Volume : 5 | Issue : 3 | March 2016 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 69.48

Effect of Aqueous Extract of Talinum Triangulare on Thiobarbaturic Acid Reactive Substances, Hydroperoxides And Histopathology of Pancreas In Alloxan Induced Diabetic Rats

Medical Science

KEYWORDS : Talinum triangulare (TT), Alloxan, Thiobarbaturic Acid Reactive Substances (TBARS).

J.Sudha Rani*

Department of Medical Biochemistry, Gayatri Vidya Parishad Health Care and Medical Technology , Marikavalasa, Maridivaly , Visakhapatnam , Pincode-48,Andhra Pradesh, INDIA.

ABSTRACT

In the present investigation the aqueous leaf extract of Talinum triangulare (TT) to alloxan induced diabetic rats decrease the plasma Thiobarbaturic Acid Reactive Substances (TBARS) and hydroperoxides to near normal levels, which could be a result of improved antioxidant status. Oral administration of TT leaf extract for 15 days effectively restored the pathological changes in alloxan induced diabetic rat pancreatic tissues.

INTRODUCTION:

Diabetes mellitus (DM) is a chief disarray of carbohydrate metabolism, which commonly involves complete or relative insulin deficiency and/or insulin opposition and finally leads to hyperglycemia. In the present situation there was an escalating demand for the exploitation of natural products with anti-diabetic activity. The adverse side effects of synthetic drugs, easier utilization or accessibility and the fact that they are not appropriate for utilization during pregnancy, have been some of the factors primary thinking to have a strong need to use hypoglycemic agents of plant source (Jelodar *et al.*, 2007; Yadav *et al.*, 2008). Some herbs and plant natural products have been revealed to have antihyperglycemic action (Elder, 2004; Badole *et al.*, 2006). Natural Plants and there extracts may act on blood glucose through diverse biochemical mechanisms. Several of them may have insulin-like substances (Collier *et al.*, 1987; Gray and Flatt, 1999); several may reduce insulin activity while others may enhance beta cells in pancreas by activating regeneration of these beta-cells (Shanmugasundaram *et al.*, 1990; Abdel *et al.*, 1997).

However, very only some of the traditional treatments for diabetes mellitus have received scientific analysis. The aim of this study was to investigate the effect of aqueous extract of *Talinum triangulare* in traditional medicine in treatment of diabetes and its possible role on pancreatic tissue.

MATERIALS AND METHODS:

Animals: Male albino rats (Wistar strain, weighing 150-200g) were purchased from Tamil Nadu Veterinary Animal Science University, Madhavaram, Chennai and housed under standard husbandry conditions (30°C ± 2°C, 60-70 % relative humidity and 12hr day night cycle) and allowed standard pelleted rat feed and water. Animal experiments were designed and conducted in accordance with the guidelines of Institutional Animal Ethical Committee (Sri Venkateswara University, Tirupati).

Plant material and extract preparation

The *Talinum triangulare* leaves were harvested and shade dried for 20 days. Then grinded mechanically and 100g of coarse powder was extracted by using water in soxhlet apparatus. Extract was concentrated to semi-solid water free material and final extract yield was 8.5%.

Induction of diabetes mellitus in rats

Diabetes was induced in male wistar albino rats by intraperitoneal injection with cold aqueous alloxan monohydrate (80 mg/kg body wt). Since alloxan is capable of producing hypoglycemic conditions the rats are fed with 15% glucose solutions. From 2nd day onwards blood sample were collected from the rats by cutting the tail and glucose estimation was made to know the induction of DM. After 12 days the rats were identified having hyperglycemic condition (blood glucose 250 mg/dl) and was selected as experimental material. All the animals were allowed to have free access to the tap water and pellet diet.

Experimental design

Animals were divided in to six groups of six animals each. Group I served as a control: group II had normal + TT(40 mg/kg bw) rats; group III had normal + TT (80 mg/kg bw) and Group IV acts as diabetic control, V as diabetic + TT (40 mg/ kg bw) and VI comprised the diabetic + TT (80 mg/kg bw) rats treated with *talinum triangulare* aqueous leaves extract 40 and 80 mg/Kg bw/day respectively for 6 weeks, by oral incubation method. Rats were sacrificed at the end of 6 weeks and the blood samples were collected to analyze the effect of TT on biochemical parameters. Collection and processing of blood for estimation of glucose and other biochemical parameters.

Lipid peroxidation parameters

Estimation of TBARS

The concentration of TBARS in the plasma, erythrocytes and tissues was estimated by the method of Nichans and Samuelson (1968). In this method, malondialdehyde and other thiobarbituric acid reactive substances (TBARS) reacts with thiobarbituric acid in an acidic condition to generate a pink: color chromophore which was read at 535 nm.

Procedure

0.5 mL of plasma was diluted to 0.5 mL with double distilled water and mixed well, and then 2.0 mL of TBA-TCA-HCl reagent was added. The mixture was kept in a boiling water bath for 15 min; after cooling, the tubes were centrifuged at 1000 g for 10 min and the supernatant was estimated. A series of standard solutions in the concentration of 2-10 n mol was treated in a similar manner. The absorbance of the chromophore was read at 535 nm against reagent blank. The values were expressed as m mol/mL of plasma or m mol/100 g of tissues or n mol/mg of protein for erythrocytes. .

Estimation of lipid hydroperoxides

Lipid hydroperoxide in the plasma, erythrocytes and tissues was estimated by the method of Jiang et al. (1992). Oxidation of ferrous ion (Fe^{2+}) under acidic conditions in the presence of xylenol orange leads to the formation of a chromophore with an absorbance maximum at 560 nm.

Procedure

0.9 mL of Fox reagent was mixed with 0.1 mL of the sample, incubated for 30 min at room temperature and the absorbance read in a Spectronic 20 at 560 nm. Lipid hydroperoxides were expressed a n mollmL of plasma or m moll/l00 g of tissues or m mol/mg of protein for erythrocytes.

Histopathological studies

Light microscopic studies-Paraffin method The light microscopic

Volume : 5 | Issue : 3 | March 2016 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 69.48

study was done by the method of Humason, (1979).

Procedure

The tissues such as, pancreas from untreated and parallel experimental groups were blotted free of mucus, washed in physiological saline, cut into pieces of desired size and fixed in Bouin-Hollande fixative for 72 hr. After fixation, the tissues were washed in 70% alcohol for two or three days to remove the excess picric acid and dehydrated in graded series of alcohol. The tissues were cleared using xylene. The cleared tissues were infiltrated with molten paraffin at 58-60°C through three changes (20-30 min each) and finally embedded in paraffin. 3-4 /l m thick sections of all the tissues were obtained using a rotary microtome (Leica, Germany) and stained in Ehrlich's hematoxylin with eosin as the counter stain. The slides were mounted using DPX mountant.

RESULTS AND DISCUSSION:

Oxidative stress that leads to an increased production of ROS and finally cellular lipidperoxidation has been found to play an important role in the development of diabetes (Pari and Latha, 2005). We observed elevated levels of thiobarbituric acid reactive substances and hydroperoxides in plasma of alloxan -induced diabetic rats (Table 1and 2). In diabetes it is thought that hypoinsulinemia increases the activity of the enzyme fatty acyl CoA oxidase, which initiates β-oxidation of fatty acids, resulting in lipid peroxidation (Rahimi *et al.,* 2005). Increased lipid peroxidation impairs membrane function by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors. In our study, significant increased levels of TBARS were observed in diabetic tissues and erythrocyte is a marker of lipid peroxidation (Table 1and 2). In the present results the formation of TBARS, a product of lipid peroxidation was significantly increased in diabetic tissues (Nandhini and Anuradha *et al.,* 2003). Similarly, Rajasekaran *et al.* (2005) have also reported the increase lipid peroxide levels in diabetic rats. Oral administrations of TT-leaf extract to diabetic rats decrease the plasma TBARS and hydroperoxides to near normal levels, which could be a result of improved antioxidant status. Inactivation or inhibition of antioxidant enzymes by glycosylation in poorly controlled diabetes may give rise to increased lipid peroxidation. Pushparaj *et al.,* (2000) also reported increased TBARS in plasma, liver and kidney of alloxan induced diabetic rats. Oral administration of TT-leaf extract decreases TBARS in alloxan -diabetic rat tissues and erythrocyte. Hydroperoxides are high toxicity molecules and have a high potential for destroying enzymic proteins and cell membranes (Kowluru *et al.,* 1996). In our study, an increase in HP in the erythrocyte and tissues of alloxan -diabetic rats was observed. The decreased activity of antioxidant enzymes is a favorable factor for uncontrolled generation of free radicals and subsequent generation of lipid hydroperoxides (Halliwell and Gutteridge, 1990). Oral administration of TT-leaf extract lowers hydroperoxides in alloxan induced plasma, tissues and erythrocyte (Table 1and 2).

Table 1and 2: Effect of TT - leaf extract on plasma lipid peroxidation levels in control and alloxan - diabetic rats.

Groups	TBARS (nmoles/ml)
Normal	2.71±0.21
Normal + TT (40 mg/kg bw)	2.47±0.24 [b]
Normal + TT (80 mg/kg bw)	2.78±0.26 [b]
Diabetic control	5.12±0.38 [a]
Diabetic + TT (40 mg/kg bw)	2.86±0.31 [b]
Diabetic + TT (80 mg/kg bw)	2.79±0.29 [b]

Groups	HP (Values X 10-5 mM/dl)
Normal	8.21±0.65
Normal + TT (40 mg/kg bw)	8.17±0.59 b
Normal + TT (80 mg/kg bw)	8.88±0.61 b
Diabetic control	19.42±1.11 a
Diabetic+ TT (40 mg/kg bw)	9.12±0.71 b
Diabetic + TT (80 mg/kg bw)	8.88±0.69 b

Each value is mean ± SD for 6 rats in each group.
a: $p<0.05$ by comparison with normal rats.
b: $p<0.05$ by comparison with alloxan diabetic rats.
- : No significance

In the present study, the histology and ultrastructure demonstrated that most of the islets were affected and showed observed changes in structures. The β-cells showed degranulation and swelling of the intracellular organelles. All these vital intracellular structures were affected thus inhibiting the synthesis and release of insulin (Fig1B). Microscopic examination shows abundant patches of β cells in the pancreas of normal rats which are absent in diabetic pancreas (Anil et al., 1996) (Fig 1A). Selective destruction of β cells is observed in alloxan induced diabetic rats (Susan Bonner and Smith 1994). Small and shrunken islets and destruction of β cells are observed in the diabetic condition (Kesavulu et al., 2000).

Oral administration of TT leaf extract for 15 days effectively restored the pathological changes in alloxan induced diabetic rat pancreatic tissues. In this context, treatment of alloxan induced diabetic animals with. (-)-epicatechin and N-acetyl-l-cysteine (NAC) well-known terpenoid, prevented hyperglycemia through reduced oxidative stress and restored β-cell function (Sheehan and Zemaitis, 1983) (Fig 1 B and D). Our results are in agreement with the above observations of Nagappa et al., (2003) showed that the regeneration of beta cells in the pancreas of Terminalia catappa fruit extract-treated diabetic rats, due to β-carotene, which is a constituent of T. catappa fruit. Sharma et al., (2003) have reported that oral administration of Eugenia jambolana seed extract reversed the abnormalities in the islet of Langerhans of alloxan-induced diabetic rabbits. The histopathological study reveals that decreased blood glucose concentration of diabetic rats by TT leaf extract treatment is due to the regeneration/proliferation in the pancreatic β-cells.

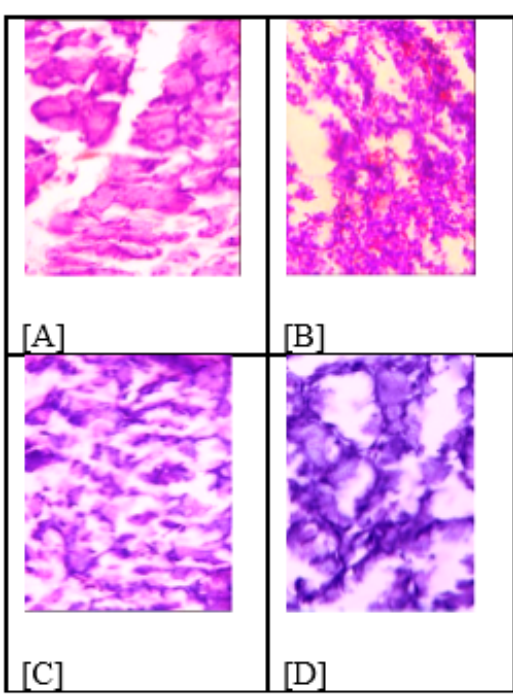

Fig.1: Histopathological panels of rat pancreas prepared from 4 different groups of albino rats **a**. Control; **b.** Diabetic; **c**. Diabetic - TT (40 mg/kg bw); **d.** Diabetic - (80 mg/kg bw).

CONCLUSION:

In the present study the leaf extract of Talinum triangulare (TT) to alloxan induced diabetic rats decrease the plasma TBARS and hydroperoxides to near normal levels, which could be a result of improved antioxidant status. Oral administration of TT aqueous leaf extract for 15 days to the diabetic rats helped in the restoration of cell mass in pancreas to improve the secretion of insulin. The observed results state that further work should be conducted to improve and identify the bioactive compound in the above said plant to overcome diabetic condition.

REFERENCES:

1. Jeloder G, Mohsen M and Shahram S (2007). Effect of walnut leaf, coriander and pomegranate on blood glucose and histopathology of pancreas of alloxaninduced diabetic rats. African J. Traditional, Complementary and Alternative Medicines (*Afr. J. Trad. CAM*, **4**(3): 299-305.
2. Yadev JP, Saini S, Kalia AN and Dangi AS (2008). Hypoglycemic activity of ethanolic extract of *Salvadora oleoides* in normal and alloxan – induced diabetes rats. *Indian J. Pharmacol*., **40**(1): 23-27.
3. Elder C (2004). Ayurveda for diabetes mellitus. A review of the biochemical literature. *Altern. Ther. Health Med.,* **10**: 44-50.
4. Badole S, Patel N, Bodhankar S, Jain B and Bhardwaj S (2006). Antihyperglycemic activity of aqueous extract of leaves of *Cocculus hirsutus* (*L.*) *Diels* in alloxaninduced diabetic mice. *Indian J. Pharmacol*., **38**: 49-53.
5. Collier E, Watkinson A, Cleland CF and Roth J (1987). Partial purification and characterization of an insulinlike material from spinach and lemna gibba G3. *J. Biol. Chem*., **262**: 6238-6241.
6. Gray AM and Flatt PR (1999). Insulin – releasing and insulin – like activity of the traditional antidiabetic plant *Coriander sativum* (coriander). *Br. J. Nutr*., **81**: 203-208.
7. Shanmugasundaram ER, Gopith KI, Radha SK and Rajendram VM (1990). Possible regeneration of the islets of Langerhans in streptozotocin diabetic rats given *Gymnema sylvestere* leaf extract. *J. Ethnopharm*., **30**: 265-269.
8. Abdel Monein A, El-Feki M and Salah E (1997). Effect of *Nigella sativa*, fish oil and gliclazide on alloxan diabetic rats. Biochemical and Histopathological studies. *J. Egy. Ger. Soci. Zool*., **23**: 237-265.
9. Nichans WG. and Samuelson B.(1968). Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. Eur J Biochem. **6**(1), 126-130.
10. Jiang Z.Y., Hunt J.V,and Wolff SP. (1992). Ferrous ion oxidation in the presence of xylenol orange for the detection of lipid hydroperoxides in low density lipoprotein. Anal Biochem. **202**: 384-389.
11. Humason GL, (1979). Animal tissue techniques. WH Freeman and Company, San Francisco.
12. Pari L. and Latha M. 2005. Antidiabetic effect of *Scoparia dulcis*: effect on lipid peroxidation in streptozotocin diabetes. *Gen. Physiol. Biophys*. **24**: 13–26.
13. Rahimi, R. Nikfer, S., Larijani, B., and Abdollahi, M. (2005). A review on the role of antioxidants in the management of diabetes and its complications. *Biomed Pharmacother*. **59**: 365-373.
14. Nandhini, T. A. and Anuradha, C.V. (2003). Inhibition of lipid peroxidation protein glycation and elevation of membrane pump activity by tarurine in RBC exposed high glucose. *Clin. Chim Acta*. **336**, 129-135.
15. Rajasekaran, S., Sivagnanam, K. and Subramanian, S. (2005). Modulatory effects of Aloe vera leaf gel extract on oxidative stress in rats treated with streptozotocin. *J Pharm Pharmacol*. **57**, 241-246.
16. Pushparaj, P., Tan, C. R. and Tan, B. K. H. (2000). Effects of A verrhoa bilimbi leaf extract on blood glucose and lipids in streptozotocin-diabetic rats. *J. Ethnopharmacol*. **72**, 69-76.
17. Kowluru, R. A., Kern, T. S., Engerman, R. L. and Armstrong, D. (1996). Abnormalities in retinal metabolism in diabetes or experimental galactosemia III. Effects of antioxidants. *Diabetes*. **45**, 1233-1237.
18. Halliwell, B. and Gutteridge, J. M. C. (1990). Free radicals in biology and medicine. oxford: clarendon Press.
19. Anil, V., Das'Padayatti, P.S., and panlose, C.S. (1996). Effect of leaf extract of *Aegle marmelos* on histological and ultra structural changes in tissues of STZ induced diabetic rats. *Indian. J. Exo. Biol*. **34**:341-345.
20. Susan Bonner,W. and Smith,F.E. (1994) Islets of langerhans: Morphological and its implications . In :Joslins Diabetes mellitus. 13th edition. Ronald khan, C and Cordon, C. Weir, (eds). Leaf Febiger. A waverly company. Philadelphia. Pp:15-26.
21. Kesavulu, M. M., Giri, R., Kameswara, R. B. and Apparao,O. (2000). Lipid peroxidation and antioxidant enzyme levels in type II diabetic with micro vascular complication. Diabet. Metabol. 26:387-392.
22. Sheehan, E.W. and Zemaitis M.A. (1983). A constituent of Pterocarpus marsupium, (-)-Epicatechin, as a potential antidiabetic agent. J. Nat. Prod. 46: 232-234.
23. Nagappa, A.N., Thakurdesai, P.A. Rao N.V. and Singh, J. (2003). Antidiabetic activity of Terminalia catappa Linn fruits. J. Ethnopharmacol., 88: 45-50.
24. Sharma SB, Nasir A, Prabhu KM, Murthy PS, Gev G. (2003). Hypoglycaemic and hypolipidemic effect of ethanolic extract of seeds of Eugenia jambolana in alloxan-induced diabetic rabbits. J. Ethnopharmacol. 85: 201-206.