



PROTECTIVE ROLE OF METHANOLIC LEAF EXTRACT OF *Holarrhena floribunda* AGAINST SODIUM ARSENITE INDUCED LUNG OXIDATIVE STRESS IN MALE ALBINO RATS



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Abstract: Plant-based dietary components and added substances are known to shield cells from oxidative stress. This research was conducted to ascertain the protective effect of methanolic extract of *Holarrhena floribunda* against arsenite-initiated oxidative stress in the lungs of male albino rats. Thirty-six (36) adult rats with normal weight of 160 g were partitioned into six (6) groups marked A-F and treated as follows; Group A (negative control) was given distilled water, while 100 and 200 mg/kg weight of the leaf extract were given by oral gavage to groups C and D, and groups E and F individually, for 14 consecutive days. 5 mg/kg weight of Sodium arsenite was intraperitoneally administered to groups B (positive control), D and F, 24 h before sacrifice. Aftereffects of Glutathione reductase (GSH), Glutathione peroxidase (GPX) and total protein on post-mitochondrial portion of the lung homogenates demonstrated that sodium arsenite decreased GSH and GPX, while increment in total protein was observed. Co-administration of extract at 100 and 200 mg/kg with sodium arsenite expanded GSH by 5 and 44.3%, and GPX by 38.6 and 10%, while total protein diminished by 10 and 7.5% separately. The investigation demonstrated that the extract can possibly improve arsenite-induced lung oxidative stress and toxicity.

Keywords: Glutathione peroxidase, Glutathione reductase, homogenate, oxidative stress

Introduction

Inorganic arsenic is a noteworthy concern all through the world to a great extent in view of its cancer-causing potential due to after work related or ecological exposure. It is designated as an IARC category 1 cancer-causing agent (IARC, 1987; NRC, 1999). Its exposure to people is related with advancement of malignancies in almost all organs, including the skin, liver, urinary bladder, lung and prostate (Smith *et al.*, 1992; Simeonova and Luster, 2000). Epidemiological examinations have likewise over and over indicated clear dose-response connections between natural arsenic levels and human cancer incidence. The essential wellspring of arsenic in most human populaces is the drinking water, where it dominantly exists in inorganic forms. Unmistakably hazardous levels of arsenic are found in the drinking water from numerous zones all through the world (Bates *et al.*, 1992; NRC, 1999; Pott *et al.*, 2001). In any case, late proof shows that the considerably more direct levels of arsenic, which can be found in drinking water may convey a critical cancer-causing hazard in human populace (Lewis *et al.*, 1999; Morales *et al.*, 2000) practically identical in greatness with that postured by presentation to ecological radon or second-hand tobacco smoke (Morales *et al.*, 2000).

Arsenicals cause cellular harm through the production of free radicals (Barchowsky *et al.*, 1996). A few investigations propose that arsenic compounds elicit their lethality through production of reactive oxygen species, for example, superoxide, hydroxyl radicals, hydrogen peroxide and nitric oxide during their metabolism, in this way assuming a basic part in intervening DNA damage and starting the cancer-causing process in the cells (Liu *et al.*, 2001; Kligerman *et al.*, 2005).

It is established that oxygen free radicals and their metabolites can actuate direct cell damage, which may trigger a course of radical responses advancing the illness process including inflammation and cancer in the lungs (Fantone and Ward, 1982; Jamieson, 1989). Enzymatic and non-enzymatic systems have advanced to secure cells against free radical harm. These defensive components incorporate cancer prevention agents, for example, ascorbic acid, tocopherol, uric acid, bilirubin, retinol and glutathione, and also a few metalloenzymes, for example, superoxide dismutase, catalase and glutathione

peroxidase (Defeng and Arthur 2003; Chaitanya *et al.*, 2011). Be that as it may, these frameworks are not adequate in circumstances of oxidative stress, contamination, UV-exposure, et cetera, where the generation of free radicals significantly increases (Badmus *et al.*, 2010).

Currently, most research work centers around the role of antioxidants and additionally cancer prevention compounds in the treatment and prevention of the maladies related with metal toxicity. The most regular antioxidants are vitamins, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-butylhydroquinone (TBHQ). Nonetheless, their handiness is constrained due to suspected toxicity (Anagnostopoulou *et al.*, 2006). Along these lines, the advancement and usage of more powerful antioxidants of natural origin is desirable.

This has prompted significant consideration towards the identification of plants with antioxidant capacity without harmfulness. A few therapeutic plants have been investigated for their biological activity and active constituents. One of such therapeutic plants is *Holarrhena floribunda* (false rubber tree), belonging to the Apocynaceae family (Letouzey, 1972). There is almost no data on the defensive impact of the plant against ROS toxicities. Henceforth, this investigation fundamentally centered on assessing the ameliorative impact of *Holarrhena floribunda* leaf against arsenite-initiated lung oxidative stress.

Materials and Methods

Plant material

Holarrhena floribunda leaves were collected in Ogbomoso and authenticated by a botanist. The leaves were air-dried in ambient temperature for about two weeks after which they were blended, weighed and kept in a cool dry place.

Preparation of plant extract

Powdered leaves (1000 g) of *Holarrhena floribunda* was soaked in 3 litres of 70% methanol and agitated vigorously. The mixture was allowed to stand for 48 h in the dark with intermittent agitation, and then filtered using Whatman (No. 1) filter paper. The filtrate was left on the bench in petri dishes to evaporate at room temperature for two weeks. The obtained solid crude extract was stored in the refrigerator until ready to use for the preparation of test solution.

Preparation of extract administered

Methanolic extract (20 g) of *Holarrhena floribunda* was dissolved in 300 ml of distilled water and stored at 4°C.

Preparation of arsenite administered

Sodium arsenite (0.01 g) was dissolved in 10 ml of distilled water as the stock solution with a concentration of 1 mg/ml.

Experimental animals

Thirty-six albino male rats with an average weight of 160 g were purchased from the Animal house, University of Ibadan. They were kept in well-ventilated cages under controlled conditions of light (12 hr-light/12 hr-dark cycle). The animals were fed with normal rat pellets purchased from Ladokun feeds Limited and water and allowed to acclimatize over a period of three weeks.

Administration of test samples

There were six treatment groups: A, B, C, D, E and F. Group A was given distilled water as negative control, while groups B, C, D, E, and F were given 5 mg/kg sodium arsenite alone, 100 mg/kg of extract alone, 100 mg/kg of extract and 5 mg/kg sodium arsenite, 200 mg/kg of extract alone, and 200 mg/kg of extract and 5 mg/kg sodium arsenite, respectively (Table 1). Both distilled water and extracts were administered orally, while 5 mg/kg sodium arsenite was administered intraperitoneally on the 14th day of the experiment. The rats were starved overnight after sodium arsenite administration and sacrificed on the 15th day.

Table 1: Grouping of rats based on the substances administered

Groups	Substances administered
Group A	0.3 ml of distilled water
Group B	5 mg/kg of sodium arsenite
Group C	100 mg/kg of extract alone
Group D	100 mg/kg of extract + 5 mg/kg of sodium arsenite
Group E	200 mg/kg extract alone
Group F	200 mg/kg of extract + 5 mg/kg of sodium arsenite

Preparation of lungs homogenate

The experimental animals were anaesthetized using mild chloroform and then fixed and dissected with the ventral side up. Lungs were harvested in each, washed in physiological saline, and transferred into film tubes containing washing buffer (1.15% KCl) to prevent interference of haemoglobin on the enzymic antioxidants. The lungs were minced and mixed with four volumes of homogenizing buffer and homogenized using a mechanically driven homogenizer. The homogenate was centrifuged at 3000 rev/min for 10 min to yield the post-mitochondrial fraction. The supernatant was collected into clean test tubes and was used for determination of enzyme activities and total protein analysis.

Estimation of reduced glutathione (GSH) activity

The assay was carried out using the method of Beutler *et al.* (1963). Homogenate sample (0.2 ml) was mixed with 1.8 ml of normal saline to give a 1 in 10 serial dilution. Precipitating reagent (3 ml) was added to the diluted sample and allowed to stand for 10 min. The mixture was centrifuged at 3000 rev/min for 5 min and 0.5 ml of the supernatant was added to 4 ml of

phosphate buffer pH 7.4. Ellman's reagent (0.5 ml) was added to the solution and the absorbance of sample was read at 412 nm within 30 min of colour development. GSH standard curve was plotted to determine GSH concentration in the homogenates.

Estimation of glutathione peroxidase (GPx) activity

Glutathione peroxidase (GPx) activity was assayed according to the method described by Rotruck *et al.* (1973).

0.2 ml of Tris-HCl was mixed with 0.1 ml of Sodium azide and 0.1 ml of hydrogen peroxide followed by 0.2 ml of lung homogenate and 0.2 ml of glutathione. The contents were incubated at room temperature for 10 minutes and the reaction quenched with 0.4 ml trichloroacetic acid and centrifuged at 3000 rev/min for 5 min. To 1 ml of the supernatant, 3 ml of phosphate buffer and 0.5 ml of Ellman's reagent were added and absorbance was read at 412 nm wavelength. GSH standard curve was plotted to determine GPx concentration in the homogenates.

Total protein analysis

Total protein assay was carried out using Biuret method (Plummer, 1974) using LABKIT reagent. The reagents and samples were pipette into the cuvette as shown in Table 2, mixed and incubated for 10 min at room temperature (15 – 25°C). The absorbances of the calibrator and samples were read against the blank at 540 nm.

Total protein was calculated as:

$$\text{Total protein (g/dL)} = \frac{\text{absorbance of sample}}{\text{absorbance standard}} \times 7 \text{ (calibrator conc.)}$$

Table 2: Protocol for the addition of reagents in total protein analysis

	Blank	Calibrator	Sample
R (ml)	1.0	1.0	1.0
Calibrator (µL)	----	25	----
Sample (µL)	----	----	25

Statistical analysis

Data were presented as Mean ± S.D. The data were subjected to one way Analysis of Variance (ANOVA) followed by Duncan's multiple range test to compare significant difference among the different groups. Thus, a confidence level exhibited at P < 0.05 was considered statistically significant.

Results and Discussion

The result as presented in Table 3 above shows a decrease in body weight in the group given arsenite alone relative to the negative control group while groups given the leaf extract showed positive percentage increase in weight except group E, which showed a non-distinct reduction in body weight. The values of relative weight of lung to the whole body weight of animals after treatment showed no distinct differences across the groups.

Table 3: Body and organ weight of the experimental animals (Mean ± S.D) and % weight change after exposure to arsenite and *Holarrhena floribunda* leaf extract

Group	Treatment	Initial body weight (g)	Final body weight (g)	%Weight Change	Weight of Lung (g)	Relative Lung wt. to Final Body wt. (x 10 ⁻³)
A	Distilled water	157.33±22.79	157.00±30.79	0.21	0.93 ± 0.07	6.16 ± 1.42
B	5 mg/kg Arsenite	165.00±7.02	158.50±16.52	3.93	1.18 ± 0.18	7.54 ± 1.59
C	100 mg/kg Extract	157.50±27.00	163.33±26.22	3.57	1.21 ± 0.22	7.51 ± 1.63
D	100 mg/kg Extract + 5 mg/kg Arsenite	150.33±23.17	150.67±30.43	0.23	1.24 ± 0.24	8.34 ± 1.62
E	200 mg/kg Extract	158.00±13.49	157.60±29.17	0.25	1.31 ± 0.50	8.50 ± 3.36
F	200 mg/kg Extract + 5 mg/kg Arsenite	155.67±13.71	172.67±15.06	10.92	1.18 ± 0.19	6.77 ± 1.02

The result as presented in Table 4 shows that arsenite reduced GSH in the positive control group by 19.2% relative to the negative control group. Co-administration of extract at 100 and 200 mg/kg with arsenite however, showed an increase (5.8 and 39.6, respectively) in the GSH reduced by arsenite-induced toxicity. Groups C and E treated exclusively with the leaf extract showed an increase in GSH compared to the control groups, while group F showed the highest increase in GSH.

The result as presented in Table 5 shows that Glutathione peroxidase was reduced by 30% in animals in the positive control group relative to the negative control group. Co-administration of extract at 100 and 200 mg/kg with 5 mg/kg arsenite however, showed a significant increase of 44.3 and 10%, respectively in the arsenite-reduced Glutathione peroxidase, while groups treated exclusively with extract showed the highest levels of glutathione peroxidase. The result as presented in Table 6 shows a significant increase in the total protein of arsenite treated (positive control) group compared to the negative control group. Co-administration of extract at 100 and 200 mg/kg with 5 mg/kg arsenite in groups D and F however showed a reduction in the arsenite induced-increase.

Table 4: Result of reduced glutathione in the lungs of *Holarrhena floribunda* treated sodium arsenite-exposed rats

Groups	Treatment	Reduced Glutathione* (Unit/100 g Tissue) x 10 ⁻¹
A	Distilled water (Negative control)	17.2 ± 2.8
B	5 mg/kg Arsenite (Positive control)	13.9 ± 0.8
C	100 mg/kg Extract	17.1 ± 6.0
D	100 mg/kg Extract + 5 mg/kg Arsenite	14.7 ± 2.9
E	200 mg/kg Extract	15.7 ± 3.0
F	200 mg/kg Extract + 5 mg/kg Arsenite	19.4 ± 5.1

*Data were expressed as Mean ± Standard deviation of 6 animals per group

Table 5: Result of glutathione peroxidase in lungs of *Holarrhena floribunda* treated sodium arsenite-exposed rats

Groups	Treatment	Glutathione Peroxidase* (Unit/100 g Tissue)x10 ⁻¹
A	Distilled water (Negative control)	10.0 ± 5.1
B	5 mg/kg Arsenite (Positive control)	7.00 ± 0.6
C	100 mg/kg Extract	15.1 ± 6.8
D	100 mg/kg Extract + 5 mg/kg Arsenite	10.1 ± 1.8**
E	200 mg/kg Extract	10.4 ± 3.4
F	200 mg/kg Extract + 5 mg/kg Arsenite	7.7 ± 1.4**

*Data were expressed as Mean ± Standard deviation of 6 animals per group; **p < 0.05

Table 6: Result of total protein in lungs of *Holarrhena floribunda* treated sodium arsenite-exposed rats

Groups	Treatment	Total Protein* (Unit/100 g Tissue) x 10 ⁰
A	Distilled water (Negative control)	10.73 ± 1.33
B	5 mg/kg Arsenite (Positive control)	16.05 ± 3.04
C	100 mg/kg Extract	14.67 ± 1.87
D	100 mg/kg Extract + 5 mg/kg Arsenite	14.43 ± 2.86
E	200 mg/kg Extract	14.97 ± 1.44
F	200 mg/kg Extract + 5 mg/kg Arsenite	14.84 ± 1.82

*Data were expressed as Mean ± Standard deviation of 6 animals per group

Arsenic has been demonstrated to initiate toxicity through oxidative stress in an assortment of tissues and organs (Chen *et al.*, 1995; Wang *et al.*, 1996; Nesnow *et al.*, 2002; Balakumar *et al.*, 2010). Oxygen free radicals (OFRs) reversibly or irreversibly damage compounds of every biochemical class, including nucleic acids, proteins and free amino acids, lipids and lipoproteins, starches, and connective tissue macromolecules. Oxygen free radicals species may disable such cell activity as membrane function and gene expression (Nesnow *et al.*, 2002).

From the outcome (Table 3), administration of arsenite decreased the body weight of rats in the positive control group. Co-administration of the leaf extract with arsenite and selective treatment with the leaf extract, demonstrated a reversal of the diminished body weight. In any case, the values of relative weight of lung to the entire body weight of animals demonstrated an unmistakable differences when contrasted with the negative control group.

Thiols in the reduced glutathione (GSH) constitute one of the primary intracellular and extracellular cancer prevention agent protection frameworks in the lung. Among antioxidants, GSH and its redox enzymes play an imperative defensive role in the lungs and intracellularly in lung epithelial cells (Cantin *et al.*, 1987; Smith *et al.*, 1992). GSH, being the most critical biomolecule against artificially induced toxicity, goes about as a conciliatory target for ROS and other products of lipid peroxidation, for example, reactive carbonyls in the presence of glutathione peroxidase (GPx). GSH hence become oxidized to its dimeric form (GSSG) or structure adducts with reactive carbonyls and other reactive xenobiotics (GS-X) (Meister, 1984; Nicotera and Orrenius, 1986). In the present investigation, a marked depletion (19%) in the levels of GSH was seen in lungs homogenates of rats exposed to sodium arsenite than the positive control (Table 4). Pre-treatment with 100 and 200 mg/kg methanolic extract of *Holarrhena floribunda* preceding sodium arsenite administration significantly (P < 0.05) enhanced the levels (by 5.8 and 39.6% individually) in respect to the sodium arsenite treated group (positive control). This outcome is in concurrence with the observation in pulmonary tuberculosis infected patients and in rats with hypoxic exposure pretreated with Seabuckthorn (*Hippophae rhamnoides* L.) (Pocernich *et al.*, 2000; Nwanjo and Oze, 2007). The alleviation of the reduction in GSH activity by *H. floribunda* increased with increment in dose. This outcome recommends that *Holarrhena floribunda* confers a significant protection against arsenite-instigated oxidative harm on the lungs, in this manner having a toxicity improving potential.

GPx isoforms are a group of selenium-containing enzymes that are available in all mammals and are particularly active in the epithelial lining fluid, epithelium, macrophages and different cells of the lungs, where there is a moderately high exposure to oxidative effect. Their significance has been shown in expand tests by Alvarez and Story (1989), in which enzyme inhibition with mercaptosuccinate (permeate to plasma membrane) increased the lipid peroxidation process 20-fold and in which glutathione was available in its reduced form, subsequently showing that functioning GPx, together with a reductive substrate, is important for counteracting lipid peroxidation. Depleting GSH or repressing GPx significantly increases lipid peroxidation. Diminished activity of the enzyme may bring about the inclusion of harmful oxidative change because of the accumulation of poisonous products (Venkateswaran and Pari, 2003). In the present work, the treatment of rats with arsenite demonstrated a 30% reduction in glutathione peroxidase action in the lungs (Table 5). The oral administration of methanolic extract of *Holarrhena floribunda* at 100 and 200 mg/kg essentially elevated the activity of pulmonary glutathione peroxidase (44 and 10%

separately) amid arsenite induced oxidative stress in rats in respect to the positive control in concurrence with the report of Lee *et al.* (2002). Subsequently, the outcomes acquired uncovered that the methanolic concentrate of *Holarrhena floribunda* leaves impacts positively the antioxidant status of arsenite inebriated lungs, which reinforces its capacity to enhance pulmonary toxicity.

Numerous prior examinations have detailed that ailing conditions are related with the leakage of highly concentrated proteinous fluid (Berg, 2004; Lorraine and Michael, 2005; Bartsch, 1997) into the lungs from the pulmonary vasculature. The aftereffects of this investigation (Table 6) additionally exhibited a stamped increment (50%) in total protein in the lungs of animals exposed to arsenite. Be that as it may, administration of *H. floribunda* leaves extract at 100 and 200 mg/kg abridged arsenite-induced increment in total protein (10 and 7.5% individually) in concurrence with the result obtained by Jayamurthy *et al.* (2011) on hypoxia-induced rats, proposing likewise that *H. floribunda* can restrain arsenite-induced oxidative stress in lungs.

Considering the antioxidant potential of *H. floribunda* as reported by Badmus *et al.* (2010) and the result of this study, it can be inferred that *Holarrhena floribunda* can ameliorate danger caused by oxidative stress and oxidant creating substances like arsenite.

Conclusion

The antioxidant properties of medicinal plants make them highly effective in the prevention and treatment of diseases which emanate as a result of oxidative stress. The result of this study establishes the ability of *Holarrhena floribunda* to ameliorate oxidative stress linked pathophysiology.

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Conflict of Interest

Authors declare there is no conflict of interest associated with the research.

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