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Metformin on Insulin, Glucagon, Oxidative Stress Markers and Pancreatic Tissue Histology in Streptozotocin-Induced Diabetic Rats

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Abstract

This study determined the effects of Metformin on plasma insulin, glucagon, oxidative stress status and histology of pancreas in diabetic rats. Grouping was carried out on 21 rats, and were assigned into 3 groups (n = 7 rats per group); non-DM, DM and DM+metformin (200 mg/kg). Diabetes Mellitus was induced and treatments were given daily by oral gavage for 4 weeks. Food intake, fasting blood glucose (FBG), glucagon, malondialdehyde and protein carbonyl were significantly higher while final body weight, insulin and total antioxidant capacity were significantly lower in Diabetes Mellitus group compared with non- Diabetes Mellitus group. There was significantly higher body weight, insulin level and total antioxidant capacity with significantly lower food intake, FBG, glucagon, malondialdehyde and protein carbonyl levels in Diabetes Mellitus +Metformin group compared with the Diabetes Mellitus group. Pancreatic tissue histology revealed islet cells regeneration in Diabetes Mellitus +Metformin group. This in vivo study provides evidence of the potential of antihyperglycemic and improved oxidants- antioxidant status which may be due to the oral hypoglycaemic nature or effects of metformin as seen in this study.

Keywords: *metformin, oxidative stress, insulin, hypoglycaemia, streptozotocin*

Introduction

Diabetes mellitus (DM) has long been described as a metabolic disorder associated with abnormal glucose metabolism (ADA, 2016). There is an increase in DM prevalence which is projected to increase up to 642 million by 2040. It's effect on the patient socio-economy, physical and medical state has become a major concern globally (IDF, 2016). Healthy life style and exercise remain a major effective strategic intervention (Zhang *et al*., 2017). Under normal physiological conditions, insulin secretion from pancreatic beta cells is involved to decrease blood glucose level. Insulin secretion is regulated by glucose, hormones and autonomic nervous system activities (Kumitoshi *et al*., 2011). Pancreatic beta cells are sensitive to reactive oxygen species (ROS) because of its low antioxidant enzyme contents (Lei and Vataminiuk, 2011). Hyperglycaemia increases ROS production which in turn alters mitochondrial function leading to tissue damage (Freed *et al*., 2017; Sakai *et al*., 2003). In the process of cellular respiration, part of the oxygen taken into living cells is changed to several harmful ROS and free radicals (Adly, 2010). Once formed, free radicals can start a chain reaction leading to formation of more ROS (Reddy *et al*., 2013; Sathya *et al*., 2013). Superoxide anion radical (O^-_2) is one of the strongest ROS among the radicals that are generated after oxygen is taken into living cells. The O^-_2 changes to other harmful ROS, which are implicated in the aetiology of many diseases including DM (Roberto *et al*., 2017; Richard and Yong, 2010).

Previous studies reported metformin to have possess antihyperglycaemic property (Usman *et al*., 2016; Usman and Mohamed, 2015). To the best of our literature searching, there is need for more study on metformin to improved insulin-glucagon ratio, pancreatic tissue histology and

diabetic induced-oxidative stress. Hence, the objective of the study was to determine the laboratory-based results on the effects of the levels of plasma insulin, glucagon, histology of pancreas and oxidative stress markers in streptozotocin-induced diabetic rats.

Materials and Methods

Animals

Twenty-one (21) female Sprague Dawley Rats of age 8 to 10 weeks (190 – 220 g) were used in this study. The animals were obtained from the VTU Research Unit, Faculty of Veterinary Medicine, UDUS. They were given food and water *ad libitum*. The study was performed in accordance with the guidelines by the Animals Ethical Committee, which was in accordance with the internationally accepted principles for laboratory animal use and care.

Induction and assessment of DM

Rats were fasted overnight for 12 hours and DM was induced using single intraperitoneal injection of streptozotocin (STZ; Sigma-Aldrich Co., St Louis, USA) at 75 mg/kg body weight. Diabetes was confirmed following 48 h of streptozotocin injection (Damascene *et al*., 2014). Animals with fasting blood glucose (FBG) level of more than 200 mg/dl (Digital Glucometer, Lifescan Inc Milpitas, USA) blood taken from the tail vein.

Experimental design

The rats were randomly assigned into 3 groups (n = 7 rats per group) as follows: (i) Non-DM group: Healthy rats received distilled water (1 ml/day) as negative control group, (ii) DM group: Diabetic rats received distilled water (1 ml/day) as positive control group and (iii) DM+metformin group: Diabetic rats on 200 mg/kg body weight metformin. Treatments were given by oral gavage daily between 7 to 8 am for 4 weeks. Total food intake, body weight, initial and final FBG levels were recorded. After 4 weeks of treatment, rats were fasted overnight and sacrificed under anaesthesia (90 mg/kg ketamine and 5 mg/kg xylazine). Plasma was analysed for the levels of FBG, insulin, glucagon and oxidative stress markers such as total antioxidant capacity (TAC), malondialdehyde (MDA) and protein carbonyl (PCO) using commercial kits (BioAssay Systems. California, USA). Pancreas was carefully dissected, fixed with 10% formalin, sectioned of 5µm and stained with haematoxylin and eosin for histological study.

Statistical Analysis

All data (numerical) are expressed as mean and standard deviation (SD). Statistical analysis was performed using Instat. Exe version 3.1. (Charleswork Publishing Services Ltd. Deighton, Huddersfield, UK). One way analysis of variance (ANOVA) followed by Turkey-Kramer test was used to assess the differences among means and $P<0.05$ was considered as significant.

Results

Food intake and body weight

In DM group, total food intake was significantly higher while final body weight was significantly lower compared with non-DM group. The total food intake was significantly lower and final body weight was higher in DM+Metformin groups compared with DM group. In DM+metformin group, the final body weight was significantly lower than non-DM group but significantly higher than DM group (Table 1).

Table 1 Food intake and body weight of all the experimental groups

Groups	Total food intake (g)	Initial body weight (g)	Final body weight (g)
non-DM	516.24 (40.03)	207.49 (6.63)	248.04 (6.11)
DM	945.56 (192.20) [a]	210.65 (8.74)	174.99 (10.86) [a]
DM+Metformin	799.56 (122.42) [a]	203.16 (4.96)	230.80 (8.12) [a b]

Values are mean (SD), n = 7/group. [a] $P < 0.05$ compared with non-DM group, [b] $P < 0.05$ compared with DM group (one way ANOVA followed by Tukey-Kramer post hoc test).

FBG, insulin and glucagon levels

FBG level in non-DM group was invariable throughout the study period. On the contrary, the final FBG level in DM group was significantly higher compared with non-DM group. The FBG was significantly lower in DM+metformin groups compared with DM group (Table 2).

Table 2 Initial and final fasting blood glucose (FBG) levels of all the groups

Groups	Initial FBG levels (mg/dl)	Final FBG levels (mg/dl)
non-DM	90.88 (2.03)	90.50 (1.07)
DM	437.13 (67.37) [a]	541.88 (62.45) [a]
DM+metformin	494.63 (50.42) [a]	284.25 (74.01) [a, b]

Values are mean (SD), n = 7/group. [a] $P < 0.05$ compared with non-DM group, [b] $P < 0.05$ compared with DM group (one way ANOVA followed by Tukey-Kramer post-hoc test).

Plasma insulin was significantly lower while glucagon level was significantly higher in DM group compared with non-DM group. However, plasma insulin level was significantly higher while glucagon level was significantly lower in DM+Metformin groups compared with DM group (Table 3).

Table 3 Plasma insulin and glucagon levels of all the groups

Groups	Insulin levels (µ IU/ml)	Glucagon levels (ng/ml)
non-DM	27.16 (0.56)	35.89 (13.30)
DM	8.24 (2.00) [a]	100.85 (18.72) [a]
DM+metformin	9.05 (2.23) [a]	92.98 (21.71) [a,]

Values are mean (SD), n = 7/group. [a] $p < 0.05$ compared with non-DM group, [b] $p < 0.05$ compared with DM group, (one way ANOVA followed by Tukey-Kramer post-hoc test).

Histology of the pancreas

The histological section of pancreas in non-DM group revealed normal morphology of pancreas with normal islet of Langerhans. DM group showed shrunken and degenerating islet of Langerhans. However, improved morphological features of islet of Langerhans were observed in DM+Metformin groups (Fig.1).

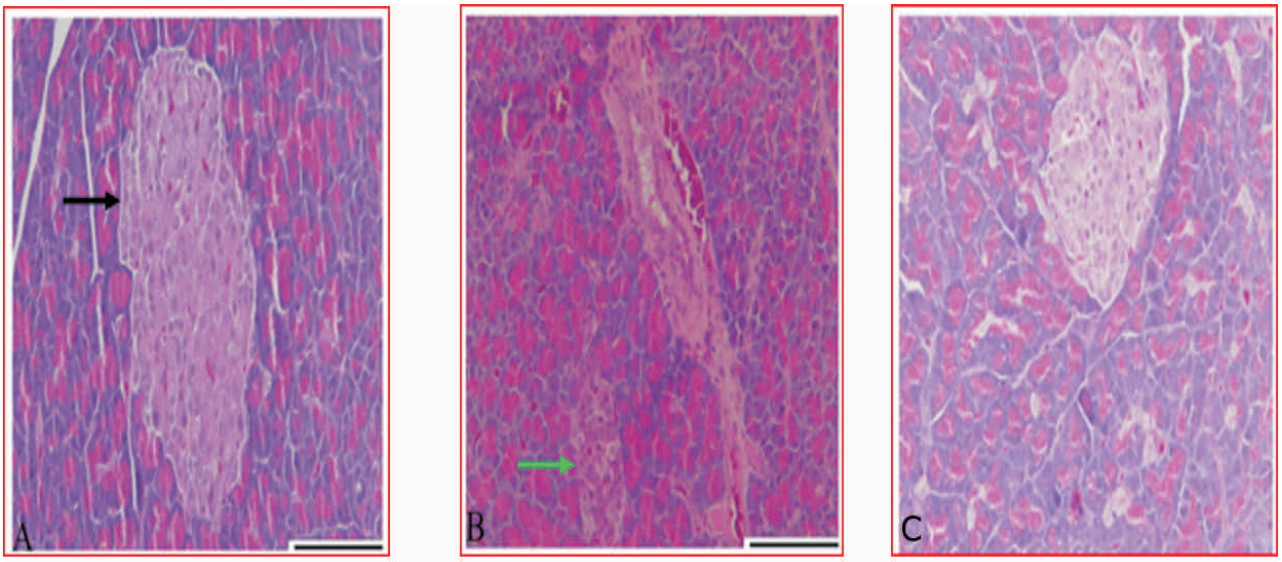

Fig. 1: Representative photomicrograph of pancreas. (A) non-DM group showing normal islet of Langerhans with normal size (dark arrow). (B) DM group showing shrunken islet of Langerhans with reduced size (green arrow). (C) DM+Metformin groups showing improvement for the size of islet of Langerhans (magnification 100x; haematoxylin and eosin staining; scale bar: 100 µm).

Antioxidant and oxidative stress markers

Plasma TAC was significantly lower while MDA and PCO levels were significantly higher in DM group compared with non-DM group. In DM+Metformin, plasma TAC was significantly higher while MDA and PCO levels were significantly lower than DM group (Fig. 2).

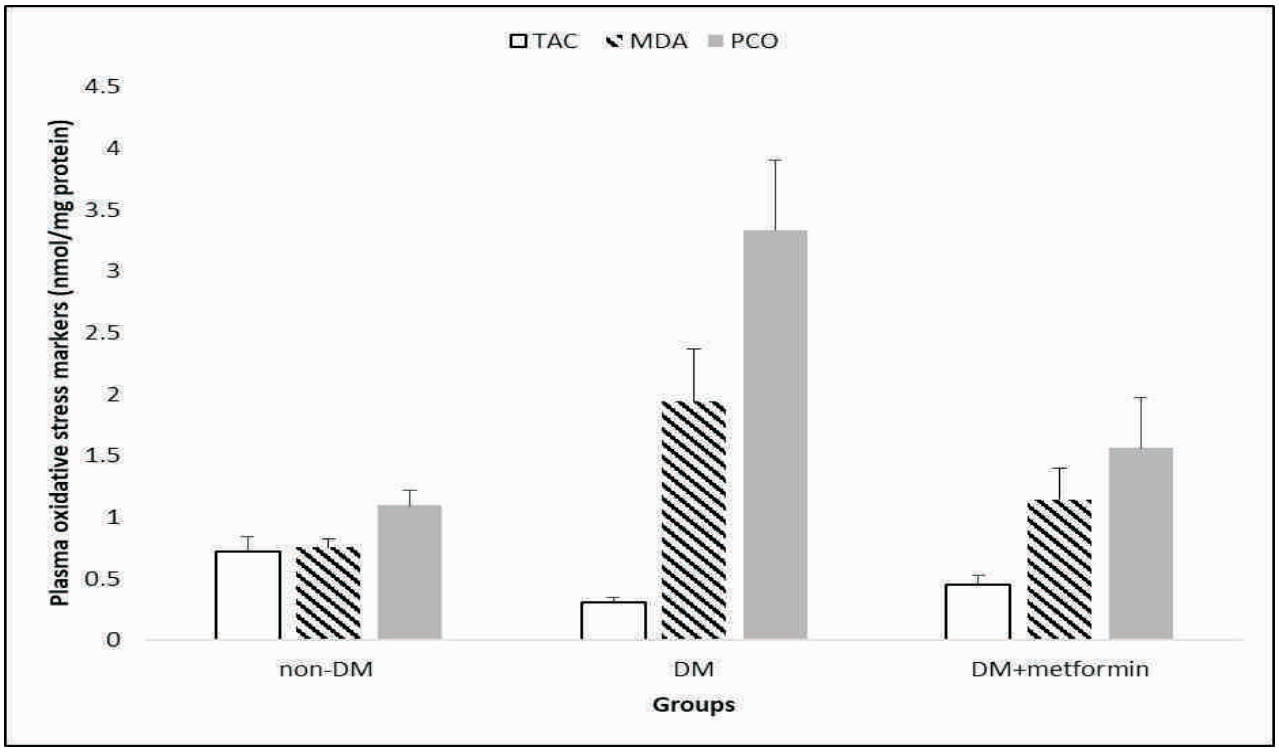

Fig. 2: Plasma oxidative stress markers. Total antioxidant capacity (TAC); malonaldehyde (MDA) and protein carbonyl (PCO). Data are presented as mean (SD) (n = 7 rats per group).
[a] $P< 0.05$ compared with non-DM group, [b] $P< 0.05$ compared with DM group (one way ANOVA followed by Tukey-Kramer post-hoc test).

Discussion

In this study DM group, total food intake was significantly higher while final body weight was significantly lower compared with non-DM group. The significantly lower food intake, higher final body weight and lower FBG in DM+Metformin groups compared with DM group suggest the antidiabetic potential of metformin. The finding on body weight is consistent with our previous pilot study on metformin (Usman *et al*., 2016). The significantly higher plasma insulin and significantly lower FBG and glucagon levels in the metformin group compared with DM group, may explain the drugs antihyperglycaemic effect as seen in this study. Previous report shows phenolic compound with antioxidant property may reduce oxidative damage, which in turn may increase insulin release from isolated islets cells (Lenzen, 2008). Moreover, the improved FBG level and, plasma insulin and reduced glucagon levels may suggest the improved function of beta cells in secreting more insulin which in turn may reduce the glucagon secretion.

Islet of Langerhan cells of the pancreas is the key organ affected in this DM model with functional loss due to the destruction by streptozotocin, which leads to impaired insulin production and secretion (Bank, 1986). Histology of pancreas section of diabetic control rat showed severe damage to the islet of Langerhans, shrunken islet cells and reduced size of islet of Langerhans compared with non-DM and DM+metformin groups. The histology of pancreas showed some improvements as characterized by increment in size of islets of Langerhans in the metformin group compared with that of control DM group. Interestingly, the improved histological finding may suggest the improved function of beta cells in producing and secreting insulin as indicated by higher level of insulin in metformin group compared to DM group.

Previous study shows that chronic hyperglycaemia leads to increased production of oxidative stress and decreased antioxidant activity or action in DM (Roberto *et al*., 2017). As a result of these events, the balance between normal reactive oxygen species or free radical production and antioxidant activity or production to protect against oxidative stress will be altered (Ayepola *et al*., 2014). This is shown in the present study where by the levels of oxidative stress markers such as MDA and PCO were significantly higher and the level of TAC was significantly lower suggesting the presence of oxidative stress in DM group compared to non-DM group. The beta-cells are highly prone to oxidative stress and damage due to its low antioxidants enzymes activity (Greenberg, 2014). Therefore, it can be suggested that the degenerative and shrunken changes on the islets of Langerhans may be due to the increased oxidative stress in DM group.

Conclusion

This *in vivo* study provides evidence of the potential of antihyperglycemic and improved oxidants- antioxidant status which may be due to the oral hypoglycaemic nature or effects of metformin as seen in this study. Further studies are needed to evaluate the exact molecular mechanism of action, and potential ameliorating effects on other organs.

Conflict of interests

The authors report no conflict of interests.

References

Adly, A.M.A. (2010) Oxidative Stress and Disease: An Updated Review. *Research Journal of Immunology*;**3**: 129-145.

American Diabetes Association (ADA, 2016). Standards of medical care in Diabetes. Diabetes Care, summary of revision. http://www.diabetes.org/diabetescare. DOI: 10.2337/dc14- SO14; **37**: 14-64.

Bank, S. (1986). Chronic pancreatitis: Clinical features and medical management. *American* Journal of *Gastroenterology*;**81**: 153-167.

Damascene, D.C., Netto, A.O., Lessi, I.L., Gallego, E.Q. and Corvino, S.B. (2014). Streptozotocin-induced diabetes models: pathophysiological mechanisms and foetal outcomes. *Biomed Research International*;**1**: 665-676.

Freed, J.K., Durand, M.J., Hoffmann, B.R., Densmore, J.C., Greene, A.S. and Guttlerman, DD. (2017). Mitochondria-regulated formation of endothelial extracellular vesicles shifts the mediator of

flow-induced vasodilation. *American Journal of Physiology. Heart and Circulatory Physiology*;17: 680-684.

Greenberg, M.M. (2014). Abasic and oxidized abasic site reactivity in DNA: enzyme inhibition, cross-linking and nucleosome catalyzed reactions. *Account of Chemical Research*;2: 646-655.

International Diabetes Federation (IDF, 2016) 7th Edition Atlas, revision. http://.

Kumitoshi, U., Katsuya, D., Boldbaata, D., Hitoshi, I., Tetsuya, S., Yasuo, M., Toshihiko, Y., Yasuhiko, M. and Makoto, T. (2011). Lack of TRPM2 impaired insulin secretion and glucose metabolism in mice. *Diabetes;*60: 119-126.

Lei, X.G. and Vataminiuk, M.Z. (2011). Two tales of antioxidant enzymes on beta cells and diabetes. *Antioxidant & Redox Signaling*;**14**: 489-503.

Lenzen, S. (2008). The mechanisms of alloxan and streptozotocin-induced diabetes. *Diabetologia*;**51**: 216-226.

Ayepola, O.R., Brooks, N.L. and Oguntibeju, O.O. (2014). Oxidative stress and diabetic complications: The role of antioxidant, vitamins and flavonoids. *IntechOpen*;**2**: 25-35.

Reddy, G.S., Rao, K.U.M. and Sundar, C.S. (2013) Neat synthesis and antioxidant activity of α- amino phosphonate. *Arabian Journal of Chemistry*;**5**: 833-838.

Richard, A.C. and Yong, T.X. (2010). Vascular oxidative stress: common link in hypertensive and diabetic vascular disease. *Journal of Pharmaceutical Sciences*;**55**: 308-316.

Roberto, T., Rita, B.A., Prattichizzo, F. and La, S.L. (2017). The "Metabolic memory" Theory and the early treatment of Hyperglycemia in prevention of diabetic complications. *Nutrients*;**5**: 437-442.

Sakai, K., Matsumoto, K., Nishikawa, T., Suefuji, M., Nakamaru, K.Y. and Hirashima, Y. (2003). Mitochondrial reactive oxygen species reduce insulin secretion by pancreatic beta- cells. *Biochemical* and *Biophysical Research Communications*;**300: 216-222.**

Sathya, R., Kanaga, N., Sankar, P. and Jeeva, S. (2013). Antioxidant properties of phlorotannins from brawn seaweed cystoseira trinodis (forsskal) C. Agardh. *Arabian Journal of Chemistry*;**5**: 1-7.

Usman, Z.U., Abu Bakar, A. and Mohamed, M. (2016). Phytochemical composition and activity against hyperglycemia of Malaysian propolis in diabetic rats. *Biomedical Research*;**27**: 46-51.

Usman, Z.U. and Mohamed, M. (2015). Analysis of phytochemical compounds in water and ethanol extracts of Malaysian propolis. International Journal *of* Pharma *and* Bio *Sciences;***6**: 374-380.

Zhang, N., Bu, S.M. and Ma, G.S. (2017). Current lifestyle factors that increase risk of T2DM in China. *European Journal of Clinical Nutrition*;**4**: 20-26.

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