Research & Reviews: Journal of Pharmacology and Toxicological Studies

e-ISSN:2322-0139
p-ISSN:2322-0120

Protective Role of Methanol Leaf Extract of Holarrhena Floribunda (*G.Don*) Against Sodium Arsenite-induced Toxicity in Wistar Rats

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Research Article

Received date: 06/9/2021
Accepted date: 20/9/2021
Published date: 27/9/2021

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Keywords: Holarrhena floribunda, sodium arsenite, oxidative stress, anti-inflammatory

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ABSTRACT

Plants bioactive components protect human from heavy metal-induced toxicities. This study investigated the protective effect of the methanol leaf extract of *Holarrhena floribunda* (MLEHF) against arsenate-induced toxicity in male Wistar rats. Animals were pre-treated with two doses of the extract (100 and 200 mg/kg body weight (b.w)) for 14 days before intraperitoneally exposure to sodium arsenide (5 mg/kg b.w.) 24 hr after the last administration. Serum TNF-α, urea and Creatinine levels were evaluated. Also, total protein (TP), reduced glutathione (GSH), malondialdehyde (MDA), lipid hydro peroxides (LHP), glutathione peroxidase (GPx) and superoxide dismutase (SOD) were estimated in rat spleen, testis and heart. The result showed that arsenate only triggers a significant increase in serum TNF-α, urea and Creatinine. Also, arsenate induced significant increase ($P<0.05$) in cardiac, testicular and splenic lipid peroxidation (MDA and LHP) levels. Contrarily, arsenate reduced significantly ($P<0.05$) heart and testis GSH SOD and GPx activities compared with un-exposed control. MLEHF prevented the disgruntled influence of arsenate on the levels of serum TNF-α, urea and Creatinine, and the activities of GSH, SOD and GPx in the testis and heart of rats. The extract also reversed the arsenate-induced increase in cardiac, testicular and splenic lipid peroxidation (MDA and LHP). The protective ability of the extract may be linked to polyphenolic compounds in the leaf extract.

INTRODUCTION

Exposures to heavy metals from different sources are known to prompt oxidative stress that could compromise the health status of an organism [1]. Scientific finding has related oxidative stress in aerobic organisms to austere degenerative conditions such as cardiovascular disease and cancers [2]. Arsenic (As) is naturally occurring metallic-elements ubiquitously present in the environment [1]. Natural, industrial and un-intentional discharges are known primarily as source of human exposure to As in the environment [2]. Water gets contaminated with arsenate due to run-off either from industry or agro-chemical waste, due to over-removal of groundwater for irrigation and from industrial operations [3]. Drinking water remains the predominant route through which human, especially growing up children exposed to arsenite [4]. Although, acute and persistent unregulated exposure to arsenic poisoning is connected with mild inflammatory response,[5] hampering organs and cellular functions, oxidative stress elevation, neurodegenerative disorders, Type-2- diabetes [6] . Also, declines observed in membrane structures and functions in human, cancer incidence and impedes reproductive functions have been connected to As exposure [7,8] . Plant is a natural gift to man, which is employed in ancient period for the wellness of human being. The pre-historic usages are traced to folkloric medicine where various parts of plants are brought together in the form of concoction/decoction use to mitigate disease conditions and its associated complications [9]. Recently, investigations have focused on the role of plant phytochemicals with antioxidant potential against metal-induced systemic maladies because of the maligned effect of synthetic antioxidants [10]. Leaf extract of *Holarrhena floribunda* has recently been shown to contain polyphenolic compounds and compelling antioxidant activity against radicals [11]. A previous study has revealed the preventive potential of the leaf against arsenite-induced hepatic injury [9]. This study, however, investigated the role of the leaf on the arsenite-induced toxicity in serum, spleen, heart and testis.

MATERIALS AND METHODS

Chemicals

Sodium arsenite [$NaAsO_3$], thiobarbituric acid (TNB), trichloroacetic acid (TCA), reduced glutathione (GSH) and 5,5'-dithiobis-2-

nitrobenzoic acid (DTNB) was obtained from Sigma Aldrich, USA. All other reagents used were of analytical grade.

Plant material

Holarrhena floribunda leaves were collected in Ogbomoso during the raining season of June, 2019 and authenticated by a botanist at the Department of Pure and Applied Biology, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Oyo State, Nigeria. The leaves were air-dried at ambient temperature for two weeks, after which they were pulverized and kept in a cool dry place until ready for use.

Preparation of plant extract

Powdered leaves (100 g) of *Holarrhena floribunda* were soaked in 3 litres of 70% methanol and agitated vigorously. The mixture was allowed to stand for 72 hr in the dark with intermittent agitation, and then filtered using Whatman (No. 1) filter paper. The filtrate was concentrated, until dryness using a rotary evaporator at a temperature of 40 ^{0}C. The obtained solid crude extract was stored in the refrigerator until ready to use for the preparation of test solution.

Preparation of administered extract

The leaf extract (10 g) of *Holarrhena floribunda* was dissolved first in 1 ml DMSO and made up with 9 ml distilled water as stock concentration. The subsequent working concentrations were further dissolved in distilled water to make 100 mg/kg and 200 mg/kg body weight. The final concentration of DMSO for the working concentration was 1%.

Experimental animals, groupings and protocols

Thirty-six male Wistar rats averagely weighing 200 g were obtained from the Animal House of College of Health Sciences, LAUTECH, Ogbomoso, Oyo State, Nigeria. The animals were handled and treated based on our Institution's guidelines on ethics and conducts for handling experimental animals which conforms to the international standards. They were housed in cages under standard laboratory conditions of light (12 hr-light/12 hr-dark cycle), fed with normal rat pellets and water *ad-libitum* and allowed to acclimatize over a period of two weeks. The animals were randomly divided into six groups with six animals each, namely A, B, C, D, E and F. The various groups received corresponding treatments has shown in **Table 1** below.

Table 1. Animal Treatment Groups.

Groups	Treatments
A	Distilled water (control)
B	5 mg/kg.bw Sodium arsenite only
C	100 mg/kg.bw extract only
D	100 mg/kg.bw extract and sodium arsenite
E	200 mg/kg.bw extract only
F	200 mg/kg.bw extract and sodium arsenite

Preparation of Tissues Homogenates and Blood Serum

The experimental animals were sacrificed after 24 hr of the last administration period using mild anaesthesia (ketamine hydrochloride (30 mg/kg b.w)). The animals were carefully open and the blood was drained from the heart using a syringe (heart puncturing). The spleen, heart and testis were excised and thoroughly washed in washing buffer to remove the haemoglobin which may inhibit the activity of enzymes. All these procedures were carried out at 4ºC. The organs (1 g) were homogenized in 9 ml of homogenizing buffer using Teflon head Homogenizer under ice to preserve enzyme activities. The homogenates were centrifuged at 9000 rpm for 10 min to obtain supernatants as post-mitochondrial fractions and stored in aliquot at 4ºC. The blood was collected in the small plain sample bottle and centrifuged at 4000 rpm for 10 min to collect serum. The serum collected was stored at 4ºC for further biochemical analysis.

Biochemical Parameters

Serum and tissue total proteins were determined according to the Biuret method of Burtis and Ashwood [12] while quantitative determination of Tumor necrosis factor-alpha (TNF-α) were determined using solid phase Enzyme Linked Immunosorbent Assay (ELISA) designed to measure TNF-α in cell culture supernatant, serum and plasma. This assay employs the quantitative sandwich enzyme immunoassay technique with an antibody specific for TNF-α pre-coated onto a micro plate using Ray Biotech diagnostic (Norcross, GA) kits based on the principle of the interaction between antibody and antigen to quantify the TNF-α in the serum [13]. Serum creatinine [14] and urea [15] were evaluated following the methods as described in the Randox kit. Also, the spleen, heart and testicular homogenates were used to study antioxidant enzymes and oxidative product of macromolecule; Determination of reduced glutathione (GSH) concentration was done using the method described by Anderson [16] while superoxide dismutase (SOD) activity was determined by the methods of Misra and Fridovich [17]. Malondialdehyde (MDA) was estimated spectrophotometrically by thiobarbituric acid-reacting substances (TBARS) as described in the procedure of Varshney and Kale [18].

Statistical protocol

The results were reported as means ± SD of six animals in each treatment group. Data were analyzed using One-Way analysis of Variance (ANOVA) followed by Tukey's post hoc analysis using GraphPad Prism version 6.05 for Windows (GraphPad Software, La

Jolla California, USA. www.graphpad. The value of P<.05 was considered statistically significant.

RESULTS AND DISCUSSION

Arsenic (As) has inherent toxicology potential to initiate oxidative stress in the tissues of arsenic-exposed human with profound health consequences [19]. The oxidative stress instigates either reversible or irreversible damage to several target organs and bio-molecules [20]. It also restricts cellular activity essential for specific membrane function and gene expression [21]. Enzymatic and non-enzymatic antioxidant system is a natural defense mechanism that counteracts deteriorating effects posed by metal-induced radical toxicity [22]. Interestingly, bioactive ingredients obtained from many plants have been established to contain vital constituents that can ameliorate and protects against metal-induced oxidative stress toxicity. Hence, this work investigated anti-inflammatory and anti-oxidative roles of a methanol leaf extract of *Holarrhena floribunda* in sodium arsenite-induced tissue oxidative stress in rats.

Tumor Necrosis Factor-alpha (TNF-α), a known intercellular chemical messenger or cytokine produced by various blood cells is responsible for vital regulation of the body's immune response [23]. The significant (P<0.05) increase in the serum TNF-α level **(Table 2)** in Group B (Sodium arsenite only) was also manifested in the groups exposed to the extract only (Group C and E). The presence of both sodium arsenite and the extract (Groups D and F) significantly depressed the levels of serum TNF-α. This result is in consonant with the *in vitro* study of Hedayati and Co that reported increase secretion of TNF-α in the presence of *Daphne mucronata* extract [24]. Plant extract with immunopotenting component has been shown to have prophylactic action against development of mammary tumours in mice [25]. The decrease in levels of serum TNF-α of both groups exposed to sodium arsenite and the extract could also be attributed to the anti-inflammatory effects of crude methanolic extract of *Holarrhena floribunda* leaves [25]. Significant reduction of sodium arsenite-induced increase in the levels of serum creatinine and urea indicated nephro-protective potential of the extract [26].

Table 2. Tumor Necrosis Factor-Alpha (TNF-α) in *Holarrhena floribunda* treated sodium arsenite-induced rats.

Serum	A	B	C	D	E	F
TNF-α (pg/ml)	21.78±12.08	35.38±3.87	36.91±17.91	25.23±9.36	31.19±21.19	27.44±4.77
Creatinine (mg/dl)	1.01±0.07	35.38±3.87	0.99±0.07	0.86±0.02	0.81±0.10	0.87±0.12
Urea (mg/dl)	56.33±6.85	72.47±12.98	57.73±6.59	43.66±3.93	53.45±8.33	47.71±3.99

Data were expressed as Mean ± standard deviation. Values with different superscripts along the same rows were significantly different (P<0.05). Group A (distilled water only), Group B (Sodium arsenite only), Group C (100 mg/kg extract), Group D (100 mg/kg extract and Sodium arsenite), Group E (200 mg/kg extract alone) and Group F (200 mg/kg extract and Sodium arsenite).

Among the different intracellular substrates altered in the presence of arsenic is a water soluble sulfur-containing tripeptide known as reduced glutathione (GSH). The availability and reducing potential of this molecule in aerobic organisms is linked to its thiol group of cysteinyl residue [27]. The significant (P<0.05) reduction in GSH levels in spleen and testis of rats of Group B (Animal treated with Sodium arsenite only) when compared with the control group. Contrarily, insignificantly reduction of the heart GSH level was observed in the group treated with sodium arsenite only (Table 3). The observed reductions in GSH levels could be attributed to either increase free radical generation or the used up of the molecule and reduce the synthesis of the same. This consequently may lead to a decreased antioxidant capacity of cells, a process which contributes to the oxidative damage of tissues [26]. The exposure of rats with the extract and sodium arsenites protected the spleen and testes of rats by reversing sodium arsenites-induced decrease in splenetic and testicular GSH levels. This result attests to the efficacy of the crude methanolic extract as a booster of the rat antioxidant status challenged with sodium arsenite toxicity. The action of the extract is probably due to its bioactive component's ability to weaken sodium arsenites binding affinity to a sulphridyl group of GSH [28]. Glutathione peroxidases (GPx) are a group of enzymes that are ubiquitously present in mammals and play active roles in cells of different tissues that are highly susceptible to oxidant effect [29,30]. Reduced activity of GPx may be responsible for the oxidative modification of biomolecules caused by continuous accumulation of noxiously toxic products [31]. The results as presented in Table 3 showed that the exposure of rats to sodium arsenite demonstrated significant (P<0.05) reduction in glutathione peroxidase activity in the testis. The oral administration of methanolic extract of *Holarrhena floribunda* leaves essentially elevated the activity of GPx in the testis amid arsenite-induced oxidative stress toxicity in rats. This result is similar to the reports of Ugbaja and Co [32] and which reinforces the capacity of the leaf extract capacity to ameliorate metal-induced testicular enzymes derangements [26]. The insignificant (P>0.05) reduction in GPx levels observed in spleen and heart of rats exposed to sodium arsenite only might either be due to the concentration of the toxicant or its effect required more time to be significant in the tissues.

Similarly, this study shows that sodium arsenites decreased the activities of SOD in the testis and heart tissues **(Table 3)**. SOD is considered the first line of defence against deleterious effects of oxyradicals in the cell by catalyzing the dismutation of superoxide anion radicals to H_2O_2 and GPx converts H_2O_2 to water by oxidation of GSH [33,34]. The result of this study corroborated previous studies indicating compromising potential of arsenic compounds against antioxidant defence system [35]. Sodium arsenites-mediated alterations in heart and testis SOD activities may be linked to elevated level of oxidative stress induced by sodium arsenites [26]. The methanol leaves extract ameliorated oxidative stress in the testis and heart tissues by protecting the sodium arsenite-induced reduction of SOD activity. The disgruntle effects of sodium arsenite marred by the extract might be due to the presence of polyphenolic compounds present in the leaves as previously reported [11] . The cellular constituents of many bio-membranes are susceptible to oxidative cleavage by free radicals. The action of these reactive radicals directly triggers cell damage

Table 3. Effects of methanol leaf extract of *Holarrhena floribunda* on reduced glutathione (GSH), oxidative enzyme parameters (SOD and GPx), lipid peroxidation (MDA) and lipid hydro peroxide (LHP) in spleen, testis and heart of rats.

Parameters (U/mgprotein)	Tissues	Group A	Group B	Group C	Group D	Group E	Group F
GSH	Spleen	1.8±0.20[a]	1.1±0.60[c]	2.5±0.60[a]	2.4±0.80[a]	2.2±0.80[a]	2.3±1.00[a]
	Testis	0.04±0.01[a]	0.02±0.01[b]	0.04±0.01[a]	0.05±0.02[a]	0.04±0.01[a]	0.04±0.02[a]
	Heart	4.2±0.71[a]	3.9±0.32[a]	3.8±0.25[a]	4.0±0.16[a]	3.7±0.37[a]	4.0±0.54[a]
GPx	Spleen	2.2±0.70[a]	2.1±0.70[a]	2.2±1.00[a]	2.30±1.10[a]	2.9±1.20[a]	2.9±1.20[a]
	Testis	24.80±4.53[a]	14.60±2.58[b]	24.60±1.50[a]	25.20±4.90[a]	29.80±4.17[a]	24.80±4.53[a]
	Heart	2.3±0.34	2.0±0.32	2.0±0.23	2.1±0.14	2.1±0.17	2.2±0.03
SOD	Spleen	ND	ND	ND	ND	ND	ND
	Testis	3.37±1.05[a]	1.27±0.05[b]	2.85±0.75[a]	3.27±0.96[a]	2.86±1.48[a]	3.28±0.72[a]
	Heart	89.0±25.00	48.5±13.70	61.8±13.00	3.27±0.96[a]	70.1±40.6	75.1±26.00
MDA	Spleen	20.3±7.8[a]	26.6±8.1[b]	17.1±2.0[a]	22.5±4.8[a]	21.1±3.4[a]	16.6±3.5[a]
	Testis	20.00±0.40[a]	34.00±0.90[b]	26.00±0.40	23.00±0.60[a]	25.00±0.50[a]	19.00±0.50[a]
	Heart	58.90±3.53	97.00±3.63	33.80±1.53	78.40±5.31	30.40±1.35	58.00±3.24
LHP	Spleen	2.40±0.60[a]	4.30±0.90[b]	2.70±0.90[a]	2.80±0.70[a]	2.80±0.80[a]	2.60±1.01[a]
	Testis	ND	ND	ND	ND	ND	ND
	Heart	3.00±0.32	2.40±0.28	2.50±0.43	2.60±0.46	2.20±0.15	2.30±0.20

by covalently binding to bio-molecules in a reaction that encourages lipid peroxidation [36] and implicated in many degenerative diseases such as neurodegenerative, carcinogenesis, inflammation and aging [37]. The degradation of lipid components of most membrane during lipid peroxidation generate different aldehyde secondary products, among which include malondialdehyde (MDA) and lipid hydro-peroxides an easily accessible biomarker of lipid peroxidation [38]. The result showed that sodium arsenites induced significant increases in MDA and lipid hydro peroxides levels in the spleen, testis and heart of rats (Table 3). The induction in MDA and LHP levels may be attributed to increased oxidative damage to lipid membrane components by harmful effect of sodium arsenites due to increased production of ROS/RNS [39]. A decrease in cellular GSH level has been shown to be inversely correlated with elevated lipid peroxides formation observed in this study [21]. However, the increase in MDA and hydro peroxide in the group treated with sodium arsenite only was obviated in the presence of the extract, which indicates its protective potential [19].

Values were expressed as mean± SD. Values with different superscript along the same row are significantly different ($P<0.05$). Group A (Distilled water only), Group B (Sodium arsenite only), Group C (100 mg/kg extract), Group D (100 mg/kg extract and Sodium arsenite), Group E (200 mg/kg extract alone) and Group F (200 mg/kg extract and Sodium arsenite). ND (not determined); GSH (reduced glutathione); Gpx (glutathione peroxidase); SOD (superoxide dismutase); MDA (malondialdehyde); LHP (lipid hydro peroxide).

CONCLUSION

This study established that the antioxidant potential of *Holarrhena floribunda* leaf extract to ameliorate sodium arsenites-induced oxidative stress in tissues. These results also suggest *Holarrhena floribunda* leaves can be of value as an effective agent to protect against metal-induced toxicity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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