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Protective effect of cycloart-23-ene-3β, 25-diol (B2) isolated from *Pongamia pinnata* L. Pierre on vital organs in streptozotocin-nicotinamide induced diabetic mice

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ABSTRACT

Objective: To evaluate the protective effect of cycloart-23-ene-3β, 25-diol (B2) on vital organs in streptozotocin-nicotinamide induced diabetic mice. **Methods:** Diabetes was induced in mice by streptozotocin (200 mg/kg, *i.p.*) injected 15 min after nicotinamide (110 mg/kg, *i.p.*). The mice were divided into following groups; group 1- non-diabetic, group 2- diabetic control and group 3- B2 (1 mg/kg, *p.o.*). At the end of 28th day, mice were sacrificed by cervical dislocation method. Liver, kidney, heart, eye and brain tissue were isolated. The isolated tissues were trimmed into small pieces and preserved in 10 % formalin for 24 h. Specimens were cut in section of 3-5 μm in thickness and stained by hematoxyline-eosin stain. **Results:** Microscopic examination of vital organs showed severe alteration in diabetic control group in contrast to absence of any changes or minimum changes in B2 treatment. **Conclusions:** It is concluded that B2 exhibits protection of vital organs by minimizing toxic effects and related abnormalities of diabetic conditions in streptozotocin- nicotinamide induced diabetic mice.

1. Introduction

The increasing worldwide incidence of diabetes mellitus in adults constitutes a global public health burden. In 2030, India, China and the United States will be the largest number of peoples with diabetes. Diabetes is a group of heterogeneous disorders characterized by hyperglycaemia and glucose intolerance due to insulin deficiency, impaired effectiveness of insulin action or both. The chronic hyperglycaemia is associated with long term damage, dysfunction and failure of various organs especially the eye, kidney, nerves, heart and blood vessels. Thus diabetes covers a wide range of heterogeneous diseases[1]. The complications of diabetes mellitus include retinopathy, nephropathy and neuropathy (both peripheral and autonomic). The risk for atherosclerotic vascular disease is also increased in persons with diabetes mellitus. The risk for microvascular and neuropathic complications is related to both duration of diabetes and the severity of hyperglycaemia[2]. Despite the great interest in the development of new drugs to prevent the burden of complications associated with this disease and the raised interest in the scientific community to evaluate either raw or isolated natural products in experimental studies[3]. Natural supplements are widely used around the world to treat diabetes[4].

Pongamia pinnata (*P. pinnata*) (L.) Pierre (Fabaceae) is commonly known as Indian beech, pongam oil tree or hongay oil tree[5]. Alcoholic and petroleum ether[7] extracts of stem bark of *P. pinnata* showed antihyperglycaemic activity in alloxan induced diabetic mice[6,7]. Petroleum ether extract of *P. pinnata* showed maximum antihyperglycaemic effect. Hence, further we have investigated petroleum ether extract concomitantly administered with synthetic oral hypoglycaemic drugs (glyburide, metformin and pioglitazone) showed synergistic effect[8]. Cycloart-23-ene-3β, 25-diol (B2) isolated from the petroleum ether extract of stem bark of *P. pinnata* showed potent antihyperglycaemic activity in alloxan induced diabetic mice in acute stud[9]. So, the experiment carried out in alloxan induced diabetic mice required to be confirmed in more specific animal models of type 2 diabetes. Recently, we have reported the antidiabetic activity of B2 in streptozotocin-nicotinamide

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induced diabetic mice. The results obtain showed potent antidiabetic activity of B2[10]. In the present study, we found that B2 showed protection of vital organs in diabetic stage. Hence, the objective of the present investigation was to evaluate the protective effect of cycloart-23-ene-3β, 25-diol (B2) on vital organs in streptozotocin-nicotinamide induced diabetic mice.

2. Materials and methods

2.1. Cycloart-23-ene-3β, 25-diol identification and characterization

Isolation and characterization of cycloart-23-ene-3β, 25-diol (B2) has been previously reported[9]. Chemical structure of B2 is shown in the Figure 1.

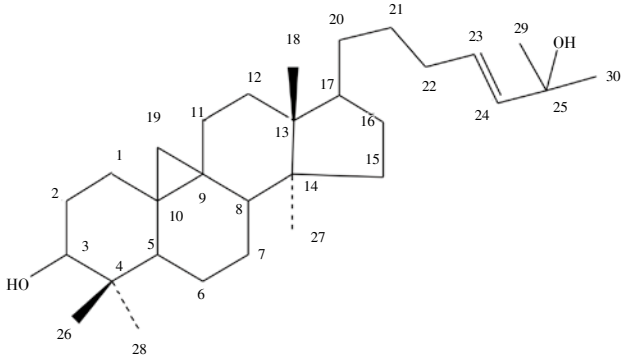

Figure 1. Structure of B2.

2.2. Drugs and chemicals

Streptozotocin (STZ), nicotinamide (NTM) (Sigma Chemical Co. USA), GOD/POD kit (Acuurex, India), Glibenclamide (Ranbaxy, India), tween-80 (Research-Lab, India) were purchased from respective vendors. All chemicals used were of analytical grade.

2.3. Animals and research protocol approval

Swiss albino male mice (25-30 g) were purchased from National Toxicology Centre, Pune, India. Animals were housed at a temperature of (25 ± 2) ℃ and relative humidity of 45% to 55% under 12-h light: 12-h dark cycle. The animals had free access to food pellets (Chakan Oil Mills, Pune, India) except when starvation was required. Water was provided *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC).

2.4. Preparation of drugs solution

B2 was emulsified with 2% tween-80. Streptozotocin was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal physiological saline.

2.5. Induction of diabetes

Diabetes was induced in mice by previously reported method by Badole and Bodhankar[10]. Overnight fasted mice were treated with nicotinamide (110 mg/kg, *i.p.*). Streptozotocin (200 mg/kg, *i.p.*) was injected 15 minute after nicotinamide injection in all the groups except group 1 which was non-diabetic. Mice were fed with glucose solution (5%) for 12 h to avoid hypoglycaemia. Hyperglycaemia was confirmed after 3 days. Steady state of hyperglycaemia was reached after 10 days. Blood was withdrawn by retro orbital puncture method. Serum glucose was determined by the glucose oxidase peroxidase method. Mice having serum glucose between 300-400 mg/dL were selected for the study. The diabetic animals were randomly divided into following groups (n=6); Group 1- Non-diabetic, Group 2- diabetic control (only streptozotocin-nicotinamide), Group 3- glibenclamide (reference drug; 10 mg/kg, *p.o.*), Group 4- B2 (1 mg/kg, *p.o.*). Chronic study involved daily administration of B2 for 28 days (once a day) at predetermined time.

2.6. Histology of vital organs

At the end of 28th day, all mice were scarified by cervical dislocation method. Liver, kidney, heart, eye and brain tissue were isolated. The isolated organs were trimmed into small pieces and preserved in 10% formalin for 24 h. Specimens were cut in section of 3-5 μm in thickness and stained by hematoxyline-eosin stain. The specimen was mounted by disterene phthalate xylene (DPX). The photomicrographs of each tissue section were observed using cell imaging software for life science microscopy (Olympus soft imaging solution GmbH, Munster, Germany).

3. Results

3.1. Histological changes in vital organs

Non-diabetic mice showed normal histological structure of liver (Figure 2A). Focal necrosis and swelling of cord were observed in the liver of diabetic control mice (Figure 2B). Glibenclamide (Figure 2C) and B2 (Figure 2D) treated mice showed minimum pathological changes.

Non-diabetic mice showed normal histological structure of kidney (Figure 3A). Tubular swelling, focal degeneration and dilated tubules were observed in kidney of diabetic control mice (Figure 3B). Glibenclamide (Figure 3C) and B2 (Figure 3D) treated mice showed minimum pathological changes.

Non-diabetic mice showed normal architecture of heart (Figure 4A). Swelling of myocardial fibers and focal degeneration were observed in the heart of diabetic control (Figure 4B). Glibenclamide (Figure 4C) and B2 (Figure 4D) treated mice showed minimum pathological changes in heart.

Non-diabetic mice showed normal histological structure of eye (Figure 5A). Necrosis and opacity of the lens were observed in the eye of diabetic control mice (Figure 5B). Glibenclamide (Figure 5C) and B2 (Figure 5D) treated diabetic mice showed minimum degeneration and vacuolisation in the lens.

Non-diabetic mice showed normal histological structure of brain (Figure 6A). Neuronal degeneration and vacuolar changes in matrix were observed in the brain of diabetic control (Figure 6B). Minimum vacuolar changes and neuronal degeneration were observed in the brain of Glibenclamide (Figure 6C) and B2 (Figure 6D) treated mice.

Non-diabetic mice showed normal histological structure of spleen (Figure 7A). Diabetic mice spleen showed moderate depopulation of the lymphocytes (Figure 7B). Glibenclamide (Figure 7C) and B2 (Figure 7D) treated mice showed minimum depopulation of the lymphocytes.

Non-diabetic mice showed normal histological structure

of lung (Figure 8A). Diabetic mice lung showed moderate emphysema and focal pneumonic Figure 8A). Glibenclamide (Figure 8C) and B2 (Figure 8D) showed minimum emphysema and focal pneumonic changes.

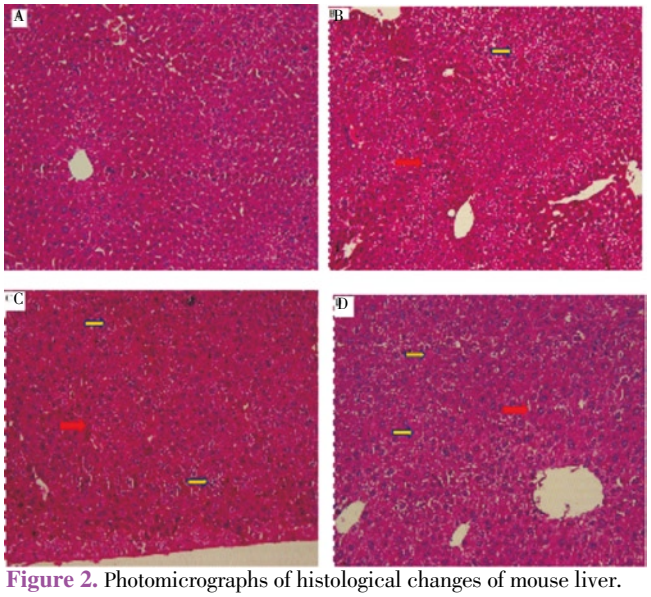

Figure 2. Photomicrographs of histological changes of mouse liver.

A) Non-diabetic: Normal (Grade --); B) Diabetic contral: Focal necrosis (yellow arrows) and swelling of cord (red arrows) (Grade +++); C) Glibenclamide (10 mg/kg) treated: Focal necrosis (yellow arrows) and swelling of cord (red arrows) (Grade +); D) B2 treated: Focal necrosis (yellow arrows) and swelling of cords (red arrow) (Magnification 100×).

Grade --: No injury; Grade +++: Severe injury; Grade ++: Moderate injury; Grade +: Mild injury.

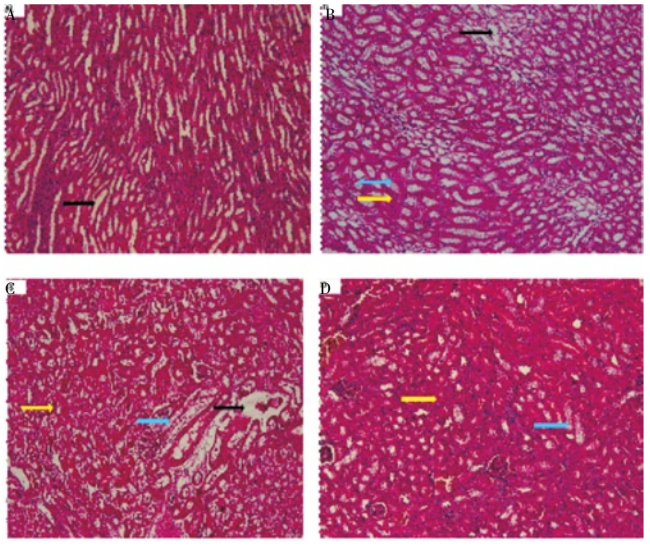

Figure 3. Photomicrographs of histological changes of mouse kidney.

A)Non-diabetic: Normal (Grade --); B) Diabetic contral: Swelling (yellow arrow), focal degeneration (blue arrow) and dilated tubules (black arrow) (Grade +++); C) Glibenclamide (10 mg/kg, *p.o.*) treated: Tubular swelling (yellow arrow), focal degeneration (blue arrow) and dilated tubules (black arrow) (Grade +); D) B2 treated: Tubular swelling (yellow arrow) and focal degeneration (blue arrow) (Grade +) (Magnification 100×).

Grade --: No injury; Grade +++: Severe injury; Grade ++: Moderate injury; Grade +: Mild injury.

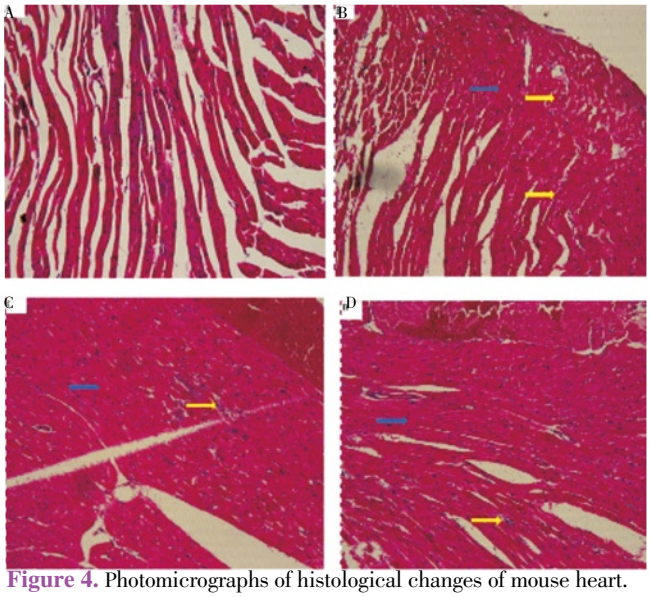

Figure 4. Photomicrographs of histological changes of mouse heart.

A)Non-diabetic: Normal (Grade --); B) Diabetic control: Swelling of myocardial fibers (blue arrow) and focal degeneration (yellow arrow) (Grade +++); C) Glibenclamide (10 mg/kg, *p.o.*) treated: Swelling of myocardial fibers (blue arrow) and focal degeneration (yellow arrow) (Grade +); D) B2 treated: Swelling of myocardial fibers (blue arrow) and focal degeneration (yellow arrow) (Grade +) (Magnification 100×).

Grade --: No injury; Grade +++: Severe injury; Grade ++: Moderate injury; Grade +: Mild injury.

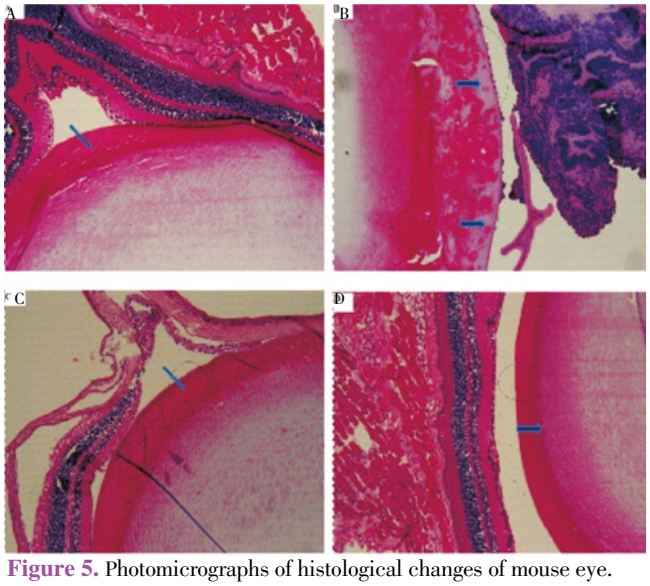

Figure 5. Photomicrographs of histological changes of mouse eye.

A)Non-diabetic: Normal (blue arrows) (Grade --); B) Diabetic controcl: Necrosis and opacity of the lens (blue arrows) (Grade +++); C)Glibenclamide (10 mg/kg, *p.o.*) treated: Minimum degeneration of lens (blue arrow) (Grade +); D) B2 treated: Minimum degeneration and vacuolation in the lens (blue arrow) (Grade +) (Magnification 100×).

Grade --: No injury; Grade +++: Severe injury; Grade ++: Moderate injury; Grade +: Mild injury.

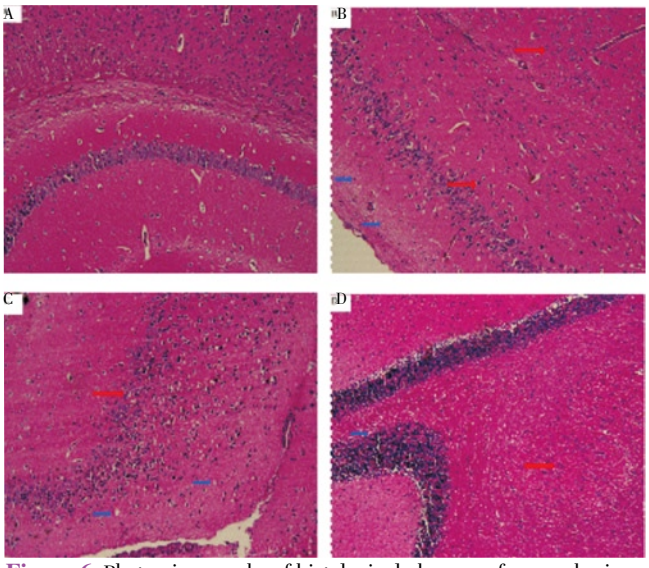

Figure 6. Photomicrographs of histological changes of mouse brain.

A) Non-diabetic: Normal (Grade --); B) Diabetic contral: Severe vacuolar changes (blue arrow) and neuronal degeneration (red arrow) (Grade +++); C) Glibenclamide (10 mg/kg, *p.o.*) treated: Minimum vacuolar changes (blue arrow) and neuronal degeneration (red arrow) (Grade +); D) B2 treated: Minimum vacuolar changes (blue arrow) and neuronal degeneration (red arrow) (Grade +) (Magnification 100×).

Grade --: No injury; Grade +++: Severe injury; Grade ++: Moderate injury; Grade +: Mild injury.

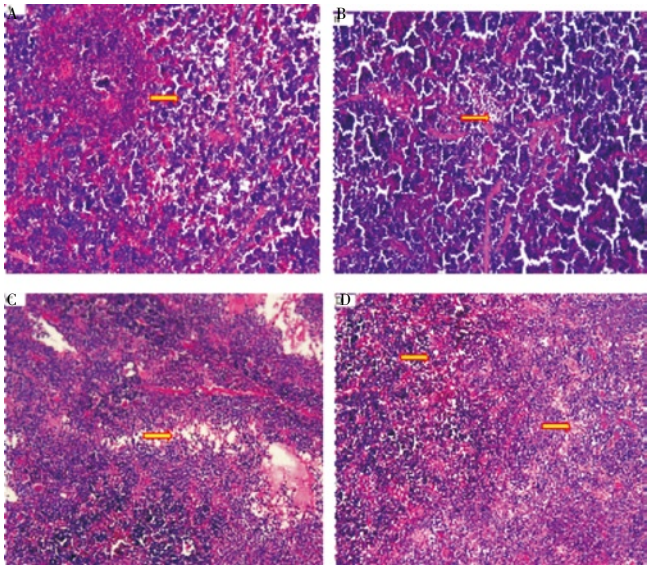

Figure 7. Photomicrographs of histological changes of mouse spleen.

A) Non-diabetic: Normal. Normal spleen showing minimum depopulation of the lymphocytes (yellow arrow) (Grade --); B) Diabetic contral: Moderate focal depopulation of the lymphocytes (yellow arrow) (Grade ++); C) Glibenclamide (10 mg/kg, *p.o.*) treated: Minimum depopulation of the lymphocytes (yellow arrow) (Grade --); D) B2 treated: Minimum depopulation of the lymphocytes (yellow arrow) (Grade --) (Magnification 100×).

Grade --: No injury; Grade +++: Severe injury; Grade ++: Moderate injury; Grade +: Mild injury.

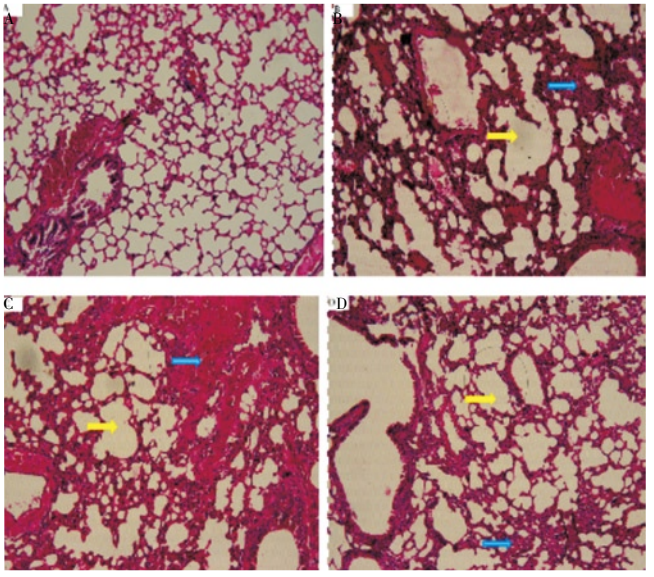

Figure 8. Photomicrographs of histological changes of mouse lungs.

A)Non-diabetic: Normal (Grade --); B) Diabetic contral: Moderate pathological changes *i.e.* emphysema (yellow arrow) and focal pneumonic changes (blue arrow) (Grade ++); C) Glibenclamide (10 mg/kg, *p.o.*) treated: Minimum emphysema (yellow arrow) and focal pneumonic changes (blue arrow) (Grade +); D)B2 treated: Minimum emphysema (yellow arrow) and focal pneumonic changes (blue arrow) (Grade +) (Magnification 100×).

Grade --: No injury; Grade +++: Severe injury; Grade ++: Moderate injury; Grade +: Mild injury.

4. Discussion

Previously we have reported B2 (1 mg/kg, *p.o.*) significantly reduced serum glucose in streptozotocin-nicotinamide induced diabetic mice. B2 improved glucose homeostasis. B2 showed peak antihyperglycaemic effect at 6 h and increased glucose utilization in diabetic mice. Decreased serum glucose and increased serum as well as pancreatic insulin concentration of B2 treated diabetic mice indicated that B2 stimulate insulin secretion. B2 treated animals showed hypertrophy of the pancreatic β cells. The number of β cells was not increased but increased size of β cells. Streptozotocin-nicotinamide diabetic control showed very little improvement in the insulin secretion and mouse pancreata showed small sized islet. The antidiabetic activity of B2 is due to increased serum and pancreatic insulin as well as decreased oxidative stress[10].

In the present study, the histology of diabetic mice liver showed focal single cell necrosis, swelling of hepatic cords, granular degeneration and perivascular aggregations of the mononuclear cells. Preservation of normal architecture of liver by B2 justified their beneficial effect on liver. Absence of any toxic effect in B2 treated mice was confirmed by previously reported data of decreased aspartate transaminase, alanin transaminase, alkaline phosphatase, bilirubin as well as enzymatic oxidative markers (malonialdehyde, reduced glutathione and superdioxide dismutase)[10].

Toxic effects of streptozotocin are not restricted to pancreatic β cells but it causes also renal injury, oxidative stress inflammation and endothelial dysfunction[11]. High doses streptozotocin has cytotoxic effect on kidney of

mice[12]. In our study, diabetic mice showed swelling, focal degeneration and dilated tubules in kidney but less damage was observed in the B2 and Glibenclamide treated mice. These results are supported by decrease in serum uric acid and urea treated with B2 and Glibenclamide[10]. The histopathology of heart of diabetic mice showed swelling of myocardial fibers and focal degenerative changes which could be attributed to the hyperglycaemia. Formation of oxygen free radicals induces degenerative changes in the heart along with cardiomyopthy[13]. Less damage in the heart of B2 and Glibenclamide treated mice may be due to reduction of oxidative stress[10].

Diabetes mellitus is one of the major risk factors for cataract development[14]. Mice showed necrosis, opacity of the lenses and the well defined cataract in the mice eye agree with earlier reports[15]. B2 and Glibenclamide treated diabetic mice did not show any abnormalities in the eyes.

In the central nervous system, oxidative stress signifies an important pathway that leads to the damage of both neuronal and vascular cells[16]. Diabetic neuropathy most likely comes from a mixture of micro vascular along with neuronal deficits, and oxidative stress participates in the breakdown of neuronal phenotype in experimental diabetic neuropathy[17]. Hyperglycemia may contribute to central nervous system malformation via oxidative stress. Diabetes accelerates maturation of neuronal damage, increases infarct volume and induces post-ischemic seizures[18]. We observed neuronal degeneration and vacuolar changes in brain matrix of diabetic mice. B2 treated and Glibenclamide diabetic mice showed minimum neuronal degeneration, which confirmed their protective activity against streptozotocin induced toxicity.

The histology of spleen in diabetic mice showed moderate depopulation of the lymphocytes while in B2 and Glibenclamide treated mice showed minimum pathological changes.

Lung damage in streptozotocin induced diabetic hamsters has been reported[19]. Hyperglycaemia affects the lungs by damaging capillaries and by non-enzymatic glycosylation of collagen[20]. Hyperglycaemia appears to cause cellular stress by a number of mechanisms which could be detrimental to the lung[21]. Emphysema and focal pneumonic changes were observed in lung of diabetic mice. B2 and Glibenclamide treated animals showed less damage of lungs.

It is concluded that B2 has ability to protect vital organs from the toxic effects persuaded in diabetic conditions in streptozotocin- nicotinamide induced diabetic mice. Further molecular and cellular level study should be performed to understand the exact mechanism of B2 which may lead to development of new antidiabetic agent.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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