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Original Research Article

IN VIVO ANTIOXIDANT ACTIVITY OF PHYLLANTHUS EMBLICUS AGAINST CISPLATIN INDUCED OXIDATIVE STRESS IN MICE

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Abstract

Background: Plants are rich source of antioxidants. They can ameliorate the oxidative stress induced complications. Cisplatin is a cytotoxic drug which produced unwanted effects in the patients due to generation of free radicals inside the body. *Phyllanthus emblicus*possessed *in vitro* antioxidant activity.

Objective: The current study was aimed to explore *in vivo* antioxidant potential of *Phyllanthus emblicus* against the oxidative stress induced by cisplatin in mice.

Method: oxidative stress was induced in micewith acute toxic dose (10 mg/kg) of cisplatin given i.p. Animals were divided into five groups (n = 5). Group I: negative control, group II: positive control group III and IV were given methanol extract of*Phyllanthus emblicus* (250- & 500 mg/kg; orally respectively) and group V was standard group receiving orally vitamin C & E (200 mg/kg each) for 20 days. On 21st day, animals were sacrificed and oxidative stress biomarkers were quantified.

Result: *Phyllanthus emblicus*extract showed vigorous *in vivo* antioxidant effect at 500mg/kg by increasing the SOD, CAT, and GSH (antioxidant enzymes) levels in heart, liver, kidney and brain homogentaes and MDA level decreased. Plant also displayed a cure against oxidative stress induced changes in renal, liver and lipid profile parameters.

Conclusion: *Phyllanthus emblicus*raised antioxidant enzyme levels in mice. It manifested hypolipidemic, hepato-renal curative effects. Its adjuvant use with standard therapies may help to resolve unwanted effects.

Key words: *In vivo* antioxidant activity, hypolipidemic, hepato-renal curative

Introduction

Phyllanthus emblicus (common name: Amla) belongs to family Euphorbiaceae. Chemical constituents include ellagic acid, gallic acid, emblicanian A and emblicanian B [1].Number of researches unveiled its various pharmacological properties such as anti-tumor [2], antidiabetic [3], antimicrobial [4], gastroprotective [5], hypolipidemic [6], *in vitro* antioxidant activity[7, 8] antitussive [9] analgesic [10], anti- inflammatory [11], memory enhancing [12] and snake venom neutralizing effect[13]. Cisplatin is a cytotoxic drug which produced unwanted effects in the patients due to generation of free radicals (oxidative stress) inside the body.Oxidative stress is one of the causative agents in number of morbidities. Antioxidants help combat oxidative stress induced diseases. The current study was aimed to explore *in vivo* antioxidant activity of *Phyllanthus emblica*against cisplatin induced oxidative stress in mice.

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MATERIALS AND METHOD

Sample collection and preparation of extract

Fresh fruits of *Phyllanthus emblica* were collected fromsuburbs of Lahore-Pakistan. They were dried under shade and ground to fine powder. Extract was prepared in 70 % methanol by maceration and solvent was removed on rotary evaporator at 40^{o}C.

Preparation of samples

Distilled water was used to prepare *Phyllanthus emblicus*and vitamin C solutions. Olive oil was used for vitamin E. All the samples were administered orally (p.o.).

Induction of oxidative stress

Acute toxic dose of cisplatin (10mg/kg) was given intraperitoneally (i.p). to induce oxidative stress.

Study Design

Twenty five mice of either sex weighing from 25 to 30 g were divided into five groups (n=5). Group I served as negative control, receiving chow and water *ad libitum*. Group IIwas positive control, received only cisplatin (10mg/kg). GroupIII and IV were given cisplatin (10mg/kg)via i.p. route three hours prior to administering plant extract at 250mg/kg and 500mg/kg dose levels respectively for 20 days. Group V was given two standard antioxidants i) vitamin C (200mg/kg p.o.) ii) vitamin E (200mg/kg p.o.) for 20 days.

Collection of blood sample

On 21st day animals were anaesthetized using chloroform and blood samples were collected by cardiac puncture. Serum was separated for analysis of renal function tests (RFT's), liver function tests (LFT's), lipid profile.

Preparation of tissue homogenates

Animals were sacrificed and organs i.e. liver, heart, kidney and brain were isolated and immediately stored in phosphate buffer (pH 7.4). Tissue homogenates were prepared by using ice cold phosphate buffer, it was then centrifuged for 10 minutes at 4000 rpm. Cellular debris was settled down and supernatant was separated and stored for quantification of antioxidant enzymes [glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) malondialdehyde (MDA)].

Quantitative analysis of oxidative stress biomarkers in tissues homogenates

GSH, CAT, SOD and MDA levels were quantified by adoptingMoron *et al.,* 1979,Aebi, 1974, Kakkar *et al.,*1984, and Ohkawa *et al*. 1979methods respectively [14-17].

Determination of renal function tests, liver function tests and lipid profilefor quantitative analysis of oxidative stress biomarkers in serum

Renal function tests (BUNand serum creatinine), liver function tests (ALT, ALP, AST & total protein) and lipid profile (cholesterol, triglycerides, LDL & HDL) were determined by using randox UK kits..

Statistical analysis

The data were presented as mean ± SD. One way ANOVA followed by post hoc duncan test was applied using SPSS version 16.0. P <0.05 was considered significant.

RESULTS

Quantification of oxidative stress biomarkers in tissue homogenates

There was significant increase (P<0.05) inGSH, SOD, and CAT levels and decrease in MDA levels at 500 mg/kg dose of *Phyllanthus emblicus* in liver, brain, kidney and heart homogenates as compared to positive control group (Table 1-4).

Table 1. Quantitative analysis of oxidative stress biomarkers in liver

Groups	Dose mg/kg	GSH µg/mg	CAT µg/mg	SOD µg/mg	MDA µg/mg
Negative control	-	3.52 ± 0.05*	9.88 ± 0.24*	16.78 ± 1.28*	34.25 ± 2.94*
Positive control	10 i.p	1.77 ± 0.09	5.54 ± 0.54	8.85± 1.03	93.64 ± 2.18
Phyllanthus emblicus	250 p.o	2.50 ± 0.08*	5.75 ± 0.12*	10.28 ± 0.28*	93.29 ± 1.84*
Phyllanthus emblicus	500 p.o	2.80 ± 0.17*	6.55 ± 0.17*	13.75 ± 1.28*	72.07 ± 1.61*
Vit. C & Vit. E	200 each p.o	2.67 ± 0.08*	6.35 ± 0.12*	13.27 ± 2.20*	82.02 ± 2.20*

*P<0.05 as compared to positive control

Table. Quantitative analysis of oxidative stress biomarkers in brain

Groups	Dose mg/kg	GSH µg/mg	CAT µg/mg	SOD µg/mg	MDA µg/mg
Negative control	-	3.52 ± 0.05*	9.76 ± 0.65*	18.60 ± 1.88	35.23 ± 1.42*
Positive control	10 i.p	2.08 ± 0.58	5.05 ± 0.95	9.09 ± 1.65	95.02 ± 2.64
Phyllanthus emblicus	250 p.o	2.41 ± 0.08*	5.96 ± 0.26*	10.71 ± 0.50 *	95.23 ± 3.51*
Phyllanthus emblicus	500 p.o	2.64 ± 0.11*	6.65 ± 0.05*	13.21 ± 0.77*	75.87 ± 4.32*
Vit. C & Vit. E	200 each p.o	2.62 ± 0.12*	6.43 ± 0.20*	12.60 ± 1.12*	83.23 ± 1.63*

*P<0.05 as compared to positive control

Table 3. Quantitative analysis of oxidative stress biomarkers in kidney

Groups	Dose mg/kg	GSH µg/mg	CAT µg/mg	SOD µg/mg	MDA µg/mg
Negative control	-	3.42 ± 0.09*	10.12 ± 0.07*	16.55 ± 0.91*	36.03 ± 0.96*
Positive control	10 i.p	1.82 ± 0.09	5.79 ± 0.62	11.02 ± 0.97	93.02 ± 3.57
Phyllanthus emblicus	250 p.o	2.6 ± 0.14*	6.57 ± 0.09*	12.27 ± 0.82*	88.07 ± 2.67*
Phyllanthus emblicus	500 p.o	3.32 ± 0.15*	8.67 ± 0.98*	17.28 ± 0.78*	55.93 ± 9.40*
Vit. C & Vit. E	200 each p.o	2.92 ± 0.18*	7.37 ± 0.20*	15.55 ± 1.0*	75.38 ± 2.54*

*P<0.05 as compared to positive control

Quantitative analysis of liver function tests

Levels of alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) were increased in the positive control group (cisplatin 10mg/kg), while in the same group level of total protein (TP) was decreased. Treatment with *Phyllanthus emblicus* at 500mg/kg showed maximum decrease in the level of these enzymes and increase in total protein content indicating the hepato curative effect of the plant (Table 5).

Table 4. Quantitative analysis of oxidative stress biomarkers in heart

Groups	Dose mg/kg	GSH µg/mg	CAT µg/mg	SOD µg/mg	MDA µg/mg
Negative control	-	3.5 ± 0.08*	9.92 ± 0.34*	19.46 ± 1.73*	36.30 ± 0.81*
Positive control	10 i.p	1.72 ± 0.17	5.51 ± 0.37	9.33 ± 1.07	92.37 ± 2.21
Phyllanthus emblicus	250 p.o	2.65 ± 0.12*	6.53 ± 0.68*	11 ± 0.95*	93.29 ± 2.46*
Phyllanthus emblicus	500 p.o	3.15 ± 0.05*	7.48 ± 0.13*	15.78 ± 1.29*	62.25 ± 2.16*
Vit. C & Vit. E	200 each p.o	2.7 ± 0.08*	7.02 ± 0.35*	13.75 ± 0.58*	79.4 ± 1.71*

*P<0.05 as compared to positive control

Table 5. Effect of various treatments on LFT's

Groups	Dose mg/kg	ALT IU/L	AST IU/L	ALP IU/L	TP mg/dL
Negative control	-	28.75 ± 1.28*	27.93 ± 1.99*	80.14 ± 2.11*	6.15± 0.84*
Positive control	10 i.p	117.22 ± 9.71	108.22 ± 1.40	139.85 ±1.52	2.69 ± 0.96
Phyllanthus emblicus	250 p.o	30.76 ± 0.57 *	31.00 ± 0.96*	118.50 ± 1.18*	4.75 ± 0.53*
Phyllanthus emblicus	500 p.o	23.03 ± 1.40*	22.74 ± 1.90*	77.25 ± 1.35*	6.51 ± 0.89*
Vit. C & Vit. E	200 each p.o	35.0 ± 1.19*	29.52 ± 1.25*	110.52 ± 1.30*	5.51± 0.93*

*P<0.05 as compared to positive control

Quantitative analysis of renal function tests

Injection of cisplatin (10mglkg) increased the level of serum creatinine and BUN in positive control group, which was significantly decreased after various treatments. Maximum decrease in the BUN and creatinine levels was observed in the group of animals treated with *Phyllanthus emblicus* 500mg/kg (Table 6).

Table 6. Effect of various treatments on renal function tests

Groups	Dose mg/kg	BUN mg/dL	CRT mg/dL
Negative control	-	14.75 ± 0.95*	0.56 ± 0.12*
Positive control	10 i.p	31.0 ± 0.81	0.86 ± 0.37
Phyllanthus emblicus	250 p.o	23.0 ± 2.11*	0.81 ± 0.45*
Phyllanthus emblicus	500 p.o	12.25 ± 1.70*	0.53 ± 0.45*
Vit. C & Vit. E	200 each p.o	13.71± 2.62*	0.66± 0.34*

*P<0.05 as compared to positive control

Quantitative analysis of lipid profile

There was increase in cholesterol, triglycerides, and LDL levels in positive control group while HDL value decreased as compare to negative control group.*Phyllanthus emblicus* (500mg/kg) showed maximum decrease in cholesterol, triglycerides, and LDLlevels whereas HDL increased. There was significant variation (P<0.05) in all lipid profile parameters of all the groups with respect to positive control.

Table 7. Effect of various treatments on lipid profile

Groups	Dose mg/kg	Cholesterol mg/dl	Triglycerides mg/dl	HDL mg/dl	LDL mg/dl
Negative control	-	117.09 ± 1.73*	108.43 ± 0.95*	26.75 ± 1.25*	48.40 ± 0.93*
Positive control	10 i.p	180.51 ± 8.61	286.33 ± 4.07	13.10 ± 0.01	71.00 ± 0.55
Phyllanthus emblicus	150 p.o	156.04 ± 5.70*	193.9 ± 4.66*	15.56 ± 0.18*	67.95 ± 1.31*
Phyllanthus emblicus	500 p.o	119.12 ± 2.08*	111.55 ± 2.33*	18.69 ± 0.18*	49.75 ± 0.57*
Vit. C & Vit. E	200 each p.o	128.07 ± 3.10*	119.6 ± 0.95*	24.78 ± 0.10*	55.22 ± 2.15*

*P<0.05 as compared to positive control

DISCUSSION

Results of current study showedsignificant *in vivo* antioxidant potential of *Phyllanthus emblicus* as it increased oxidative stress biomarkers in tissue homogenates. Biochemical tests on serum also revealed hepato-renal curative effect and hypolipidemic effect. From the results, it had been observed that *Phyllanthus emblicus* antioxidant activity was greater than the combined effect of vitamin E and C.It may be concluded from the findings that *Phyllanthus emblicus* extract at the dose of 500mg/kg could be an effective and novel approach for treating oxidative stress induced pathological conditions.

CONCLUSION

*Phyllanthus emblicus*raised antioxidant enzyme levels in mice. It manifested hypolipidemic, hepato-renal curative effects. Its adjuvant use with standard therapies may help to resolve unwanted effects.

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