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Prevention of Alloxan Induced Diabetes Mellitus in Rats by Vitamin a Dietary Supplementation

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Abstract: The aim of the study was to examine whether vitamin A dietary supplementation can prevent alloxan induced diabetes mellitus. Alloxan was used to destroy the β-cells of pancreas by generation of reactive oxygen species in Wistar albino rats. In this study, the blood glucose level and histological architecture of the pancreatic beta cells were examined. Three groups (n = 5) and were fed on commercial diet as lib. Group one was control diabetic, group two was fed with vitamin A supplementation in the diet (824 IU daily) before and after diabetic induction with alloxan. Group three was control non diabetic. The rats were made diabetic by intra peritoneal injection of 100 mg kg^{-1} b.wt. with alloxan once per week for three weeks. Fasting blood glucose was measured seven days after diabetic induction to determine the severity of blood glucose elevation. Histochemical analysis using hematoxylin stain (magnification X40) of the vitamin A administered rats revealed remarkable normal pancreatic beta cells, unlike the diabetic rats whose beta cell were necrotic and little in number. Vitamin A effect on serum glucose level was significantly different compared to diabetic control (5.53±0.40 vs. 18.64±3.92 mmol L^{-1}, $p<0.0001$), respectively. Through treatment with vitamin A for one week after induction did not have any significant effect, but has reduced the serum level by about 3 mmol compared before treatment (16.81±2.88 vs. 19.60±2.95 mmol L^{-1}, $p<0.96$), respectively. Taken together, the result suggests that Vitamin A protects against alloxan induced diabetes in rats.

Key words: Antioxidant, vitamin A, oxidative stress, free radical, alloxan

INTRODUCTION

Diabetes mellitus is a disorder characterized by hyperglycemia. It is a heterogeneous primary disorder of carbohydrate metabolism (Anonymous, 2009), with varied aetiology culminating in absolute relative insulin deficiency or insulin resistance or both. There is a reservoir of basic information that suggests the involvement of oxidative stress in the pathogenesis of diabetes mellitus. It is now recognized that sustained hyperglycemia in diabetic patients, causes protein glycation and generates free radicals through auto oxidation and polyol pathways (Ramakrishna and Rama, 2007; Sharm *et al*., 2000). High levels of free radicals with concurrent decline of antioxidant defence mechanisms may lead to the damage of cellular organelles and enzymes (Ottaviano *et al*., 2008). This can culminate in increased lipid peroxidation and development of insulin resistance, which may consequently promote the development of complications of diabetes mellitus (Demozay *et al*., 2008).

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Alloxan is used to induce experimental diabetes by selectively destroying pancreatic β-cell. Alloxan is taken up by pancreatic β-cell and subsequently generates Reactive Oxygen Species (ROS), which contributes to DNA fragmentation and evoke other deleterious changes in the cells (Dunn and McLetchie, 1943; Lankin *et al*., 2004) and cell death (Dunn and McLetchie, 1943; Heikkila *et al*., 1976).

It is well known that vitamin A is a lipid soluble antioxidant vitamin. As lipid antioxidant, it acts by protecting unsaturated fatty acid, a main component of cell membrane from the attack of oxygen derived free radicals.

Though, in the recent past it has been shown that retinyl palmitate administered interperitoneally prior to alloxan administration, conferred protection against alloxan induced diabetes (Chertow *et al*., 1989), there is yet paucity of information whether oral diet supplementation with vitamin A could prevent chemical induced diabetes. The aim of the this study was to evaluate the potential of oral dietary supplementation of Vitamin A in the prevention of chemical induced diabetes.

MATERIALS AND METHODS

Chemical Agents

All chemicals unless otherwise stated were purchased from Sigma Chemical Company (St Louis, MO) USA. Retinyl palmitate 1,600,000 usp units per gram.

Animals

The experiments were performed on male Sprague Dawley Albino rats (approx 180 g) obtained from Physiology Department Faculty of Medicine, Bayero University Kano, Nigeria. All aspects of animal care complied with the ethical guidelines and technical requirements approved by the Institutional Animal Ethics Committee.

Animals were housed individually in cages in an environmentally controlled animal facility (room temperature, 12 h light: 12 h dark cycle) with free access to a standard commercial diet and water *ad libitum*. The weight gain, food and water intake were determined daily in the morning. The experiment was conducted for a period of 5 weeks.

Induction and Treatment of Diabetes

All animals were fed on normal diet for 7 days of acclimatization. Diabetes was included by an intraperitoneal (IP) injection of 100 mg alloxan kg^{-1} b.wt.

Diabetes was induced by a single injection of alloxan 100 mg alloxan kg^{-1} b.wt. by IP to rats fasting for at least 16 h in freshly prepared 10 mmol L^{-1} sodium citrate, pH 4.5. Blood glucose levels were measured daily 3 days prior and 7 days after alloxan administration. Development of diabetes mellitus was proven by sustained hyperglycaemia and glycosuria [diabetic rats had glycaemia >11.11 mmol and glucosuria. This was based on the observation that only diabetic rats that had a fasting glucose greater than 200 mg dL^{-1} would be included in the study (Stanley and Venogopal, 2001).

Experimental Design

The rats were randomly divided into 4 groups (n = 5) as follows:

Group I: Control animals non-diabetic received normal diet (Saline solution, i.p.)

Group II: Animals treated with alloxan (100 mg kg^{-1} i.p. diabetic control animals). The rats developed diabetes after alloxan injection as evidenced by sustained hyperglycaemia and glycosuria 7 days after the induction

Group III: Vitamin A (824 IU per 100 g diet) treatment for seven days followed by alloxan administration

Group IV: Animals treated with alloxan (100 mg kg^{-1} i.p.). The rats developed diabetes after alloxan injection as evidenced by sustained hyperglycaemia and glycosuria 7 days after the induction, then treated with vitamin A (824 IU per 100 g diet) for 7 days

Glucose Estimation

Animals were starved for 16 h before blood collection. Fasting blood glucose was estimated by glucose oxidase method according manufacturer's procedure (Randox laboratories Ltd. Ardmore, United Kingdom). Urine was collected in cage urine separator bottle containing 1 mL of 10% thymol and glucose determined using Combostik (DFI Co. Ltd., Gimhae, Gyuang-Nam, Korea).

Histochemical Analysis

Histochemical analysis was done according to Bancroft and Stevens (1982).

Sacrificing Procedure

The Rats were made unconscious with carbon (IV) oxide before sacrifice by surgical dislocation of neck.

Statistical Analysis

Mean±SD was used to describe variables. Two-Sample Independent student t-test as well ANOVA using OpenEpi Version 2 program was used in the comparison of group means to determine the significance of the result.

RESULTS

Table 1 shows the fasting blood glucose one week before and one week after induction of diabetic mellitus. Blood glucose level of vitamin A group was compared with diabetic control. The effect of vitamin A on glucose level in the serum showed significant difference compared to diabetic control ($p<0.0001$). Histochemical analysis suggested that vitamin A protected the integrity of pancreas tissue against allaxon induced damage. Glucosuria increased significantly in diabetic animals compared with vitamin A treated group where there was no glucosuria. Though treatment with vitamin A one week after alloxan induced diabetes did not have any significant effect compared with result before treatment with Vitamin A (16.81±2.88 vs. 19.60±2.95 mmol L^{-1}, $p<0.96$, respectively), it however reduced the level of glucose by 3 mmol L^{-1}, suggesting that the normal level of serum glucose seem in vitamin A treated group before induction was achieved by the protection of the pancreatic tissue against alloxan induced damage rather than reversing the effect by vitamin A (Table 1).

Table 1: Fasting blood sugar (mmol L^{-1}) of rats

Groups	Before induction of diabetes	Seven days after induction of diabetes	Seven days after treatment with Vitamin A
Non-diabetic control (I)	5.12±0.29	5.62±0.22	-
Diabetic control (II)	5.02±0.88	18.64±3.92[a]	-
Vitamin A (III)	5.05±0.79	5.53±0.40[b]	-
Vitamin A (IV) after induction	4.99±1.02	19.60±2.95[c]	16.81±2.88

Result Mean±SD for n = 5, [a]$p<0.0001$ for comparing diabetic control and non diabetic control after induction of diabetes [b]$p<0.001$ for comparing diabetic control (Group II) and vitamin A (III) after alloxan treatment, [c]$p<0.0001$ for comparing non-diabetic control and group (IV) before vitamin A supplementation

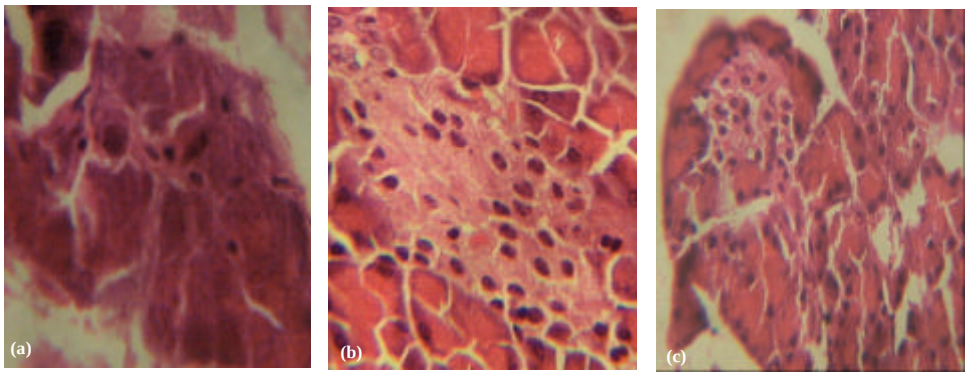

Fig. 1: Histochemical structure of pancreatic cells of albino rats stained with haemtoxylin, (a) Allaxon treated showing cell necrosis in which beta cell are severely a ffected, (b) vitamin A treated in which the cells integrity is not affected and (c) control non diabetic fed on normal diet *ad libitum*

Table 2: Weekly mean body weight of rats (g)

Weeks	Control non diabetic	Control diabetic (Allaxon treated)	114.90±6.00	Vitamin A treatment one week after Alloxan induced diabetes
0	102.27±1.05	101.50±1.01	119.65±1.25	104.23±0.983
1	104.37±1.33	103.30±1.23	126.95±1.00	108.22±0.93
2	116.00±2.84	104.20±4.00	129.00±1.00	110.11±2.01
3	120.99±3.20	106.20±7.66	126.95±1.00	115.00±2.66
4	123.33±5.77	100.00±17.32	129.00±1.00	115.00±2.66

Result Mean±SD for n = 5

Histochemical study of pancreatic tissue is shown in Fig. 1a-c. Vitamin A group were compared to both controls (diabetic and normal control).

The diabetic control group II showed necrotic beta cells, suggesting that the integrity of pancreatic tissue was compromised following diabetes induction (Fig. 1a). Vitamin A group revealed normal and remarkable pancreatic cells, indicating that the integrity of pancreatic tissues was protected by vitamin A against alloxan induced damage (Fig. 1b).

The normal control group showed normal pancreatic tissue architecture (Fig. 1c). Table 2 shows the weekly body weights one week before and 4 weeks after induction of diabetes mellitus. The weekly mean body weights are in grams. Group 1 and Group 3 appeared to have weekly increase in weight but Group 2 has reduction in weight at week four this indicates that the antioxidant do not retard growth of the animals. In the case of diabetes incidence there could be lost of weight due to mobilization of protein store.

DISCUSSION

Several lines of evidence have shown that diabetes mellitus and complication thereof from the disorder can arise due to oxidative stress. Similarly increased lipid peroxides have been seen in many animal models of diabetes mellitus (Anjaneyulu and Chopra, 2004).

Since the understanding that the selective destruction by alloxan of pancreatic beta cells is mediated via generation of oxidative stress (Dunn and McLetchie, 1943), interest has been stimulated in the use of antioxidant to prevent chemically induced damage of pancreatic cells. Toxicity of alloxan is elicited through its reduction by glutathione to dialuric acid, in which redox recycling process generates ROS that damages the beta cells (Malaisse, 1982).

Furthermore, transition metals such as iron and copper, which are potentially involved in the generation of hydroxyl free radical are also involved in alloxan mediated killing of beta cells (Malaisse, 1982; Wolff, 1993; Wilson *et al*., 1984). It has been known that vitamin E prevented alloxan induced diabetes in rats (Fatemi *et al*., 2008). This study suggested that vitamin A consumption has beneficial effect and dose used in this study was effectively potent to inhibit the toxicity of alloxan on β-cells of pancreas. In the recent past, it has been demonstrated that intravenous administration of retinyl paltimate prior to that of alloxan administration, protected against alloxan induced diabetic in rats (Chertow *et al*., 1989). However, there is paucity of information as to whether vitamin A supplementation in the diet can protect against alloxan induced damage of beta cells. Though Siefter *et al*. (1981) have demonstrated the efficacy of using twice the recommended daily allowance of vitamin A in rodent with streptozotocin induced diabetes to improve wound healing process caused by steptozotocin, the work did not look at the potential of vitamin A dietary supplementation in the prevention of chemically induced diabetes. Hence, this work provided insight as to the potential of vitamin A dietary supplementation at human RDA in the prevention of chemically induced diabetes mellitus in rodent. Similarly, the study corroborated with the Siefter *et al*. (1981) finding, that once beta cells are destroyed, vitamin A does not alter, carbohydrate metabolism, but has provided a reserviour of information about protection of chemical induced diabetes mellitus using food supplemented with vitamin A. This was in variance with Chertow *et al*. (1989) study, where administration of Vitamin A at twice human RDA was used some hours prior to alloxan administration. Treatment with dietary vitamin in this study did not alter hyperglacaemia and glucosuria in animals with alloxan induced diabetes. It is thought possible that since vitamin E has been demonstrated to protect against chemically induced destruction of beta cell, that vitamin A which shares the same mechanism of free radical scavenging may have similar effect. In this study, we were able to show that vitamin A supplementation at daily recommended dose given to human can protect against alloxan induced diabetes in rats. The addition of vitamin A to the diet is computed based on the amount of diet taken by the rats over a period of 7 days. The daily recommended allowance of vitamin A for adult is 700 μg (considering the average body weight is 77 kg; (Olson, 1987). Hence base on that, 824 IU per 100 g diet was given to the experimental animals. It is also interesting to note that the histological architecture of the pancreatic cells was highly protected by vitamin A. This study suggested that vitamin A supplementation may have an effect on chemical induced diabetes mellitus. Furthermore in view of its antioxidant property it may as well delay complication arising from the disease. Future work should look at the potential of combination of vitamin A and E at lower dose in preventing oxidative stress induced diabetes. This would be more therapeutically valuable in view of the low income of the mass of Nigerians. Diabetes mellitus is becoming a major global health (Anonymous, 1993) and societal problem, costing the United States approximately $132 billion annually (Hogan *et al*., 2003). In Sub-Saharan Africa, there are no enough financial resources to adequately carter for this disease, while urban migration and increasing sedentary lifestyle in sub-Saharan Africa are increasing the incidence of the condition here Nigeria. While the war on substandard and fake drugs, including antidiabetics is yet to be won, the collapse of the power sector in Nigeria has made sustained potency of hypoglycemics including insulin near impossible in most cases. These negative factors collectively have escalated the development of diabetic complications which are known to be difficult and costly to manage even in the developed world. Hence, since, complication arising from diabetes mellitus is strongly associated with generation of reactive oxygen species, it is thought possible that use of sustain antioxidants at concentration that may not

be toxic, may provide a better way in augmenting the managing of the condition as well as protecting against oxidative stress induced diabetes mellitus. The pathogenesis of type I diabetes mellitus is invariably associated with oxidative stress, hence alloxan induced diabetic condition is elicited through generation of free radical, it is likely dietary supplementation with Vitamin at RDA dosage may likely attenuate the effect of free radicals in the induction of type I diabetes mellitus.

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REFERENCES

Anjaneyulu, M. and K. Chopra, 2004. Nordihydroguairetic acid, a lignin, prevents oxidative stress and the development of diabetic nephropathy in rats. Pharmacology, 72: 42-50.

Anonymous, 1993. Investing in Health: World Development Indicators. Oxford Press, Oxford.

Anonymous, 2009. International expert committee report on the role of the A1C assay in the diagnosis of diabetes. Diabetes Care, 32: 1327-1334.

Bancroft, J.D. and A. Stevens, 1982. Theory and Practice of Histological Techniques. Churchill Livingstone, Edinburgh, pp: 41-120.

Chertow, B.S., W.D. Webb, J.W. Leidy Jr. and M.B. Cordle, 1989. Protective effects of retinyl palmitate on streptozotocin- and alloxan-induced beta cell toxicity and diabetes in the rat. Res. Commun. Chem. Pathol. Pharmacol., 63: 27-44.

Demozay, D., J.C. Mas, S. Rocchi and E. van obberghen, 2008. FALDH reverses the deleterious action of oxidative stress induced by lipid peroxidation product 4-hydroxynonenal on insulin signaling in 3T3-L1 adipocytes. Diabetes, 57: 1216-1226.

Dunn, J.S. and N.G.B. McLetchie, 1943. Experimental alloxan diabetes in the rat. Lancet, 2: 384-387.

Fatemi, T.S.R., A.A. Papahn, M.R. Jalali and L. Rahimi, 2008. Effect of oral Vitamin E on induction and consequence of experimental diabetes mellitus in rat. Pak. J. Biol. Sci., 11: 633-637.

Heikkila, R., B. Winston and G. Cohen, 1976. Alloxan induced-diabetes evidence for hydroxyl radical as a cytotoxic intermediate. Biochem. Pharmacol., 25: 1085-1092.

Hogan, P., T. Dall and P. Nikolon, 2003. Economic costs of diabetes in the US in 2002. Diabetes Care, 26: 917-932.

Lankin, V.Z., V.I. Korchin, G.G. Konovalova, M.O. Lisina, A.K. Tikhaze and I.G. Akmaev, 2004. Role of antioxidant enzymes and antioxidant compound probucol in antiradical protection of pancreatic beta-cells during alloxan-induced diabetes. Bull. Exp. Biol. Med., 137: 20-23.

Malaisse, W.J., 1982. Alloxan toxicity of the pancreatic B-cell a new hypothesis. Biochem. Pharmacol., 22: 3527-3534.

Olson, J.A., 1987. Recommended dietary intakes (RDI) of vitamin A in human. Am. J. Clin. Nutr., 45: 704-716.

Ottaviano, F.G., D.E. Handy and J. Loscalzo, 2008. Redox regulation in the extracellular environment. Circ. J., 72: 1-16.

Ramakrishna, V. and J. Rama, 2007. Evaluation of oxidative stress in Insulin Dependent Diabetes Mellitus (IDDM) patients. Diagn. Pathol., 2: 22-22.

Sharm, A., S. Khar, S.N. Chugh, R. Kakkar and G.P. Singh, 2000. Evaluation of oxidative stress before and after control of gylcemia and after vitamin E supplementation in diabetic patients. Metabolism, 49: 160-162.

Siefter, E., G. Rettura, J. Padawer, D. Kambosos and S.M. Levenson, 1981. Impaired wound healing in streptozotocin diabetes: Prevention by supplemental vitamin A. Ann. Surgery, 194: 42-50.

Stanley, M.P. and M.P. Venogopal, 2001. Antioxidant action of Tinospora, Cordiofolia root extract in alloxan-induced diabetic rats. Phytother. Res., 15: 213-218.

Wilson, G.L., N.J. Patton, J.M. McCord, D.W. Mullins and B.T. Mossman, 1984. Mechanisms of streptozotocin- and alloxan-induced damage in rat B cells. Diabetologia, 27: 587-591.

Wolff, S.P., 1993. Diabetes mellitus and free radicals. Free radicals, transition, metals and oxidative mellitus and complication. Br. Med. Bull., 49: 264-652.