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EFFECT OF ACUTE ARSENIC TRIOXIDE TOXICITY AND ITS TREATMENT ON HSP70 AND CATALASE mRNA IN RENAL TISSUES

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ABSTRACT

The induction of heat shock protein (HSP70) in tissues relayed on tissue type, damage induced by stress and metal specific as arsenic trioxide (As2O3) toxicity which considered the biggest inducer of HSP70 which represents biochemical finger prints of exposure and provides protective mechanism against subsequent lethal effect of arsenic trioxide toxicity. Forty adult male albino rats (180 ± 20gm) were segregated into four groups, 10 animals each. First group (control group) rats were injected s/c with single dose of saline. Second group, rats were injected s/c with arsenic trioxide (0.043mM).Third group, rats were injected I/P with 0.7mM of meso 2,3di mercaptosuccinic acid (DMSA). Fourth group, rats were injected s/c with arsenic trioxide (0.043mM) followed by I/P injection with (DMSA) (0.7mM) after 30 minutes of arsenic

trioxide dose. The results revealed significant increase in renal lipid peroxidation (measured as malondialdehyde, MDA) which was associated with a significant decrease in the antioxidant systems such as reduced glutathione (GSH) levels and catalase activity in arsenic trioxide intoxicated group. On the other hand, treatment of rats with DMSA after arsenic trioxide led to a significant decrease in

MDA concentration and increase levels of GSH and the activity of catalase when compared with those of arsenic trioxide intoxicated group. Furthermore, total protein and total globulins showed significant decrease in arsenic trioxide intoxicated group than DMSA with arsenic trioxide treated group. At the level of gene expression, we found marked elevation in HSP70 mRNA in arsenic trioxide-intoxicated group while in arsenic trioxide group treated with DMSA its level decreased. Catalase mRNA exhibited a decrease in its level in arsenic trioxide-intoxicated group meanwhile, arsenic trioxide-treated group with DMSA returned catalase mRNA levels to normal. Our results concluded that decreased reduced glutathione concentration and catalase activities in arsenic trioxide intoxicated group were the main inducers for HSP70 mRNA in renal homogenate and DMSA was considered as an effective treatment for acute As2O3 toxicity through amelioration of its oxidative stress especially in the first 30 minutes.

Key word: As_2O_3, DMSA, CAT mRNA, HSP70 mRNA, RT-PCR, GSH

INTRODUCTION

Arsenic **(As)** is one of the metalloid compounds widely distributed in environment where it can be used to treat acute promyelocytic leukemia[(1)] but, on the other hand, inorganic arsenic is also known as human carcinogen as its long term exposure through water consumption is associated with an increased risk for tumors of skin, kidney, liver and bladder [(2,3)].Inorganic **As** exists predominantly in trivalent **(As^{+3})** and pentavalent **(As^{+5})** forms where trivalent compounds are more toxic than pentavalent ones[(4)].

Arsenates **(As^{+3})** are reported to act at a molecular level by inactivation of sulfhydryl group (SH) of oxidative enzymes and inactivation of SH group of glutathione or other essential monothiols and dithiols [(5)] where during their metabolism in cells generate reactive oxygen species (ROS) like superoxide, hydroxyl radical and hydrogen peroxide which were responsible for some of their toxic effects [(6)].

Meso 2,3-dimercaptosuccinic acid (DMSA) has been reported to be effective in reducing lethality of trivalent arsenicals in laboratory animals due to its lower toxicity, easy oral administration and the enhanced biliary clearance of As poisoning as DMSA combine in the body with **As** produced stable and rapidly excreted compounds via kidneys[7].

Heat shock proteins (HSPs) are a family of highly conserved proteins that are induced by a number of stressors including toxic metals, and they are considered the early and sensitive biomarkers of cell stress, especially HSP70 which was induced as a result of reactive oxygen species (ROS) generated in **As** toxicity [8].There were three sources for ROS generations. Firstly, the intermediary arsinic species that have been formed [9].Secondly, methylated arsenic species can release redox-active iron from ferritin and this free iron could play a role in generating reactive oxygen species by promoting conversion of $O_2^{\cdot -}$ and H_2O_2 into highly reactive $^{\cdot}OH$ radical through the Haber-Weiss reaction[10].Thirdly, ROS may also be formed during oxidation of arsenite to arsenate[11].The role of thiol compounds (glutathione and proteins) in **As** disposition and toxicity is multifunctional. It probably includes a cofactor for enzymes in the metabolism of **As**, a reducing agent to form **AsIII**, and to form free thiols needed as binding agents to form **As III** complexes. The complexes can have several functions and could include reducing the free concentration of **AsIII**, changing the redox potential of **AsIII** (either oxidation or reduction because **As** is a metalloid), or altering the transport of **AsIII** across membranes[12].The potency of **As** to induce HSP depends on its capacity to reach the target, its valence and type of exposure, as arsenite is considered the biggest inducer of HSP70 mRNA in the kidney [13].Thus, the objective of this study was to follow up the transcriptional response and biochemical changes after exposure to single acute arsenic trioxide **(As_2O_3)** and its treatment with DMSA. Gene expression changes were studied in the kidney using reverse transcriptase polymerase chain reaction (RT-PCR).

MATERIAL AND METHODS

CHEMICALS:

Reduced glutathione (GSH),Dithiobis-nitrobenzoic acid (DTNB), Meso 2,3-dimercaptosuccinic acid (DMSA), Trichloroacetic Acid, thiobarbituric acid and arsenic trioxide (As_2O_3) were purchased from Sigma-Aldrich (Chemie GmbH St. Louis, MO, USA). Total RNA was extracted with RNeasy Mini Kit (QIAGEN).RT-PCR was performed using RobusT II RT-PCR Kit from Finnzymes Oy, Finland.

ANIMAL TREATMENTS

Forty adult male albino rats (180±20gm) were obtained from the central animal's house of faculty of Veterinary Medicine, Zagazig University. Animals were maintained in stainless steel cages at the biochemistry department under hygienic condition for 2 weeks before experiment at 25±0.5°C, under a 12:12 light/dark cycle. The animals received standard rat pellet diet and water *adlibtum*. The animals were segregated into four equal groups as follow: First group (-ve control) rats were injected s/c with single dose of saline. Second group (II) rats were injected s/c with As_2O_3 (0.043mM/kg B.w) according to [14].Third group (+ve control) rats were injected I/P with DMSA (0.7 mM/kg B.w) according to [14]. Fourth group (IV): rats were injected s/c with As_2O_3 (0.043 mM /kg B.w) followed by I/P injection with DMSA (0.7 mM/kg B.w) after 30 minutes of As_2O_3.

At the end of the experimental period, all rats were anesthetized and sacrificed by cervical dislocation after four hours of last treatment.

Blood was collected by cardiac puncture in labeled tubes without anticoagulant for obtaining serum while kidney tissues were isolated, cleaned from of adhering matters, washed with saline solution, where small portions immediately kept in liquid nitrogen until be used for determination of HSP70 mRNA and catalase mRNA and another portions were homogenized using tissue homogenizer in 100mM potassium phosphate buffer containing 1mM EDTA, pH 7.4,

centrifuged at 3000 rpm/ 10 min and stored at −20 ◦C for the biochemical studies

1 – DETERMINATION OF BIOCHEMICAL PARAMETERS:

Catalase activities were determined according to method by[15].Reduced glutathione (GSH) were measured by[16].Determination of MDA (Malondialdehyde) concentration as a marker of lipid peroxidation according the method adapted by [17].Levels of total protein and albumin were estimated colorimeterically according to the manufacturer's instructions and confirmed by [18]. Globulins were estimated by substracting total proteins from albumin as adopted by [19].

2-DETERMINATION OF CATALASE AND HSP70 GENE EXPRESSION IN RENAL TISSUES using a semi-quantitative RT-PCR according to [20].

A-PROTOCOL OF RNA EXTRACTION FROM RENAL TISSUES:

Total RNA was extracted with RNeasy Mini Kit (QIAGEN).

B-PROTOCOL OF REVERSE TRANSCRIPTASE

POLYMERASE CHAIN REACTION:

Total RNA of 1µg was reverse transcripted to cDNA followed by PCR amplification by using RobusT II RT-PCR kits from Finnzymes Oy,Finland and the protocol according to instruction provided with kit. The primer designed for HSP70 gene and catalase gene to amplify PCR products that cross introns to avoid confusion between mRNA transcript and genomic DNA and sequence were sense: 5-CAA GAT CAG CGA GGC TGA CAA G-3. antisense: 5-AAC TGT ACA CAG GGT GGC AGT G-3 & sense: 5-TTC TGG CCC ACC AAC TTC-3 antisense: 5- CCC ACA GAC TCG GCA

CTC -3 for HSP70 and catalase respectively. Band intensity was measured with a Storm 840 PhosphorImager and quantified with Image Quant software (Molecular Dynamics). PCR products were separated on a 1% agarose gel using 100bp DNA ladder for electrophoresis of PCR product of HSP70, Catalase from Biobasic, Canada.

STATISTICAL ANALYSIS: The data obtained from this work were statistically analyzed with one way ANOVA [21].

RESULTS

Table (1) Effect of administration of As_2O_3 and As_2O_3 with DMSA on Catalase activity, reduced glutathione concentration and Malondialdehyde levels in kidney homogenate of male albino rats compared to control groups.

Groups	Catalase activity in kidney homogenate (µM H_2O_2 decomposed/gm tissue)	Reduced glutathione concentration in kidney homogenate (mg/gm tissue)	Malondialdehyde (MDA) in kidney homogenate (nmol/g tissues)
Control(I)	12.56 ± 1.08 [a]	10.67±0.50 [a]	70.35 ± 0.83 [c]
As_2O_3(II)	6.08 ± 0.88 [b]	4.26±0.42 [c]	167.73 ± 1.03 [a]
DMSA(III)	10.42 ± 0.62 [a]	10.12 ±0.53[a]	68.12 ±1.07 [c]
$As_2O_3$and DMSA(IV)	12.70 ± 0.56 [a]	7.31±0.31 [b]	145.08 ± 1.30 [b]

Means bearing different superscript in a column differ significantly at **P** ≤0.05.

Table (2) showed that highly significant decrease of serum total protein and total globulins in As_2O_3intoxicated group than other groups while albumin showed no significant difference between As_2O_3 intoxicated group and other groups.

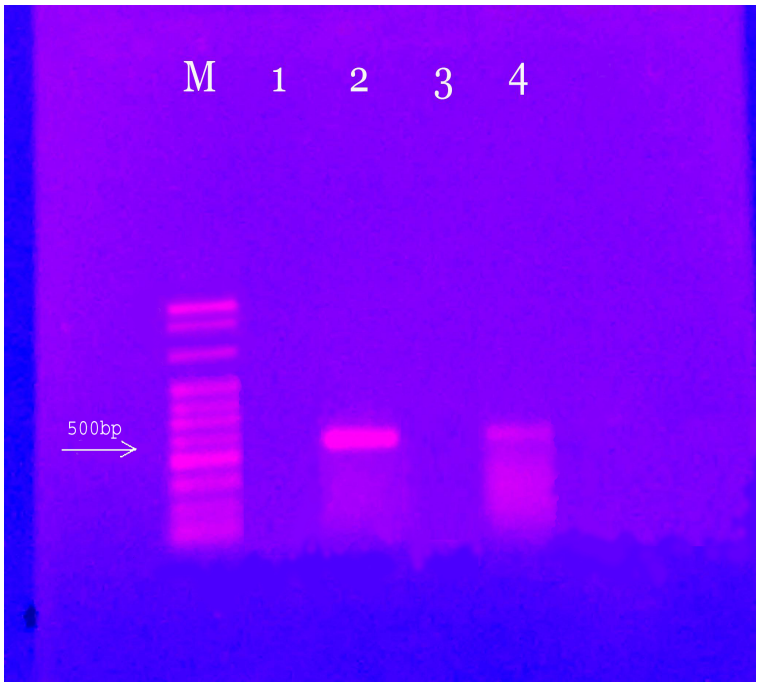

Fig. (1) The electrophoretic photograph of HSP70 mRNA (500bp) expression in kidney of male albino rats. Lane 1: Control group. Lane 2: expression of HSP70 mRNA in male albino rats treated with acute S/C As2O3.Lane 3: expression of HSP70 mRNA in male albino rats treated with I/P injection DMSA group. Lane 4: expression of HSP70 mRNA in As2O3 plus DMSA group.

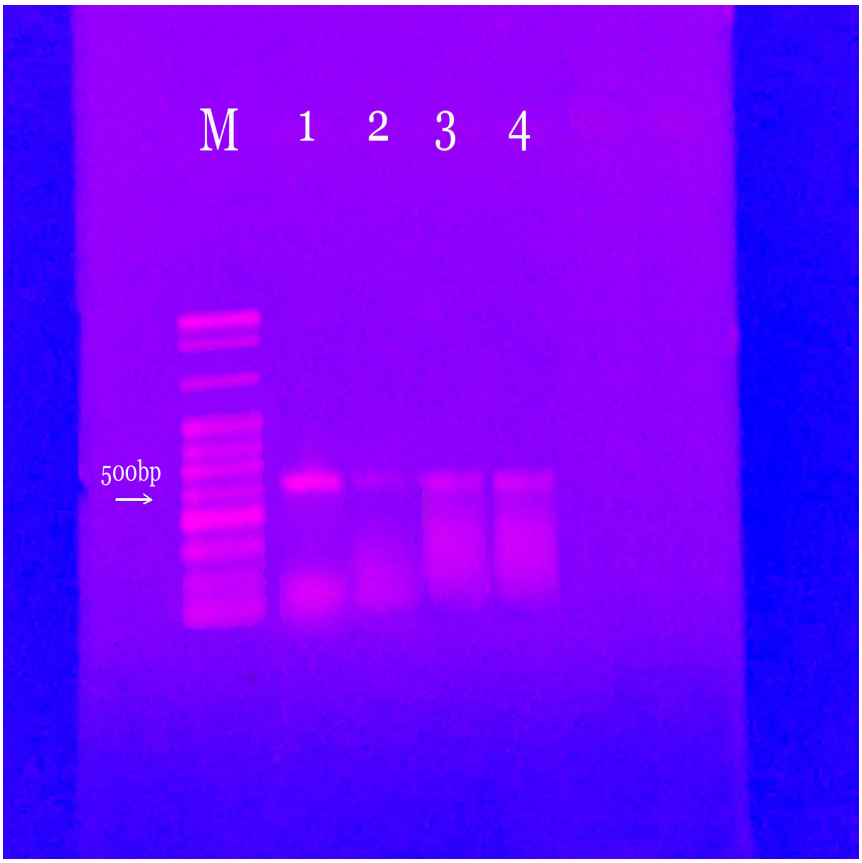

Fig. (2) The electrophoretic photograph of catalase mRNA (500bp) expression in Kidney of male albino rats. Lane 1: Control group. Lane 2: expression of catalase mRNA in male albino rats treated with acute S/C As2O3. Lane 3: expression of catalase mRNA in male albino rats treated with I/P injection of DMSA group. Lane 4: Expression of catalase mRNA in As_2O_3 plus DMSA group

Means bearing different superscript in a column differ significantly at **P** ≤0.05.

Table (1) showed that highly significant decrease of renal catalase activity and reduced glutathione concentration in As_2O_3 intoxicated group than other groups while there were no significant difference in catalase activity between DMSA with As_2O_3treated group and control indicating that As_2O_3 oxidative stress were ameliorated by DMSA that return catalase activity to control level and reduced glutathione reach near to control.Renal Malondialdehyde exhibited highly significant increased in As2O3 intoxicated group compared to control group.

Table (2) Effect of administration of As2O3 and As2O3 with DMSA on total proteins, albumin and total globulins concentration in serum of male albino rats compared to control groups.

Groups	Total protein concentration (gm/dl)	Albumin Concentration (gm/dl)	Total globulins concentration (gm/dl)
Control(I)	6.74±0.30 [a]	2.69±0.22 [b]	4.05±0.31[a]
As_2O_3(II)	4.12±0.22 [c]	3.01 ±0.19 [ab]	1.11 ±0.12 [d]
DMSA(III)	5.56 ±0.25 [b]	3.34 ±0.14 [a]	2.22 ±0.24 [c]
$As_2O_3$and DMSA(IV)	6.50±0.25 [a]	3.43±0.19 [a]	3.07±0.33 [b]

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DISCUSSION

This study was designed to follow up the oxidative stress of acute As_2O_3 toxicity and the role of DMSA treatment in ameliorating this effect on the level of HSP70 mRNA, catalase mRNA, ROS scavenger antioxidant.

The lipid peroxidation end product, MDA, was significantly elevated in renal tissues of rats treated with arsenic trioxide compared to control group. These results were supported by [22] who showed that **As** increased ROS levels in blood and thiobarbituric acid reactive substance (TBARS) in liver and kidney suggested oxidative stress following free radical generation. Reactive oxygen species (ROS) were involved in the initiation of lipid peroxidation and oxidative stress in different tissues [23]**.**In the present study we found significant reduction of GSH concentration after As_2O_3toxicity while in As_2O_3-treated group with DMSA we found amelioration of GSH concentration near the control. These results were in agreement with [24] who demonstrated that the reduction of GSH concentration in acute As_2O_3 - intoxicated group attributed to binding of **As** with the various intracellular sulfhydryl groups with increase in the level of GSSG or this rapid decrease in the GSH concentration may be due to the formation of the tris complex where low level AsIII caused oxidative stress from increased activity of NADH and/or NADPH oxidase[25]**.**Other explanation of reduction of renal GSH after acute **As** toxicity was recorded by [26] who showed that trivalent arsenicals inhibit the action of GSH reductase which form GSH and this inhibition may alter intracellular redox status that eventually leads to cytotoxicity while in As_2O_3-treated group with DMSA we found amelioration of GSH concentration near the control values and this result came in harmony with [27] who recoded that DMSA act as antioxidant that scavenging ROS due to its thiol groups and these explain our result about restoring of GSH levels after treatment of arsenite-intoxicated group with DMSA. This finding supported by [28]

who showed that loss of GSH content in **As**-treated rats and then was partially restored by DMSA but not to the level of control. This present study showed a significant reduction of renal catalase activity and their mRNA in As_2O_3exposed rats while in As_2O_3- treated group with DMSA led to increased catalase activity near to control. Our findings agreed with those reported by [29] who explained reduction of catalase activity after acute As_2O_3toxicity by increase free radical production as superoxide anion radicals and hydrogen peroxide which inhibit catalase and make tissues more susceptible to biochemical injury**.** Furthermore, overproduction of ROS in the tissue cells lead to reduced catalase mRNA expression and as a result, catalase protein expression was decreased and catalase activity was inhibited [30] and other explanation that catalase activity inhibited after As_2O_3toxicity due to NADPH accumulation, as NADPH is required for the activation of catalase from its inactive form [31]**.**

On the other hand we found intoxicated rats treated with DMSA revealed increased catalase activity which was supported by [28] who reported that treatment with DMSA along with **As** restored the loss of catalase activity.Our results showed that As_2O_3 intoxicated group revealed significant reduction of total protein however, DMSA showed a significant elevation to control level.

Our findings were confirmed by the results of [32] who found reduction in the protein content after **As** exposure and attributed this reduction to high affinity of **As** towards different amino acid residues of proteins, which is considered as the premier biochemical parameter for early indication of stress or other explanation by changes in the secondary structure of protein resulted from completely disappeared helical structure and bends in secondary structure after **As** exposure might be of some important structural alterations in the existing proteins and /or expression of new types of proteins [33]**.**Other explanation due to free radicals that released as result of **As** treatment could cause reduction in protein synthesis [34, 35]**. As** exerts its toxic effects by reversible reaction with sulfhydryl groups, especially vicinal dithiols of proteins and enzymes leading to

inhibiting of enzymes activities which responsible for protein biosynthesis [36].Furthermore, we found the treatment with DMSA restored total protein to control levels and this result was supported by [32] who found that DMSA treatment reduces the toxic effects of **As** and helps the recovery of total protein in the tissues and its return to the level of the control as DMSA act by enhancing the excretion of **As** and reducing the tissue concentrations to such low levels that the effects of **As** would be obviated.

Our results showed that As_2O_3 intoxicated group revealed elevation of HSP70 mRNA expression and when treated with DMSA showed significant reduction.

Our results came in accordance with those recorded by [37] who showed that arsenite is ubiquitous inducer of heat shock response in mammalian cells, that it activated heat shock transcription factor 1(HSF1), enhanced induction of HSP70 mRNA and this induction appear to provide a protective mechanism against oxidative stress**.**

As_2O_3 induced HSP70 through release of ROS causing activation and hyperphosphorylation of HSF1[38] while the elevation of HSP70 after As toxicity attributed to inhibition of Pyruvate dehydrogenase enzyme complex (PDH) that leads to decreased production of ATP[39]. On the other hand we found decreased of HSP70 mRNA after treatment with DMSA as this was supported by [8] who found that HSP70 may be potential biomarker for monitoring cellular damage induced by **As** with other markers of oxidative injury to provide an excellent fingerprint for the early stages of injury**,** while a thiol-containing general antioxidant, completely inhibited the induction of HSP70 to the control level and this also came in agreement with [40] who reported that antioxidant significantly reduced expression of HSP70 via decreased phosphorylation of HSF1through inhibiting ROS activity. Our results concluded that acute As2O3 toxicity caused oxidative stress that increased HSP70 mRNA while decreased catalase mRNA with change in redox status in renal tissues and DMSA protect these adverse effects.

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