

## EVALUATION OF RENOPROTECTIVE ACTIVITY OF MESOZEAXANTHIN IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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### ABSTRACT

Diabetic nephropathy (DN) is one of the most common complications of a common prevalent chronic endocrine disorder – diabetes mellitus. It involves a great deal of damage to the renal anatomy and physiology. The following mentioned study was undertaken to investigate the renoprotective effect, if any, of mesozeaxanthin, an antioxidant in experimentally induced diabetic nephropathy in rats. A single dose of Streptozotocin (STZ) (50 mg/kg, i.p.) was incorporated to precipitate the induced-condition of diabetes mellitus in the concerned rats. Diabetic nephropathy developed after 8 weeks of STZ administration and was assessed by the measurement of various

parameters viz., levels of serum creatinine, blood urea nitrogen (BUN) and creatinine clearance, renal collagen content. Furthermore, changes in renal TBARS (Thiobarbituric acid reactive substances) and reduced glutathione levels were measured as markers of oxidative stress besides measuring the levels of glucose and lipid content for also observing the possibility of the drug action via some other therapeutic activity. Mesozeaxanthin treatment for the first time has been demonstrated to significantly attenuate STZ-induced DN, as evidenced by a significant decrease in serum creatinine, BUN, renal collagen content and a significant increase in creatinine clearance. Mesozeaxanthin treatment dose-dependently decreased the renal oxidative stress in diabetic rats. It is, thus, concluded that the renoprotective effect of mesozeaxanthin is due to its antioxidant property as no significant

changes were observed in blood glucose and lipid profile, confirm that mesozeaxanthin is a pure antioxidant.

**KEY WORDS:** - diabetic nephropathy, reactive oxygen species, oxidative stress, serum creatinine, blood urea nitrogen.

## INTRODUCTION

Diabetes mellitus represents a group of diseases which is characterized by chronic hyperglycemia and other metabolic abnormalities, which are due to absolute or relative deficiency of insulin. After long duration of metabolic derangement, specific complications of diabetes such as retinopathy, nephropathy, neuropathy & cardiomyopathy may occur <sup>[1]</sup>. Diabetic nephropathy (DN) is considered to be one of the major complications of diabetes mellitus. DN is a leading cause of chronic kidney disease and end - stage renal failure and accounts for significant morbidity and mortality in diabetic patients <sup>[2]</sup>. The pathological changes such as expansion of mesangial cells, accumulation of extracellular matrix protein, thickening of glomerular and tubular basement membranes, tubulointerstitial fibrosis, glomerulosclerosis and renal endothelial dysfunction are noted to occur in the diabetic kidney <sup>[3, 4]</sup>. The reactive oxygen species (ROS) are considered as the common activators of various signaling cellular pathways implicated in diabetic nephropathy <sup>[5]</sup>. They are continuously produced and degraded normally to maintain homeostasis, but generation of high concentrations of certain specific ROS such as superoxide anion, H<sub>2</sub>O<sub>2</sub>, hydroxyl radicals and peroxynitrite by hyperglycemia may cause injury to various organs, including kidney. Most of the ROS are generated during mitochondrial oxidative phosphorylation and small amounts via NADPH-oxidase system <sup>[6]</sup>. ROS is also held responsible for the activation of protein kinase C (PKC), mitogen activated protein kinase (MAPK), Janus kinase/signal transducer and activator of transcription (JAK/STAT) and several transcription factors such as nuclear factor-kappa B (NF-kB) and activating protein-1 (AP-1). It also plays a role in the upregulation of transforming growth factor – beta 1 (TGF-β1). All the above listed activated pathways, transcription factors and upregulated growth factors lead to severe deterioration of the functioning of diabetic kidney. Many conducted studies, in this aspect, give evidences which suggest that ROS-regulated signaling pathways play a key role in certain mechanism of the pathophysiology which further lead to extracellular matrix (ECM) deposition in diabetic kidney <sup>[5]</sup>.

Recently a carotenoid, meso-zeaxanthin [(3R, 30S)-b, carotene-3, 30-diol] was found to be effective in reducing oxidative stress<sup>[7]</sup>. Meso-zeaxanthin was instituted to be involved in the process of scavenging superoxide radicals, hydroxyl radicals and the inhibition of in-vitro lipid peroxidation<sup>[8]</sup>. Various evidences from numerous researches have been well reported that the mesozeaxanthin drug also shows protective behavior in AMD (age related macular degeneration) by quenching the triplet state of photosensitizers; however no significant results has been shown in this regard with Vit A & Vit C [9]. Single oral administration of meso-zeaxanthin to rats is sufficient to significantly increase the catalase, superoxide dismutase, glutathione and glutathione reductase levels<sup>[10]</sup>. Therefore, mesozeaxanthin can be considered as “pure” antioxidant because it exhibits little or no pro-oxidative behavior, even at high carotenoid concentration and at high oxygen tension<sup>[11]</sup>. Extensive literature search revealed that, although the role of oxidative stress in other important complications of diabetes mellitus, such as diabetic retinopathy, diabetic neuropathy and diabetic microangiopathy, is well established by a number of researches and studies, the protective effect of mesozeaxanthin on them has not been explored as yet. Confining the designation of the present study to the investigation of the renoprotective effect of mesozeaxanthin in experimentally induced-DN in rats, the following mentioned experiment was performed.

## MATERIALS AND METHODS

The experimental protocol used in the present study was approved by the Institutional Animal Ethical Committee. Age matched young Wistar rats of either sex, weighing about 230-260g were employed. Rats were fed on standard chow diet and water ad libitum. They were acclimatized in animal house and were exposed to normal day and light cycle.

### Experimental protocol

The present study consists of five groups and each group comprises of 6 rats of either sex. Mesozeaxanthin was dissolved in sunflower oil.

#### Group 1

(Normal Control): Rats were maintained on standard food and water regimen and no treatment was given.

#### Group 2

(Mesozeaxanthin *per se* in normal rats): Rats were administered mesozeaxanthin (200 mg/kg) for 4 weeks.

**Group 3**

(Diabetic Control): Rats were administered STZ (50 mg/kg, *i.p.* once) dissolved in citrate buffer (pH 4.5).

**Group 4**

(Mesozeaxanthin low dose treated diabetic group): The diabetic rats after 4 weeks of STZ administration were treated with mesozeaxanthin low dose (100 mg/kg, orally/*i.p.*) for 4 weeks.

**Group 5**

(Mesozeaxanthin high dose treated diabetic group): The diabetic rats after 4 weeks of STZ administration were treated with mesozeaxanthin high dose (200 mg/kg, orally/*i.p.*) for 4 weeks. All parameters were assessed at the end of 8 weeks in both normal and STZ-treated rats.

**Induction of DN**

Diabetes mellitus was induced by single injection of STZ (45 mg/kg, *i.p.*) dissolved in freshly prepared ice cold citrate buffer (pH 4.5). After 1 week of STZ administration animals having random serum glucose more than 240 mg/dl were considered as diabetic. The pathological changes starts after 4 weeks of STZ administration and DN developed after 8 weeks of STZ administration as reported earlier <sup>[12]</sup>.

**Assessment of STZ-induced Diabetes****Estimation of serum glucose and serum lipid profile**

At the end of the experimental protocol, the blood samples were collected from retroorbital Sinus under light ether anaesthesia and serum was separated and stored in -20°C until analysis of biochemical parameters. The serum glucose concentration was estimated by glucose oxidase-peroxidase (GOD-POD) method <sup>[13]</sup>. Total cholesterol in serum was estimated by cholesterol oxidase peroxidase (CHOD-PAP) method <sup>[14]</sup>. Serum triglycerides was estimated by glycerophosphate method <sup>[13]</sup>. Serum HDL was estimated by Polyethylene glycol (PEG) precipitation method using commercially available kits from Coral Clinical System, Goa, India <sup>[15]</sup>.

### **Assessment of Diabetic Nephropathy**

#### **Estimation of BUN, Serum creatinine, Creatinine clearance**

Serum creatinine and urinary creatinine concentrations were estimated with the alkaline picrate method <sup>[16]</sup>. Creatinine clearance was estimated with a formula using serum creatinine and urinary creatinine, BUN was estimated by berthelot method <sup>[17]</sup>, Renal hypertrophy was assessed morphologically by measuring KW/BW % and biochemically by measuring total renal collagen content <sup>[18-20]</sup>. Kits for these measurements were purchased from Coral Clinical System, Goa, India.

#### **Assessment of renal oxidative stress**

The development of oxidative stress in the kidney was assessed by estimating renal thiobarbituric acid reactive substances (TBARS) and reduced form of glutathione content (GSH) as reported earlier <sup>[21-22]</sup>.

#### **Drugs and chemicals**

Mesozeaxanthin was obtained from Omni Active Health Technologies Private Limited, Mumbai as an Ex-gratia. STZ was obtained from Sigma Aldrich Ltd, St. Louis, USA. All other chemicals used in present study were of analytical grade.

#### **Statistical analysis**

All values were expressed as mean  $\pm$  S.D. The data obtained from various study parameters were statistically analyzed using one way ANOVA followed by Tukey's multiple comparison test. The p value  $< 0.05$  was considered to be statistically significant.

### **RESULTS-**

All animals completed the study. Administration of mesozeaxanthin for 4 weeks to normal rats did not produce any significant *per se* effects on various parameters assessed in the present study.

#### **Effect of mesozeaxanthin low and high dose on serum glucose level and serum lipid profile in diabetic rats**

Data in Table 1 demonstrate that there was a significant increase in serum glucose level noted in diabetic rats as compared to normal control. However, treatment with both low and high dose of Mesozeaxanthin did not produce any significant anti hyperglycemic effect in STZ treated rats. The increase in serum total cholesterol, triglycerides and decrease in HDL were

also observed in diabetic rats. Treatment with both low dose and high dose of mesozeaxanthin did not produce any significant change in case of serum lipids in diabetic rats.

#### **Effect of mesozeaxanthin on Kidney weight/body weight %, renal collagen content, renal oxidative stress**

Table 2 demonstrate that there was a significant increase in KW /BW % and renal collagen content, noted in STZ treated rats, as compared with normal rats. Treatment with both low and high dose of mesozeaxanthin showed no significant decrease in case of kidney weight/body weight, but a significant decrease was observed in renal collagen content in diabetic rats. A significant increase in TBARS and significant decrease in reduced glutathione was observed in diabetic rats. Treatment with low dose and high dose produced a dose dependent significant increase in TBARS and a dose dependent significant decrease in reduced glutathione in diabetic rats.

**Effect of mesozeaxanthin on BUN, serum creatinine and creatinine clearance:** The BUN and serum creatinine were noted to be significantly increased in diabetic rats, as compared to normal control. Treatment with both low and high dose of mesozeaxanthin significantly decreased BUN and serum creatinine in STZ treated rats. The creatinine clearance was also noted to be significantly decreased in diabetic rats as compared with age matched normal rats. Treatment with both low and high dose mesozeaxanthin significantly increased creatinine clearance in diabetic rats. However, in all above these parameters, there was no significant difference between low and high dose mesozeaxanthin treated diabetic rats (Figure 1, 2, 3, 4).

**Table 1: Effect of mesozeaxanthin low dose and high dose on Serum Glucose, Serum cholesterol, Serum triglycerides, Serum HDL on 56<sup>th</sup> Day.**

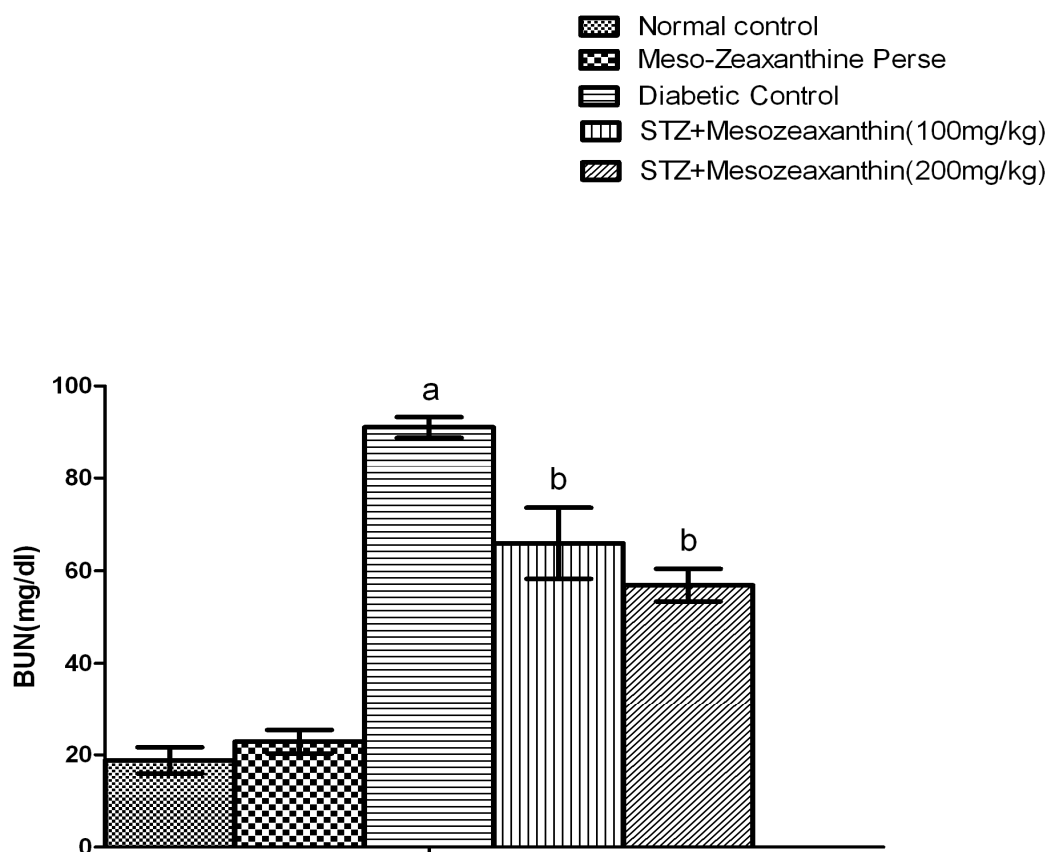
Groups	Serum Glucose (mg/dl)	Serum cholesterol (mg/dl)	Serum triglycerides (mg/dl)	Serum HDL (mg/dl)
Normal control	92.77±7.25	42.7±4.70	71.2 ± 7.50	33.8 ± 4.27
Mesozeaxanthin perse in normal rats	95.66±7.79	44.6±4.62	80.9±6.75	36.67±4.13
Diabetic control	451.81±50.7 <sup>a</sup>	51.05±6.97	188.08±24.21 <sup>a</sup>	24.20±3.64 <sup>a</sup>
Mesozeaxanthin (100mg/kg)	400 ±25.92	46.49 ± 6.68	171.78± 24.9	27.21± 3.86
Mesozeaxanthin (200mg/kg)	377.5±27.13	43.72±5.93	167.38±24.85	28.48± 4.45

All values are expressed as mean ± S.D. a = p<0.05 vs. normal control.

**Table 2 : Effect of mesozeaxanthin low dose and high dose on renal TBARS, Glutathione, Kw/Bw % and renal collagen content on 56<sup>th</sup> day.**

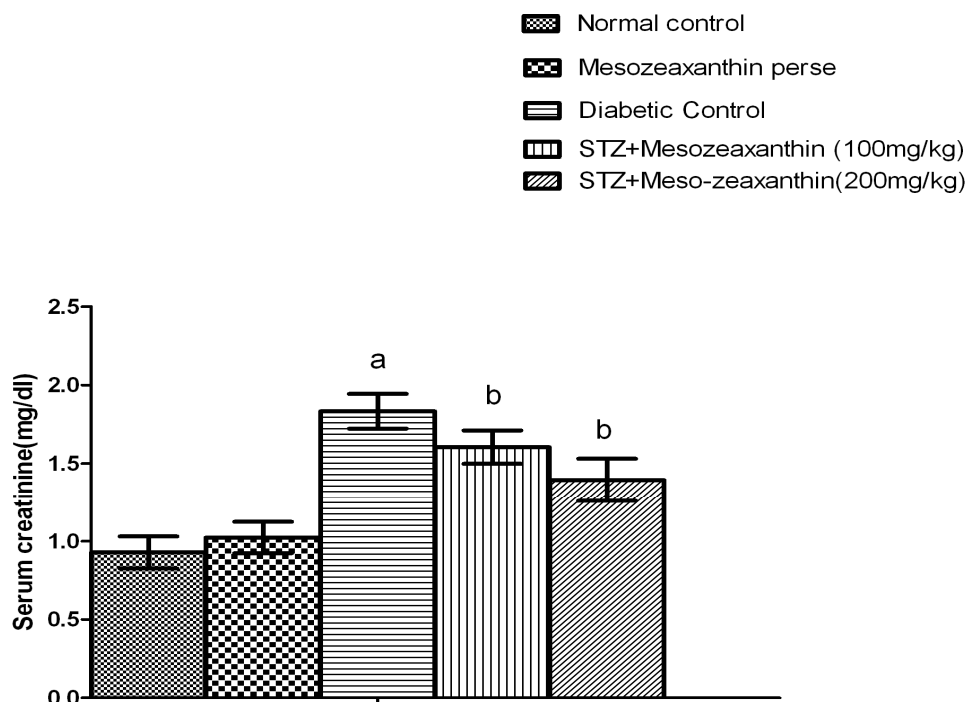
Groups	TBARS (nmol/mg tissue)	Glutathione (nmol/mg tissue)	Kidney weight / Body weight%	Renal collagen content (mg/g of renal cortex)
Normal control	0.53 ± 0.09	22.33 ± 1.21	0.26 ± 0.04	2.94 ± 0.58
Mesozeaxanthin perse in normal rats	0.54±0.09	22.42±1.18	0.29±0.04	3.03 ± 0.64
Diabetic control	1.15±0.11 <sup>a</sup>	12.41±1.33 <sup>a</sup>	0.60±0.08 <sup>a</sup>	5.58 ± 0.32 <sup>a</sup>
Mesozeaxanthin (100mg/kg)	0.82 ± 0.03 <sup>b</sup>	17.05± 0.68 <sup>b</sup>	0.59 ± 0.06	4.80 ±0.30 <sup>b</sup>
Mesozeaxanthin (200mg/kg)	0.68±0.06 <sup>c</sup>	19.29±0.66 <sup>c</sup>	0.51± 0.06	04.61± 0.38

All values are expressed as mean ± S.D. a = p<0.05 vs. normal control; b = p<0.05 vs. diabetic control, c=p<0.05 vs. low dose

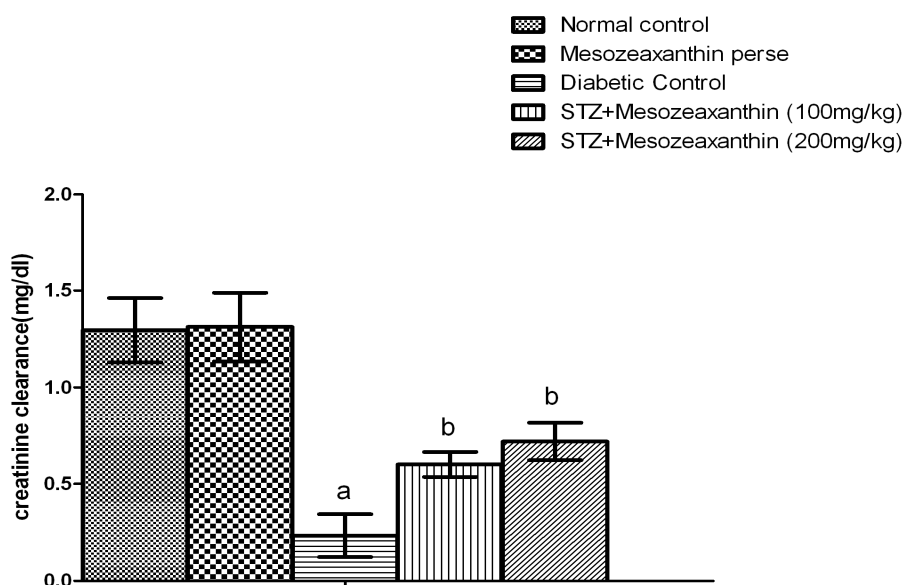


**Figure 1: Effect of low dose and high dose mesozeaxanthin on Blood urea nitrogen (BUN).** One group was fed with normal diet other with mesozeaxanthin perse, Diabetic control, Low dose mesozeaxanthin and High dose of mesozeaxnthin. All values are expressed as mean ± S.D. a = p<0.05 vs. normal control, b = p<0.05 vs. diabetic control.





**Figure 2: Effect of low dose and high dose mesozeraxnthin on Serum creatinine.** One group was fed with normal diet other with mesozeaxanthin perse, Diabetic control, Low dose mesozeaxanthin and High dose of mesozeaxnthin. All values are expressed as mean  $\pm$  S.D. a =  $p < 0.05$  vs. normal control, b =  $p < 0.05$  vs. diabetic control.



**Figure 3: Effect of low dose and high dose mesozeraxnthin on Creatinine clearence.** One group was fed with normal diet other with mesozeaxanthin perse, Diabetic control, Low dose mesozeaxanthin and High dose of mesozeaxnthin. All values are expressed as mean  $\pm$  S.D. a =  $p < 0.05$  vs. normal control, b =  $p < 0.05$  vs. diabetic control.



## DISCUSSION

The major findings of this study are that treatment with mesozeaxanthin for the first time attenuates STZ- induced DN in wistar rats as evidenced by a decrease in serum creatinine, BUN and increased creatinine clearance. Further, mesozeaxanthin treated also reduced renal fibrosis and renal oxidative stress in diabetic rats. It is well documented that hyperglycemia-induced oxidative stress in diabetes plays independent role for development and progression of diabetic microvascular complications such as nephropathy <sup>[23]</sup>. They are continuously produced and degraded normally to maintain homeostasis, but generation of high concentrations of ROS such as superoxide anion, H<sub>2</sub>O<sub>2</sub>, hydroxyl radicals and peroxynitrite by hyperglycemia may cause injury to various organs, including kidney. Most of the ROS are generated during mitochondrial oxidative phosphorylation and small amounts via NADPH-oxidase system <sup>[6]</sup>. ROS activates PKC <sup>[24]</sup>, ERK/MAPK <sup>[25]</sup>, JAK/STAT <sup>[26]</sup> and transcription factors NF-κB <sup>[27]</sup>, activating protein-1 (AP-1) <sup>[28]</sup>, and upregulates TGF-β1 leading to deterioration of the function of diabetic kidney <sup>[29-30]</sup>. Antioxidants, effectively inhibit high glucose-induced over expression of TGF-β and reduce the oxidative stress by increasing the levels of intracellular antioxidants such as superoxide dismutase, catalase etc <sup>[31-32]</sup>. Various evidences suggest that ROS-regulated signaling pathways lead to extracellular matrix (ECM) deposition in diabetic kidney which consequently produced DN <sup>[5]</sup>. Meseaxanthin, treatment for the first time significantly attenuate various markers of DN such as increase in BUN and serum creatinine and decrease in creatinine clearance possibly through its antioxidant potential. The above study concludes that single administration of STZ produced hyperglycemia, dyslipidemia and oxidative stress which subsequently produced nephropathy in rats and mesozeaxanthin shows renoprotective effect due to its antioxidant property, since this protective effect was not associated with any significant decrease in serum glucose, triglycerides and cholesterol levels or an increase in HDL levels.

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