Hematological and hepatoprotective effects of aqueous extract of *Phyllanthus amarus* in streptozotocin induced-diabetic male Wistar rats

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ABSTRACT

The main objective of the present study was to investigate the hematological and hepatoprotective activities of aqueous extract of *Phyllanthus amarus* in streptozotocin (STZ) induced diabetic rats. The male Wistar albino rats were randomly divided in to four groups of 10 animals each: Control group (C), PA-treated control group (C+PA), Diabetic control group (D), PA treated diabetic group (D+PA). Diabetes was induced by intraperitoneal injection of STZ (55 mg/kg/bwt) and after ascertaining, the diabetic rats were treated with aqueous extract of *Phyllanthus amarus* for 60 days. The biochemical estimations like hematological parameters, transaminases and antioxidant potential of the hepatic tissue were performed. The altered hematological parameters like hemoglobin concentration, red blood cells, mean cell volume, packed cell volume, mean cell hemoglobin and mean cell hemoglobin concentration (11.1, 7.6, 12.9, 20, 3.6 and 11.2%) in D-group were restored to normal level by the co-administration of *Phyllanthus amarus* aqueous extract (PAAEt) at 200 mg/kg bw to diabetic rats. The activities of hepatic transaminases viz., glutamate pyruvate transaminase, glutamate oxaloacetate transaminase increased significantly while the activities of hepatic antioxidant enzymes such as glutathione peroxidase, glutathione-S-transferase, glutathione reductase, superoxide dismutase and catalase decreased significantly (p<0.05) and depletion of reduced glutathione illustrates increased lipid peroxidation (107.13%) which were restored to control level in D+PA group. In conclusion, our results clearly indicate the therapeutic efficacy of *P. amarus* aqueous extract against altered hematological parameters, transaminases and oxidative stress of hepatic tissue in STZ-induced diabetes. Hence this plant could be used as an adjuvant therapy for the prevention and/or management of diabetes.

KEYWORDS: Hepatic tissue, Oxidative stress, *Phyllanthus amarus*, Streptozotocin
INTRODUCTION

Diabetes mellitus is probably the single most important metabolic disease and is widely recognized as one of the leading causes of death and disability. It affects every cell in the body and the body’s essential biochemical processes, and it is a major public health problem in developed as well as developing countries (Kameswara Rao et al., 2003). Diabetes arises due to a deficiency of insulin secretion from the beta cells of the pancreas or a deficiency of insulin action. The treatment of diabetes depends on insulin injections, diet, and exercise. Insulin injection is the major therapy for type 1 diabetes. Defective insulin secretion is the major cause for chronic hyperglycemia resulting in impaired function or serious damage to many of the body’s systems like eyes, kidneys, nerves, heart and blood vessels (Arun et al., 2012). Hematological complications consist mainly of abnormalities in the function, morphology and metabolism of erythrocytes, leukocytes and platelets (Comazzi et al., 2004). Heart rate of diabetics is higher, but red and white blood cell counts are lower than non-diabetics (Mansi et al., 2006). Glutamate pyruvate transaminase (GPT) is a cytosol enzyme, more specific to the liver so that a rise only occurs with liver disease (Kumar and Clark, 2001). Its levels are tested to look for and evaluate damage to the liver. It is also measured to check medical treatments that may lead to liver inflammation. Glutamic oxaloacetate transaminase (GOT) is also specific to the liver and indicates derangement in liver metabolism. Since diabetes mellitus is a multifactorial disease, the treatment is aimed at not only controlling blood sugar level to normal limit but also correcting the associated metabolic defects (Hattersley, 2002). Streptozotocin (STZ) induces diabetes mellitus by destroying pancreatic β-cells, possibly through generating excess reactive oxygen species (ROS). Exaggerated production of these reactive species in diabetes can lead to very serious problems including cardiovascular disease, liver and kidneys failure, blindness, and nerve injury (Neyenwe et al., 2011). Thus antioxidant therapy is one of the strategies for diabetes treatment. Prevalence of diabetes mellitus is now ubiquitous, affecting 150 million people worldwide. According to International Diabetes Federation (IDF), more than one fifth (33 million) of them are Indians and hence, India has been declared as the “diabetic capital of the world” (Kochhar et al., 2007). Throughout the world, many traditional plant treatments for diabetes exist, and therein lies a hidden wealth of potentially useful natural products for the control of diabetes (Maiti et al., 2008). Natural plant drugs are frequently considered to be less toxic and free from side effects than synthetic ones (Sunil et al., 2009).

Phyllanthus amarus (Euphorbiaceae) is found in all tropical regions of the world including southern India and China. It can grow to 30-60 cm in height and bloom with yellow flowers. All parts of the plant are used in ayurvedic medicine because of their medicinal properties. Leaves of this plant are reported to contain lignans, alkaloids, flavonoids, galloantnoids and glycosides (Dhalwal et al., 2006). P. amarus has been claimed to be an excellent remedy for infective hepatitis (Thyagarajan et al., 1988). It was reported to have anti plasmodial (Tran et al., 2003), antiviral (Notka et al., 2004), anti-diarrheal (Odetola and Akojenu, 2000), anti-inflammatory (Klemer, 2003) immunostimulatory (Annalakshmi et al., 2013) and kidney stones dissolution properties (Rajeshkumar and Kuttan, 2000). P. amarus exhibited protective action against CCl₄ induced mitochon-
drial dysfunction (Padma and Setty, 1999), and hepato protective potential against ethanol (Toyin et al., 2008), alloxan (Raphael et al., 2002,) and cyclophosphamide-induced oxidative stress in rats (Kumar and Kuttan, 2005). Methanol extract of P. amarus was reported to have hypoglycemic effect on alloxan induced diabetes mellitus (Das and Bhattacharjee, 1970). Earlier work in our laboratory revealed antioxidant potential of aqueous extract of Phyllanthus amarus in rats (Karuna et al., 2009) and Protective effect of Phyllanthus amarus aqueous extract against renal oxidative stress in Streptozotocin-induced diabetic rats (Karuna et al., 2011). Therefore, the present investigation was undertaken to study the hematological and hepatoprotective effects of Phyllanthus amarus aqueous extract in streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Chemicals

The chemicals used in the current study were procured from Sigma Chemical Company (St. Louis, MO, USA), and SISCO Research Laboratory Pvt. Ltd, Mumbai, India).

Plant material

Phyllanthus amarus aqueous extract (PAAE) whole herb was purchased from Chemiloids (manufacturer and exporter of herbal extract, Vijayawada, A.P, India). Prior to preparation of the plant extract, the plant was authenticated by Dr. Narasimha Reddy, Taxonomist, Laila Implex R&D Centre, Vijayawada. The extract was stored in an air-tight container and dissolved in water just before use. Herb to product ratio was 10:1.

Induction of diabetes mellitus

Male Wistar rats of 2-3 month old weighing (125-150 g) were procured from Sri Venkateswara Enterprises (Bangalore, India). They were acclimatized for 7 days two to three animals were housed per cage sized (41x28x14 cm) in our animal house (Regd. No.470/01/a/CPCSEA, dated 24th August, 2001) and maintained under standard conditions of temperature and relative humidity with 12 h light/dark cycles. Diabetes was induced in overnight fasted rats by a single intraperitoneal injection of freshly prepared STZ (55 mg/kg body weight) in 0.05 M ice-cold citrate buffer (pH 4.5) in a volume of 0.1 ml per rat. After 72 hours of STZ administration, the plasma glucose level of each rat was determined for confirmation of diabetic induction. Rats with plasma glucose level above 230 mg/dL were considered as diabetic and used subsequently.

Experimental design

In the present experiment, a total of 40 rats (20 diabetic rats; 20 normal rats) were used. The rats were divided into following four groups of 10 each: Control (C); Control rats treated with P. amarus (C+PA); Diabetic (D); and Diabetic rats treated with P. amarus (D+PA). Diabetic treated group and Control treated group rats received an aqueous extract of the P. amarus in 3 ml of distilled water per rat at once by orogastric tube at a dose of 200 mg/kg body weight for 60 days, whereas, distilled water was administered to control and diabetic rats. The dose was fixed based on the results from preliminary experiments.

Animal sacrifice and organ collection

After the experimental period of 60 days, the animals from each experimental group were starved for 12 h and sacrificed by cervical dislocation and immediately the whole liver was dissected out and washed with ice-cold saline and used for analysis. Ten percent homogenate of liver was prepared by us-
ing Potter-Elvehjem homogenizer with Teflon pestle at a 4°C in 0.15 M KCl. The whole homogenate was used for estimation of reduced glutathione (GSH) and lipid peroxidation (LPO). The homogenate was centrifuged in cooling centrifuge (12000 rpm for 45 min at 0-4°C) to remove the debris and supernatant was used for enzyme assays. The total protein content of the liver in the whole homogenate was estimated by the method of Lowry et al. (1951).

**Hematological studies**

**Red blood cell count**

Red blood cells were counted by the method of Davidson and Peter (1963). Blood was diluted in red blood cell pipette using grower’s erythrocyte diluting fluid (12.5 g sodium sulphate, 33.3 ml glacial acetic acid in 200 ml water). Improved Neubauers counting chamber was charged carefully, and red blood cells were counted under high power of microscope in the four cornered and central squares of the central large square and the number of red blood cells in 1 mm³ of blood was calculated using the formula:

\[
\text{Number of cells/mm}^3 = \frac{\text{Cells counted} \times \text{Blood dilution} \times \text{Chamber depth}}{\text{Area of chamber counted}}
\]

**Packed cell volume**

The hematocrit of blood was the ratio of the volume of erythrocyte to that of the whole blood and expressed as percentage. The microcapillary tube was filled with a well-mixed blood by capillary action and the empty end was sealed. The filled capillary tubes were centrifuged in a microhematocrit (Centrikan Model CIC6SO) for 10 min. After centrifugation, the whole blood and red cell columns were measured with the measuring device provided with the instrument and PCV was expressed as percentage (Henry, 1984).

**Hemoglobin**

Hemoglobin in the blood was estimated by cyanomethemoglobin method as outlined by Samuel (1989). To 5 ml of Drabkin solution, 20 ul of blood was added, mixed well and intensity was read at 540 nm. By treating the cyanomethemoglobin standard (5-15 mg) in the same manner, a graph was plotted from which the concentration of Hb in the blood sample was calculated and expressed as g%.

**Erythrocyte indices**

MCV and MCH were calculated from concentration of hemoglobin, PCV and red blood cell count (Chessbrough and Arthur, 1976; Henry, 1984). MCV is the average volume of the red blood cell and calculated from the PCV and red blood cell count.

\[
\text{MCV (μm}^3\) = \frac{\text{PCV} (%) \times 10}{\text{Red cell count (millions/mm}^3\)}
\]

MCH is the content (weight) of hemoglobin of average red cells and is calculated from hemoglobin concentration and the red blood cell count.

\[
\text{MCH (pg) = } \frac{\text{Hb (g %)}}{\text{Red cell count (millions/mm}^3\)}
\]

MCHC is the average concentration of hemoglobin in a given volume of packed cells and is calculated from the hemoglobin concentration and the PCV.

\[
\text{MCHC (g %) = } \frac{\text{Hb (g %) \times 100}}{\text{PCV ( %)}}
\]

**Hepatic transaminases**

Glutamate pyruvate trasaminase (GPT) (EC 2.6.1.2) and Glutamate oxaloacetate transaminase (GOT) (EC 2.6.1.1) were assayed by the method of Reitman and Frankle (1957). To 1 ml of buffered substrate solution, 0.2 ml of enzyme source was added and incu-
bated for 60 min at 37°C. The reaction was arrested by the addition of 1 ml of DNPH. After 20 min, 10 ml of 0.4N NaOH was added and left at room temperature for another 10 min. A series of pyruvate standards (10-50 µg) were also treated in a similar manner. The reddish brown color developed was read at 520 nm against the reagent blank. The enzyme activities are expressed as µg of pyruvate liberated/min/mg protein.

**Estimation of lipid peroxidation**

Lipid peroxidation in liver was estimated colorimetrically by measuring the thiobarbituric acid reactive substances (TBARS) by the method of Utley et al. (1967). To 0.1 ml of 10% tissue homogenate, 2 ml of 10% TCA and 4 ml of 0.67% TBA were added and heated in a water bath for 30 min and cooled. Absorbance of the supernatant was read at 535 nm. The extent of LPO was expressed as nmoles of malondialdehyde (MDA) formed/g tissue, using a molar extinction coefficient of MDA as 1.56 x 10^5 M^-1 cm^-1.

**Estimation of reduced glutathione**

Reduced glutathione was estimated in liver homogenate (10%) using a disulfide compound, 5,5′-dithio-bis-nitrobenzoic acid (DTNB), which readily gets reduced by sulfhydryl compounds forming a highly colored anion having maximum absorbance at 412 nm as outlined by the method of Ellman (1959). The activity was expressed as µg of GSH consumed/min/mg protein.

**Assay of enzymes in liver homogenate**

**Glutathione reductase**

Glutathione reductase (GR) catalyzes the reduction of oxidized glutathione (GSSG) by NADPH to GSH. The activity of GR in liver was measured by the method of Pinto and Bartley (1969). In brief, the assay mixture consists of 0.5 ml of phosphate buffer (0.25 M, pH 7.4), 1.0 ml of EDTA (25 mM), 0.1 ml of NADPH(1 mM), 0.96 ml of distilled water and 0.1 ml of liver homogenate. The reaction was initiated by the addition of 0.024 ml of GSSG (50 mM). The activity was expressed as µmoles of NADPH oxidized/min/mg protein by using milli molar extinction coefficient of NADPH as 6.22.

**Glutathione peroxidase**

Glutathione peroxidase (GPx) in liver was measured by the method of Rotruck et al. (1973). Briefly, the reaction mixture consists of 0.5 ml of phosphate buffer (0.2 M, pH 7.0 containing 0.4 mM EDTA, and 10 mM sodium azide), 0.2 ml of liver homogenate, 0.2 ml of GSH (2 mM) and 0.1 ml of H₂O₂ (0.2 mM) and incubated for 10 min at room temperature along with blank containing all reagents except tissue homogenate. The reaction was arrested by the addition of 0.5 ml of TCA (10%), centrifuged at 4000 rpm for 5 min and GSH content in 0.5 ml of supernatant was estimated by following the method of Ellman (1959). The activity was expressed as µg of GSH consumed/min/mg protein.

**Glutathione-S-transferase**

Glutathione-S-transferase (GST) in liver was measured by the method of Habig et al. (1974). The assay mixture consists of 1.7 ml of sodium phosphate buffer (0.14 M, pH 6.5), 0.2 ml of GSH (30 mM) and 0.04 ml of homogenate. The reaction was initiated by addition of 0.06 ml of 1-chloro-2,4-dinitrobenzene (CDNB) (0.01 M dissolved in 50% ethanol). The activity was calculated by using mill molar extinction coefficient of CDNB-GSH conjugate as 9.6. The activity was expressed as µmoles of CDNB-GSH conjugate formed/min/mg protein.

**Catalase**
Catalase (CAT) activity was assayed by the method of Beers and Sizer (1952). The assay mixture consists of 1.9 ml of sodium phosphate buffer (0.05 M, pH 7.0), 1.0 ml of \( \text{H}_2\text{O}_2 \) (0.059 M) and 0.1 ml of liver homogenate. The activity was expressed as moles of \( \text{H}_2\text{O}_2 \) decomposed/min/mg protein.

**Superoxide dismutase**

Superoxide dismutase (SOD) activity in liver was determined according to a modified procedure adopted by Soon and Tan (2000). Superoxide dismutase activity was measured based on the ability of the enzyme to inhibit the oxidation of pyrogallol. The assay mixture consists of 0.5 ml of phosphate buffer (50 mM, pH 7.8 containing 1 mM EDTA), 0.02 ml of brain homogenate and 0.86 ml of distilled water. The reaction was initiated by the addition of 0.02 ml of pyrogallol (10 mM, in 0.01N HCl). One unit of SOD was defined as the amount of enzyme required to inhibit the oxidation of pyrogallol by 50% in standard assay system of 3 ml. The activity was expressed as Units/min/mg of protein.

**Statistical analysis**

Data were expressed as the mean ± SE for the number, \( n = 10 \) of animals in the group as indicated in (Tables 1–3). The data were subjected to statistical analysis by Duncan’s Multiple Range (DMR) (Duncan, 1955) test. Values of \( p < 0.05 \) were considered statistically significant.

**Table 1:** Effect of *P. amarus* treatment on hematological parameters in STZ induced diabetic rats.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>C</th>
<th>C + PA</th>
<th>D</th>
<th>D + PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g %)</td>
<td>10.775 ± 0.059&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.375 ± 0.059&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.575 ± 0.070&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.550 ± 0.050&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>R.B.C count (millions/mm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>8.713 ± 0.077&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.700 ± 0.091&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.050 ± 0.027&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.375 ± 0.067&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>33.125±0.350&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.250 ±0.366&lt;sup&gt;c&lt;/sup&gt;</td>
<td>26.500 ±0.463&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.125 ±0.398&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MCV(µm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>37.815±0.453&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38.404 ±0.338&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.925 ±0.632&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.559±0.499&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MCH(pg)</td>
<td>12.333±0.087&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.725 ±0113&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.894±0.116&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.596±0.108&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MCHC (g %)</td>
<td>32.561±0.464&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.353 ±0.359&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.194±0.583&lt;sup&gt;c&lt;/sup&gt;</td>
<td>31.256±0.492&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.EM (n=10). Values with different superscripts across the row are significantly different at \( P<0.05 \) (Duncan’s multiple range test). C: control, C+PA: control treated with *Phyllanthus amarus*, D: diabetic, D+PA: diabetic treated with *Phyllanthus amarus* aqueous extract, Hb: hemoglobin concentration; RBC: red blood cells; PCV: packed cell volume; MCV: mean cell volume; MCH mean cell hemoglobin; MCHC: mean cell hemoglobin concentration.

**RESULTS**

**General observation and characteristics**

No visible side effects and variations in animal behavior (respiratory distress, abnormal locomotion and cathelepsy) were observed in C+PAAE group of rats indicating the non-toxic nature of *P. amarus* administration.

**Effect of *P. amarus* aqueous extract (PAAEt) on hematological parameters**

Data presented in Table 1 indicate significant decrease in the activity like Hb, RBC count; PCV, MCV and MCH while a significant increase in MCHC was observed in STZ induced diabetic rats compared to Control rats.
Co-administration with PAAE enhanced the activities of hematological parameters like Hb, RBC and MCV significantly but not normalized whereas PCV, MCH, and MCHC were rectified and reached the normal levels.

**Effect of PAAE on hepatic transaminases**

Table 2 depicts the significant increase in the activities of both hepatic transaminases in STZ induced diabetic rats when compared to control rats. Co-administration with PAAE restored the activity of GOT to the normal value whereas GPT was significantly lesser than control rats. Hepatic transaminases decreased significantly in control treated rats compared to control rats.

**Effect of PAAE on oxidative stress markers and antioxidant enzymes**

Table 3 illustrates the significant decrease in the activities of hepatic oxidative stress markers viz., GSH and LPO and antioxidant enzymes glutathione dependent enzymes (GPX, GST, and GRx), anti-lipid peroxidative enzymes (SOD and CAT) in STZ induced diabetic rats in comparison to normal control rats. This indicates decreased levels of tissue antioxidant status and increased lipid peroxidation, reduced glutathione in STZ induced diabetic rats. Co-administration with PAAE significantly (p<0.05) prevented these adverse changes in hepatic tissue and maintained near normal levels.

**Table 2:** Effect of *P. amarus* treatment on hepatic tissue transaminases in STZ-induced diabetic rats.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>C</th>
<th>C + PA</th>
<th>D</th>
<th>D + PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPT (µg of pyruvate formed/min/mg protein)</td>
<td>3.439 ±0.102^c</td>
<td>2.908± 0.042^a</td>
<td>4.869± 0.052^d</td>
<td>3.125± 0.063^b</td>
</tr>
<tr>
<td>GOT (µg of pyruvate formed/min/mg protein)</td>
<td>3.983 ±0.030^b</td>
<td>3.377 ±0.049^a</td>
<td>4.980 ±0.041^c</td>
<td>3.930 ±0.043^b</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.EM (n=10). Values with different superscripts across the row are significantly different at P<0.05 (Duncan’s multiple range test). C: control, C+PA: control treated with *Phyllanthus amarus*, D: diabetic, D+PA: diabetic treated with *Phyllanthus amarus* PAAE: *Phyllanthus amarus* aqueous extract, GPT: Glutamate pyruvate trasaminase, GOT: Glutamate oxaloacetate transaminase.

**DICUSSION**

STZ is generally used in rat models of diabetes because it has lower toxicity than alloxan, (Liu et al., 2008). The cytotoxic action of STZ is associated with the generation of ROS causing oxidative damage (Szkudelski, 2001). Several hematological abnormalities have been identified in patients with diabetes mellitus like short life span of erythrocyte (Lahrman, 1977), oxidative stress mediated defects in maturation process of reticulocyte to erythrocytes (Sailaja et al., 2003), abnormalities of leucocytes (Tan et al., 1975) and platelet function (Kwaan et al., 1972). Prolonged hyperglycemia seen in uncontrolled diabetes mellitus leads to glucose oxidation which is believed to be the main source of free radicals. The free radical induced oxidative damage may be more prominent in red blood cells compared to other tissues due to their high content of iron and poly unsaturated fatty acids, their role as an oxygen transporter, and due to protection of the host *in vivo* by neutralizing exogenously competing
with other tissues for the toxic effect of free radicals (Richards et al., 1998). Reduced RBC count, Hb and hematocrit, in untreated diabetic rats indicate the progression towards anemia, which may be the result of impaired red blood cell production (Cheeke, 1998). The occurrence of anemia in diabetes mellitus was reported due to the increased non-enzymatic glycosylation of RBC membrane proteins (Oyedemi et al., 2011). Oxidation of these proteins and hyperglycaemia in diabetes mellitus causes an increase in the production of lipid peroxides that lead to hemolysis of RBC (Arun and Ramesh, 2002). In the present investigation, the RBC membrane lipid peroxide levels in diabetic rats were not measured. However, the red blood cells indices as RBC, Hb, PCV, MCV, MCH and MCHC were studied to investigate the beneficial effect of PAAE extract on the anemic status of diabetic rats. The levels of RBC, Hb in the diabetic animals were drastically reduced which may be attributed to the infections on the normal body systems. This observation is in agreement with the reported anti-hyperglycemic activity of aqueous root extract of Rubia cordifolia in streptozotocin-induced diabetic rats in our laboratory (Baskar et al., 2006). And it also supports the restoration of the altered hematological parameters with treatment of PAAE. A significant increase in Hb and RBC count with no significant alteration in other hematological parameters of control treated rats compared to control rats reflects the beneficial and non-toxic nature of the P. amarus. This hematological correction may be attributed to various phytochemicals present in PAAEt.

Liver tissue contains 3 times the amount of GPT as heart muscle or kidney. Alanine and aspartate were elevated in diabetes while co-administration with PAAEt resulted in significant decrease. Thus it may protect liver from free radical damage.
Table 3: Effect of *P. amarus* treatment on hepatic oxidative stress markers and antioxidants in STZ-induced diabetic rats.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>C</th>
<th>C+PA</th>
<th>D</th>
<th>D+PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid peroxidation (nmoles of MDA/mg protein)</td>
<td>15.071 ±0.0321b</td>
<td>12.282 ±0.106a</td>
<td>31.217 ±0.123d</td>
<td>16.964± 0.08c</td>
</tr>
<tr>
<td>Reduced glutathione (µg of GSH/mg protein)</td>
<td>1.309± 0.019c</td>
<td>1.449± 0.014d</td>
<td>0.552±0.008a</td>
<td>1.148± 0.037b</td>
</tr>
<tr>
<td>Superoxide dismutase (U/min/mg protein)</td>
<td>6.302± 0.229b</td>
<td>29.005± 0.257c</td>
<td>20.025 ±0.112a</td>
<td>30.315 ±0.275d</td>
</tr>
<tr>
<td>Catalase (nmols of H₂O₂ decomposed/min/mg protein)</td>
<td>22.441± 0.171c</td>
<td>33.468± 0.225d</td>
<td>13.224 ±0.123a</td>
<td>19.172± 0.179b</td>
</tr>
<tr>
<td>Glutathione peroxidase (µg of GSH consumed/min/mg protein)</td>
<td>0.793±0.006c</td>
<td>1.061±0.014d</td>
<td>0.250±0.005a</td>
<td>0.454±0.012b</td>
</tr>
<tr>
<td>Glutathione reductase (µmols of NADPH oxidized/min/mg protein)</td>
<td>10.774±0.085c</td>
<td>11.527±0.143d</td>
<td>6.700±0.058a</td>
<td>10.031±0.041b</td>
</tr>
<tr>
<td>Glutathione-S-transferase (µmols of GSH-CDNB conjugate formed/min/mg of protein)</td>
<td>0.653±0.005c</td>
<td>0.707±0.006d</td>
<td>0.494±0.005a</td>
<td>0.552±0.006b</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.EM (n=10). Values with different superscripts across the row are significantly different at P<0.05 (Duncan’s multiple range test). C: control, C+PA: control treated with *Phyllanthus amarus*, D: diabetic, D+PA: diabetic treated with *Phyllanthus amarus* PAAEt: *Phyllanthus amarus* aqueous extract.

Indices of oxidative stress like enhanced LPO, and decreased GSH content observed in the liver of D-rats, confirm the existence of oxidative stress under diabetic conditions. Peroxides produced in a cell can be detoxified by the action of GPx and CAT. GPx is specific for its hydrogen donor GSH, and non-specific for the hydroperoxides ranging from H₂O₂ to organic peroxides, thus offering a major defense role in cells against peroxidative damage of complex biochemical compounds such as lipids and nucleic acids. GST is a multifunctional protein with a broad specificity for organic hydroperoxides but not for H₂O₂. It also plays an important role in detoxification of xenobiotic compounds thereby protecting the cell from peroxidative damage (Uday et al., 1999). Glutathione, SOD and CAT protect the cell constituents from oxidative damage. Despite these extensive defense systems, biomolecular damage may still occur and persist within the cell. An increase in a combination of different eliminating enzymes, rather than an increase in any single enzyme, is generally more effective in the inhibition of radical induced cellular damage (Halliwell and Gutteridge, 1990). It is thought that PAAE extracts augment the activity of SOD, CAT, GSH-Px, and GR enzymes to eliminate harmful radical oxygen species produced by STZ administration, resulting in protecting the body from cellular damage. The significant
increase in the activities of SOD, CAT, GPx, GST, GR and GSH suggests a greater level of endogenous antioxidant associated with the PAAE treatment resulting in an enhanced free radical scavenging activity by decreasing the LPO. Plants are the sources for a wide variety of compounds like flavonoids and polyphenols. Aqueous extract of P. amarus contained high content of phenolic compounds which were found to have strong and significant positive correlation with 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-O-glucoside) and tannins (amarin, repandusinıc acid and phyllanthusiiın D) (Lim and Murtijaya, 2007; Londhe et al., 2008). These compounds may be responsible for increasing antioxidant status.

CONCLUSION

Biochemical findings of the present study suggested that PAAE possesses antioxidant properties in hepatic tissue and protects against STZ induced diabetes. In addition, the extract could improve some hematological parameters. The most important protective mechanism offered by PAAE is through its ability to decrease liver marker enzymes, lipid peroxidation and increased levels of antioxidant enzymes. Thus, PAAE has been shown to possess hepatoprotective activity against STZ induced oxidative stress in rats.

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REFERENCES


