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Research Article

Histopathological Effects of Metformin on Splenic Tissue in Alloxan-Induced Diabetic Rats

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Abstract

Introduction: Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from an absolute or relative deficiency of insulin secretion by pancreatic β-cells. In addition to its systemic metabolic effects, diabetes induces structural and functional alterations in various organs, including the spleen. Alloxan is a well-established diabetogenic agent that selectively damages pancreatic β-cells and produces experimental diabetes in animals. Metformin is a widely used antihyperglycemic drug with beneficial effects on glucose homeostasis and tissue injury. The present study was undertaken to evaluate the histopathological effects of metformin on splenic tissue in alloxan-induced diabetic rats.

Methods: This experimental study was conducted on 24 rats divided into three groups of 8 animals each: healthy control, diabetic control, and diabetic rats treated with metformin. At the end of 28 days, the animals were sacrificed, and body weight, blood glucose levels, and histopathological changes in the spleen were assessed.

Results: Diabetic control rats showed progressive hyperglycemia and loss of body weight compared with healthy controls. Histopathological examination of the spleen in diabetic control rats showed disturbed architecture, atrophy of the white pulp, reduction in lymphocytes, dilated and congested sinusoids, focal inflammatory infiltrate, and thickening of the capsule and trabeculae. Metformin-treated diabetic rats showed a progressive reduction in blood glucose levels and improvement in splenic histology, with restoration of white and red pulp, a relatively preserved capsule, mild inflammatory infiltrate, and an increased lymphocyte population compared with diabetic control rats.

Conclusion: Metformin ameliorated diabetes-induced histopathological lesions in the spleen, likely through restoration of normoglycemia. These findings suggest a protective role of metformin against splenic damage associated with diabetes mellitus.

Keywords: Diabetes mellitus, metformin, spleen, histopathology, alloxan

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels due to impaired insulin secretion, impaired insulin action, or both. Persistent hyperglycemia produces progressive structural and functional changes

in multiple organs and is associated with oxidative stress, inflammation, immune dysregulation, and tissue injury. [1] Experimental diabetes models are widely used to study these changes in different organs.

Alloxan monohydrate is a toxic glucose analogue commonly used to induce diabetes in laboratory animals. It preferentially accumulates in pancreatic β-cells and causes their destruction through reactive oxygen species generation and related oxidative mechanisms, leading to insulin deficiency and sustained hyperglycemia. [2] This makes it a useful model for evaluating diabetes-induced organ damage and the possible protective effects of therapeutic agents.

The spleen is an important secondary lymphoid organ involved in blood filtration, removal of damaged erythrocytes, and immune regulation. Diabetes-associated oxidative and inflammatory injury may disrupt normal splenic architecture and affect the white pulp, red pulp, vascular sinusoids, and lymphocyte populations. Previous experimental studies have shown that diabetes can alter immune tissues and induce oxidative damage in the spleen. [3,4]

Metformin is one of the most widely used oral antihyperglycemic agents. Its glucose-lowering effects are mainly related to reduced hepatic glucose production, improved insulin sensitivity, and broader effects on cellular energy pathways. Experimental evidence also suggests tissue-protective, anti-inflammatory, and antioxidant benefits beyond glycemic control. [5,6] The present study was therefore undertaken to evaluate the histopathological effects of metformin on the spleen in alloxan-induced diabetic rats.

Aim

To evaluate the histopathological effects of metformin on the spleen in alloxan-induced diabetic rats and to correlate these changes with blood glucose and body weight observations.

Materials and Methods

The present experimental study was carried out on wisteria albino rats weighing approximately 120-160 g. The animals were procured from the Central Animal House, Department of Pharmacology, Government Medical College, Jammu. The study was conducted after obtaining clearance from the Institutional Animal Ethics Committee.

Twenty-four rats were divided into three groups of 8 rats each as follows:

Group 1: Healthy control rats received normal saline (0.5 mL/day) orally for 28 days.

Group 2: Diabetic control rats were made diabetic with alloxan and received no antidiabetic treatment.

Group 3: Diabetic + metformin rats were made diabetic with alloxan and treated with metformin orally for 28 days.

The animals were kept in clean plastic cages in a well-ventilated room and maintained at approximately 25 ± 2°C. Rice husk was used as bedding material. Standard rat feed and water were provided ad libitum throughout the study period, and cages were cleaned daily.

Diabetes mellitus was induced by intraperitoneal injection of alloxan dissolved in distilled water at a dose of 150 mg/kg. Blood glucose was measured 75 hours after alloxan administration using a glucometer and glucose test strips. Rats with blood glucose levels of 250 mg/dL or above were considered diabetic. Metformin was administered orally

at a dose of 500 mg/kg/day for 28 days. (Table 1)

Blood glucose and body weight were recorded at baseline and at weekly intervals. At the end of the 28-day period, the rats were sacrificed and the spleen was removed for histopathological examination. Splenic tissue was processed, sectioned, stained with hematoxylin and eosin, and examined under light microscopy.

Table 1. Study design

Group No.	Drug	Dose	Period and route of administration
1	Normal saline	0.5 mL/day	28 days, orally
2	Alloxan	150 mg/kg	Single dose, intraperitoneally
3	Diabetic+Metformin	500 mg/kg/day	28 days, orally

Results

Blood Glucose Estimation

The healthy control group maintained near-normal blood glucose values throughout the study period, ranging from 86 to 90 mg/dL. The diabetic control group showed a progressive rise in blood glucose from 260 mg/dL on day 0 to 325 mg/dL on day 28. In contrast, the diabetic + metformin group showed a progressive reduction in blood glucose from 262 mg/dL on day 0 to 106 mg/dL on day 28, indicating a marked antihyperglycemic effect of metformin (Table 2)

Table 2. Blood glucose estimation (mg/dL)

Group	Day 0	Day 7	Day 14	Day 21	Day 28
Healthy control	88	86	89	88	90
Diabetic control	260	275	305	312	325
Diabetic + metformin	262	225	200	162	106

Body Weight Estimation

The healthy control group showed a progressive gain in body weight from 150 g on day 0 to 230 g on day 28. The diabetic control group showed a decline in body weight from 156 g to 125 g. The diabetic + metformin group also showed a reduction in body weight from 156 g on day 0 to 120 g on day 28 over the experimental period (Table 3).

Table 3. Mean body weight estimation (g)

Group	Day 0	Day 7	Day 14	Day 21	Day 28
Healthy control	150	162	187	210	230
Diabetic control	156	154	140	132	125
Diabetic + metformin	156	150	138	127	120

Light Microscopic Examination of the Spleen

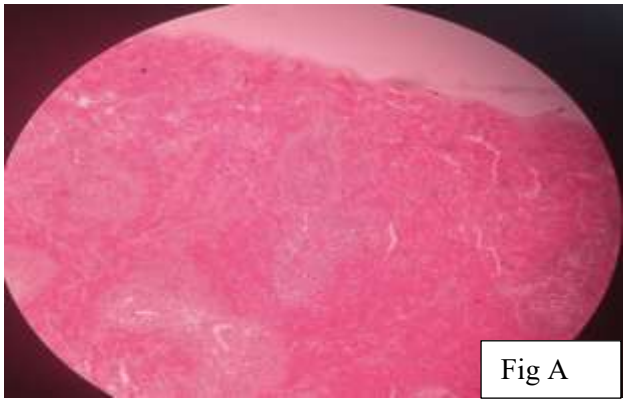
Group 1: Healthy control. Light microscopic examination of hematoxylin and eosin-stained sections of the spleen from healthy control rats revealed normal splenic architecture. The white pulp appeared as small circular lymphoid areas composed predominantly of lymphocytes. The red pulp consisted of a meshwork of splenic cords of Billroth and vascular sinusoids containing erythrocytes, lymphocytes, plasma cells, and macrophages (Fig A).

Group 2: Diabetic control. Histological examination of the spleen from diabetic control rats showed disturbed splenic architecture. The white pulp showed atrophy with a reduction in lymphocyte population. The sinusoids were dilated and congested with focal inflammatory infiltrate. Thickening of the capsule and trabeculae was also observed(Fig B and Fig C)

Group 3: Diabetic + metformin. Spleen sections from diabetic rats treated with metformin showed improvement in histological architecture compared with the diabetic control group. The white and red pulp appeared closer to normal, the capsule was relatively preserved, inflammatory infiltrate was mild, and the lymphocyte population was increased. These findings suggest amelioration of diabetes-induced splenic injury by metformin (Fig D).

Discussion

Diabetes mellitus is a systemic metabolic disorder characterized by elevated blood glucose due to an absolute or relative deficiency of insulin secretion from pancreatic β-cells or impaired insulin action. Persistent hyperglycemia leads to structural and functional changes in multiple target organs through oxidative stress, inflammatory injury, and metabolic derangement. [1] In the present study, alloxan monohydrate was used for the induction of diabetes in albino rats. This approach is supported by established evidence showing that alloxan selectively damages pancreatic β-cells and induces experimental diabetes through reactive oxygen species-mediated toxicity. [2]



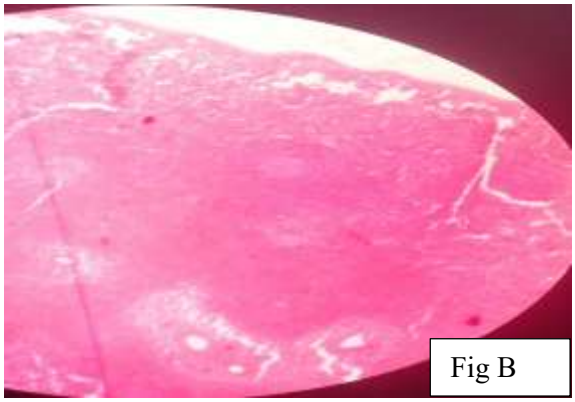
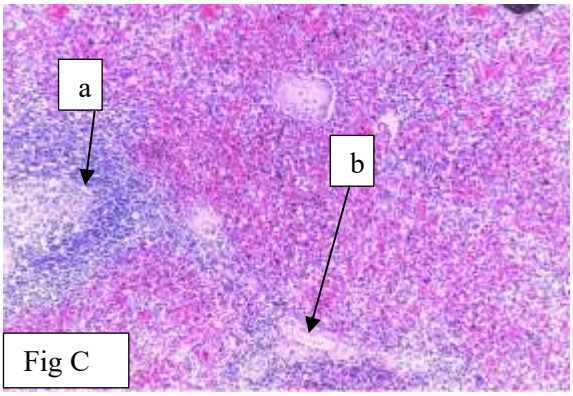


Fig. A. Photomicrograph of spleen from the healthy control group showing normal splenic architecture with distinct white pulp and red pulp. Hematoxylin and eosin stain, ×10.

Fig. B. Photomicrograph of spleen from the diabetic control group showing disturbed splenic architecture with atrophy of white pulp, reduced lymphocyte population, dilated and congested sinusoids, and focal inflammatory infiltrate. Hematoxylin and eosin stain, ×10.



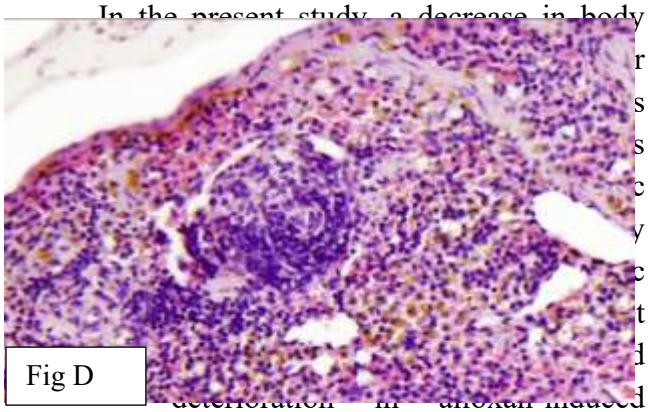


Fig. C. Photomicrograph of spleen from the diabetic control group showing (a) atrophy of white pulp with reduction of lymphocytes (b) congested sinusoids and focal inflammatory infiltrate. Hematoxylin and eosin stain, 40x.

Fig. D. Photomicrograph of spleen from the metformin-treated diabetic group showing improvement in splenic histology with near-normal white pulp and red pulp, normal capsule, mild inflammatory infiltrate, and increased lymphocytic population. Hematoxylin and eosin stain, 40x.

Effect on Body Weight:

In the present study, a decrease in body [illegible] deterioration in alloxan induced diabetic rats. [7,8] Although metformin improved glycemic control,

In the present study, treatment of alloxan-induced diabetic rats with metformin (500 mg/kg/day, orally for 28 days) improved glycemic status but did not restore body weight body weight did not recover during the study period and remained lower than both baseline and healthy control values. The loss of body weight in diabetic animals may be attributed to increased proteolysis and lipolysis caused by insulin deficiency and reduced utilization of glucose by tissues. Similar metabolic improvement without full

normalization of all parameters has been reported in experimental diabetic studies evaluating metformin. [9,10]

Effect on Blood Glucose:

In the present study, metformin-treated diabetic rats showed a marked reduction in blood glucose levels compared with the diabetic control group. This observation is in agreement with the well-established antihyperglycemic effect of metformin. Mechanistically, metformin reduces hepatic glucose production and modulates energy pathways through both AMPK-dependent and AMPK-independent mechanisms. [5,6] Similar reductions in blood glucose in metformin-treated diabetic rats were reported by Kianifard et al. and Khadre et al. in earlier experimental studies. [11,12]

Histopathological Findings in the Spleen:

The spleen is an important lymphoid organ, and diabetes-associated oxidative stress and immune injury can adversely affect its architecture. Previous studies have shown altered immune status in experimental diabetes and oxidative damage in splenic tissue. [3,4] In the present study, the spleen of alloxan-induced diabetic rats showed white pulp atrophy, reduction in lymphocytes, dilated and congested sinusoids, focal inflammatory infiltrate, and thickening of the capsule and trabeculae. These findings indicate significant diabetes-induced histopathological injury in splenic tissue.

Comparable histological changes in diabetic experimental tissues have been described by Sunil et al., who reported histological abnormalities in alloxan-induced diabetic rats, and by other experimental studies showing tissue injury associated with hyperglycemia and oxidative stress. [13,14] The present findings therefore support the view that uncontrolled diabetes can cause substantial injury to immune and parenchymal tissues.

In the present study, daily administration of metformin for 28 days improved histological changes in the spleen. The white pulp showed less atrophy than in the diabetic control group, inflammatory infiltrate was mild, and the lymphocyte population was increased. These observations are consistent with the broader tissue-protective effects reported for metformin in diabetic animal models. Experimental work by Ismail et al. and Almuttairi demonstrated histological improvement in diabetic tissues following metformin treatment. [9,10] In addition, older experimental reports such as the study by Njagi et al. support the overall glucose-lowering and protective role of antihyperglycemic interventions in alloxan-induced models. [15]

The beneficial effect of metformin in the present study is likely related primarily to improved glycemic control, together with anti-inflammatory and antioxidant actions described in the literature. [5,6] By reducing hyperglycemia and limiting downstream tissue injury, metformin appears to preserve splenic architecture in diabetic rats.

A limitation of the present study is that formal statistical testing for histological variables was not presented. Future studies incorporating morphometric analysis, immunohistochemistry, and biochemical markers of oxidative stress would further strengthen the evidence for the protective role of metformin in splenic injury associated with diabetes.

Conclusion

The present study demonstrates that alloxan-induced diabetes causes significant histopathological changes in the spleen, including white pulp atrophy, lymphocyte depletion, sinusoidal congestion, inflammatory infiltration, and thickening of the capsule and trabeculae. Metformin treatment improved blood glucose levels and ameliorated these splenic lesions. These findings suggest that metformin may have a protective effect against splenic damage associated with diabetes mellitus.

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