for 30 min at 37°C. After incubation thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were added. The samples were kept for 45 min in boiling water. The colored complex formed was extracted with pure butanol-pyridine (15 : 1, v/v) mixture and absorbance was measured at 532 nm. The extinction coefficient of malondialdehyde color complex was 1.5×10^{-5} cm/M. Protein content in each sample was measured by the method of Lowry *et al.* (1951).

Determination of liver glycogen: The liver glycogen content of animals treated with STZ, B-20 drops and gliclazide was determined using the method of Rex Montgomery, 1957.

Other methods: Samples were analyzed for the measurement of blood glucose level by glucose oxidase-peroxidase (GOD-POD) method. (Braham and Trinder, 1972) using glucose kits (Span Diagnostics).

Histological studies

Liver and pancreatic tissues were collected from the rats treated with STZ, B-20 drops, and gliclazide and fixed in 10% buffered neutral formalin routinely. Hematoxylin and eosin stained preparation of processed sections were studied microscopically.

Statistical analysis

Results are expressed as mean ± S.E.M. The significance of difference was assessed by using students '*t*' test and ANOVA. F ratio was also calculated. $P < 0.05$ was regarded as significant.

RESULTS

A significant ($P < 0.01$) increase in blood glucose levels was observed in animals injected with STZ. Treatment with B-20 drops in two doses for 15 days reduced the blood glucose levels significantly ($P < 0.01$) in a dose-dependent manner. Administration of gliclazide (25 mg/kg) for 15 days also reduced the blood glucose level significantly ($P < 0.01$) when compared to the animals treated with only STZ (Table 1).

The concentration of malondialdehyde (MDA) that reflect the tissue lipid peroxides in animals treated with STZ alone (group II) was significantly increased ($P < 0.01$) as compared to normal saline treated rats (group I). Simultaneous treatment with B-20 drops at two concentrations reduced the level of TBARS in pancreas (group III and IV). The standard oral hypoglycemic agent, gliclazide also had lowering effect on TBARS in STZ diabetic animals (group V). The lowering effect on TBARS in pancreas by B-20 drops and gliclazide was comparable.

The liver glycogen content was reduced significantly ($P < 0.01$) in STZ diabetic rats as compared to the level in normal control rats, substantiating depletion of liver glycogen and failure of utilization by hepatocytes in STZ induced diabetes. It was

Table 1. Effect of B-20 drops and gliclazide on blood glucose, pancreatic lipid peroxides and liver glycogen in STZ induced diabetic rats

Groups	Treatment	Blood glucose (mg/dl)	Lipid peroxides (nmoles/mg of protein)	Liver glycogen (mg/100mg)
I	Normal Saline (1.0 ml/kg, i.p)	70.72 ± 5.30	0.93 ± 0.15	5.44 ± 0.34
II	STZ (100 µg/g, i.p)	273.57 ± 19.35**	1.93 ± 0.34**	2.42 ± 0.28**
III	STZ + B-20 Drops (0.2 ml/200g, p.o)	87.62 ± 1.71**	1.10 ± 0.05**	4.18 ± 0.34**
IV	STZ + B-20 Drops (0.2 ml/200g, p.o)	72.66 ± 1.74**	1.02 ± 0.08**	4.57 ± 0.36**
V	STZ + gliclazide (25 mg/kg, p.o.)	82.46 ± 1.30**	1.00 ± 0.07**	4.74 ± 0.24**
	F-Ratio	93.49	5.66	12.85

Values represent mean ± S.E.M. n = 6. Group II is compared with group I and group III, IV and V are compared with group II. Where **$P < 0.01$.

further observed that diabetic rats treated with B-20 drops (in two doses) showed a gradual replenishment of glycogen stores in the liver as is seen from the values of 4.18 ± 0.34 and 4.57 ± 0.36 (group III and IV) as against 2.42 ± 0.28 (group II). Re-establishment of glycogen content was also observed in animals treated with gliclazide.

Multi-focal areas of necrosis associated with lymphomononuclear cells infiltration, severe degeneration of hepatic parenchyma, periportal lymphocytic infiltration were observed in STZ diabetic animals (Fig. 1B). Parenchymal necrosis, degeneration and infiltrative changes were more or less absent in animals treated with B-20 drops 2 (3 drops diluted with 1 ml) (Fig. 1C). Histologically, the liver was near normal, an observation consistently seen in all the animals in this group. However, in B-20 drops 1 (2 drops diluted with 1 ml) treated group, presence of necrotic foci was seen in rats. The histological picture of liver improved in animals treated with gliclazide, however, presence of necrotic foci and mild to moderate periportal infiltrates were evident in liver parenchyma (Fig. 1D). STZ diabetes resulted in degenerative and lytic changes in the islets of Langerhans of pancreas (Fig. 2B). In some of the sections, there was depletion of various cells i.e. β cells and the dimension of the islet was also considerably

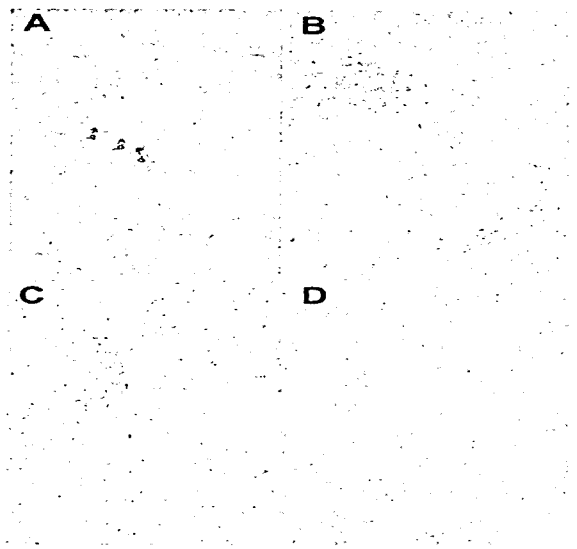

Fig. 1. Histopathology of liver samples from rats induced diabetes with STZ and protected by B-20 drops and gliclazide **(A)** Represents the section of rat liver showing (a) central vein (b) normal hepatocytes with nucleus and nucleoli as well as cytoplasm.; **(B)** Represents the section of STZ treated rat liver showing multi-focal necrosis and leucocytic infiltration.; **(C)** Represents the section of B-20 drops (3 drops diluted with 1 ml) treated rat liver section showing (a) perfectly normal hepatocytes, especially in the periportal areas (b) normal portal triad.; **(D)** Represents the section of gliclazide (25 mg/kg) treated rat liver showing tiny lymphocytic focus and mild periportal infiltration. (A, B, C and D-H and EX200).

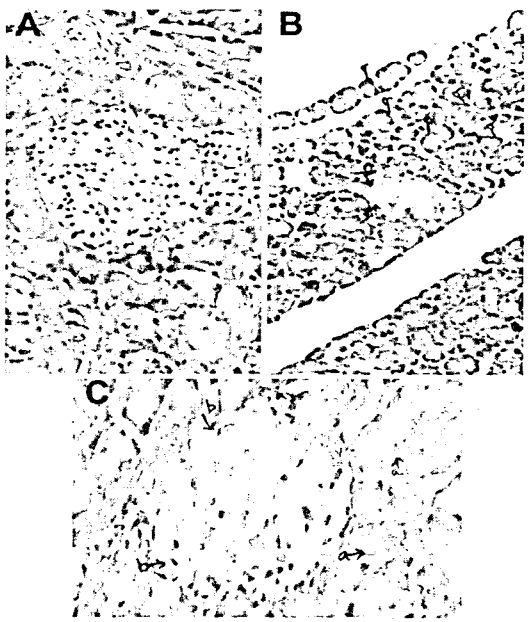

Fig. 2. Histopathology of pancreas samples from rats induced diabetes with STZ and protected by B-20 drops **(A)** Representative section of rat pancreas from normal control group showing (a) pancreatic acini (b) islets of Langerhans comprising β cells and blood vessels.; **(B)** Representative section of pancreas from rat with STZ induced diabetes revealing (a) pancreatic acini (b) islets of Langerhans with depletion of various cells i.e. β cells revealing the damage to these islets by STZ. Note the shrunkness of islet.; **(C)** Representative section of pancreas from diabetic rat treated with B-20 drops (3 drops diluted with 1 ml) (a) well brought out pancreatic acini (b) Increased size of pancreatic islets comprising β cells thereby revealing the protective effect of B-20 drops. (A and B–H and EX200; C-H and EX400).

reduced and shrunken. The histological picture of pancreas was improved in animals treated with B-20 drops, as evidenced by the increase in the dimension of the islet thereby revealing the well protective effect of B-20 drops (Fig. 2C).

DISCUSSION

Type II (non-insulin dependent) diabetes mellitus is primarily characterized by hyperglycemia and increased oxidative stress (Baynes, 1991; Ceriello, 2000). LPO has been implicated in the pathogenesis of naturally occuring or induced diabetes (Wolff *et al.*, 1990). The major mechanism involved in STZ cytotoxicity is OFR induced LPO and DNA strand breaking in pancreatic islet cells (Halliwell, 1989). STZ injection in rats causes hyperglycemia and increased LPO in pancreas. B-20 drops are a homoeopathic formulation with known potential of correcting diabetes and are used clinically. The above fact has also been convincingly substantiated in our experiment. Takasu *et al.*, 1991 have also demonstrated that STZ generates some types of oxygen radicals that facilitate H_2O_2 generation and the peroxides causes fragmentation of DNA.

The LPO resulted from the oxidative degeneration of polyunsaturated fatty acids of the cell membranes, may produce tissue damage and finally causes various diabetes-induced complications (Barnes, 1990). A marked increase in the concentration of TBARS is observed in pancreatic tissue of diabetic rats. Co-administration of B-20 drops in two doses resulted in lowering of TBARS in the pancreas. This observation demonstrates the protective effect of B-20 drops on free radicals produced by pancreas in response to STZ toxicity. The TBARS lowering effect could be attributed to *Syigium cumini*, one of its main ingredients which is known for its hypoglycemic and antioxidant activity. The aqueous extract (2.5 ml/ kg body wt.) of *Syzigium cumini* results in the decreased free radical formation in the various tissues (Prince *et al.*, 1998). Seeds of *Syzigium cumini* contain glycoside jumboline which prevents the coversion of starch into sugar (Nadkarni, 1979). Oral administration of aqueous extract of seeds has been shown to significantly reduce the blood glucose and increased Hb content (Prince *et al.*, 1998).

In this study the effect of B-20 drops treatment, on the glycogen level in the liver of rats in group III and Group IV, when compared with normal rats (Group I), supported an interpretation that B-20 sugar drops at lower doses treatment made the liver cells to build up glycogen that was not available in the STZ induced diabetic rats (Group II). Though the levels of glycogen build up had not touched those observed in normal rats (Group I) within the short duration of this experiment, it has provided a dependable observation that the capacity to rebuild glycogen reserve has been achieved by B-20 drops treatment. Biochemical observations were in keeping with the morphological changes in liver and pancreas. The necrotic and infiltrative changes of liver and depletion of various cells of islets of Langerhans which were consistently observed in animals treated with STZ were reduced to a minimum with B-20 drops co-treatment, further substantiating the protective effect of B-20 drops in the liver and pancreas of rat.

The hypoglycemic effect of B-20 drops could be attributed to *Syzygium Cumini*, one of its main ingredients, which is known for lowering the blood sugar levels. Jambul is prescribed by herbal practitioners to counter the effect of diabetes where the islet cells of pancreas cease to produce sufficient insulin. In India, powdered jambul seeds or occasionally the tincture, are given for diabetes and the frequent urination that accompanied it (Encyclopedia, 1996). The protective effect of B-20 drops was comparable to gliclazide, an oral hypoglycemic agent. Numerous studies have been conducted highlighting the anti diabetic property of *Syzygium Cumini* (Jamun). Prince and co-workers studied the hypoglycemic and antioxidant property of aqueous extract of *Syzygium Cumini* in alloxan diabetic rats (Prince *et al.*, 1998). Another

important component of B-20 drops *Phaseolus Vulgaris* (French beans) have been used since antiquity to treat diabetes. Powdered or infused pod have hypoglycemic activity and used in the treatment of diabetes (Encyclopedia, 1996). In summary, the present study demonstrates the antihyperglycemic activity of B-20 drops in STZ diabetes. Analytical studies determining the active component responsible for hypoglycemic activity are of present interest. Detailed mechanisms of action on different free radicals by B-20 drops require further study.

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