Indian Journal of Natural Products and Resources
Vol. 5 (2), June 2014, pp. 129-133

Protective effect of *Psidium guajava* L. leaves ethanolic extract on doxorubicin-induced nephrotoxicity in rats

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Received 30 July 2013; Accepted 12 February 2014

The objective of the present study was to investigate the protective effect of ethanolic extract of *Psidium guajava* L. leaves against doxorubicin-induced nephrotoxicity in rats. Animals treated with doxorubicin (8mg/kg, i.p) once daily for 2 days significantly (P<0.05) increased Serum Urea, BUN, creatinine, total protein, LPO and significantly (*P*<0.05) decreased CAT, SOD, GSH levels as compared to vehicle treated rats. Treatment with *P. guajava* (100 & 300 mg/kg, p.o) showed significant (*P*<0.05) decrease in Serum Urea, BUN, creatinine, total protein, LPO and significant (*P*<0.05) increase in CAT, SOD, GSH levels as compared to doxorubicin treated group. Histopathological examinations of kidney tissue showed that doxorubicin changed the renal architecture significantly which was less evident in *P. guajava* (100 mg/kg & 300 mg/kg) pre-treated rats. Results suggest that *P. guajava* extract has the potential to ameliorate doxorubicin induced nephrotoxicity and might serve as a novel combination agent with doxorubicin to limit renal damage.

Keywords: Antioxidant, Doxorubicin, Nephrotoxicity, *Psidium guajava*

IPC code; Int. cl. (2014.01)–A61K 36/00

Introduction

Nephrotoxicity is one of the important side effects of anthracycline antibiotics. A number of other antibiotics including penicillins, cephalosporins, tetracyclines as well as aminoglycosides and sulphonamides are potential nephrotoxicants[1]. Development of nephrotoxicity can further increase load on the kidney leading to severe complications. Doxorubicin (DOX) is a quinine containing anthracycline antibiotic and has been used for the treatment of cancer since 1969. In spite of its high antitumor efficacy, DOX use in chemotherapy has been largely limited due to its cardiac, renal, pulmonary, testicular and haematological toxicities[2,3]. DOX causes imbalance between free oxygen radicals and antioxidants. The disturbance in oxidant-antioxidant system results in tissue injury[4]. Although the exact mechanism of DOX-induced nephrotoxicity remains unknown, it is believed that the toxicity may be mediated through free radical formation, iron dependent oxidative damage of biological macromolecules, membrane LPO, and protein oxidation[5]. Synthetic agents including Vitamin E, Captopril and Amlodipine have been reported for the treatment of nephrotoxicity, but are associated with several side effects such as nausea, diarrhoea, stomach cramps, fatigue, weakness, blurred vision, and bleeding.

Since time immemorial medicinal plants have curative properties due to the presence of various complex chemical substances [6]. The use of drugs from natural origin is encouraging in the present times. Some of the medicinal plants used in treating nephrotoxicity include *Portulaca oleracea* L[7], *Pedalium murex* L[8], *Sida cordifolia* L[9], *Bauhinia purpurea* L[10] and *Ocimum gratissimum* L[11].

Psidium guajava L. is a fruit-bearing tree commonly known as guava, which belongs to the family Myrtaceae. Guava grows nearly throughout India up to 1500 m in height and is cultivated commercially in almost all states. Its leaf contains copious amounts of phenolic phytochemicals which inhibit peroxidation reaction in the living body, and therefore can be expected to prevent various chronic diseases such as diabetes, cancer, heart-disease[12]. Furthermore, decreasing free-radicals has antioxidizing effect in the body, meaning these guava leaf polyphenols can prevent arterial sclerosis, thrombosis, cataract, and inhibit senescence of the body and skin[13]. Many people habitually take medicinal decoction of guava leaf for

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long treatment of diarrhoea[14] and therefore, the safety of guava leaves have empirically been confirmed[15]. People in China use guava leaf as anti-inflammatory and haemostatic agent[16]. Guava is rich in tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins, vitamins, fibre and fatty acids. It is reported that the leaves of *P. guajava* L. contain an essential oil rich in cineol, tannins and triterpenes. In addition, three flavonoids (quercetin, avicularin and guaijaverin) have been isolated from the leaves[17]. The antioxidative activity is conventionally used to indicate the ability of antioxidant to scavenge some radicals. The phenolic compounds from its leaves are oxygen scavengers in foods and have been evaluated by several methods[18]. Scientific studies on the nephroprotective potential of guava leaves are lacking. Therefore, this study was planned to investigate the ethanolic extract of the leaves of against doxorubicin induced nephrotoxicity.

Materials and Methods

Drugs and chemicals

Doxorubicin (Adrim 2 mg/mL) was purchased from the Medplus Pharmacy, Hyderabad. All other chemicals used for the study were purchased locally.

Animals

Wistar albino male rats weighing (150-200 g) were obtained from Mahavira Enterprises, Hyderabad, India. They were housed in ventilated rooms at a temperature of 24±2 ºC with a 12 h light/dark cycle and 54±5% relative humidity, maintained on standard pellet and water *ad libitum* through the experimental period. The animals were acclimatized for a period of one week. The experiments were according to the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), New Delhi, India and approved by the Institutional Animal Ethical Committee (IAEC)

Plant material collection

The fresh leaves of *P. guajava* were collected in the month of December from Sainikpuri, Secunderabad and authenticated from Department of Pharmacognosy, Priyadarshini College of Pharmaceutical Sciences, Hyderabad.

Plant material extraction

The leaves were cleaned, shade dried and grounded to coarse powder. The dried powder (100 g) obtained was defatted by maceration with petroleum ether (60-80°C). The marc was dried and extracted with ethanol. Finally ethanolic extract was air dried at room temperature. 5.6 %w/w extract thus obtained was subjected for TLC evaluation using ethylacetate: methanol (7:3) solvent system. The TLC plate showed a mixture of components having Rf values as 0.35, 0.44, 0.51, 0.67, 0.89. The test samples of ethanolic extract were made in appropriate concentrations using distilled water prior to its use for animal studies.

Phytochemical screening

The preliminary phytochemical screening of ethanolic extract showed positive results for flavonoids, alkaloids, tannins and saponins[19].

Experimental procedure

The rats were randomly divided into four of five animals in each group: Group 1: Vehicle-Distilled water (1 mL/kg, p.o); Group 2: Doxorubicin (8 mg/kg, i.p) once daily for 2 days before the sacrifice of the animal; Group 3*: P. guajava* (100 mg/kg, p.o) daily for
14 days + Doxorubicin (8 mg/kg, i.p) once daily for 2 days before sacrifice; Group 4: *P. guajava* (300 mg/kg, p.o) daily for 14 days + Doxorubicin (8 mg/kg, i.p) once daily for 2 days before sacrifice[20]. On the 17th day, the animals were anaesthetised. Blood was withdrawn immediately through retro orbital puncture for evaluating biochemical parameters. The animal was sacrificed and kidney tissue was rapidly excised for antioxidant and histopathological studies.

Preparation of serum and tissue homogenate

Blood was collected and serum was separated using refrigerated centrifuge (REMI, Mumbai) at 3000 rpm for 10 min. Serum was used for estimation of Urea[21], BUN[22], Creatinine[23] and Total protein[24]. The kidney tissue was washed with ice cold 0.9% saline and homogenised quickly with ice cold 0.1 M Tris HCL buffer, (pH 7.5) to give a 10% homogenate. The homogenate was centrifuged at 10,000 rpm for 20 min and the supernatants were used for estimation of antioxidant enzyme levels.

Antioxidant parameters

Superoxide dimutase activity (SOD)

The assay of SOD was based on ability of SOD to inhibit spontaneous oxidation of adrenaline to adrenochrome. 0.05 mL of supernatant was added to 2.0 mL of carbonate buffer and 0.5 mL of 0.01 Mm EDTA solution. The reaction was initiated by addition of 0.5 mL of epinephrine and autoxidation of adrenaline to adrenochrome was measured at 480 nm.

The change in absorbance for every minute was measured against blank. The results are expressed as unit of SOD activity (mg/wet tissue)[25].

Catalase activity (CAT)

The reaction mixture consisted of 2 mL of phosphate buffer (pH 7.0), 0.95 mL of hydrogen peroxide (0.019M) and 0.05 mL of supernatant. Absorbance was recorded at 240 nm every 10 sec for 1 min. One unit of CAT was defined as the amount of enzyme required to decompose 1µmol of peroxide per min at 25°C. The results were expressed as units of CAT U/g of wet tissue[26].

Reduced Glutathione (GSH)

1 mL of homogenate was added to 1 mL of 10% TCA and centrifuged. 1 mL of supernatant was treated with 0.5 mL of Ellman's reagent (19.8 mg of 5, 5'-dithiobisnitro benzoic acid (DTNB) in 100 mL of 1% sodium citrate) and 3 mL of phosphate buffer (pH-8). The colour developed was measured at 412 nm[27].

Lipid peroxidation (LPO)

0.1 mL of homogenate (Tris HCL buffer, pH 7.5) was treated with 2 mL of (1:1:1) TBA-TCA-HCL reagent and placed in water bath for 15 min, cooled and centrifuged at room temperature for 10 min at 1000 rpm. The absorbance of supernatant was measured against reference blank at 535 nm[28].

Histopathalogical examination

The kidney tissues were fixed in 10% formalin. The specimens were processed for standard procedure and were embedded in paraffin wax. The blocks were sectioned according to hematoxylin and eosin method[29]. Five-micrometer thick histological sections were obtained from the paraffin blocks. The sections were examined under the light microscope and photographs were taken under 100 X.

Statistical Analysis

All data were expressed as the mean ± SEM. The group means were compared by One-way Analysis of Variance (ANOVA) followed by Dunnett´s test, $P<0.05$ was considered significant.

Results and Discussion

Biochemical study

The biochemical results of renal tissue are illustrated in Table 1. Results showed that levels of Urea, BUN, Creatinine and Total protein were increased significantly ($P<0.05$) in Doxorubicin (8 mg/kg) treated group as compared to vehicle treated group. The animals treated with *P. guajava* (100 and 300 mg/kg) showed significant ($P<0.05$) decrease in Urea, BUN, Creatinine and Total protein as compared to Doxorubicin treated group.

Antioxidant study

Antioxidant results of renal tissue are illustrated in Table 2. The levels of CAT, SOD, GSH were decreased significantly ($P<0.05$) and the levels of LPO were increased significantly ($P<0.05$) in

Table 1— Effect of *Psidium guajava* (100 mg/kg and 300 mg/kg) on kidney function test in rats treated with DOX

S. No	Treatment (mg/kg)	Urea (mg/dl)	BUN (mg/dl)	Creatinine (mg %)	Total protein (g %)
1	Vehicle (1 mL/kg)	12.95±1.51	20.77±0.55	1.43±0.37	6.30±0.52
2	DOX (8)	205.2±35.61*	154.2±27.13*	9.4±2.32*	19.17±0.65*
3	PG (100)+DOX(8)	49.35±5.81#	61.61±3.64#	4.00±0.16#	12.57±0.91#
4	PG (300)+DOX (8)	27.94±2.65#	49.06±3.17#	2.08±0.54#	10.46±0.64#
	F (3,16)	5.66	13.75	8.96	58.85

N=5. The observations are Mean ± SEM. *P<0.05 as compared to control and #P<0.05 as compared to DOX (one way ANOVA followed by Dunnett's test). PG = Ethanolic extract of *Psidium guajava*; DOX = Doxorubicin; BUN= Blood Urea Nitrogen

Table 2— Effect of *Psidium guajava* (100 mg/kg and 300 mg/kg) on antioxidant levels of kidney tissue in Rats treated with DOX

S. No	Treatment group (mg/kg)	Cat (U/Mg Wet Tissue)	Sod (U/Mg Wet Tissue)	Gsh (Nmoles/Mg Wet Tissue)	LPO (µM/MG Wet Tissue)
1	Vehicle (1 mL/kg)	7.19±O.24	46.34±1.88	10.51±0.86	1.49±0.05
2	DOX (8)	0.30±0.22*	29.03±5.48*	4.26±0.35*	5.58±0.52*
3	PG(100)+DOX(8)	1.62±0.21#	47.28±4.79#	6.52±0.35#	0.47±0.06#
4	PG(300)+DOX(8)	2.4±0.23#	57.15±2.14#	8.44±0.23#	0.72±0.01#
	F(3,16)	171.79	8.92	26.83	80.69

N=5. The observations are Mean ± SEM. *P<0.05 as compared to control and #P<0.05 as compared to DOX (one way ANOVA followed by Dunnett's test); PG = Ethanolic extract of *Psidium guajava*; DOX = Doxorubicin; CAT= Catalase; SOD= Superoxide dismutase; GSH= Reduced glutathione; LPO= Lipid peroxidation.

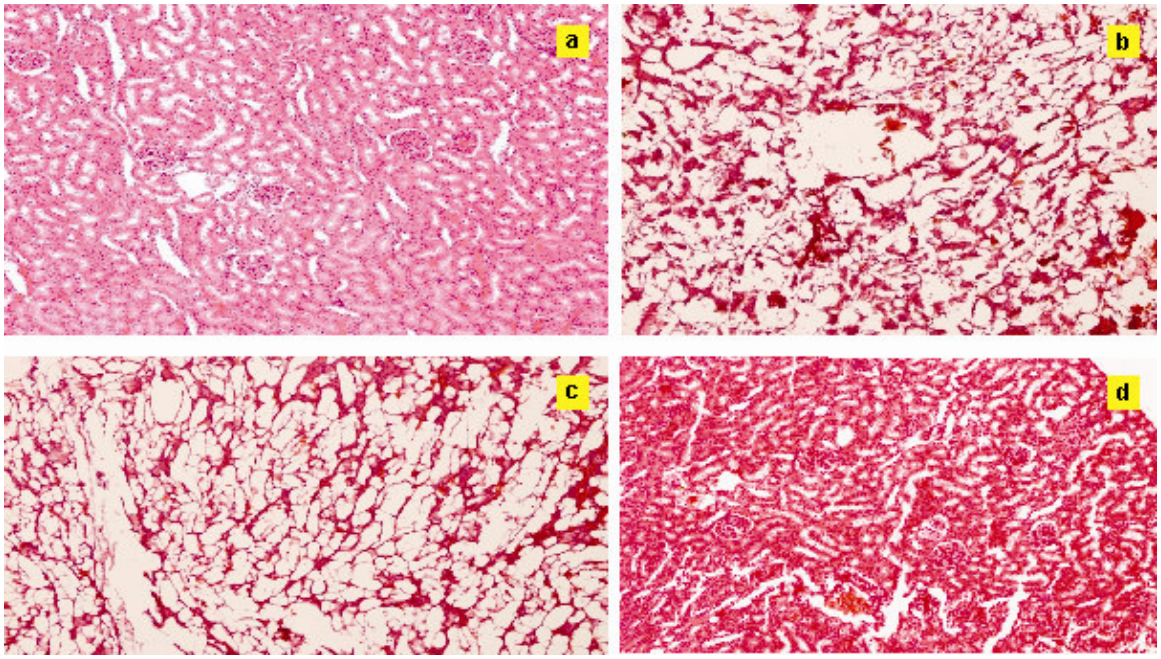

Plate 1 (a-d)- Photographs of histopathological examination (100X) of kidney tissue: a. Control group treated with vehicle showed normal architecture,b. Group treated with doxorubicin (8mg/kg): architecture was destroyed; c. Group treated with *P. guajava* extract (100 mg/kg): showing damaged architecture; d. Group treated with *P. guajava* extract (300 mg/kg): showed normal architecture.

Doxorubicin (8 mg/kg) treated group as compared to vehicle treated group. The animals treated with *P. guajava* (100 and 300 mg/kg) showed significant (P<0.05) increase in CAT, SOD, GSH levels and significant (P<0.05) decrease in LPO levels as compared to doxorubicin group.

Histopathological study

The photomicrographs of histopathological examination (100 X) of kidney tissue of groups treated with doxorubicin showed that the architecture was destroyed whereas those treated with *P. guajava* extract (300 mg/kg) showed normal architecture (Plate 1 a-d).

The present study illustrates the amelioration of ethanolic extract of leaves of *P. guajava* (100 and 300 mg/kg) on doxorubicin induced nephrotoxicity as evident by its biochemical, antioxidant and histopathological data. The nephroprotective mechanisms also appear to be through modulation of various antioxidant parameters thereby improving the overall antioxidant defence of renal tissue[30]. Phenolic compounds and flavonoids are major constituents of most of the plants reported to possess antioxidant and free radical scavenging activity[31] including antimicrobial activity[32]. In our study, *P. guajava* extract (100 and 300 mg/kg) treatment significantly (P<0.05) reversed the changes in antioxidant levels induced by doxorubicin treatment. These findings correlated with the biochemical and renal histopathological examination.

Conclusion

The present study suggests that ethanolic extract (100 and 300 mg/kg) of *P. guajava* leaves has nephroprotective activity against doxorubicin (8 mg/kg i.p.). However, further studies are required to establish role of particular active principles.

Acknowledgements

Authors are grateful to Dr. Uday Kumar Putcha, Head of Pathology Department, NIN Laboratories, Tarnaka for assisting in histopathological study.

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