Negligible alterations in hematological parameters during sub-acute diabetic condition in wounded rats

Kant Vinay[1*], Sharma Maneesh[2], Jangir Babu L.[3] and Kumar Vinod[1]

1. Department of Veterinary Pharmacology and Toxicology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar 125 004 (Haryana), INDIA
2. Department of Veterinary Clinical Complex, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar 125 004 (Haryana), INDIA
3. Department of Veterinary Pathology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar 125 004 (Haryana), INDIA

*drvinaykantluvas@gmail.com

Abstract

This study aimed to evaluate effect of quercetin in hematological parameters after topical application on wounds of diabetic rats. Diabetes was induced by streptozotocin in rats. Full thickness cutaneous wound was created on the back of the non-diabetic and diabetic rats. Wounded rats were divided into three groups viz. healthy control (non-diabetic), diabetic control and quercetin treated diabetic groups. Ointment base was topically applied once daily for 21 days in healthy and diabetic control groups. 0.3% quercetin was applied topically in quercetin treated diabetic group. Blood samples were collected on the last day of experiment and processed for various hematological parameters.

There were no significant changes between healthy control, diabetic control and diabetic treated rats for haemoglobin, packed cell volume, red blood cell count, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and white blood cell count. Lymphocytes count was significantly increased and granulocyte count significantly decreased in healthy control as compared to diabetic control and diabetic treated rats. In conclusion, sub-acute diabetic condition in this study failed to induce significant changes in most of the hematological parameters and topical application of quercetin (0.3%) on wounds of diabetic rats was also ineffective to improve the haematology.

Keywords: Diabetes, Hematology, Quercetin and Streptozotocin.

Introduction

Diabetes Mellitus is a chronic metabolic disorder that affects the metabolism of carbohydrates, fats, proteins and electrolytes in the body, leading to severe complications which are classified into acute, sub-acute and chronic[12,14]. It is caused by a decrease or deficiency in insulin secretion and increased cellular resistance coincident with the induction of oxidative stress[10]. Inspite of continuous discovery of new drugs and chemicals for therapy, it is still the leading cause of morbidity and mortality in India and abroad. Streptozotocin (STZ) is a naturally occurring nitrosourea that induces insulin dependent *diabetes mellitus* in experimental animals because of its toxic effects on islet beta cells[4]. The diabetogenic action of STZ is the direct result of irreversible damage and necrosis to the pancreatic beta cells resulting in degranulation and loss of capacity to secrete insulin[2,5]. In several experimental studies, different doses of streptozocin via intraperitoneal route in rat model were given viz. 40 mg/Kg, I/P[12]; 45 mg/kg body weight, I/P[18]; 50 mg/kg body weight, I/P[17] and 60 mg/kg body weight, I/P[8] for induction of hyperglycemia.

Several hypoglycemic drugs and conventional insulin therapy are currently available to reduce hyperglycemia in *diabetes mellitus.* However, in the present scenario, alternative use and therapeutic potentiation of many herbal medicines have been studied and recommended on diabetes and its complications. Flavonoids are a large group of naturally occurring compounds having variable phenolic structure that are found in plants and are frequently consumed as part of the human diet as tea and wine[1]. These are receiving much attention nowadays for their potential pharmacological properties. One of these therapeutic agents is quercetin which acts as flavonoid and determines a wide range of biological functions including anti-inflammatory, anticarcinogenic and antiviral activities.

Moreover, it inhibits lipid peroxidation, platelet aggregation and capillary permeability and is reported to prevent oxidant injury, cell death by scavenging of oxygen radicals, protection from lipid peroxidation and chelation of metal ions[7]. Several reports of antidiabetic effect of quercetin on *diabetes mellitus* induced rats model were conducted to check the efficacy of this compound at different doses and by different routes like 15 mg/Kg body weight, I/P[8]; 5-50 mg/Kg body weight, Gavage[11] and 30 mg/kg body weight, I/P[17]. Hence, the present study was aimed to determine the effects of quercetin on some hematological parameters in diabetic rats induced experimentally with streptozotocin.

Material and Methods

Experimental design and animals: The present study was conducted on healthy adult albino rats weighing 150-200 gms selected from an inbred group housed in specially designed cages and maintained under standard conditions for at least one week before start of experiment. The research experiment and animals housing conditions were approved

by Institutional Animals Ethics Committee. Diabetes was induced by streptozotocin (60 mg/kg b.wt.) in rats and kept for seven days. Full thickness cutaneous wound (≈400 mm^2) was created on the back (thoraco-lumber) region of the non-diabetic and diabetic rats under ketamine (50 mg/kg, i.p.) + xylazine (5mg/kg, i.p.) anaesthesia.

The wounded rats were divided into three groups viz. healthy control (non-diabetic), diabetic control and quercetin treated diabetic groups. Ointment base was topically applied once daily for 21 days in healthy control and diabetic control groups. 0.3% quercetin in ointment base was similarly applied topically in quercetin treated diabetic group rats.

Blood examination: To study the effect of locally applied topical application of quercetin (0.3%) on wounds of diabetic rats, blood sample was collected for haemtaological analysis from healthy control (non-diabetic), diabetic control and quercetin treated diabetic groups. Haematological analysis was performed on an automated hematology analyzer (Blood cell counter machine, MS4Se, HD Consortium) by using EDTA as anticoagulant mixed with blood sample.

Statistical analysis: Data generated from different parameters among control and exposed groups were presented as Mean ± SE by employing one way analysis of variance (ANOVA) and the differences between the different treatment groups were considered statistically significant at $P \leq 0.05$ or lower.

Results and Discussion

Diabetes mellitus is a complex metabolic disorder involving several integrated mechanisms that can cause pancreatic β-cell dysfunction including chronic inflammation, oxidative stress, excessive hyperglycemia and nutrient levels, lipotoxicity, endoplasmic reticulum stress[6,13].

According to World Health Organization, around 422 million people worldwide have diabetes, particularly in low- and middle-income countries and 1.6 million deaths are directly attributed to diabetes each year. Both the number of cases and the prevalence of diabetes have been steadily increasing over the past few decades[16]. Hematological complications in diabetic patients have been observed in various studies. Antioxidant supplementations have been shown to reverse hematological abnormalities to near control values or significantly improve hematology and its function in diabetes.

Our earlier studies have shown that quercetin on topical applications has antioxidant effect at wound site and improves the wound healing in rats. In the present study, haematological parameters were evaluated by use of qurecitin (0.3%, topical application) as antioxidant in STZ induced diabetes rats. Blood parameters analyzed of each group were presented in table 1 and fig. 1.

There were no significant changes observed in haemoglobin (Hb), HCT/PCV (Hematocrit/packed cell volume) and red blood cell count (RBC). Red blood cell indices including mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) also showed no significant difference in healthy control as compared to diabetic control and diabetic treated rats. Some studies reported that quercetin (15 mg/kg I/P) produces significant decrease in blood parameters like Hb, PCV, RBC and platelet count but no significant changes were observed in MCV, MCH and MCHC in diabetic rats as compared with control and qurecitin treatment groups[8].

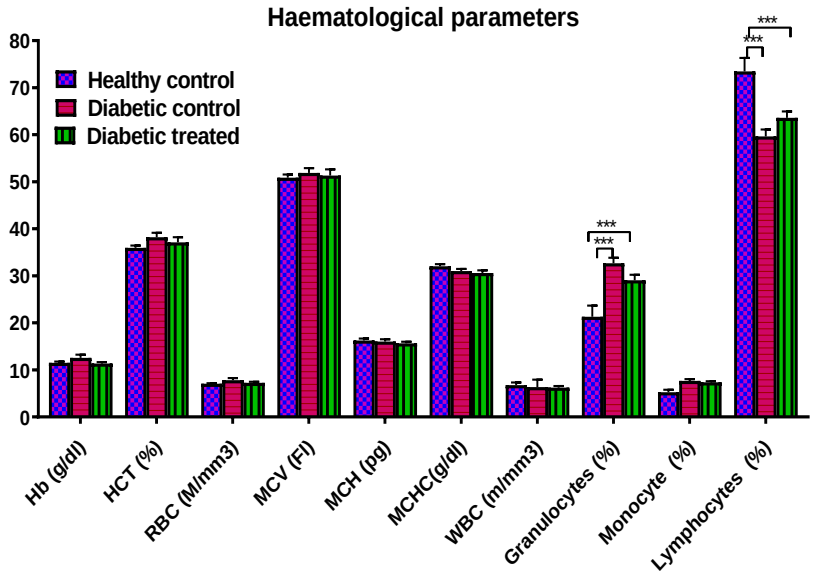

Fig. 1: Haematological parameters in healthy control (non-diabetic), diabetic control and quercetin treated sub-acute diabetic condition in wounded rats

Table 1
Haematological parameters in healthy control (non-diabetic), diabetic control and quercetin treated sub-acute diabetic condition in wounded rats

Parameters	Healthy control	Diabetic Control	Diabetic treated
Hb (g/dl)	11.52±0.25	12.56±0.68	11.36±0.28
HCT (%)	35.94±0.51	38.20±0.97	37.10±1.11
RBC (M/mm3)	7.08±0.08	7.82±0.44	7.25±0.24
MCV (Fl)	50.86±0.70	51.90±1.00	51.32±1.32
MCH (pg)	16.26±0.40	16.04±0.47	15.66±0.34
MCHC(g/dl)	32.02±0.48	31.00±0.46	30.60±0.56
WBC (m/mm3)	6.75±0.57	6.34±1.60	6.25±0.30
Granulocytes (%)	21.30±2.37	32.68±1.18[a]	29.04±1.17[a]
Lymphocytes (%)	73.46±2.87	59.64±1.49[a]	63.60±1.36[a]
Monocytes (%)	5.24±0.54	7.68±0.34	7.36±0.23

Data are expressed as mean ± SE, (n=5). The values in the row are compared and the values having same superscript are non-significantly different

Decreased levels of RBC, HGB, PCV as well as red blood cell indices including MCV, MCH and MCHC were also observed in intraperitoneal injection of alloxan induced diabetic rats as compared with normal control rats[3]. Non-significant changes in the blood parameters in the present study might be due to low dose of locally applied topical application of quercetin (0.3%) on wounds of diabetic rats and duration of the study.

In our study, lymphocytes count was significantly increased and granulocyte count significantly decreased in healthy control as compared to diabetic control and diabetic treated rats. Significant changes observed in our results are in corroboration with earlier reports[8,9] that observed increased neutrophilic count in diabetic control rats as compared with healthy control rats. Although, no significant changes in the white blood cell count (WBC) in healthy control rats, diabetic control and diabetic treated rats were observed in present study.

However, reports of researchers revealed moderate neutrophilic leukocytosis as observed in diabetic rats[8,9]. Some reports are contraindicated as compared to present study as significant leucopenia and neutropenia in diabetic rats in streptozotocin-induced diabetes mellitus[11].

Earlier findings also revealed involvement of peripheral polymorphonuclear leukocytes in oxidative stress and inflammation in type 2 diabetic patients[15]. Hence, our study concluded that quercetin (0.3%) did not produce significant alteration in the haematological parameters. So, further study was needed regarding its effects on the diabetes with amount and duration.

Acknowledgement

We are thankful to Science and Engineering Research Board, (SERB), New Delhi, India for providing financial assistance (Project No. SB/YS/LS-221/2013).

References

1. Aguirre L., Arias N., Macarulla M.T., Ana M.T., Gracia A. and Portillo M.P., Beneficial effects of quercetin on obesity and diabetes, *The Open Nutra. J.*, **4**, 189-198 **(2011)**

2. Arora S., Ojha S. and Vohora D., Characterisation of streptozotocin induced diabetes mellitus in swiss albino mice, *Global J. Pharmacol*., **3**, 81-84 **(2009)**

3. Erukainure O.L., Ebuehi O.A.T., Adeboyejo F.O., Aliyu M. and Elemo G.N., Hematological and biochemical changes in diabetic rats fed with fiber-enriched cake, *J. Acute Med*., **3**, 39-44 **(2013)**

4. Fadillioglu E., Kurcer Z., Parlakpinar H., Iraz M. and Gursul C., Melatonin treatment against remote open injury induced by renal ischemia reperfusion injury in diabetes mellitus, *Arch. Pharm. Res.*, **31(6)**, 705-712 **(2008)**

5. Gu D., Arnush M. and Sarvetnic N., Endocrine/exocrine intermediate cells in Streptozotocin treated Ins-IFN gamma transgenic mice, *Pancreas.*, **15(3)**, 246-250 **(1997)**

6. Houstis N., Rosen E.D. and Lander E.S., Reactive oxygen species have a causal role in multiple forms of insulin resistance, *Nature*, **440**, 944-948 **(2006)**

7. Kanter M., Altan M.F., Donmez S., Ocakc A. and Kartal M.E., The effects of quercetin on bone minerals, biomechanical behavior, and structure in streptozotocin-induced diabetic rats, *Cell Biochem. Function*., **25**, 747-752 **(2007)**

8. Keskin E., Donmez N., Kilicarslan G. and Kandir S., Beneficial effect of quercetin on some haematological parameters in streptozotocin-induced diabetic rats, *Bull. Env. Pharmacol. Life Sci*., **5(6)**, 65-68 **(2016)**

9. Kozlov I., Novitski V. and Baìkov A., Kinetics of blood leukocytes in mice with alloxan diabetes, *Biull. Eksp. Biol. Med*., **120**, 33-35 **(1995)**

10. Machha A., Achike F.I., Mustafa A.M. and Mustafa M.R., Quercetin, a flavonoid antioxidant, modulates endothelium-derived nitric oxide bioavailability in diabetic rat aortas, *Nitric Oxide*, **16**, 442-447 **(2007)**

11. Maciel R.M., Costa M.M., Martins D.B., Franca R.T., Schmatz R., Graca D.L., Duarte M.M.M.F., Danesi C.C., Mazzanti C.M., Schetinger M.R.C., Paim F.C., Palma H.E., Abdala F.H., Stefanello N., Zimpel C.K., Felin D.V. and Lopes S.T.A., Antioxidant and anti-inflammatory effects of quercetin in functional and morphological alterations in streptozotocin-induced diabetic rats, *Res. Vet. Sci.*, **95**, 389-397 **(2013)**

12. Nagarchi K., Ahmed S., Sabus A. and Saheb S.H., Effect of streptozotocin on glucose levels in albino wister rats, *J. Pharm. Sci. Res.*, **7(2)**, 67-69 **(2015)**

13. Poitout V. and Robertson R.P., Glucolipotoxicity: fuel excess and beta-cell dysfunction, *Endocr. Rev*., **29,** 351-366 **(2008)**

14. Rang H.P., Dale M.M. and Ritters J.M., The endocrine pancreas and the control of blood glucose; in Pharmacology, eds., Simmons B. and Beasley S., U.K., Longman group Ltd., 403-410 **(1991)**

15. Shurtz-Swirski R., Sela S., Herskovits A.T., Shasha S.M., Shapiro G. and Nasser L., Involvement of peripheral polymorphonuclear leukocytes in oxidative stress and inflammation in type 2 diabetic patients, *Diabetes Care*, **24**, 104-110 **(2004)**

16. World Health organization Diabetes-Key facts, https://www.who.int/news-room/fact sheets/detail/diabetes **(2018)**

17. Yang D.K. and Kang H.S., Anti-Diabetic effect of cotreatment with quercetin and resveratrol in streptozotocin-induced diabetic rats, *Biomol Ther.*, **26(2)**, 130-138 **(2018)**

18. Zafar M., Naeem-ul-Hassan N.S., Ahmed M. and Kaim Khani Z.A., Altered liver morphology and enzymes in streptozotocin induced diabetic rats, *Int. J. Morphol.*, **27(3)**, 719-725 **(2009)**.

(Received 27th May 2020, accepted 30th July 2020)