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Alternations of cardiac biomarkers in White Pekin ducks intoxicated with arsenic and its amelioration by use of ginger

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ABSTRACT

Cardiotoxicity is an imperative issue in the assessment of heavy metal consumption and inorganic arsenic (As). These have a cardiotoxic effect which is evaluated by biochemical, and oxidative-antioxidant tests, and by the Nrf2-HO-1 pathway. Dried ginger powder is recognized for its efficient antioxidant activities and as a protector of the cardiovascular system from toxic damage caused by heavy metals. However, the possible function of ginger against As in heart via heme oxygenase-1 (HO-1) and nuclear factor erythroid 2-related factor (Nrf2) is unclear. A total of 120 White Pekin ducks were randomly distributed into groups comprising 24 birds in each. Each group comprised 3 replicates having 8 birds in each replicate. The time period of this study was 90 days. The groups were the control [Group I] whereas groups II to IV were fed a basal diet including arsenic at 28 mg/L. Dried ginger powder as an ameliorative agent was mixed with the basal diet and fed at 0.1, 0.3 and 1 g/kg feed to groups III, IV and V, respectively. In the current experiment, dried ginger powder decreased As-induced reactive oxygen species (ROSs) production, oxidative injury and pathological modifications. In addition, cardiac dysfunction factors, intracellular calcium (Ca^{2+}), As accumulation and cAMP deficiency levels were noticed in ducks; these alternations were attenuated by ginger. Furthermore, ginger significantly altered the down regulation of both HO-1 and Nrf2 gene expressions caused by As. Thus, the proven protective role of ginger against As-induced cardiotoxicity may be a consequence of the maintenance of redox homeostasis, i.e. the Nrf2-HO-1 pathway and by enabling As efflux.

Key words: arsenic; duck; Nrf2-HO1 gene; cardiac biomarkers; oxidative-antioxidant

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Introduction

In certain regions of many countries, including India, Bangladesh and China, groundwater contamination is an important route of exposure to As (MANDAL and SUZUKI, 2002) and contaminated drinking water is an important source of As intoxication in poultry (EFSA, 2005). Since As shows toxic effects in myocardial cells, it is well known as a cardiovascular toxicant (VINEETHA et al., 2015). In addition, it generates ROS by decreasing the activities of membrane-based enzymes (BINU et al., 2017). The consumption of As for a prolonged period of time leads to deposition of As in myocardial tissues and ultimately decreases the level of cAMPs which function as intracellular messengers (VARGHESE et al., 2017; ZACCOLO et al., 2009). The activation of Nrf-2 is due to the affinity of As for sulfhydryl, and results in an anti-inflammatory and anti-oxidant response by which detoxification enzymes are activated. Nrf-2 regulates superoxide dismutase (SOD) which gives NADPH to glutathione reductase (GR), and HO-1 regulates apoptosis by generation of anti-oxidant molecules. Moreover the reduction of glutathione disulfide (GSSG) to reduced glutathione (GSH) is catalyzed by GR (ZHANG et al., 2013; LOBODA et al., 2016). The cardiac markers which indicate heart damage, such as creatine kinase (CK) and creatine kinase from the muscle and brain (CK-MB), troponin-T, lactate dehydrogenase (LDH) are significantly increased by As exposure (YU et al., 2017; ADIL et al., 2016; HEMMATI et al., 2018)

Anti-oxidant phytonutrients may prevent the adverse effects of As in cardiac tissues (MANDAL, 2017). The rhizome ginger (*Zingiber officinalis*) is cultivated in India and other parts of the world. From ancient times, it has been consumed worldwide as a spice, a flavouring agent, and it is attributed with many medicinal properties (SHUKLA and SINGH, 2007). It contains active ingredients such as gingerdiol, shogaols, dehydrogingerdiones, gingerdione, acetoxy gingerols, diacetoxy gingerdiol, paradols, acetoxy gingerdiols, and (6)-gingerol, which have anti-oxidant activities and regulate cAMP levels (JIANG et al., 2005). The functional ingredients of ginger have shown many pharmacological effects, including cardio-protective, antioxidant and anti-inflammatory properties (DUGASANI et al., 2010).

However, there are only limited studies that demonstrate the protective properties of dried ginger powder on cardiac biomarkers in ducks exposed to As toxicity. Moreover, there is no drug that has a protective effect on the heart to protect against As toxicity in clinical practice (ZHANG et al., 2013). Therefore, we proposed this study to investigate the effect of dried ginger powder on As-induced myocardial damage related to changes in cardiac biomarkers. Whether ginger can ameliorate the As-induced cardiotoxicity mediated by boosting cardiac function through redox signalling pathways and decrease As accumulation has not previously been determined. The current experiment was undertaken to investigate this problem. The goal of this study was to investigate the cardiotoxicity of As and the ameliorative effect of a dried ginger powder combination in ducks, focusing on the oxidative changes, the Nrf-2-HO-1 pathway, and alterations in cardiac biomarkers.

Materials and methods

Ethical approval. The experiments were carried out at the Central Avian Research Institute (CARI, Bhubaneswar, Odisha, India) in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Environment, Forests and Climate Change, Government of India.

Experimental Designs. A total of 120 seven day old White Pekin ducks (*Anas platyrhynchos*) were procured from CARI. After one week of acclimatization, these birds were arbitrarily distributed into groups comprising 24 birds in each. Each group comprised 3 replicates having 8 birds in each replicate. The time period of this study was 90 days. These birds were cared for and managed in accordance with the ethical considerations for handling birds, and prior to its commencement, the experiment was assessed by the staff of CARI.

Experimental treatments included:

Group-I: Birds were fed with the recommended basal feed and drinking water ad libitum

Group-II: Birds were fed with the recommended basal feed and drinking water treated with arsenic trioxide at 28 mg/L

Group-III: Birds were fed with the recommended basal feed and drinking water treated with arsenic trioxide at 28 mg/L plus dried ginger powder at 0.1 g/kg basal feed

Group-IV: Birds were fed with the recommended basal feed and drinking water treated with arsenic trioxide at 28 mg/L plus dried ginger powder at 0.3 g/kg basal feed

Group-V: Birds were fed with the recommended basal feed and drinking water treated with arsenic trioxide at 28 ppm/L plus dried ginger powder at 1 g/kg basal feed

Arsenic trioxide, in the form of sodium-meta arsenite, and dried ginger powder at different dose rates were fed to the different groups of birds with drinking water and basal feed daily for 90 days respectively. The composition of the basal diet was as per the recommendations of the Bureau of Indian Standards, 2007. The maximum tolerance level of inorganic As for poultry has been set at 50 mg/L, and at 11.36 mg/L it causes fatty liver without showing toxic signs (CHEN and CHIOUT, 2001). As at 30 mg/L causes marked alterations in duck (DAS et al., 2021).

Estimation of cardiac biomarkers. From 2 ml of blood which was collected from puncturing the wing vein, 1 ml was used for separation of serum and the other 1 ml for collection of plasma. LDH and aspartate aminotransferase (AST) were measured using a commercial kit (CORAL, India) following the manufacturer's instructions using a nanospectrophotometer (Eppendorf). Plasma CK, CK-MB, and cTn-T were estimated using a Bio-Rad Microplate reader, and its accompanying commercial kits (My BioSource).

Measurement of oxidative and anti-oxidant biomarkers. At the end of the experiment 3 ml of blood was collected and was used for serum, plasma and DNA separation. After the collection of blood, the birds were sacrificed and their hearts were dissected out. The collected hearts were cleaned in ice-cold 1X PBS (pH 7.4) and stored in separate boxes, labelled and stored immediately at -80 °C awaiting homogenization.

From the homogenized tissue, lipid peroxidation (LPO), superoxide radical anion ($O_2^{\cdot-}$), myeloperoxidase (MPO), nitric oxide (NO), catalase (CAT), and SOD were calculated according to the methods of previous researchers (REHMAN, 1984; WANG et al., 1998; GRISHAM et al., 1994; PATRIARCA et al., 1977; SASTRY et al., 2002; AEBI, 1984; MADESH and BALASUBRAMANIAN, 1993). The ferric reducing antioxidant power (FRAP) assay was performed using an ENZAssayTM antioxidant activity estimation kit according to the manufacturer's protocol. By 5,5′-dithiobis-(2-nitrobenzoic acid) [DTNB] (LAKRITZ et al., 1997), GSH was estimated. Intracellular production of reactive oxygen species (ROSs) by cardiac tissue was determined using cell-permeant 2', 7'-dichlorodihydrofluorescein diacetate (H2DC FDA) assay (Invitrogen) (MAXWELL et al., 1999).

Estimation of Arsenic (As) Accumulation in the Heart: Cardiac tissue samples were wet digested (HERSHEY et al., 1988) and the concentration of As in the digested samples was estimated using a hydride generation atomic absorption spectrophotometer (AAS, ECIL-4141, India) as per the method described by previous researchers (TAGGART et al., 2006), and the results were given in µg/mg wet tissues.

Estimation of Cytosolic Free Calcium Ion (Ca^{2+}) Level and cAMP Concentration: Cytosolic free Ca ions (Ca^{2+}) from cardiac tissue were estimated according to the methods described by previous researchers (ZHANG et al., 2013; KIMURA, 2007). From the supernatant of the homogenized cardiac tissue, cAMP was estimated using a cyclic AMP competitive ELIA kit (Invitrogen) following the manufacturer's instructions.

Estimation of Heme Oxygenase-1 (HO-1) and Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2) mRNA Level by Quantitative Real-Time PCR Assay: Total RNA was isolated from the cardiac tissue samples using a RNAqueous™ total RNA isolation kit (Invitrogen). Quantification of extracted total RNA was done using a nanospectrophotometer

(Eppendorf). From 5 μL of total RNA, cDNA was synthesized using the M-MLV reverse transcriptase (Invitrogen), as described in the manufacturer's system. Quantitative real-time PCR was carried out using a SYBR Green PCR kit (Applied Biosystem) and PCR amplification was conducted on an ABI PRISM 7500 Sequence Detector System (Perkin-Elmer Applied Biosystems). The primer sequences for the genes are as follows:

Nrf-2 Forward: 5'-CCTGGATCTTGATGGTTTCG -3',

Nrf-2 reverse: 5'-AGCCACTTTATTCTTGCCTCT -3';

HO-1 Forward: 5'-CGTTCATGTCGCCGTTGATG -3',

HO-1 Reverse: 5'-GGTCTCCCAGATAGCGAGT GT-3';

GAPDH Forward: 5'-TGCTAAGCGTGTCATCAT CT-3',

GAPDH Reverse: 5'-AGTGGTCATAAGACCCTC CA-3'.

The expression of mRNA levels in each sample was normalized against its GAPDH mRNA level.

Statistical Analysis: All values are presented as mean ± S.E. Statistical analysis was done using Tukey; comparison of all pairs of columns by One-way ANOVA using the Graph Pad Prism v4.03 software program (San Diego, CA, USA), and the differences between the experimental and control groups were considered statistically significant at $p \leq 0.01$ or lower.

Results

There was a significant ($P<0.01$) reduction in body weight in Group II as compared to Group I. The ameliorative groups (Groups IV and V) revealed significant ($P<0.01$) improvement in body weight on comparison with Group-II. It was found that there was a significant ($P<0.01$) decrease in the absolute weight of the hearts in Group II on comparison with Group I. The ginger fed groups showed significant ($P<0.01$) improvement in the absolute weight of the hearts in Group V in comparison with Group-II. The respective data are presented in Table 1.

Table 1. The effect of ginger on the growth performance of White Pekin ducks exposed to arsenic toxicity

Parameters	Group-I	Group-II	Group-III	Group-IV	Group-V
Bodyweight (kg)	2.864 ± 0.290^{e}	2.366 ± 0.193^{a}	2.455 ± 0.371^{b}	2.554 ± 0.254^{c}	2.755 ± 0.233^{d}
Absolute heart weight (g)	19.50 ± 0.89^{b}	14.25 ± 0.44^{a}	14.98 ± 0.38^{a}	15.75 ± 0.38^{a}	18.67 ± 0.60^{b}
Relative heart weight (g)	0.0068 ± 0.0003^{b}	0.0060 ± 0.0002^{a}	0.0061 ± 0.0001^{a}	0.0062 ± 0.0001^{a}	0.0068 ± 0.0002^{b}

Mean±SE values bearing different superscripts within rows differ significantly ($P \leq 0.01$)

In the biochemical parameters there were significant ($P<0.01$) increases in the values of cardiac biomarkers such as AST, LDH, CK, CK-MB, and cTn-T in Group II in comparison with the control Group I. The AST, and cTn-T values showed a significant ($P< 0.01$) decrease in Groups IV and V in comparison with Group-II. Also, LDH, CK, and CK-MB values showed a significant decrease in Groups IV and V ($P<0.05$ and $P<0.01$, respectively), as compared to Group II. The respective data are presented given in Table 2.

It was observed that the As caused a significant ($P<0.01$) rise in levels of ROSs, LPO, MPO, NO and O_2^{-} activities in the cardiac tissue of Group II in comparison to the control. The ROSs, LPO and MPO activities of the heart showed a significant ($P<0.01$) decrease in Groups IV and V compared to Group II. O_2^{-}, and NO activities decreased

significantly in Groups III, IV and V ($P<0.05$, $P<0.01$ and $P<0.01$, respectively), in comparison with Group II. On the other hand, GSH, SOD, CAT and FRAP activities were found to have decreased significantly ($P<0.01$) in Group II. GSH and SOD activities increased significantly ($P<0.01$) in Group V when compared to Group II but FRAP activities increased significantly ($P<0.01$) in Groups IV and V compared with Group II. Also, CAT activity showed a significant ($P<0.05$ and $P<0.01$) increase in Groups IV and V in comparison with Group II. The data pertaining to these parameters are presented in Table 3.

Table 2. The effect of ginger on the cardiac specific biomarkers of White Pekin ducks exposed to arsenic toxicity

Parameters	Group-I	Group-II	Group-III	Group-IV	Group-V
AST (U/L)	157.48±10.97^{a}	296.57±10.67^{c}	250.31±16.86^{b}	227.96±8.08^{b}	184.25±13.13^{a}
LDH (U/L)	374.61±13.73^{a}	557.76±33.34^{d}	505.16±27.71^{cd}	450.98±18.21^{bc}	412.60±18.93^{ab}
CK (U/L)	311.78±37.65^{a}	985.15±98.85^{d}	845.54±54.22^{cd}	785.48±57.11^{bc}	548.46±45.24^{ab}
CK-MB (U/L)	185.25±23.54^{a}	655.57±38.58^{d}	586.27±36.54^{cd}	486.64±18.57^{bc}	325.14±25.12^{ab}
cTn-T (pg/ml)	34.25±5.84^{a}	118.12±22.54^{c}	106.78±14.65^{b}	95.87±12.05^{b}	57.25±9.84^{a}

Mean±SE values bearing different superscripts within rows differ significantly ($P \leq 0.01$)

Table 3. The effect of ginger on oxidative stress and antioxidant parameters in the cardiac tissue of White Pekin ducks exposed to arsenic toxicity

Parameters	Group-I	Group-II	Group-III	Group-IV	Group-V
SOD (units/mg of protein)	53.07±3.15^{c}	30.40±2.10^{a}	32.34±1.60^{a}	36.69±2.59^{a}	44.75±3.48^{b}
CAT (µmole of H_2O_2/mg protein)	8.80±0.52^{c}	3.91±0.27^{a}	4.43±0.24^{a}	5.88±0.27^{b}	6.53±0.66^{b}
FRAP (Fe^{II} iron equivalents in µmole)	344.51±14.01^{d}	199.79±13.35^{a}	235.40±8.46^{b}	278.25±10.56^{c}	309.58±9.61^{c}
GSH (µmole DTNB-GSH conjugate/g wet tissue)	10.87±0.85^{d}	3.60±0.35^{a}	4.43±0.49^{ab}	5.99±0.71^{bc}	7.85±0.87^{c}
ROSs (pmol/mg protein)	4.11±0.74^{a}	22.25±2.45^{d}	19.75±3.64^{c}	15.87±2.76^{c}	9.64±1.05^{b}
LPO (nmol MDA/g of tissue)	270.91±19.57^{a}	604.58±17.34^{c}	538.44±16.55^{c}	422.39±32.13^{b}	369.89±27.09^{b}
MPO (in u/mg of protein in 1min)	6.51±0.51^{a}	19.71±0.99^{d}	16.26±0.83^{c}	13.83±1.39^{c}	10.17±0.62^{b}
NO (µmole/g tissue)	8.84±0.67^{a}	40.77±2.48^{e}	32.42±2.04^{d}	24.33±1.19^{c}	18.14±1.04^{b}
$O_2^{\cdot-}$ (pmol/min/mg protein)	0.88±0.07^{a}	4.08±0.25^{e}	3.11±0.16^{d}	2.49±0.29^{c}	1.86±0.13^{b}

Mean±SE values bearing different superscripts within rows differ significantly ($P \leq 0.01$)

There were significant (P<0.01) increases in the values of total As and Ca^{++} in Group II in comparison with the control, whereas these values showed significant (P<0.01) decreases in Groups IV and V in comparison with Group II. cAMP showed a significant (P<0.01) decrease in Group II as compared to Group I but it showed a significant increase in Groups IV and V (P<0.05 and P<0.01, respectively) compared to Group II. The respective data are presented in Table 4.

There was significant (P<0.01) down-regulation of HO-1 and Nrf2 in Group II in comparison to Group I, whereas, these values showed significant (P<0.01) up-regulation in Groups IV and V in comparison with Group II (Table 5).

Table 4. The effect of ginger on total As, Ca^{2+}, and cAMP parameters in the cardiac tissue of White Pekin ducks exposed to arsenic toxicity

Parameters	Group-I	Group-II	Group-III	Group-IV	Group-V
Total As (μg/g)	0^{a}	2.5 ± 0.14^{e}	2.17 ± 0.34^{d}	1.64 ± 0.57^{c}	0.078 ± 0.002^{b}
Ca^{++} (nmol/L)	85.48 ± 8.47^{a}	165.25 ± 15.34^{e}	141.47 ± 12.27^{d}	134.81 ± 11.32^{c}	113.82 ± 11.03^{b}
cAMP (pmol/mg of protein)	15.84 ± 2.64^{e}	4.14 ± 0.32^{a}	5.12 ± 0.14^{b}	7.14 ± 0.25^{c}	11.78 ± 0.74^{d}

Mean±SE values bearing different superscripts within rows differ significantly ($P \leq 0.01$)

Table 5. The effect of ginger on As-induced HO-1 and Nrf2 on the mRNA level in the cardiac tissue of White Pekin ducks exposed to arsenic toxicity

Parameters	Group-I	Group-II	Group-III	Group-IV	Group-V
HO-1 mRNA/ GAPDH mRNA	124.42 ± 3.14^{e}	54.14 ± 7.21^{a}	65.24 ± 5.47^{b}	81.54 ± 7.65^{c}	111.25 ± 11.24^{d}
Nrf2 mRNA/ GAPDH mRNA	138.84 ± 5.17^{e}	62.17 ± 3.78^{a}	73.14 ± 3.15^{b}	91.87 ± 6.32^{c}	124.34 ± 10.87^{b}

Mean±SE values bearing different superscripts within rows differ significantly ($P \leq 0.01$)

Discussion

In this experiment, we studied cardiac function associated with the Nrf-2-HO-1 pathway and As accumulation in relation to the protection of provided by ginger against As-induced cardiac injury in White Pekin ducks, in vivo.

It is known that in response to As treatment LDH, AST, CK, and CK-MB levels increase as the most important bio-makers of cardiac cell injury, disorder and necrosis (ZHANG et al., 2013), and especially CK-MB, which is a more sensitive marker of cardiac cell injury than total CK activity. In the present study, both LDH and AST concentrations increased significantly following As intoxication. Elevated cytosolic enzymes reveal the ability of As to interact with membrane proteins, which then leads to the modification of cell membrane integrity, and thereby its release into circulation (KANAN et al., 2004). Increased myocardial damage in As intoxicated birds seems to be a consequence of elevation of serum LDH and AST concentrations (BALASUBRAMANIYAN and NALINI, 2007). The ginger combination treatment suppressed the release of both LDH and AST. It is well known that excessive ROS eventually attack the cell membrane and destructively damage cellular architecture (LINDROS, 1995). In this scenario, the potential

antioxidant property of ginger may eliminate the excessive ROS, thereby preventing tissue damage. Troponin is not usually seen in serum but is usually released when necrosis occurs, making it an important indicator in the diagnosis of cardiac problems (THYGESEN et al., 2007). Free radicals may increase the calcium mediated proteases activity that could damage the cellular proteins and release cytosolic enzymes into the serum (PATEL et al., 2001; REN and WOLD, 2008). Reports are available on 6-Gingerol antioxidant activity which prove that it potentially ameliorates cardiac damage by inhibiting the free radical mediated peroxidation of lipids and the discharge of cytosolic enzymes from cardiac tissue in rats (MANSOUR et al., 2008**;** ATTYAH and ISMAIL, 2012). Therefore, ginger altered the cardiac biomarkers levels in plasma under As intoxication, which may be a consequence of protection provided by ginger against lipid peroxidative stabilization of cardiac membranes, and thus preventing leakage of cardiac cytosolic enzymes via their hypolipidaemic and antioxidant activity (AL-AZHARY, 2011). Our study revealed that ginger in the diet of birds reduced the activities of cTn-T, CK-MB, LDH, ALT, and AST dose dependently, by maintaining not only the structural and functional integrity but also the permeability of the myocardial membrane, therefore limiting the escape of these indicative enzymes.

Our data showed that intracellular calcium accumulation after exposure to As is at least partially due to ROS formation (YANG et al., 2011) induced by As, which is in accordance with previous studies (ZHANG et al., 2013). Cellular antioxidants are reduced because of the formation of ROS by arsenic. The body's anti-oxidant system, such as SOD, CAT, and GSH, scavenge ROS and ultimately prevent the increased production of ROS and their harmful effects (ARCHANA et al., 2014). Arsenic causes MDA production because of the impairment of each cell's natural protective system, and also due to GSH depletion (WANG and XU, 2006). It has also been found that there is a decrease in levels of LPO in G-V, indicating the ameliorative effect of ginger. This finding is in agreement with the findings of HERVE et al. (2019) in birds. Chronic arsenic exposure increases both the serum activity of CAT and MPO, as a consequence of increased ROS (BANERJEE et al., 2010). These levels were found to be significantly decreased in Group III indicating the protective effect of ginger. These findings agree with the studies of EL-SHARAKY et al. (2009) in rats. Non-enzymatic anti-oxidant GSH, which is tripeptide in nature, has an important role in the protection of cells by ROS generated by arsenic (MASELLA et al., 2005). There was a significant reduction in the activities of SOD, GSH, CAT and FRAP, indicating the oxidative stress in arsenic treated Group II. According to WANG and XU (2006), the decrease in CAT activity in arsenic intoxicated pigs resulted in the impaired ability to detoxify H_2O_2 and so accumulation of H_2O_2. In the current study the rise in SOD, GSH, CAT and FRAP activities in Group V shows the ameliorating effect of ginger on arsenic toxicity. These findings may be correlated with the findings of HABIBI et al. (2014) and AN et al. (2019) in birds.

The biological status of a cell may be correlated with its glutathione redox state (BASSENGE et al., 2000), whereas Nrf2 has been shown to be a critical transcription factor that binds to the antioxidant response element in the promoter region of a number of genes (HOU et al., 2011). Hence, the Nrf2 pathway is probably the most central pathway in cells dealing with oxidative stress generated from exposure to the exogenous chemical As. The HO-1 enzyme possesses antioxidant and protective properties during oxidative stress (SHI and ZHOU, 2010). After exposure to As, the antioxidant defense system in birds cannot maintain the depletion. Consequently, we observed that there was significant down-regulation of Nrf2 and HO-1. In addition, there were various forms of oxidative damage in the White Pekin ducks' hearts indicated by the increase in ROS.

Ginger shows anti-inflammatory and anti-oxidant effects by Nrf-2 induction (NAKAMURA et al., 2004; CHUN et al., 2014). Our findings showed that dried ginger powder in the diet causes up-regulation of Nrf-2. It has been described that ROS production is one of the most important factors for expression of this gene. The active ingredients in ginger, such as 6-shogaol and 6-gingerol, are able

to increase the expression of the Nrf-2 gene through modification of cellular signalling pathways (BAK et al., 2012; SCHADICH et al., 2016). It was also stated that ginger administration led to increased expression of the Nrf-2/HO-1 pathway in rats (VIPIN et al., 2017). SUMI et al. 2011 stated that cardiac myocytes have a weak ability to excrete As into the extracellular space, which was attributed to the modest activation of Nrf-2, indicating a decrease in the metabolism and excretion of As. It is plausible that ginger can facilitate As efflux by suppression of the As_2O_3-induced down-regulation of Nrf-2.

Phosphodiesterases (PDEs) play a major role in regulating cAMP-mediated signalling by hydrolyse phosphodiester bonds in the second messenger cAMP, resulting in the nucleoside 5' -monophosphates AMP (BOSWELL-SMITH et al., 2006; GRESELE et al., 2011). The active ingredients of ginger help the inhibition of PDEs and ultimately raise the cAMP level (RÖHRIG et al., 2017). In our studies, treatment with ginger attenuated As-induced calcium overload and the deficiency of cAMP which might be attributed to maintenance of Ca^{2+} homeostasis. However, several studies have researched the cAMP production potential of ginger, but the results are inconsistent (GHAYUR et al., 2005; CHANG et al., 2011).

Conclusions

In this study, cardiotoxicity developed following 90 days treatment with As in the form of sodium-(meta) arsenite. In conclusion, the protective role of ginger against As-induced cardiotoxicity was established in the preservation of redox homeostasis via the Nrf-2-HO-1 pathway and facilitation of As efflux. To the best of our knowledge, this finding indicates that co-administration of dried ginger powder in As-treated birds may be a novel therapeutic approach. Additional investigation is necessary in future to explain other potential means by which ginger protects from As-induced cardiac injury, using other bird or animal models. The antioxidant property of ginger has the potential to improve the cardiovascular health of birds chronically exposed to elevated As concentrations through contaminated water supplies. While consuming dried ginger powder in basal feed is a healthy and useful approach for the prevention of cardiovascular disease, additional research is needed into the proper compounds and nanoformulations that preclude antioxidant degradation, and to lessen As-induced cardiovascular toxicity.

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SAŽETAK

Kardiotoksičnost je vrlo važna u procjeni problema koji nastaju konzumacijom teških metala. Anorganski arsen (As) ima kardiotoksičan učinak koji se procjenjuje na temelju biokemijskih, oksidacijsko-antioksidacijskih nalaza te na temelju puta Nrf2-HO-1. Sušeni đumbirov prah poznat je po svojoj učinkovitoj antioksidacijskoj aktivnosti i zaštitnom djelovanju u slučaju intoksikacije kardiovaskularnog sustava teškim metalima. Nejasna je međutim uloga đumbira u odnosu na arsen u srčanom mišiću putem hem-oksigenaze 1 (HO-1) i faktora 2 povezanog s nuklearnim eritroidnim faktorom. Ukupno je 120 bijelih pataka pasmine pekinška patka nasumično podijeljeno u skupine koje su sadržavale po 24 jedinke. U svakoj skupini provedena su 3 ponovljena postupka (replikacije) na po 8 jedinki. Ukupno je vrijeme istraživanja bilo 90 dana. Skupina I bila je kontrolna skupina koja nije primila ni arsen ni đumbir. Pokusnim skupinama od II do V je, uz osnovnu prehranu, u različitim kombinacijama dodavan arsen u dozi od 28mg/L i sušeni đumbirov prah u dozama od 0,1g/kg 0,3g/kg i 1 g/kg. Rezultati su pokazali da je sušeni đumbirov prah smanjio prisutnost reaktivnih vrsta kisika (ROS) uzrokovanu arsenom, oksidacijsko oštećenje i patološke promjene. Osim toga uočene promjene, kao što su su faktori srčane disfunkcije, unutarstanični kalcij (Ca^{2+}), nakupljanje arsena i deficijencija razine cAMP-a, ublažene su đumbirovim prahom. Đumbir je, nadalje, znakovito utjecao na smanjenje genske ekspresije i HO-1 i Nrf2 uzrokovane arsenom. Zaključeno je da bi zaštitna uloga đumbira u slučaju kardiotoksičnosti uzrokovane arsenom mogla biti posljedica održavanja redoks homeostaze, odnosno puta Nrf2-HO-1 i omogućavanja eliminacije arsena.

Ključne riječi: arsen; patka; gen Nrf2-HO1; srčani biomarkeri; oksidacija i antioksidans