



Paeonol (Extracted from *Paeonia suffruticosa* Root) Pretreatment Lends Antioxidant Protection against Arsenite Stress in Rice (*Oryza sativa* L.) Seedlings

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10.18805/IJArE.A-5951

ABSTRACT

Background: Arsenic toxicity has become a growing concern for rice growers in South East Asian countries due to the extensive use of arsenite-contaminated groundwater in rice paddies. The presence of arsenic in soil and water from irrigation results in impaired crop growth and development. The effect of exogenous pretreatment of paeonol on hydroponically grown rice seedlings was examined by investigating the antioxidant systems under arsenic stress.

Methods: In the experiment, 7-day old rice seedlings (IR-64) were exposed to 10, 50, 100 µM of arsenite separately and co-treatment with 10, 50, 100 µM paeonol (extracted from *Paeonia suffruticosa* root) in a hydroponic medium for 7 days. The activity of lipid peroxidation [malondialdehyde] antioxidants [APX, CAT, SOD, GPX] in root and shoot tissue were determined by standard protocol.

Result: Arsenite treatments inhibited growth correlating with increased arsenic accumulation in rice seedlings. Oxidative stress was induced by overproduction of reactive oxygen species (ROS) and disruption of antioxidant defense systems measured in terms of increased activity of antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase, glutathione peroxidase, catalase and lipid peroxidation (malondialdehyde) in root and shoot tissues separately. Paeonol pretreated seedlings along with arsenic exposure substantially decreased the level of arsenic uptake in plants resulting in comparatively higher plant growth as well as biomass substantial reduction in ROS overproduction and MDA content was observed in paeonol pretreatment as compared to arsenic-exposed seedlings alone. These findings indicate that paeonol has an ameliorating effect on arsenite-induced oxidative stress, suggesting that paeonol enhances the activity of antioxidants and prevents oxidative stress damage by transforming ROS to a neutral and non-toxic end product. Such natural ameliorative methodology will enhance agricultural production in arsenic stressed paddy field environments.

Key words: Antioxidant enzymes, Arsenic, Arsenite, Hydroponic medium, Paeonol.

INTRODUCTION

Arsenic, one of the world's most serious ecological toxicants entering the cell cycle through topographical and anthropogenic activities (Chowdhury *et al.*, 2018) with an elevated concentration up to up to 3200 µg/L in drinking water (McCarty *et al.*, 2011) particularly in South East Asia, causing carcinogenicity (Chandrakar *et al.*, 2016; Mehmood, *et al.*, 2017) and other chronic diseases to millions of human beings and animals *via* plants (Zhu *et al.*, 2019). Constant arsenic toxicity is related to a few sorts of chronic infections including skin, bladder, lung, liver, kidney malignant growth (Waalkes *et al.*, 2004) and arsenicosis which is answerable for some serious infections (Rahman *et al.*, 2018a) in people, while in plants arsenic up-take adversely affects the plant metabolism and lead to bring about different physiological and biochemical variations from the norm (Li *et al.*, 2006). and hinders the development of plants in various manners (Singh *et al.*, 2015), for example, restraint of seed germination, lessened development in the root and shoot (Tang and Miler, 1991), Low yield of foods grown from the ground (Marin *et al.*, 1992). Arsenic is among the toxic metals concentrated to the generation of ROS such as superoxide radical (O₂⁻), hydroxyl radical (OH) and hydrogen peroxide (H₂O₂) in different cell frameworks (Shahid *et al.*, 2017) and

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How to cite this article: Mairaj, S., Nagar, R.D., Rehman, F., Punakkal, A. and Jindal, T. (2022). Paeonol (Extracted from *Paeonia suffruticosa* Root) Pretreatment Lends Antioxidant Protection against Arsenite Stress in Rice (*Oryza sativa* L.) Seedlings. Indian Journal of Agricultural Research. 56(3): 331-336. DOI: 10.18805/IJArE.A-5951.

Submitted: 08-12-2021 **Accepted:** 11-03-2022 **Online:** 02-05-2022

causes oxidative stress (Shi *et al.*, 2004), which bringing about cell damage by enacting oxidative flagging pathways. These reactive oxygen species can be constrained by increasing the production of various enzymes such as SOD, APX, GPX, CAT, glutathione reductase and externally supplied antioxidants especially polyphenolic compounds.

Paeonol is a major bioactive ingredient of cortex moutane (*Paeonia suffruticosa*) which is utilized as a homegrown cure in treating different infections. It has an assortment of corrective impacts including its antibacterial (Rehman and Mairaj, 2013), antifungal (Mairaj *et al.*, 2016), mitigating, cancer prevention agent, (Sun *et al.*, 2004), analgesic (Kim S *et al.* 2004), invulnerable framework strengthening, anti-mutagenic activities mutagenic activities (Hsieh *et al.* 2006), antidiabetic (Valensi *et al.*, 2005). It improved liver capacity in hepatotoxic rodents (Bendong *et al.*, 2012), upgraded blood flow through its inhibitory consequences for both platelet conglomeration and blood coagulation (Abraham *et al.*, 2003), brought down histopathological scores and lessened myeloperoxidase-receptive cells of polymorphonuclear neutrophils accumulation.

In the current examination, we study the role of pre-treatment of rice seeds with paeonol extracted from the root of paeony *suffruticosa* plant to modulate oxidative stress induced by arsenic toxicity in *Oryza sativa* L.

MATERIALS AND METHODS

Chemical and reagent

Chemicals used for the analysis were of analytical grade and purchased from Sigma-Aldrich. Paeonol was extracted from the root of paeony *suffruticosa* plant and identified by element detection, IR, NMR and other parameters.

Isolation of paeonol (2-hydroxy-4-methoxy acetophenone)

The root of the *Paeonia suffruticosa* plant is sterilized with 70% ethanol followed by 0.1% mercuric chloride, shade dried and powdered. 50 g amount of the dried powdered root was transferred into a conical flask containing a sufficient amount of ethanol and macerated for about seven days. The extract was collected, concentrated and the residue dried in a vacuum desiccator (Rehman *et al.*, 2016). The dried plant extract was analyzed for the qualitative analysis of its secondary metabolites by standard protocols (Singh *et al.*, 2012). The active phenolic component (Paeonol) was isolated from other secondary metabolites of dried root extract by steam distillation (Chen *et al.*, 2017). The distillate is collected set at 4°C overnight. The crystal was dried in a silica gel desiccator (m.p. 50°C) and characterized by different parameters and spectral analysis.

2-Hydroxy-4-methoxy acetophenone

Anal found : C 65.32; H 6.19; O 28.49.

C₉H₁₀O₃ required : C 65.06; H6.03; O 28.91.

IR (KBr) : 3360 (OH), 1650 (C=O), 1250 (C-OCH₃) and 830 (Benzene ring substitution) cm⁻¹.

NMR (CDCl₃) : δ 7.5 (s, 3H, -CH₃), 6.2 (s, 3H, -OCH₃), 3.65 (d, 2H, ArH), 2.45 (s, 1H, ArH), 4.7 (bh, 1H, -OH).

Hydroponic medium and treatments

Oryza sativa L. seeds of specific genotype (IR 64) were obtained from IARI, New Delhi. For the experimental procedure, seeds were sterilized with 0.1 per cent solution

of HgCl₂ for about 0.5 minutes, after that seeds were washed 3-4 times by distilled H₂O and divided into two groups. One group of seeds that will later be treated with only arsenic were soaked in double distilled water while another group of seeds was soaked in paeonol (10, 50 and 100 μ M) for pretreatment for one day. These seeds were then transferred to Petri-dishes (3-4 days) kept in the culture room at 26.93°C in dark for proper germination. The seedlings were grown in a hydroponic medium (Dave *et al.*, 2013) for 10 days before treatment and then exposed to AsIII (NaAsO₂; 0, 10, 50 and 100 μ M) for 7 days at 27°C in a humidified culture chamber. All treatments were in triplicate. After seven-day treatment, plants were harvested, rinsed by double-distilled water and used for different parameters.

Antioxidant enzymes assay and oxidative stress markers assay

Treated and untreated rice seedling samples were homogenized in a chilled mortal with 3 ml 0.1 M Na₃PO₄ buffer at seven pH having 1.0% polyvinyl polypyrrolidone, 1.0 mM disodium-EDTA and 0.5 M sodium chloride. The amalgamate were centrifuged at 10,000 rpm about 15 minute at 4°C and the supernatant was utilized to determine APX, SOD, CAT, GPX activities.

The ascorbate peroxidase was estimated according to Nakano and Asada (1987) The superoxide dismutase was measured as per Beauchamp-Fridovich (1971) *via* assessing the inhibition of the reduction of NBT-dye by superoxide dismutase anion. The GPX-activity was estimated as per Hammer Schmidt *et al.*, 1982. The catalase was assessed according to Aebi (1984) The malondialdehyde content was assessed according to the improved method of Hodges (1999).

Statistical analysis

All investigations were conducted triplicate in structure. Analysis of variance (ANOVA) was done with all the data to confirm the variability of data and validity of results and Duncan's multiple range test (DMRT) was performed to determine the significant difference between treatments at (p<0.05).

RESULTS AND DISCUSSION

The activity results of lipid peroxidation [malondialdehyde] antioxidants [APX, CAT, SOD, GPX] of seven days old paeonol pre-treated rice seedling, exposed to applied concentrations (100 μ M, 50 μ M and 10 μ M) of arsenite, paeonol and their dose treatment of paeonol with As III exposure were shown in Fig 1 and 2.

Malondialdehyde [MDA]

The MDA increased by 98%, 75%, 26% in root while by 90%, 70%, 22% in shoots at 7 day pre-treatment by 100 μ M, 50 μ M, 10 μ M As III respectively as compared to control and positively correlated with As accumulation (R=0.974). Upon paeonol pretreatment under the same day and same concentration as As III treatment, the MDA increased by

27%,15% and 6% in root while in shoot 28%, 14%, 5% as compared to control and was positively correlated with As accumulation ($R=0.959$). Upon dose treatment of paeonol with As III the increased level of MDA in root was reduced by about 36%, 37%, 38% while in shoot 33%, 35% and 36% as compared to As III treated rice seedling under the same concentration only and was positively correlated with As accumulation ($R=0.932$) was shown in (Fig 1).

Effect of antioxidant

The APX increased by 79%, 38% and 31% in roots while by 68%, 0 μ M,10 μ M As III respectively as compared to control with As accumulation ($R=0.948$). Upon paeonol pretreatment under the same day and same concentration as As treatment, the APX decreased by 34%, 22%,12% in root while in shoot 28%,18%, 8% as compared to control and positively correlated with As accumulation ($R=0.939$). Upon dose treatment of paeonol along with As III the increased level of APX in root was reduced by about 51%, 44%13% while the shoot by about 44%, 37%, 9% as compared to only As III treated rice seedling under the same concentration only and was positively correlated with As accumulation ($R=0.998$) was shown in Fig 2(a). The CAT increased by 189%,140%, 55% in root while by 125%, 93%, 43% in the shoot at 7 d pre-treatment with 100 μ M, 50 μ M,10 μ M As III respectively and was positively correlated with As accumulation ($R=0.956$). Upon paeonol pretreatment under the same concentration as As III treatment, the CAT increased by 42%, 27%, 20% in roots while in shoots 44%, 36%, 31% as compared to control and was positively correlated with As III accumulation ($R=0.914$). Upon dose treatment of paeonol along with As III the increased level of CAT in root was reduced by about 55%, 52%, 49% while in shoot 53%, 43%, 32% as compared to only As III treated rice seedling under the same concentration only and was positively correlated with As accumulation ($R=0.975$) was shown in Fig 2(b). The GPX increased by 42%, 29%, 20%

in root while by 35%, 26%,17% in the shoot at 7 d pre-treatment 100 μ M, 50 μ M,10 μ M As III respectively as compared to control and was positively correlated with As accumulation ($R=0.913$). Upon paeonol pretreatment under the same day and same concentration as As III treatment, the GPX increased by 29%, 15% and 13% in roots while in shoots 26%, 11% and 9% as compared to control and was positively correlated with As accumulation ($R=0.918$). Upon dose treatment of paeonol along with As III the GPX activity in root was decreased on an average, by about 50%, 44%, 20%, while in shoot 45%, 38%, 17% as compared to only As III treated rice seedling under the same concentration only and was positively correlated with As accumulation ($R=0.925$) were shown in Fig 2(c) The SOD increased by 34%, 28%, 15% in root while by shoot 30%, 22% and 13% at 7 d pre-treatment with 100 μ M, 50 μ M, 10 μ M As III respectively as compared to control and was positively correlated with As accumulation ($R=0.982$). Upon paeonol pretreatment under the same day and same concentration as As III treatment, the SOD increased by 34%, 28%, 15% in root while in shoot 30%, 22% and 13% as compared to control and was positively correlated with As accumulation ($R=0.930$). Upon dose treatment of paeonol along with As III, the increased level of SOD in root was reduced by about 27-50% while in shoot 18-37% as compared to only As III treated rice seedling under the same concentration only and was positively correlated with As accumulation ($R=0.999$) were shown in Fig 2(d).

In the present study, it has been concluded that the MDA level increased with arsenic exposure in rice seedling, which may be attributed to the excessive production of reactive oxygen species such as O_2^- , OH , H_2O_2 under stressed condition by the interaction of arsenic with intracellular components and supported by Chandrakar *et al.* (2017b). From the present study, it has also be concluded that the increased amount of MDA content in rice seedlings

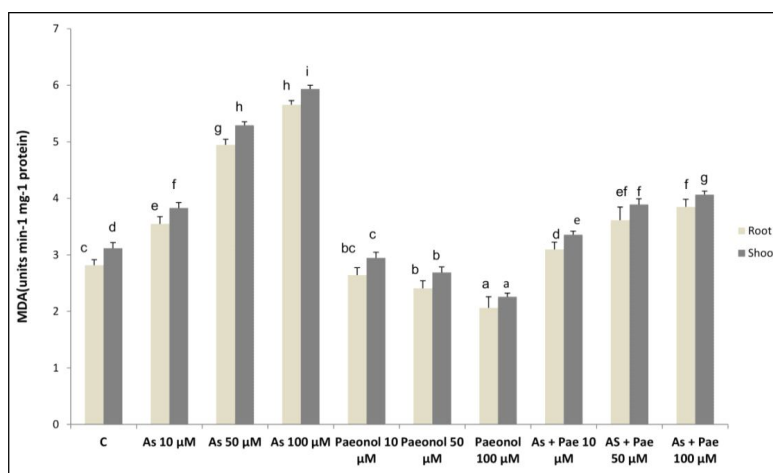


Fig 1: Changes in the level of malondialdehyde (MDA) in roots and shoots of the rice seedlings after the seventh day of treatments with (100 μ M, 50 μ M, 10 μ M Paeonol, 100 μ M, 50 μ M, 10 μ M As and 100 μ M, 50 μ M, 10 μ M As + 100 μ M, 50 μ M, 10 μ M Paeonol); All results are average of three ($n=3$) \pm SD replicates. Significant ANOVA at $P\leq0.05$.

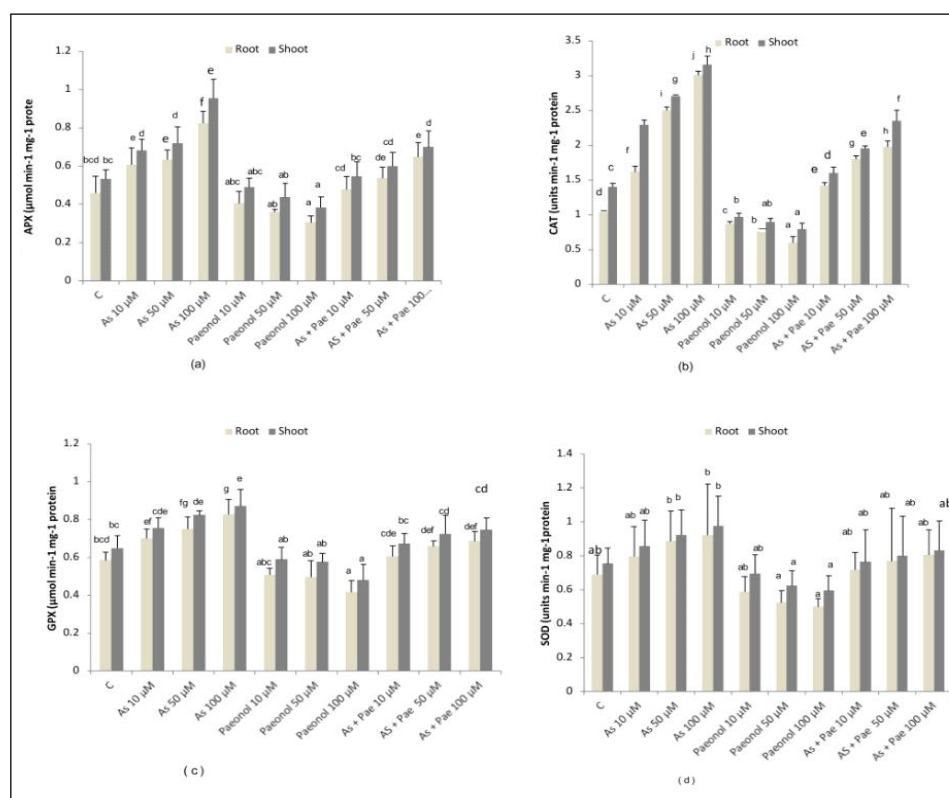


Fig 2: Changes in the level of malondialdehyde (MDA) in roots and shoots of the rice seedlings after the seventh day of treatments with (100 µM, 50 µM, 10 µM Paeonol, 100 µM, 50 µM, 10 µM As and 100 µM, 50 µM, 10 µM As + 100 µM, 50 µM, 10 µM Paeonol); All results are average of three (n=3)±SD replicates. Significant ANOVA at P≤0.05.

significantly decreased on exposure to paeonol alone and jointly with arsenite.

Significant enhancement of APX activity upon arsenic exposure depends upon more availability of H₂O₂ because of efficient break down in ASC-GSH cycle, while the altogether bringing down the estimation of APX, when pretreatment with paeonol, may be due to neutralizing toxic effect of arsenic by the formation of complex and by the reduction (Gautam *et al.* 2019) of O₂⁻ to molecular oxygen resultantly H₂O₂ levels reduced. Upregulation of APX action in mitigating the harmful impacts of H₂O₂ has likewise been reported in tomatoes, maize, *Vigna mungo* L. (Srivastava *et al.*, 2017), GPX activity was reported in rice seedling plants treated with arsenic and paeonol, suggesting that pretreatment with paeonol induces a significant reduction of oxidative stress caused by arsenic (Gautam *et al.*, 2019). The CAT activity in root and shoot were decreased on an average as recorded in arsenite treated rice seedlings, which indicate that paeonol pretreatment with arsenic causes a substantive reduction of oxidative stress, which was produced by toxic arsenic. The up-regulation of CAT activity under the introduction of various protocols of arsenic has additionally been accounted for in *Zea may* (Gautam *et al.* 2019), *Vigna mungo* L. (Srivastava *et al.*, 2017), Results inferred that higher SOD activity during arsenic stress can be a reason for generating H₂O₂. Ameliorate this stress by

diminishing the level of H₂O₂ when exposed to paeonol. Therefore paeonol plays a protective role against the oxidative stress brought about by arsenic. High dose treatment of paeonol in a hydroponic medium decreases the enzyme's activity in arsenic-exposed rice plants and is useful in diminishing arsenic expose rice plant toxicity. The upregulation of SOD under the exposure of various concentration of arsenic has additionally been accounted for in *Vigna mungo* L. (Srivastava, 2017), *Pteris vittata*, *Vetiveria zizanioides* (Tiwari *et al.*, 2017), *Solanum melongena* L. (Singh *et al.*, 2015), maize (Requejo *et al.*, 2006) and well supported by (Dave *et al.*, 2013).

CONCLUSION

In view of the outcomes, it inferred that the assimilation of As by rice plant roots and shoots was fundamentally decreased within the sight of paeonol, prompting an expansion in plant growth and biomass, which may clarify on the formation of a non-poisonous complex with paeonol. and the oxidative stress caused by arsenic may increase the activities of antioxidative enzymes like ascorbate peroxidase (APX), glutathione peroxidase (GPX) catalase (CAT) superoxide dismutase (SOD) and oxidative stress marker, for instance, lipid peroxidation. Paeonol supplementation with arsenite causes a significant decrease in H₂O₂ levels, which shows the mitigating impact of paeonol

on oxidative stress achieved by arsenic. Therefore paeonol plays a protective role against the oxidative stress brought about by arsenic. It concluded that toxic arsenic III, liable for oxidative stress may be converted to the non-lethal complex by the interaction paeonol.

ACKNOWLEDGEMENT

Amity University Uttar Pradesh is highly acknowledged for providing the infrastructure and facilities for the work done.

Conflict of interest: None.

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