ABSTRACT

Increased number of population with renal damage is attributed to sedentary life style and consumption of high-sugar diets especially fructose. The purpose of present study is to investigate the renoprotective activity of aqueous extract of *Phyllanthus amarus* (PAAEt) against high fructose diet (HFD) induced renal damage (RD) in Wistar rats. High fructose diet (66% fructose) alone and in combination with PAAEt (200 mg/kg/body wt/day) was given simultaneously to group-F and group-F+T rats respectively for a period of 60 days. Functional markers of renal tissue such as, urea, uric acid and creatinine levels in plasma were quantified on initial and final day of the experiment. Activities of transaminases, gluconeogenic enzymes such as, glucose-6-phosphatase (G-6-Pase), and fructose-1, 6-bisphosphatase (F-1,6-BPase) and polyolpathway enzymes such as, aldose reductase and sorbitol dehydrogenase were assayed in kidney. Oxidative stress (OS) markers such as, lipid peroxidation and protein oxidation and antioxidants status were measured in renal tissue. Renal histopathological changes were also examined. Co-treatment with PAAEt to group-F+T prevented the rise in the levels of functional markers and elevated activities of transaminases, gluconeogenic and polyolpathway enzymes of group-F (\(P < 0.05\)). OS developed in group-F by elevated stress markers and depletion of antioxidants were prevented in group-F+T. The observed histological changes in group-F were protected in group-F+T. Group-C+T showed no histological changes with group-C. Thus, PAAEt effectively alleviated fructose diet induced RD which may be due to its antioxidant activity. Hence this plant could be used as an adjuvant therapy for the prevention and/or management of HFD induced RD.

KEYWORDS: High-fructose diet, Oxidative stress, *Phyllanthus amarus*, Renal damage.
INTRODUCTION

Fructose (monosaccharide) consumption has largely increased over the last three decades most likely as a result of increased use of high fructose corn syrups (HFCS) which contain 55 to 90% fructose. HFCS are quite commonly found in soft drinks and juice beverages and are incorporated into many convenient pre-packaged foods (Bray et al., 2004). HFCS are inexpensive, sweeter and mixes well with many foods which make them to prefer over sucrose by processed-food manufacturers. Its consumption increased from less than 0.5 g/day in 1970 to more than 40 g/day in 1997 (Gaby, 2005).

Kidneys are dynamic organs which represent major control system in maintaining the body homeostasis. After the liver, kidney is the second most frequently affected organ by any compound (Marshall, 2000) and in metabolic disorders. High-fructose consumption has been reported to facilitate renal damage (RD) in normal rats (Gersch et al., 2007; Rajasekhar et al., 2008). In such rats high-fructose diet has a dverse effects on renal morphology and biochemical function leading to enhanced levels of urea, uric acid and creatinine in the plasma. The diabetic kidney disease affects about 15 to 25% of type 1 diabetic patients (Hovind et al., 2003) and 30 to 40% of patients with type 2 diabetes (Ritz et al., 1997; Yokoyama et al., 2000). The costs attributed to RD have risen from 4.8% a decade ago to 6.3% of all Medicare expenditures, consuming a total of $22.83 billion currently (El-Atat et al., 2004). Therefore, intervention for preventing and delaying the development and progression of diabetic nephropathy causing RD is not only a medical concern but also a social issue. The exact underlying mechanism for RD caused by fructose consumption remains unclear. However, many diseases associated with changes in the oxidative status in human beings are well documented. The antioxidants are effective in preventing majority of the abnormalities induced by HFD (Faure et al., 1997). Clinical trials suggest that there is no effective treatment for diabetic nephropathy and hence efforts have been focused on traditional herbal medicine to find novel therapeutic agents for treatment of diabetic nephropathy (Kang et al., 2006).

Phyllanthus amarus (P. amarus) belonging to Euphorbiaceae family, is a small, annual, many branched herb commonly known as Stone breaker in English, which is distributed in tropical and subtropical regions worldwide including Southern India and China. It has been widely used in folk medicine for the treatment of various diseases and disorders, such as jaundice, kidney ailments and diabetes etc. It is reported to possess diuretic, hypotensive, hypoglycemic effect, antihyperlipidemic, antihepatotoxic and antioxidant activity (Calixto et al., 1998). Moreover, aqueous extract of P. amarus exhibited significant strong antioxidant potential than ethanolic, methanolic and acetone extracts (Calixto et al., 1998; Krithika et al., 2009; Kumaran and Karunakaran, 2007; Shokunbi and Odetola, 2008). Hence PAAEt might protect against RD caused by high fructose consumption. However this has not been studied so far. Therefore we investigated the effects of PAAEt on renal function and histology as well as antioxidant status in rats fed with high-fructose diet.

MATERIALS AND METHODS

Animal maintenance and experimental setup

Six to eight week old (170-180g) male Wistar rats (n = 24) were procured from Sri Venkateswara Enterprises (Bangalore, India). Animals were kept (3 rats per cage, 41x28x14
cm) in an animal facility in a room with a 12-h light/dark cycle at an average temperature of 22 ± 2° C and humidity of 60% (Regd. No. 470/01/a/CPCSEA). Animals were assigned to a control group (n = 6) that consumed a control diet, a high fructose-fed group (n = 6) that received 66% fructose, a high fructose-fed treated group (n = 6) that consumed 66% fructose and PAAEt administered orally by gastric intubation (200 mg/kg body wt/day) and control treated group (n = 6) that received control diet and PAAEt (200 mg/kg wt/day) for sixty days. Animals had free access to food and water throughout the study. The composition of control diet contained 66% starch (replaced with fructose in fructose diet), 15% protein, 8% fat, 4% cellulose, 1% each of mineral and vitamin mix supplied by National Centre for Laboratory Animal Sciences, National Institute of Nutrition (Hyderabad, India). All the procedures were performed in accordance with the Institutional Animal Ethics Committee recommendations.

**Plant extract**

An aqueous extract of *Phyllanthus amarus* (brown, dry powder with Lot No.L5111031) was procured from Chemiloids (manufacturers and exporters of herbal extracts, Ms. Plantex Pvt. Ltd.), Vijayawada, Andhra Pradesh (India). Prior to preparation of the plant extract, the plant was authenticated by Dr. Narasimha Reddy, Taxonamist, Laila Implex R&D Centre, Vijayawada. The extract was stored in an air-tight container and dissolved in water just before use.

**Blood and Kidneys collection**

On initial and final day of the experiment, blood was collected from 12-h fasted rats with capillary tube from retino-orbital plexus, after adding EDTA (10 mg/ml) was centrifuged. Plasma was collected and used for the estimation of creatinine, urea and uric acid. At end of the experimental period, the animals were fasted overnight and killed by cervical decapitation. The kidneys were excised into ice cold saline and then thoroughly rinsed.

**Functional markers and transaminases determination**

Renal functional markers such as, urea, uric acid and creatinine were quantified using commercial kits. (Excel Diagnostic kits, Hyderabad, India). The renal transaminases such as glutamate oxalo acetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) were measured spectrophotometrically at 520 nm using 2, 4-dinitrophenyl hydrazine (Reitman et al., 1957).

**Activities of renal gluconeogenic and Polyol pathway enzymes**

Kidney homogenate (10%) was prepared in ice cold 0.1 M Tris-HCl buffer pH 7.4, and centrifuged (12,000 rpm for 45 min). From the obtained supernatant, activities of Glucose-6-phosphatase (E.C 3.1.3.9 ) and Fructose 1,6 bis phosphatase (E,C 3.1.3.11) were determined (King, 1965; Sadava et al., 1997). By the method of Hayman and Kinoshita, (1965) aldose reductase (AR; E.C 1.1.1.21) and by the method of Asada and Galiambos, (1963) sorbitol dehydrogenase (SDH; E.C 1.1.1.14) activities were measured spectrophotometrically at 340 nm.

**Stress markers (lipid peroxidation and protein oxidation) estimation**

The extent of lipid peroxidation (LPO) was determined by assaying malondialdehyde (MDA) formation according to method of Utley et al, (1967). Protein carbonyl (protein oxidation, PO) content was measured by formed labeled hydrazone derivatives using 2, 4-
DNPH which were quantified spectrophometrically at 370 nm according to the method of Levine et al, (1990).

**Enzymatic antioxidants and reduced glutathione (GSH) determination**

Tissue homogenate (10%) was prepared in ice-cold 0.15 M KCl, and centrifuged (12,000 rpm for 45 min). The clear supernatants thus obtained were used for the assay of antioxidant enzymes namely superoxide dismutase (SOD; Soon and Tan, 2002), catalase (CAT; Beers and Sizer, 1952), glutathione peroxidase (GPx; Rotruck et al., 1973), glutathione-S-transferase (GST; Habig et al., 1974) and glutathione reductase (GR; Pinto and Bartley, 1969). Reduced glutathione (GSH) was determined according to the method of Ellman (1959) by using 5,5-Dithiobis (2-nitrobenzoic acid). The protein concentration of tissue homogenate was measured by the method of Lowry et al, (1951).

**Histological examination**

From each group, kidney samples were prepared for histopathological assessment by placing in 10% formalin. Each specimen was fixed in 10% formalin solution embedded in paraffin wax. Sections of 4-5μm in thickness were stained with hematoxylin and eosin and later examined under a light microscope.

**Statistical analysis**

All the data presented are means ± SEM for 6 rats per group as indicated in the figures and tables. To determine the statistical significance of clinical and laboratory findings Duncan Multiple Range test (DMRT) was used. A difference with P <0.05 was considered statistically significant.

**RESULTS**

**Renal functional markers and transaminases levels**

No significant variation was found in plasma urea, creatinine and uric acid concentrations among the four experimental groups of rats at base line (initial day) values of experimentation whereas on the final day of the experiment only group-F showed significantly higher concentrations of urea (31.30%), creatinine (18.02%) and uric acid (58.49%) compared to group-C (Table 1).

**Table 1:** Levels of renal functional markers in experimental groups of rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Days</th>
<th>C</th>
<th>C+T</th>
<th>F</th>
<th>F+T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>Initial</td>
<td>31.59±0.16</td>
<td>32.35±0.31</td>
<td>32.45±0.42</td>
<td>31.57±0.25</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>32.50±0.30</td>
<td>31.88±0.18</td>
<td>42.61±0.43*</td>
<td>33.05±0.40</td>
</tr>
<tr>
<td>Uricacid mg/dl</td>
<td>Initial</td>
<td>2.01±0.05</td>
<td>2.05±0.06</td>
<td>2.04±0.03</td>
<td>2.02±0.04</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>2.53±0.06</td>
<td>2.48±0.09</td>
<td>4.01±0.1*</td>
<td>2.64±0.08</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>Initial</td>
<td>0.703±0.04</td>
<td>0.691±0.03</td>
<td>0.688±0.05</td>
<td>0.700±0.02</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>0.701±0.01</td>
<td>0.692±0.03</td>
<td>0.812±0.04*</td>
<td>0.698±0.02</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM (n = 6). C, control rats; C+T, control rats treated with PAAEt; F, fructose fed rats; F+T, fructose fed rats treated with PAAEt; * Significant as compared to group-C (P < 0.05 DMRT).
Group-F illustrated significantly increased activities of renal GOT (52.38%) and GPT (69.09%) when compared with group-C. Treatment with PAAEt to fructose fed rats (group-F+T) decreased the activities of transaminases significantly in kidney and brought to control level where the activities of these enzymes in group-C+T were not significantly altered when compared to control rats (P < 0.05; Fig. 1).

Activities of renal gluconeogenic and polyol pathway enzymes

As compared with group-C, fructose fed group showed significantly higher activities of gluconeogenic enzymes i.e. G6-Pase (25.11%) and F1, 6-Bpase (61.84%) and polyol pathway enzymes i.e., AR 20.09% and SDH 22.40% when compared with groups C. Co-treatment of fructose-fed rats with PAAEt (group-F+T) prevented the rise in the activities of both gluconeogenic and polyol pathway enzymes whereas group-C+T did not deviate from group-C (P < 0.05; Table.2).

Renal stress markers and antioxidants levels

Table 3 summarizes the levels of MDA, GSH, protein carbonyl groups and activities of enzymatic antioxidants SOD, CAT, GST, GPx and GR in the kidney of all experimental animals. Group-F showed significantly higher levels of TBARS (55.12%) and protein carbonyl groups (21.67%) when compared to group-C rats. In F+T-group, elevated LPO was limited to the depletion of only 11.91% whereas protein oxidation was brought to normal when compared to group-C. Group-F showed depleted renal GSH (21.30%) levels when compared to control group, but treatment with PAAEt completely restored to control level in group-F+T. The activities of enzymatic antioxidants SOD, CAT, GST, GPx and GR were significantly lowered (23.807%, 28.25%, 35.73%, 28.07% and 19.84%, respectively) in group-F rats compared to group-C rats. In F+T-group, enzymatic antioxidants were restored to near normal level when compared to control group. Stress markers and antioxidants of group-C+T did not differ significantly when compared with C-group.

Figure 1: Activities of renal transaminases in four experimental groups of rats. Values are presented as mean ± SEM (n = 6 rats/group). C, control rats; C+T, control rats treated with PAAEt; F, fructose fed rats; F+T, fructose fed rats treated with PAAEt; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; * Significantly different from group-C (P < 0.05 DMRT). # Significantly different from group-C (P < 0.05 DMRT).
Table 2: Activities of gluconeogenic and polyol pathway enzymes in kidney of four experimental groups of rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C</th>
<th>C+T</th>
<th>F</th>
<th>F+T</th>
</tr>
</thead>
<tbody>
<tr>
<td>F 1,6 BPase (nmol of F6P formed /min/mg protein)</td>
<td>3.04±0.03</td>
<td>2.93±0.02</td>
<td>4.92±0.08*</td>
<td>3.04±0.06</td>
</tr>
<tr>
<td>G 6 Pase (nmol of Pi formed/ min/mg protein)</td>
<td>13.26±0.33</td>
<td>13.02±0.39</td>
<td>16.59±0.62*</td>
<td>13.97±0.60</td>
</tr>
<tr>
<td>AR (µmol of NADPH oxidized/min/mg protein)</td>
<td>2.19±0.08</td>
<td>2.21±0.1</td>
<td>2.83±0.09*</td>
<td>2.29±0.01</td>
</tr>
<tr>
<td>SDH (µmol of NADH oxidized/min/mg protein)</td>
<td>4.33±0.11</td>
<td>4.15±0.12</td>
<td>5.30±0.11*</td>
<td>4.44±0.19</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM (n = 6 rats/group). C, control rats; C+T, control rats treated with PAAEt; F, fructose fed rats; F+T, fructose fed rats treated with PAAEt; F1,6 BPase, fructose 1,6 bis phosphatase; G6 Pase, glucose 6 phosphatase; AR, aldose reductase; SDH, sorbitol dehydrogenase; *Significant as compared to group-C (P < 0.05 DMRT).

Figure 2: Histological examination of sections of kidney in four experimental groups of rats (10X). Group-C: Kidney of control rats showing normal appearance of Bowman’s capsule (BC) and renal tubule (RT). Group-C+T: Kidney of control rats treated group (PAAEt 200 mg/kg) showing no histological change with control group, Glomeruli (G). Group-F: Kidney of fructose fed rats showing degenerative changes in Bowman’s capsule, formation of congestion (C) and vocalization (V). Group-F+T: Kidney of fructose fed rats treated with PAAEt (200 mg/kg) showing recovery to near normal appearance.
Renal histology

Results from the histological examination are shown in Fig. 2. The histological section of the control group contained normal Bowman’s capsule, glomeruli and tubules with clear delineated basement membranes and good definition of capillary loops versus mesangial structures. On the other hand, group-F showed pathological changes i.e., formation of vacuolization, congestion and deterioration of Bowman’s capsule in the kidney. These pathological changes were prevented in group-F+T and no histological alterations were observed in group-C+T when compared with group-C.

Table 3: Oxidative stress markers and antioxidants in renal tissue of all experimental groups of rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C</th>
<th>C+T</th>
<th>F</th>
<th>F+T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid peroxidation (nmol of MDA formed /mg protein)</td>
<td>16.11±0.27</td>
<td>14.91±0.34</td>
<td>24.99±0.41*</td>
<td>18.03±0.14</td>
</tr>
<tr>
<td>Protein oxidation (µmol/mg protein)</td>
<td>2.86±0.11</td>
<td>2.71±0.09</td>
<td>3.48±0.06*</td>
<td>2.82±0.08</td>
</tr>
<tr>
<td>GSH (µg/mg protein)</td>
<td>4.60±0.16</td>
<td>4.85±0.21</td>
<td>3.62±0.08*</td>
<td>4.50±0.17</td>
</tr>
<tr>
<td>Catalase (mmol H₂O₂ decomposed/ min/mg protein)</td>
<td>29.80±0.65</td>
<td>30.51±1.18</td>
<td>21.38±0.78*</td>
<td>30.03±0.36</td>
</tr>
<tr>
<td>Superoxide dismutase (U/mg protein)</td>
<td>24.11±0.68</td>
<td>24.36±0.84</td>
<td>18.37±0.50*</td>
<td>23.12±0.28</td>
</tr>
<tr>
<td>Glutathione reductase (µmol NADPH oxidized/mg protein)</td>
<td>30.53±0.26</td>
<td>31.75±1.01</td>
<td>24.47±0.43*</td>
<td>30.12±0.59</td>
</tr>
<tr>
<td>Glutathione peroxidase (µg GSH consumed/mg protein)</td>
<td>7.73±0.11</td>
<td>7.82±0.20</td>
<td>5.56±0.18*</td>
<td>7.76±0.19</td>
</tr>
<tr>
<td>Glutathione-S-transferase (µmol CDNB-GSH conjugate formed/min/mg protein)</td>
<td>129.64±1.15</td>
<td>130.62±0.6</td>
<td>83.31±1.24*</td>
<td>128.23±0.89</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM (n = 6 rats/group). C, control rats; C+T, control rats treated with PAAEt; F, fructose fed rats; F+T, fructose fed rats treated with PAAEt; MDA, Malondialdehyde; ‡, Amount of enzyme which gave 50% inhibition of pyrogallol autooxidation/min; * Significant as compared to group-C (P < 0.05 DMRT).

DISCUSSION

Several studies reported that a diet high in fructose (66%) accelerates renal damage in normal rats (Gersch et al., 2007; Palanisamy et al., 2008). Impaired renal function is associated with enhanced functional markers, altered morphology and damage of renal tissue.

The significant elevation of plasma urea, creatinine and uric acid by chronic feeding of high-fructose diet is an indication of defective kidney function in group-F compared to group-C. Earlier reports are in agreement with our findings (Palanisamy et al., 2008; Choi et al., 2011). Elevated renal functional markers in group-F are attributed to enhanced catabolism of proteins and/or decreased renal clearance (Table 1). Plasma creatinine estimation is a significantly more reliable screening index of renal function, primarily of glomerular filtration. Increased plasma level of creatinine and urea can be attributed to the damaged nephron structural integrity which resulted in impaired renal function (Ghodkar, 1994; Khan and Siddique, 2012). An elevation in circulating uric acid, rather than being a surrogate biomarker of kidney dysfunction, is actually an active player in the pathogenesis of renal dis-
ease (Inaba et al., 2013). The rise in uric acid may cause an afferent arteriolopathy resulting in glomerular hypertension (Sanchez-Lozanda et al., 2007). Hyperuricemia is a common metabolic disorder and a well-established causative factor for development of gouty arthritis, tophi formation, uric acid kidney stones and acute kidney failure (Edwards, 2008). In addition, hyperuricemia is commonly associated with obesity, hypertriglyceridemia, diabetes mellitus, development and progression of coronary artery disease (Madsen et al., 2005; Short et al., 2005), non-alcoholic fatty liver disease (Xu et al., 2010) and hypertension (Sanchez-Lozanda et al., 2007). Treatment with PAAEt to fructose fed rats (group-F+T) completely prevented the rise in the functional markers of renal tissue whereas control rats treated with PAAEt showed no deviation from the group-C. This protective action of PAAEt might be due to free radical scavenging activity and antihyperuricemic activity (Karuna et al., 2011; Murugaiyah and Chan, 2006).

In order to understand the tissue damage, transaminase activities were analyzed and found that enhanced activities of renal GOT and GPT in group-F in comparison with group-C indicate an increased protein degradation and amino acid catabolism (Fig.1). Vlssara and Palace (2002) demonstrated enhanced non-enzymatic glycation and/or oxidation of protein under hyperglycemic condition decreased the half-life of proteins by enhanced protein degradation. The increased levels of transaminases are observed in the absence of insulin/insulin function because of increased availability of amino acids in diabetics (Felig et al., 1970) which may be responsible for the increased gluconeogenesis and ketogenesis observed in high-fat fed conditions. The fact that high fructose intake induces hyperglycemia was established in our laboratory (Reddy et al., 2009). Hyperglycemia was positively correlated with gluconeogenesis and aldose reductase. Hyperglycemia and polyol pathway are the major important factors for the development of OS and driving force for many pathological diseases including RD (Chung et al., 2003). In this study we found that group-F exhibited enhanced activities of gluconeogenic enzymes such as, F-1, 6-BPase and G-6-Pase and enhanced operation of polyol pathway as reflected by significant increase of activities of AR and SDH compared to group-C (Table 2). The increased activities of transaminases were associated with an enhanced gluconeogenesis. Ineffective utilization of glucose in fructose fed state leads to enhanced breakdown of proteins thereby providing substrates for gluconeogenesis. Increased AR activity activates signal transduction pathways such as PKC, P38MAPK and JNK, which result in overproduction of inflammatory substances leading to some of the pathophysiological changes associated with diabetic nephropathy (Chaturvedi et al., 2002; Li et al., 2002; Price et al., 2004). Furthermore, increased AR activity causes substantial depletion of NADPH and consequently significant decrease in the GR activity and GSH level (Cheng and Gonzalez, 1986; Gonzalez et al., 1986). In group-F+T, treatment with PAAEt prevented the increase in the activities of renal transaminases, gluconeogenic and polyol pathway enzymes. It might be due to antihyperglycemic activity of PAAEt (Karuna et al., 2011) and aldose reductase inhibition activity (Shimizu et al., 1989). Aldose reductase inhibitor, tolrestat prevented glomerular hyperfiltration, renal hypertrophy, extracellular matrix accumulation and mesangial cell hypocontractility in STZ-induced diabetic rats (Donnelly et al., 1996). Activities of transami-
nases, gluconeogenic and polyol pathway enzymes of group-C+T showed no deviation from the group-C.

Although the exact mechanisms for renal damage caused by fructose treatment have not yet been established, much emerging evidence shows that the chronic consumption of HFD contributes to excessive formation of reactive oxygen species (ROS), leading to perpetuation of OS in renal tissue (Faure et al., 1997; Sakai et al., 2002; Palanisamy et al., 2010). Kidney is vulnerable to oxidative attack in fructose fed rats (Rajasekar et al., 2008). Adequate intake of antioxidant vitamins C and E reduced the susceptibility of LDL to oxidation and scavenge free radicals within the body, reducing the overall oxidative status (Pryor, 2000; Annuk et al., 2003; Nosratola, 2008). Despite regular exercise and weight control, consumption of a diet rich in natural antioxidants is desirable in combating oxidative stress and promoting good health (Pryor, 2000; Nosratola, 2008). In the present study, we found increased levels of LPO and PO in group-F compared to group-C (Table 3). Peroxidative deterioration of lipids is evident from the increased levels of malondialdehyde, while the increased protein carbonyl groups signify protein damage. Enhanced LPO may also play a potential role in progressive reduction in glomerular filtration rate. Decreased levels of GSH were observed in group-F than in group-C. Similar trend of depleted levels of renal GSH in fructose fed animals was reported by previous workers (Rajasekar et al., 2005). It might be due to generation of oxygen radicals by fructose catabolism causing utilization of GSH and/or decreased generation of GSH from oxidized glutathione by reduced GR activity. It increased the sensitivity of this organ to oxidative damage and may play a role in the development of diabetic nephropathy. Reactive oxygen species reduce the activity of CAT and GPx (Datta et al., 2000) and reduction in the activities of GST and GR are suggestive of reduced scavenging potential in insulin resistant rats (Nandhini et al., 2005). Decreased activity of SOD in group-F may result from a number of deleterious effects due to the accumulation of super oxide radicals and H2O2. Together these findings clearly indicate the development of OS in group-F compared to group-C. Rajasekar et al, (2008) investigated that the high fructose intake increases OS in the kidney and our findings are in accordance with this. The observed OS of group-F was prevented and maintained at normal level in group-F+T indicating the antioxidant effect of PAAEt against fructose diet induced OS. Diminished LPO, and PO and increased GSH signify reduced free radical scavenging activity (Guha et al., 2010). This may explain increase in the activities of antioxidant enzymes and / or decrease in the synthesis of ROS. Aqueous extract of P. amarus contain high content of phenolic compounds which were found to have strong and significant positive correlations to free radical scavenging potential and inhibition of lipid peroxidation (Halliwell, 1994). The strong antioxidant potential of P. amarus is due to the presence of lignans, flavonoids (rutin and quercetin 3-Oglucoside) and tannins (amariin, repandusinic acid and phyllanthusiin D) (Lim and Murtijaya, 2006; Londhe et al., 2008). Group-C+T exhibited no deviation from group-C throughout the study, indicating nontoxic nature of PAAEt. Therefore, PAAEt could help make the kidney less susceptible to oxidative damage.

In the present study, kidney damage of group-F was noticed by observing histopathological changes such as formation of vacuolization, congestion and deterioration of Bow
man’s capsule compared with group-C (Fig. 2). Previous reports demonstrated that rats fed with fructose enriched diet developed kidney hypertrophy, glomerular hypertension, cortical vasoconstriction and deterioration of glomerular and tubular structures (Choi et al., 2011; Sanchez-Lozada et al., 2007). It may be due to generation of higher levels of ROS (Chung et al., 2003) and/or fructose which may also be filtered into the urine where it is taken up in the S3 segment of the proximal tubule, leading to local intracellular generation of uric acid with oxidative stress and local inflammation (Cirillo et al., 2009). Co-treatment with PAAEt to fructose fed rats for sixty days protected the kidney in group-F+T from damage. No such changes were found in control rats. These findings clearly indicate the renoprotective effect of PAAEt which might be due to suppression of renal OS.

CONCLUSION

Our results indicate that high fructose consumption accelerates RD. Co-administration of PAAEt to fructose fed rats prevented the rise in OS and then protected the damage of kidney. It clearly indicates that PAAEt exhibited renoprotective activity which may be due to its antioxidant activity. Hence, it would seem useful as an adjuvant for the prevention and/or management of RD. Further studies are necessary to elucidate the underlying mechanisms of the renoprotective effects of Phyllanthus amarus.

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