



#### History:

Received: October 6, 2015  
Accepted: November 13, 2015  
First Published: January 1, 2016  
Collection year: 2015  
Confirmation of publication: Published

#### Identifiers and Pagination:

Year: 2016  
Volume / Issue: 8/1  
First Page: 1  
Last Page: 6  
Publisher Id: JAppPharm-8-1  
DOI: <http://dx.doi.org/10.21065/19204159>

#### Corresponding author:

Dr. Uzma Saleem, Assistant  
Professor, Faculty of Pharmaceutical  
Sciences, G. C. University,  
Faisalabad, Pakistan.  
E. [uzma95@gmail.com](mailto:uzma95@gmail.com)

#### Citation:

Mamuna Naz, Uzma Saleem, Bashir  
Ahmad. In vivo antioxidant activity of  
phyllanthus emblicus against cisplatin  
induced oxidative stress in mice.  
J App Pharm (2016) 8:1. 1-6

### Original Research Article

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

Mamuna Naz<sup>1</sup>, Uzma Saleem<sup>\*2</sup>, Bashir Ahmad<sup>3</sup>

1. Faculty of Pharmacy, The University of Lahore, Lahore-Pakistan.
2. Assistant Professor, Faculty of Pharmaceutical Sciences, G. C. University, Faisalabad-Pakistan.
3. Director / Professor, Riphah Institute of Pharmaceutical Sciences, Riphah International University, Lahore-Pakistan.

### 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 mice with 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 21<sup>st</sup> 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 homogenates 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

© Mamuna *et al.*; Licensee Consortium Publisher (ICDTD Inc.)

### Introduction

*Phyllanthus emblicus* (common name: Amla) belongs to family Euphorbiaceae. Chemical constituents include ellagic acid, gallic acid, emblicanin A and emblicanin 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.

**Reviewing editor:**

Taha Nazir, Ph.D. Scientific Executive  
ICD TD Inc. Saskatoon Saskatchewan  
Canada.: E. [taha@icdttdi.ca](mailto:taha@icdttdi.ca)

**Funding:**

The authors received no direct  
funding for this research.

**Competing Interests:**

The authors declare no competing  
interests

Additional information is available at  
the end of the article.

## MATERIALS AND METHOD

### Sample collection and preparation of extract

Fresh fruits of *Phyllanthus emblica* were collected from suburbs 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°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 II was positive control, received only cisplatin (10mg/kg). Group III 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 21<sup>st</sup> 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 adopting Moron *et al.*, 1979, Aebi, 1974, Kakkar *et al.*, 1984, and Ohkawa *et al.* 1979 methods respectively [14-17].

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

Renal function tests (BUN and 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  $\pm$  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) in GSH, 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 $\mu\text{g/mg}$ | CAT $\mu\text{g/mg}$ | SOD $\mu\text{g/mg}$ | MDA $\mu\text{g/mg}$ |
|-----------------------------|--------------|----------------------|----------------------|----------------------|----------------------|
| Negative control            | -            | 3.52 $\pm$ 0.05*     | 9.88 $\pm$ 0.24*     | 16.78 $\pm$ 1.28*    | 34.25 $\pm$ 2.94*    |
| Positive control            | 10 i.p       | 1.77 $\pm$ 0.09      | 5.54 $\pm$ 0.54      | 8.85 $\pm$ 1.03      | 93.64 $\pm$ 2.18     |
| <i>Phyllanthus emblicus</i> | 250 p.o      | 2.50 $\pm$ 0.08*     | 5.75 $\pm$ 0.12*     | 10.28 $\pm$ 0.28*    | 93.29 $\pm$ 1.84*    |
| <i>Phyllanthus emblicus</i> | 500 p.o      | 2.80 $\pm$ 0.17*     | 6.55 $\pm$ 0.17*     | 13.75 $\pm$ 1.28*    | 72.07 $\pm$ 1.61*    |
| Vit. C & Vit. E             | 200 each p.o | 2.67 $\pm$ 0.08*     | 6.35 $\pm$ 0.12*     | 13.27 $\pm$ 2.20*    | 82.02 $\pm$ 2.20*    |

\*P<0.05 as compared to positive control

Table. Quantitative analysis of oxidative stress biomarkers in brain

| Groups                      | Dose mg/kg   | GSH $\mu\text{g/mg}$ | CAT $\mu\text{g/mg}$ | SOD $\mu\text{g/mg}$ | MDA $\mu\text{g/mg}$ |
|-----------------------------|--------------|----------------------|----------------------|----------------------|----------------------|
| Negative control            | -            | 3.52 $\pm$ 0.05*     | 9.76 $\pm$ 0.65*     | 18.60 $\pm$ 1.88     | 35.23 $\pm$ 1.42*    |
| Positive control            | 10 i.p       | 2.08 $\pm$ 0.58      | 5.05 $\pm$ 0.95      | 9.09 $\pm$ 1.65      | 95.02 $\pm$ 2.64     |
| <i>Phyllanthus emblicus</i> | 250 p.o      | 2.41 $\pm$ 0.08*     | 5.96 $\pm$ 0.26*     | 10.71 $\pm$ 0.50 *   | 95.23 $\pm$ 3.51*    |
| <i>Phyllanthus emblicus</i> | 500 p.o      | 2.64 $\pm$ 0.11*     | 6.65 $\pm$ 0.05*     | 13.21 $\pm$ 0.77*    | 75.87 $\pm$ 4.32*    |
| Vit. C & Vit. E             | 200 each p.o | 2.62 $\pm$ 0.12*     | 6.43 $\pm$ 0.20*     | 12.60 $\pm$ 1.12*    | 83.23 $\pm$ 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 $\mu\text{g/mg}$ | CAT $\mu\text{g/mg}$ | SOD $\mu\text{g/mg}$ | MDA $\mu\text{g/mg}$ |
|-----------------------------|--------------|----------------------|----------------------|----------------------|----------------------|
| Negative control            | -            | 3.42 $\pm$ 0.09*     | 10.12 $\pm$ 0.07*    | 16.55 $\pm$ 0.91*    | 36.03 $\pm$ 0.96*    |
| Positive control            | 10 i.p       | 1.82 $\pm$ 0.09      | 5.79 $\pm$ 0.62      | 11.02 $\pm$ 0.97     | 93.02 $\pm$ 3.57     |
| <i>Phyllanthus emblicus</i> | 250 p.o      | 2.6 $\pm$ 0.14*      | 6.57 $\pm$ 0.09*     | 12.27 $\pm$ 0.82*    | 88.07 $\pm$ 2.67*    |
| <i>Phyllanthus emblicus</i> | 500 p.o      | 3.32 $\pm$ 0.15*     | 8.67 $\pm$ 0.98*     | 17.28 $\pm$ 0.78*    | 55.93 $\pm$ 9.40*    |
| Vit. C & Vit. E             | 200 each p.o | 2.92 $\pm$ 0.18*     | 7.37 $\pm$ 0.20*     | 15.55 $\pm$ 1.0*     | 75.38 $\pm$ 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 hepatocurative effect of the plant (Table 5).

Table 4. Quantitative analysis of oxidative stress biomarkers in heart

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

\*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            | -            | $28.75 \pm 1.28^*$ | $27.93 \pm 1.99^*$ | $80.14 \pm 2.11^*$  | $6.15 \pm 0.84^*$ |
| Positive control            | 10 i.p       | $117.22 \pm 9.71$  | $108.22 \pm 1.40$  | $139.85 \pm 1.52$   | $2.69 \pm 0.96$   |
| <i>Phyllanthus emblicus</i> | 250 p.o      | $30.76 \pm 0.57^*$ | $31.00 \pm 0.96^*$ | $118.50 \pm 1.18^*$ | $4.75 \pm 0.53^*$ |
| <i>Phyllanthus emblicus</i> | 500 p.o      | $23.03 \pm 1.40^*$ | $22.74 \pm 1.90^*$ | $77.25 \pm 1.35^*$  | $6.51 \pm 0.89^*$ |
| Vit. C & Vit. E             | 200 each p.o | $35.0 \pm 1.19^*$  | $29.52 \pm 1.25^*$ | $110.52 \pm 1.30^*$ | $5.51 \pm 0.93^*$ |

\*P<0.05 as compared to positive control

#### Quantitative analysis of renal function tests

Injection of cisplatin (10mg/kg) 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            | -            | $14.75 \pm 0.95^*$ | $0.56 \pm 0.12^*$ |
| Positive control            | 10 i.p       | $31.0 \pm 0.81$    | $0.86 \pm 0.37$   |
| <i>Phyllanthus emblicus</i> | 250 p.o      | $23.0 \pm 2.11^*$  | $0.81 \pm 0.45^*$ |
| <i>Phyllanthus emblicus</i> | 500 p.o      | $12.25 \pm 1.70^*$ | $0.53 \pm 0.45^*$ |
| Vit. C & Vit. E             | 200 each p.o | $13.71 \pm 2.62^*$ | $0.66 \pm 0.34^*$ |

\*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 LDL levels 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            | -            | $117.09 \pm 1.73^*$ | $108.43 \pm 0.95^*$ | $26.75 \pm 1.25^*$ | $48.40 \pm 0.93^*$ |
| Positive control            | 10 i.p       | $180.51 \pm 8.61$   | $286.33 \pm 4.07$   | $13.10 \pm 0.01$   | $71.00 \pm 0.55$   |
| <i>Phyllanthus emblicus</i> | 150 p.o      | $156.04 \pm 5.70^*$ | $193.9 \pm 4.66^*$  | $15.56 \pm 0.18^*$ | $67.95 \pm 1.31^*$ |
| <i>Phyllanthus emblicus</i> | 500 p.o      | $119.12 \pm 2.08^*$ | $111.55 \pm 2.33^*$ | $18.69 \pm 0.18^*$ | $49.75 \pm 0.57^*$ |
| Vit. C & Vit. E             | 200 each p.o | $128.07 \pm 3.10^*$ | $119.6 \pm 0.95^*$  | $24.78 \pm 0.10^*$ | $55.22 \pm 2.15^*$ |

\*P<0.05 as compared to positive control

## DISCUSSION

Results of current study showed significant *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.

## REFERENCES

1. Pozharitskaya ON, Ivanova SA, Shikov AN, Makarov VG. Separation and evaluation of free radical-scavenging activity of phenol components of *Emblica officinalis* extract by using an HPTLC-DPPH method. *J. Sep. Sci.* 2007; 30:1250-1254.
2. Ngamkitidechakul C, Jaijoy K, Hansakul P, Soonthornchareonnon N, Sireeratawong, S. Antitumour effects of *Phyllanthus emblica* L. Induction of cancer cell apoptosis and Inhibition of *in vivo* tumour promotion and *in vitro* invasion of human cancer cells. *Phytother. Res.* 2010; 24 (9): 1405-1413.
3. Rudnick MR, Goldfarb S, Tumlin J. Contrast-induced nephropathy: is the picture any clearer? *Clin. J. Am. Soc. Nephrol.* 2008; 3(1):261-262
4. Firoj A, Amirul IM, Mustafizur R M. Antibacterial activity of *Leonurus sibiricus* aerial parts. *Fitoterapia.* 2006; 77(4):316-317.
5. Chatterjee A, Chattopadhyay S, Bandyopadhyay SK. Biphasic Effect of *Phyllanthus emblica* L. Extract on NSAID-Induced Ulcer: An Anti-oxidative Trail Weaved with Immunomodulatory Effect. *Evid. Based Complement. Alternat. Med.* 2011; 146808: 1-13.
6. Mathur R, Sharma A, Dixit VP, Varma M. Hypolipidemic effect of fruit juice of *Emblica officinalis* in cholesterol – fed rabbits. *J. Ethnopharmacol.* 1996;50:61-68.
7. Naik GH, Priyadarsini KI, Bhagirathi RG, Mishra B, Mishra KP, Banavalikar MM, Mohan H. *In vitro* antioxidant studies and free radical reactions of triphala, an ayurvedic formulation and its constituents. *Phytother Res.* 2005; 19:582-586.
8. Kusirisin W, Srichairatanakool S, Lertrakarnnon P, Lailerd N, Suttajit M, Jaikang C, Chaiyasut C. Antioxidative activity, polyphenolic content and anti-glycation effect of some Thai medicinal plants traditionally used in diabetic patients. *Med. Chem.* 2009; 5(2):139-147.
9. Nosalova G, Mokry J, Hassan KM. Antitussive activity of the fruit extract of *Emblica officinalis* Gaertn. (Euphorbiaceae) *Phytomedicine.* 2003; 10(6-7):583-589.
10. Saha A, Masud MA, Bachar SC, Kundu JK, Datta BK, Nahar L, Sarker SD. The analgesic and anti-inflammatory activities of the extracts of *Phyllanthus reticulatus* in mice model. *Pharm. Biol.* 2007; 45(5):355-359.
11. Ahmed F, Selim MS, Das AK, Choudhuri MS. Anti-inflammatory and antinociceptive activities of *Lippia nodiflora* Linn. *Pharmazie.* 2004; 59(4):329-330.
12. Vasudevan M, Parle M. Memory enhancing activity of Anwala churna (*Emblica officinalis* Gaertn.): An ayurvedic preparation. *Physiol. Behav.* 2007; 91: 46-54.

13. Moros W B, Nascimento M C, Pereira B M, Pereira NA. Plant natural products active against snake bite-the molecular approach. *Phytochemisry*. 2000; 55: 627-642
14. Moron M J, Depierre JW, Mannlrvik B. Levels of GSH, GR and GST activities in rat lungs and liver. *Biochem. Biophys. Acta*. 1979; 582 (1): 67-78.
15. Aebi H. Catalase. *Methods in enzymatic analysis*. New York: Academic press. 1974; 276-289.
16. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. *Ind. J. Biochem. Biophys*. 1984; 21:130-132
17. Ohkawa H, Ohishi N, Yagi K: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem*. 1979; 95: 351-358.



© 2016 The Author(s). This open access article is distributed under a Creative Commons Attribution (CC-BY) 4.0 license.

You are free to:

Share — copy and redistribute the material in any medium or format

Adapt — remix, transform, and build upon the material for any purpose, even commercially.

The licensor cannot revoke these freedoms as long as you follow the license terms.

Under the following terms:

Attribution — You must give appropriate credit, provide a link to the license, and indicate if changes were made.

You may do so in any reasonable manner, but not in any way that suggests the licensor endorses you or your use.

No additional restrictions

You may not apply legal terms or technological measures that legally restrict others from doing anything the license permits