Nephroprotective effects of β-carotene on ACE gene expression, oxidative stress and antioxidant status in thioacetamide induced renal toxicity in rats

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Abstract: β-carotene is one of carotenoid natural pigments, which are produced by plants and are accountable for the bright colors of various fruits and vegetables. These pigments have been widely studied for their ability to prevent chronic diseases and toxicities. This study was designed to evaluate the effects of β-carotene on angiotensin converting enzyme (ACE) gene expression, oxidative stress and antioxidant status in thioacetamide induced renal toxicity. Total 24 albino wistar rats of male sex (200-250gm) were divided into 6 groups as Group-1: The control remained untreated; Group-2: Received thioacetamide (200mg/kg b.w; i.p) for 12 weeks; Group-3: Received β-carotene orally (200mg/kg b.w), for 24 weeks; and Group-4: Received thioacetamide (200mg/kg b.w; i.p) for 12 weeks + received β-carotene orally (200mg/kg b.w), for further 12 weeks. The expression of ACE gene in thioacetamide induced renal toxicity in rats as well as supplemented with β-carotene was investigated and compared their level with control groups by using the quantitative RT-PCR method. The ACE gene expression was significantly increase in TAA rats as compare to control rats specifies that TAA induced changes in ACE gene of kidney, elevated renal ACE has been correlated with increase hypertensive end organ renal damage. The quantity of ACE gene were diminish in our rats who received β-Carotene after TAA is administered, for this reason they seemed to be defended against increased ACE levels in kidney bought by TAA. In pre- and post-treatment groups, we studied the role of β-Carotene against thioacetamide in the kidney of Wistar rats. Experimental confirmation from our study illustrates that β-Carotene can certainly work as a successful radical-trapping antioxidant our results proved that TAA injury increased lipid peroxidation and diminish antioxidant GSH, SOD and CAT in renal tissue. Since β-Carotene administration recover renal lipid peroxidation and antioxidants, it give the impression that β-Carotene protects renal tissue against thioacetamide-induced oxidative damage.

Keywords: β-carotene, ACE gene expression, ACE gene expression, oxidative stress, cirrhosis.

INTRODUCTION

Thioacetamide (TAA), a particular hepatotoxin, to provoke hepatic failure (Albrecht *et al*., 1990) within a short phase of time following the administration of the TAA. Thioacetamide-*S*-dioxide is a very extremely reactive compound (Hunter *et al*., 1977). Its binding to tissue macromolecules possibly will induce hepatic necrosis (Porter and Neal, 1978), hyperammonemia (Butterworth, 2002) along with oxidative stress (Bruck *et al*., 1999). Toxicity of thioamide in mammals is reliant on metabolism of the complex by means of chronological oxygenations of the thioacetamide sulfur atom through flavoprotein mono-oxygenases or cytochromes P4502. Free radical development is claim on a variety of compounds together with thioacetamide (Buko *et al*., 1997) the kidney is extremely at risk to toxicants for two reasons. A high quantity of blood flows all the way through kidney and it sort outs bulky amounts of toxins capable of concentrate within kidney tubules end results in complete toxicity causing: diminish capability to emit body wastes, lack of ability to uphold body fluid as well as electrolyte balance furthermore reduce synthesis of fundamental hormones (Oduola *et al*., 2010).

Over 600 carotenoids are found in nature, beta-carotene being the most excellent recognized (Woutersen *et al*, 1999) Around 50 carotenoids has been investigated in the human diet and 20 have been identified in plasma along with other tissues (Faure *et al*., 1999). Researches are being accomplished internationally to spot defensive molecules with the intention of defending the kidney in addition to organs with a small number of refusal end products (Zaher *et al*., 2007). B-Carotene has been reflection of significance to humans and additional species not simply as precursor to vitamin A, but furthermore for having admirable antioxidant properties also perform as an immune modulator, quench singlet oxygen, and lessen peroxyl radicals at a low partial oxygen pressure (Wang and Russell, 1999).

Angiotensin converting enzyme (ACE), known as dipeptidyl carboxypeptidase, is a important enzyme of the renin angiotensin system (RAS) with the purpose of converting angiotensin (Ang) I to II. (Crackower *et al*., 2002) The significance of this enzyme is also evident

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from the current researches with the ACE knockout explaining that male rat developed renal pathological injuries (Oudit *et al*, 2006). Additional studies describes important role of this enzyme in defending against lung injury (Imai *et al*., 2005) and an exaggerated blood pressure increase after Ang II infusion. (Gurley *et al*., 2006) For the reason that the RAS participates a key responsibility in renal injury, (Taal and Brenner, 2000) and are concentrated within distinct glomerular structures. (Wysocki *et al*, 2006) The wide distribution and multifunctional properties of these peptides recommend that ACE possibly will be engaged in various pathophysiological states. (Tabatabaei *et al*, 2006). Enhanced renal ACE, in both rodents and human, has been comprehensively deliberate in association through the progression of renal as well as cardiovascular disease (Pedersen *et al*., 2009). In rats, high renal ACE has been associated with the increased defenselessness to hypertensive end organ renal damage. (Mizuiri *et al*., 2001). Hence, in continuation of investigations designed for potential modulators of thioacetamide mediated renal damage, we have observed the effects of β-Carotene, a naturally occurring carotenoids on experimentally induced rats by estimating renal function test (urea and creatinine), oxidative stress (MDA), antioxidant status (SOD, CAT and GSH) and to discover the ACE gene expression following treatment with β-Carotene.

MATERIALS AND METHODS

Animal and diet

Adult male albino wistar rats weighing 200-250gm were utilized in this study which were obtained from animal house ICCBS (International Center for Chemical and Biological Center, University of Karachi). They were kept in separate polypropylene cages and permit food and water. They were reserved in appropriate air flow and temperature for the period of whole experimentation.

Study design

Experimental phase of our research was 24 weeks. Animals were split into 4 groups (n = 6) and received the following treatments

Group 1: The control remained untreated.

Group 2: Received thioacetamide (200mg/kg b.w; i.p) for 12 weeks, twice in a week.

Group 5: Received β-Carotene orally (200mg/kg b.w), for 24 weeks, 4 times a weeks.

Group 6: Received thioacetamide for 12 weeks, twice in a weeks + received β-Carotene orally, four times a weeks.

Sample collection

By the closing stages of the dealing rats were decapitated and blood was sampled in the lithium heparin coated tubes. A part of blood was taken in the separate test tube to gather serum. Kidney were removed, trimmed, soak by means of deionized water to get rid of blood infectivity, dried out by blotting with filter paper and weighed. The tissues were than reserved in -70°C freezer until examination.

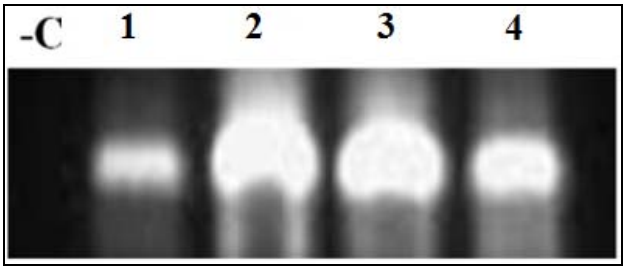

Fig. 1: Agarose gel electrophoresis showing the amplification for ACE gene in kidney tissue of: Controls (Group 1), TAA induced cirrhotic (Group 2), β-carotene treated (Group 3) and TAA+β-carotene treated rats (Group 4).

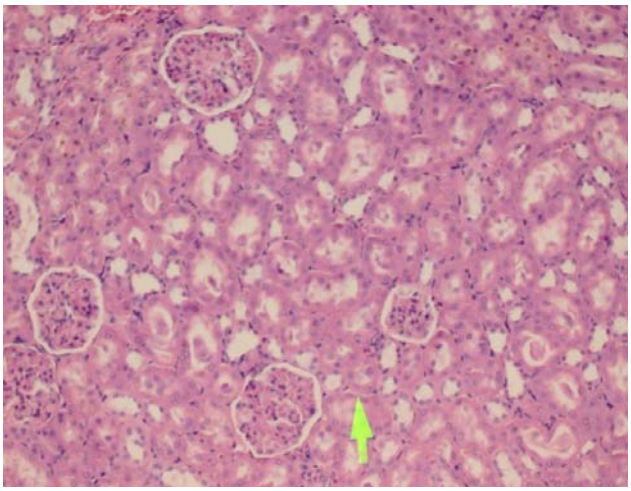

Fig. 2: Slice in kidney of control rat showing normal structure of kidney.

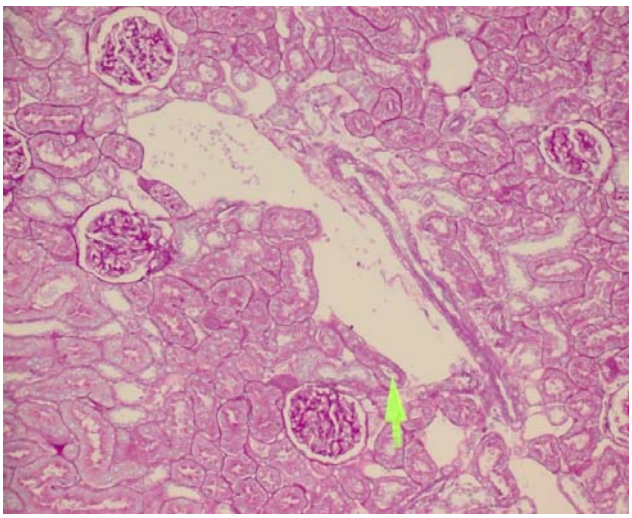

Fig. 3: Slice in kidney of rat treated with TAA showing area of internal hemorrhage with hemolysed blood corpuscles.

Table 1: Effect of β-Carotene and TAA on body and kidney weights.

Weights	Control	TAA	β-Carotene	TAA + β-Carotene	ANOVA	
					F	P
Body	230.20±26.11	171.75±19.5[a]	222.50±14.82[b]	188.16±7.17[c]	55.35	<0.001
Kidney	0.63±0.08	0.59 ±0.15[a]	0.61±0.07[b]	0.61±1.2[c]	82.62	<0.001

Table 2: Effects of β-Carotene and TAA on renal functions.

Renal Functions (mg/dl)	Control	TAA	β-Carotene	TAA+ β-Carotene	ANOVA	
					F	P
Urea	0.304±0.05	0.326±0.01[a]	0.216±0.03[b]	0.323±0.01[c]	13.79	<0.001
Creatinine	0.293±0.03	0.370±0.01[a]	0.261±0.01[b]	0.318±0.02[c]	17.46	<0.001

Table 3: Effects of β-Carotene and TAA on oxidative stress and antioxidant status of renal tissues.

Parameters	Control	TAA	β-Carotene	TAA+ β-Carotene	ANOVA	
					F	P
MDA (nmol/gm/sec)	10.70+0.85	27.74+1.18[a]	46.01+0.84[b]	22.61+0.98[c]	1386.27	<0.001
SOD (μ/gm)	98.09+1.07	75.9+1.61[a]	89.02+4.34[b]	91.5+1.62[c]	141.897	<0.001
GSH (nm/mg)	0.90+0.03	0.51+0.03[a]	0.91+0.04[b]	0.85+0.04[c]	610.48	<0.001
CAT (mmol/gm)	4.73+0.29	3.00+0.15[a]	4.12+0.06[b]	3.51+0.04[c]	102.89	< 0.001

Values are mean ±SEM; n=6 animals per group, 24 days of treatment. a= significantly different as compare to control, b= significantly different as compare to control, c= significantly different as compare to TAA

Table 4: ACE gene expression in kidney tissues.

Control	TAA	β-Carotene	TAA+ β-Carotene
0.66±0.05	3.39±1.65	0.98±0.06	2.24±0.33

Histological examination (figs. 2-5)

Left kidney was speedily eliminated, submerged in 10% formalin. The kidneys were then fixed for 2hours in a solution containing (150ml ethanol, 60ml formaldehyde, 15ml acetic acid, and 1g picric acid). As a result, the samples were included in phosphate-buffered formaldehyde until they were fixed in paraffin, sectioned at 3μm, stained with hematoxylin and eosin (H/E), and estimated by light microscope to study the constitution of the kidney (Ricardo *et al*, 2005).

Assessment of oxidative stress

The malondialdehyde, (MDA), a quantify of lipid per oxidation, was assayed in the form of thiobarbituric acid reacting substance (TBARS) by the use of tissue homogenate. (Ohkawa *et al*, 1976).

Assessment of antioxidant status

Level of superoxide dismutase (SOD) in the cell free supernatant was quantify by (Kono, 1978) Level of glutathione peroxidase (GSH) was estimated by (Calberg and Mannervik, 1985). Level of catalase (CAT) activity was assayed by (Sinha, 1972).

DNA isolation and genotyping

Genomic DNA was cut off from tail tips as formerly illustrated by (Korstanje *et al*. 2004). To resolve the ACE genotypes, primers were utilized as explain by (Hilbert *et al*. 1991). They magnify the micro satellite position at the 50 end of the intron between exons 13 and 14.

ACE gene expression in kidney

The expression of ACE gene in kidney tissue was assessed by means of the quantitative RT-PCR technique. RNA was separated by using entire RNA Prep Plus. In short, amplification reaction was carry out in 12.5μl total volume, holding a couple of exact primer: 5'CAGCTTCATCATCCAGTTCC3' and 5'CTAGGAAGAGCAGCACCCAC. PCR program consisted 30 cycles at an annealing temperature of 52-64°C. Restriction remains were afterward analyzed in 2% agrose gel stained with the assist of ethidium bromide (Sulikowski *et al*, 2011)

STATISTICAL ANALYSIS

Statistical analysis was achieved via the statistical software package (SPSS®). To evaluate differences in statistics, one-way analysis of variance (one-way ANOVA) was carry out followed by Student's t-test using the Bonferroni correction for multiple comparisons. The level of statistical significance was set at $P<0.05$. All results are expressed as mean ±SEM.

RESULTS

The result of supplementary β-Carotene increases body weight, food intake and physical activity as compare to control group. No significant increase in kidney weights of control β-Carotene and its treatment, it shows that β-Carotene does not produce much effect on kidney weights (table 1). Significant results are found ($P<0.001$) in urea and creatinine levels after pretreatment of β-Carotene in TAA treated rats (table 2). Decreased antioxidant enzymes level SOD was observed in this study after thioacetamide administration (table 3). This study was broadly aimed to find out the expression of ACE in rats distress from TAA and their cure with β-Carotene along with evaluate their level with control groups by means of the quantitative RT-PCR method. In present study, it is found that ACE gene expression was significantly increase in TAA group as compare to control group shows that TAA induced alteration in ACE gene of kidney, producing bulky quantity of ACE protein then the standard gene In rats (table 4, fig. 1) high renal ACE has been correlated with the increased susceptibility to hypertensive end organ renal damage (Liu *et al*., 2009). Whereas for the duration of nephrosis development, a positive correlation existed involving renal ACE activity, proteinuria, glomerular and interstitial injury. The number of total ACE were decreased in our rats who received β-Carotene after TAA is administered, hence they seemed to be protected against increased ACE levels in kidney bought by TAA (Oosten *et al*, 2002).

DISCUSSION

It is sound initiate that human patients as well as investigational mammals bare thioacetamide exhibit biochemical indications of oxidative damage. Facts have point towards those free radicals or ROS such as SOD, GSH and CAT are involved in TAA stimulated oxidative tissue injury. Increase lipid per oxidation combine with depletion of antioxidants in liver and kidney is a characteristics inspection in TAA induced rats. (Al-Bader *et al*, 1999) Renal failure has been examined in end-phase of cirrhosis due to severe renal vasoconstriction that often develops in patients having cirrhosis. (Schrier *et al*, 1998), (Natarajan *et al*, 2006).

In the present work, the effect of β-Carotene on the development of TAA-induced liver fibrosis in rats were studied. β -Carotene, in addition to having the strongest provitamin A activity of all carotenoids, is an efficient scavenger of peroxyl radicals, especially in low oxygen tension (Kennedy and Liebler, 1991).

TAA administration shows increase in urea and creatinine level, this increase be evidence for insufficiency of renal function. Studies have established that tubular injury plays a fundamental role in the decline of glomerular filtration rate in severe tubular necrosis (Ozen *et al*, 2004). Malondialdehyde (MDA), a biomarker of lipid per oxidation. Assays were built up to quantify MDA as a thiobartituric acid in the study of cancer. β-Carotene has been exposed to TAA induced rats to restrain lipid peroxidation by working as a scavenger of singlet oxygen and quencher of peroxy radicals at low oxygen concentrations. It confirms that β-Carotene was functioning as an antioxidant in the current study (Burton and Ingold, 1984).

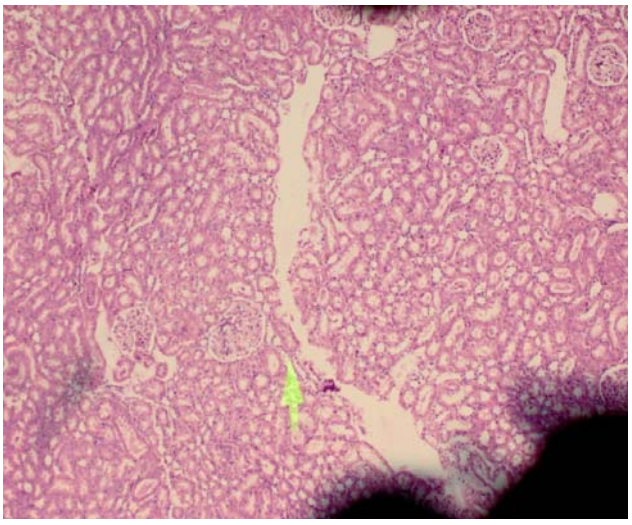

Fig. 4: Slice in kidney of rat treated with β-Carotene showing faint staining glomeruli with less stained Bowman's capsules and convoluted tubules.

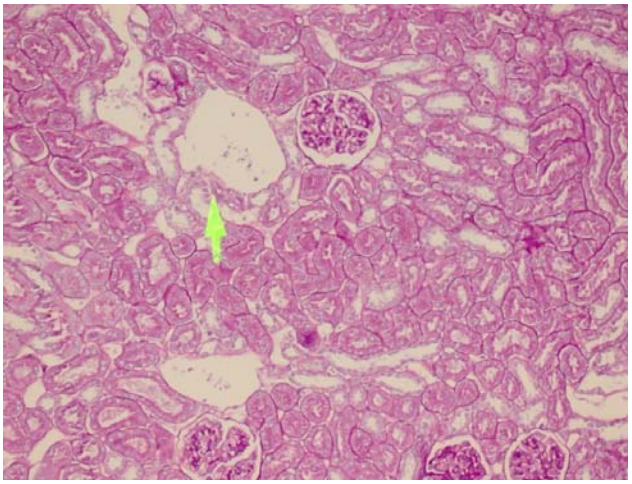

Fig. 5: Slice in kidney of rat treated with β-Carotene, shows reaction in most tubules and glomeruli.

In living creatures a mixture of enzymes (catalase, glutathione, and peroxidase) functions as defensive antioxidants through destroying hydrogen peroxides devoid of producing free radicals (Burton and Ingold, 1984). Super oxide dismutase (SOD) is generated in intracellular enzymes present in every cell in the body. SOD is considered fundamental in the route of removing

ROI by reducing superoxide to form H_2O_2 (Stief, 2003). β-Carotene treatment reverses thioacetamide induced decline in SOD activity in the kidney of rats. Catalase and glutathione peroxidase search for hydrogen peroxide and transfer it to water and diatomic oxygen. (Wasim *et al*, 2008). There is increase in CAT activity of β-Carotene on TAA induced kidney as compare to control which indicates antioxidant status and increase organism antioxidant system efficiency. It have been revealed that thioacetamide lower the activity of the GSH in the renal mitochondria following prolonged treatment results in increase GSH levels in the group supplemented with β-Carotene.

Experimental confirmation from our laboratory illustrates that β-Carotene can certainly work as a successful radical-trapping antioxidant our results proved that TAA injury increased lipid per oxidation and diminish antioxidant GSH, SOD and CAT in renal tissue. Since β-Carotene administration recover renal lipid per oxidation and antioxidants, it give the impression that β-Carotene protects renal tissue against thioacetamide-induced oxidative damage.

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