Morphological and histological changes induced by arsenic trioxide in mice offspring

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ABSTRACT

Introduction: Arsenic is a standout amongst the most lethal metals derived from the natural environment. The major reason for human arsenic toxicity is tainting of drinking water. Arsenic trioxide is inorganic arsenic that is classified by the US Environmental Protection Agency as a known human carcinogen. Aim: Illustration of morphological and histological effects of arsenic trioxide on mice offspring. Methods: Adult female albino mice were divided into five groups: Control, negative control treated with Hcl (arsenic trioxide solvent), and three groups daily treated orally with different doses of arsenic trioxide (0.3, 0.7 and 1 mg/kg). Offspring of 21 day old mice were morphologically examined, weighed and morphometric measurements were carried out. Liver and kidney were histopathologically examined. Results: Data showed a significant decrease in body weight and crown rump length in the arsenic high dose treated group and a significant decrease in head circumference, thigh and a significant increase in foot length of all treated groups. Statistics revealed a significant increase in tail measurements with the lowest dose, while the higher doses showed a significant decrease. Severe degenerative histopathological changes in different treatments were also observed. Discussion: Exposure of mice embryos to arsenic trioxide before and during pregnancy-induced morphological and histological abnormalities. It is suggested that the inhibitory effects of arsenic trioxide on embryonic development and body measurement might be attributed to elevating proapoptotic and decreasing antiapoptotic gene activity. The increase of foot and tail measurements in some cases might be explained as a result of increased proliferation rate and mutation in developing systems.

INTRODUCTION

It is known that arsenic is a naturally occurring metalloid that is ubiquitous in the crust of the earth and biosphere. Arsenic and other heavy metals cause cytotoxicity by causing oxidative stress [1]. When Arsenic produces reactive oxygen species in both natural and inorganic structures, oxidative stress develops [2]. It is widely distributed in nature and released into the environment through agricultural usage and industrial processes, arsenic occurs in two oxidative forms; a trivalent form, arsenite (As_2O_3; As III) and a pentavalent form, arsenate (As_2O_5; As V). As III is 60 times more toxic than As V. Organic arsenic is not toxic as inorganic arsenic. Arsenic toxicity inactivates up to 200 enzymes, most notably those involved in DNA replication and repair and cellular energy pathways [3].

Arsenic exposure dose and duration critically influence growth retardation and fetal death [4 & 5]. The effects of severe arsenic poisoning include gastrointestinal [6], skin and respiratory tract neoplastic alterations, haematopoietic, cardiovascular, nervous, hepatic, endocrine, and kidney damage [7]. Experiments have shown that heavy metal exposure causes severe gonadal damage in many species during spermatogenesis [8]. Arsenic affects the male and female sex organs and may cause fertility issues in these organs. In males, arsenic may induce gonad dysfunction through blocking testosterone synthesis, apoptosis and necrosis [9 & 10]. Human may be exposed to inorganic arsenic via ingestion through drinking water as a major route, or via inhalation and skin absorption as a minor route, accumulation of arsenic in the liver following repeated exposures is relatively higher and prone to increased hepatic toxicity [6 & 11]. Arsenic is also a well-known human cancer-causing agent [12].

Epidemiological studies also revealed a casual association between arsenic exposure and kidney diseases. Furthermore, chronic kidney disease has shown a gradual growth in the recent past as a notable outcome in arsenic- induced disease complications [13]. It has been reported that arsenic exposure through drinking water caused pregnancy complications (i.e. fetal loss and premature delivery) [14].

The present study aims to illustrate the morphological and histological effects of arsenic trioxide on mice offspring. Also, the study aims to explore the possible mechanisms of arsenic effects during embryonic development that results in abnormalities.

MATERIALS AND METHODS

Arsenic trioxide preparation:

Stock solutions of Arsenic trioxide were prepared by dissolving it into hydrochloric acid (0.12 N HCL, 0.5 mg/kg b.w., arsenic trioxide solvent) for in vivo experiments. Different doses of arsenic trioxide were used (0.3, 0.7 and 1 mg/kg). These solutions were kept at room temperature.

Experimental Animals:

Adult females and males albino mice (CD1) of average body weight "22-25 gm" were purchased from Theodor bilharzia institute (Giza, Egypt). The suitable temperature of 23 ± 2 °C and a lighting cycle of 12 hours light/dark were taken into consideration for accommodation. Experiments were carried out in strict compliance with the ethics prepared by INSA and (WHO/UNESCO).

Adult females albino mice (n.= 40) were grouped into five groups: Control, negative control treated with 0.12 N HCL (0.5 mg/kg b.w.), and three groups treated with different doses of arsenic trioxide (0.3, 0.7 and 1 mg/kg), females were given oral daily doses for 15 days before and 15 days after mating with males. All animals were given free access to standard chow and tap water.

Morphological investigations:

Young mice were preserved at age of 21 days after birth in formalin (5 % formaldehyde) solution and then were examined to investigate morphological abnormalities.

Morphometric measurements: Body weight, crown-rump, head length, head height, head circumference, fore limb length, leg (thigh, shank, and foot) parts lengths, and tail length were measured for minimum 25 young mice from each group.

Histological and histopathology score investigations studies:

Pieces of liver and kidney of 21 day old mice were fixed in 10 % neutral buffered formalin pH 7.2 for histology and histopathological tests. Haematoxylin and eosin stains were used to stain paraffin sections with a thickness of 5 micrometers according to Drury & Wallington [15]. For each animal group, sections of different animals were investigated. According to Heijnen's method [16], five tissue injury parameters (tissue vacuolization, cytoplasmic color fading, nuclear condensation, nuclear fragmentation, and erythrocyte-stasis) were used to score the tissues injury.

Analysis of data of the results was estimated by column statistics and analysis of variance by one-way ANOVA and the test of Newman-Keuls multiple comparisons. Data were estimated as mean $\pm$ SE. The used analytical software was Windows Prism software, version 5.0 and Excel. The data significant value difference was considered regarding $P < 0.05$, 0.01 or 0.001.

RESULTS

Morphological abnormalities:

Young mice had all characteristic features of the external complete development but some changes were observed in treated groups with arsenic trioxide.

In the lower dose (0.3 mg/kg) of arsenic trioxide, the specimens were relatively similar to normal except for a few abnormalities as the curvature of the hand, elongation of the ear compared to the head size, increase in foot length and tail elongation than the

normal tail length (Figs. 1 & 5). Specimens of the mid- dose (0.7 mg/kg) of arsenic trioxide group showed the small size and dwarfed body, elongation of the ear compared to the head size, long foot length and short tail compared to the control group (Figs. 2 & 6). Also, It was observed that specimens in the high dose group (1 mg/kg) of arsenic trioxide group have small size and dwarfed body, small head in size, long foot length, and short kinked and pygostyle tail compared to the control group (Figs. 3, 4 & 7).
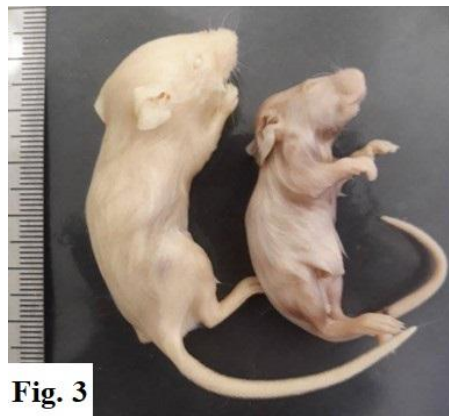
Fig. 1: A Photograph of 21 days mice treated with (0.3 mg/kg) arsenic trioxide (right) compared to control (left), showing the curvature of the hand, elongation of the ear compared to the head size, increases in foot length and tail elongation.

Fig. 2: A Photograph of 21 days mice treated with (0.7 mg/kg) arsenic trioxide (right) compared to control (left), showing elongation of the ear compared to the head size and decrease in tail length.

Fig. 3: A Photograph of 21 days mice treated with (1 mg/kg) arsenic trioxide (right) compared to control (left), showing small body size and short kinked and pygostyle tail.

Fig. 4: A Photograph of 21 days mice treated with (1 mg/kg) arsenic trioxide (right) compared to control (left), showing the small head size of the treated group.
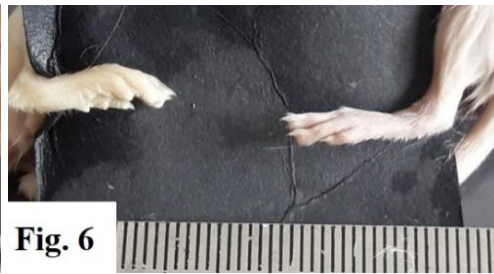

Figs. 5 - 7: Photographs of 21 days mice limbs that are treated with (0.3, 0.7 & 1 mg/kg respectively) arsenic trioxide (right) compared to control (left), showing elongation of the foot length.

Data analysis of morphometric measurements:

Weight and crown-rump were significantly decreased in the higher dose (1 mg/kg) of arsenic trioxide group compared to the control group, while the two lowest doses (0.3 & 0.7 mg/Kg) of arsenic trioxide were insignificantly compared to the control group (Figs. 8, 9 & table 1).
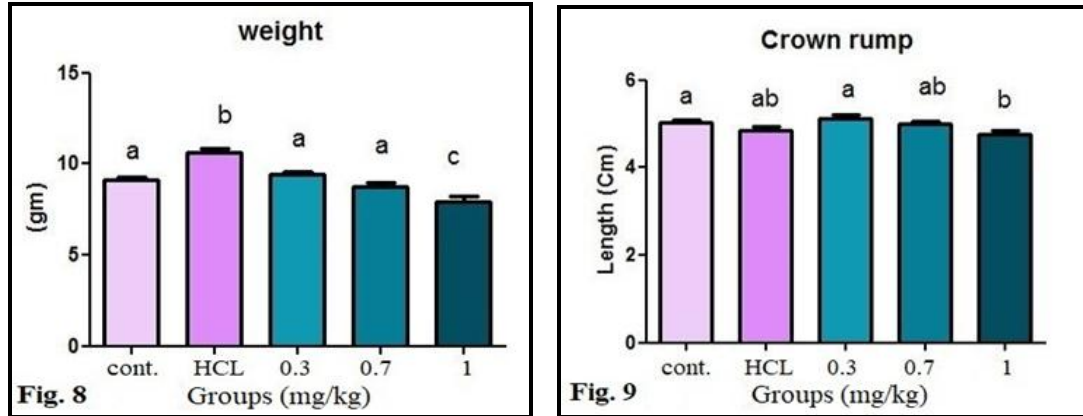
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Fig. 8: A statistical comparison of weight (gm) between different treated groups. For all figures a, b, c & d: significant difference between groups. 0.3, 0.7, 1 mg/Kg different doses groups treated with arsenic trioxide. Means ± SE is presented by columns

Fig. 9: A statistical comparison of crown-rump length (cm) between all the different treated groups.

Statistical analysis of the head length showed a significant decrease in all treated arsenic trioxide groups compared to control (Fig. 10 & table 1). The head circumference showed a significant decrease in the lowest and highest doses (0.3 & 1 mg/Kg) of arsenic trioxide treated groups, while the middle dose (0.7 mg/Kg) showed an insignificant decrease compared to control (Fig. 11 & table 1). The head height measurements showed an insignificant decrease in the lowest and highest doses (0.3 & 1 mg/Kg) of arsenic trioxide treated groups, while the middle dose (0.7 mg/Kg) showed a significant decrease compared to control (Fig. 12 & table 1).
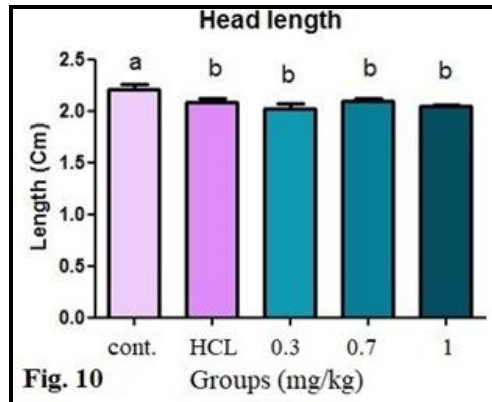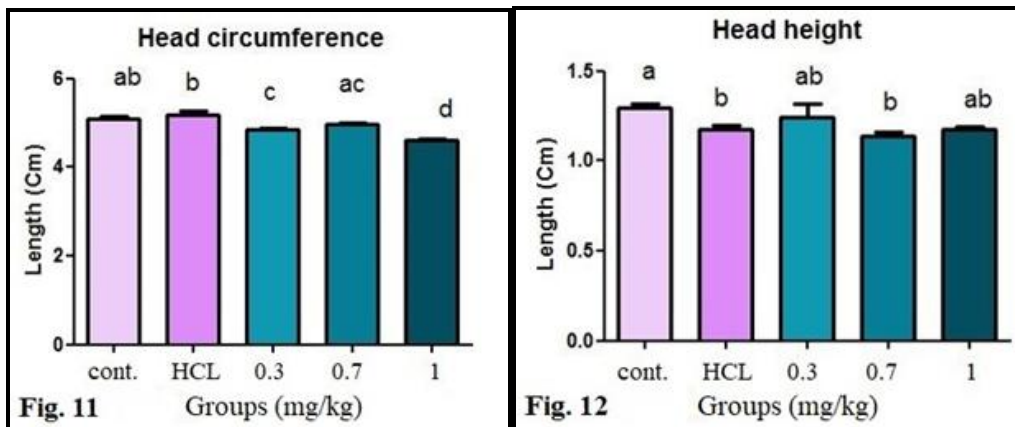
Fig. 10

Fig. 11

Fig. 12

Fig. 10: A statistical comparison of different measurements of head length between all the different treated groups.

Fig. 11: A statistical comparison of different measurements of head height between all the different treated groups.

Fig. 12: A statistical comparison of different measurements of head circumference between all the different treated groups.

The two lower doses (0.3 & 0.7 mg/Kg) of arsenic trioxide showed insignificant differences in the forelimb measurements compared to the control, while the highest dose (1 mg/Kg) showed an insignificant decrease compared to the control (Fig. 13 & table 1).
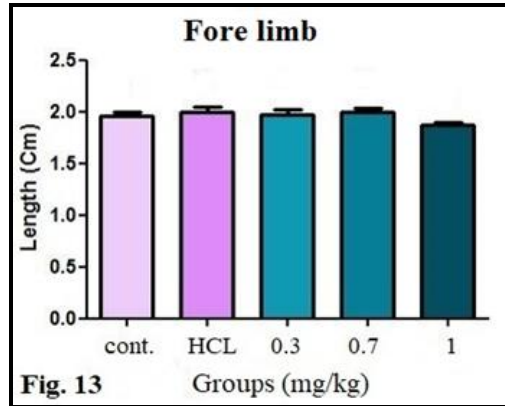
Fig. 13: A statistical comparison of fore limb length (cm) between all the different treated groups.

Statistical analysis of the different parts of the leg showed a significant decrease in the thigh part of the leg (Fig. 14 & table 1), while there were insignificant differences in the shank part of the leg (Fig. 15 & table 1) and a significant increase in the foot part of the leg in the three treated groups (0.3, 0.7 & 1 mg/Kg) of arsenic trioxide in comparison with the control (Fig.16& table 1).

There was an insignificant decrease in leg length in the arsenic trioxide treated groups (0.3, 0.7 & 1 mg/Kg) compared to the control (Fig. 17) and also (table 1).
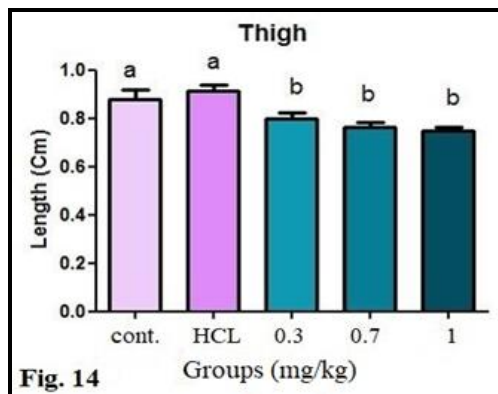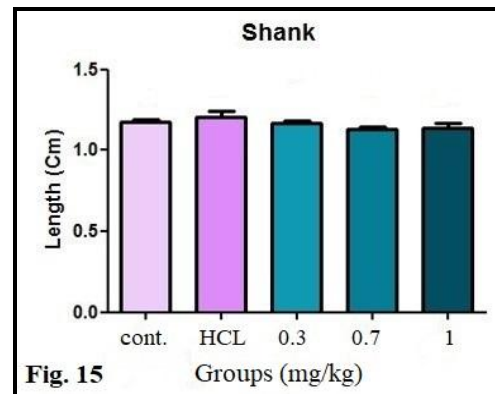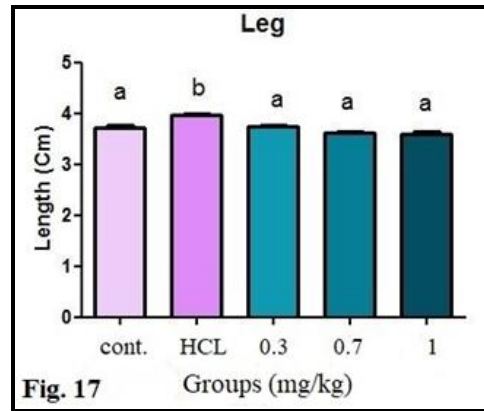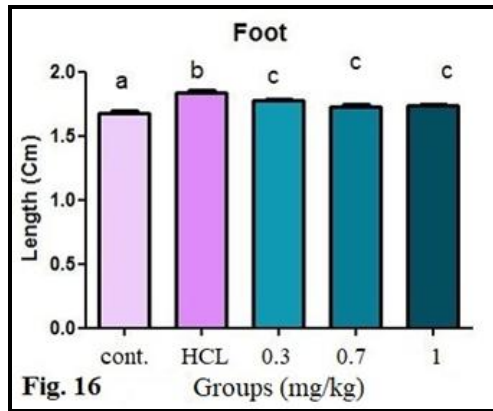
Fig. 16

Fig. 17

Fig. 14: A statistical comparison of different measurements of thigh between all the different treated groups.

Fig. 15: A statistical comparison of different measurements of shank between all the different treated groups.

Fig. 16: A statistical comparison of different measurements of foot between all the different treated groups.

Fig. 17: A statistical comparison of leg length (cm) between all the different treated groups.

There was a decrease in the significance of the tail measurements of the two highest doses (0.7 & 1 mg/Kg) of arsenic trioxide compared to the control, while there was significant increase in tail measurements of the lower dose (0.3 mg/Kg) of arsenic trioxide in comparison with the control (Fig.18 & table 1).
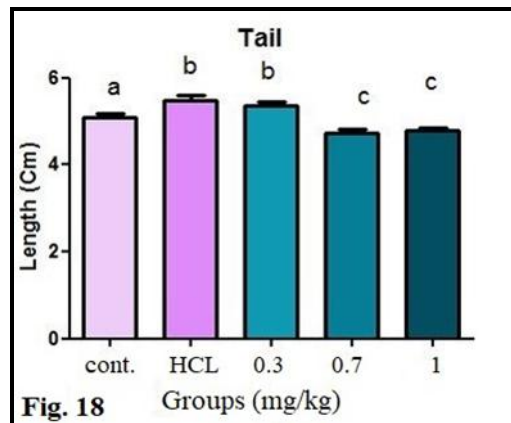

Fig. 18

Fig. 18: A statistical comparison of tail length (cm) between all the different treated groups.

Table 1: Different doses effect of Arsenic trioxide on the body parts lengths and different weights of mice at age of 21 day. a, b, c, d: the difference of significant between treated groups.

| Groups \ Parameters	Control	HCL	0.3 mg/ kg	0.7 mg/ kg	1 mg/ kg
Body weight	9.126 ± 0.1450^a	10.61 ± 0.2020^b	9.404 ± 0.1821^a	8.737 ± 0.2197^a	7.951 ± 0.2439^c
Crown-rump	5.038 ± 0.06314^a	4.863 ± 0.08049^{ab}	5.163 ± 0.09437^a	4.997 ± 0.07587^{ab}	4.767 ± 0.07245^b
Head length	2.213 ± 0.05115^a	2.079 ± 0.03611^b	2.021 ± 0.04623^b	2.091 ± 0.02707^b	2.040 ± 0.02426^b
Head height	1.297 ± 0.02396^a	1.175 ± 0.02019^b	1.246 ± 0.07198^{ab}	1.138 ± 0.02490^b	1.173 ± 0.01511^{ab}
Head circumference	5.091 ± 0.04864^{ab}	5.171 ± 0.08544^b	4.838 ± 0.04577^c	4.956 ± 0.04708^{ac}	4.610 ± 0.03694^d
Fore limb	1.963 ± 0.03830^a	2.000 ± 0.04214^a	1.975 ± 0.04554^a	2.000 ± 0.02944^a	1.877 ± 0.02233^a
Thigh	0.8781 ± 0.04009^a	0.9125 ± 0.02645^a	0.8000 ± 0.02085^b	0.7656 ± 0.01884^b	0.7467 ± 0.01417^b
Shank	1.172 ± 0.01917^a	1.208 ± 0.03237^a	1.167 ± 0.01667^a	1.128 ± 0.01364^a	1.140 ± 0.02473^a
Foot	1.672 ± 0.02300^a	1.838 ± 0.02317^b	1.775 ± 0.01621^c	1.725 ± 0.01739^c	1.733 ± 0.01207^c
Leg	3.722 ± 0.05212^a	3.958 ± 0.04253^b	3.742 ± 0.03607^a	3.619 ± 0.03403^a	3.603 ± 0.02690^a
Tail	5.078 ± 0.09331^a	5.471 ± 0.1363^b	5.346 ± 0.09008^b	4.738 ± 0.07025^c	4.780 ± 0.08043^c

Data are presented as means ± SE.

Histological examination:

1- Kidney:

Histopathological examination in the Kidney of 21 day mice in the control and the negative control treated with HCL groups; showed normal architecture of the Kidney with renal corpuscles (RC), distal (DT) and proximal (PT) tubules (Figs. 19 & 20). In As III low dose group (0.3 mg/Kg) showed a mild degenerated and abnormality structure of the kidney with necrosis (N) and hydropic degeneration (HD) in tissues and dilated Bowman's space (S) were observed in the renal corpuscle (Fig. 21).

Microscopic examination of As III middle dose group (0.7 mg/Kg) appeared, a moderate case of hydropic degeneration (HD), and pyknotic nuclei (arrows). Also, notably hypertrophication of the tufts of renal corpuscles leads to bulge into the first convoluted tubule (Fig. 22). In As III high dose (1 mg/Kg) there was severe case of hydropic degeneration (HD), bulge of tufts of renal corpuscles, blood congestion (Cg), hemolysis (Hy) and necrosis (N) in the kidney (Figs. 23 & 24). Kidney histophathological score was assessed by Heijnen's score, As III high group recorded highest scores than As III low and middle groups compared to control groups (Fig. 25).
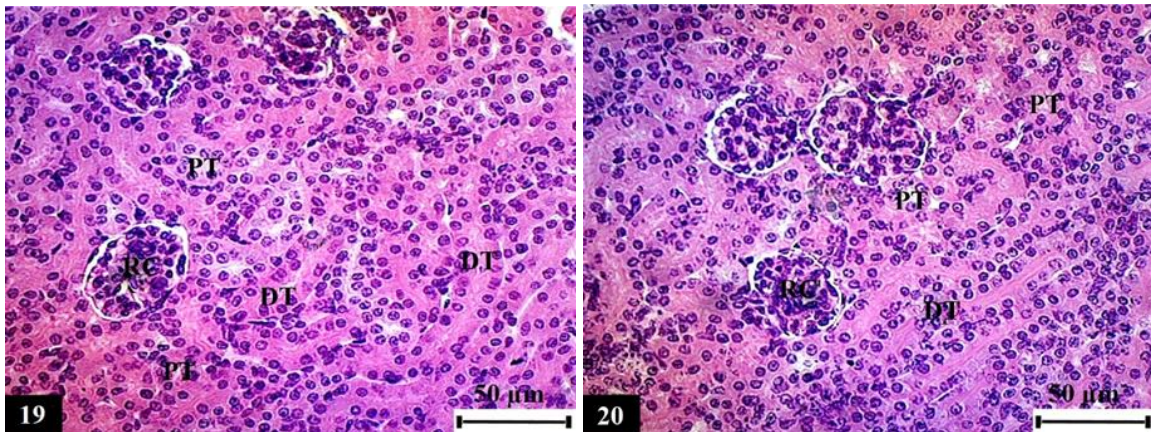
Figs. 19 & 20: Photomicrographs of kidney section of 21 day mice of control and negative control treated with HCL groups, showing renal corpuscle (RC), distal (DT) and proximal (PT) tubules.
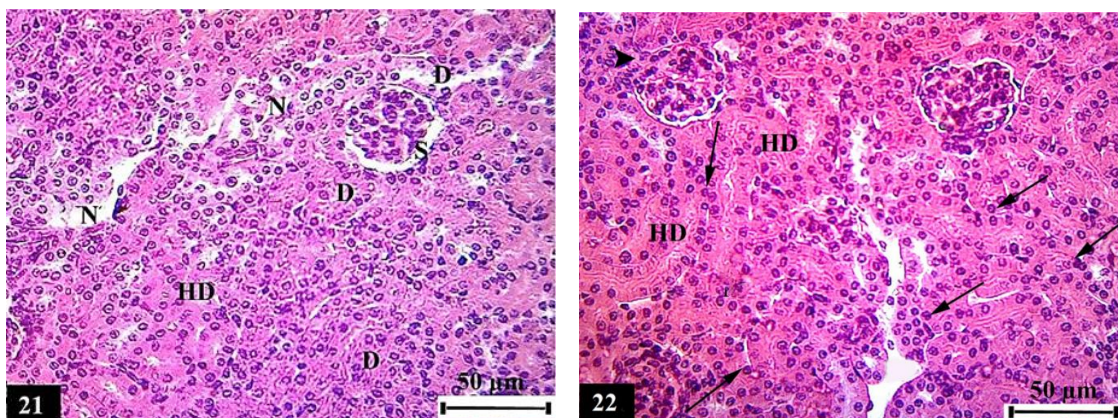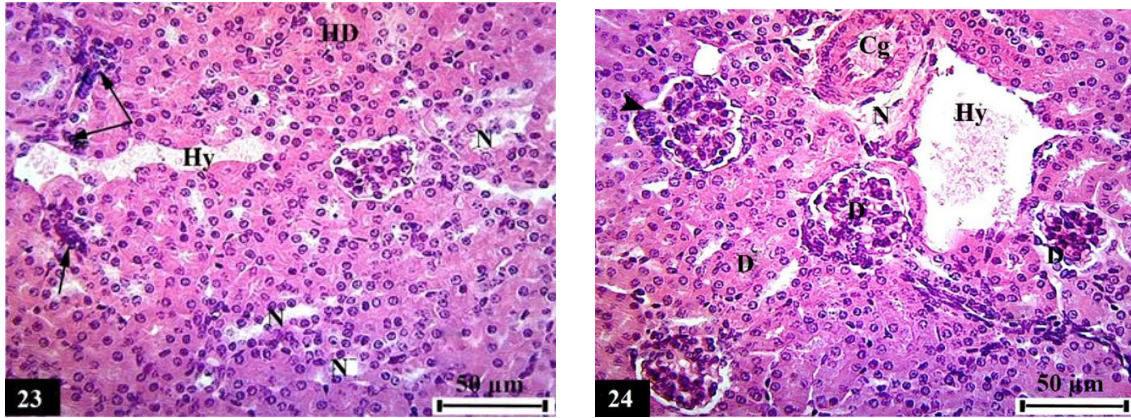

Fig. 21: A photomicrograph of kidney section of 21 day mice of As III low dose (0.3 mg/kg) showing mild degeneration (D) in tissues, hydropic degeneration (HD), necrosis (N) and dilated Bowman's space (S).

Fig. 22: A photomicrograph of kidney section of 21 day mice of As III middle dose (0.7 mg/kg) showing hydropic degeneration (HD) and pyknotic nuclei (arrows) and bulge of tufts of renal corpuscles (arrow head).

Figs. 23& 24: Photomicrographs of kidney section of 21 day mice of As III high dose (1 mg/kg) showing degeneration (D), sever hydropic degeneration (HD), blood congestion (Cg), hemolysis (Hy), plenty of areas of necrosis (N), pyknotic cells (arrows), and bulge of tufts of renal corpuscles (arrow head).
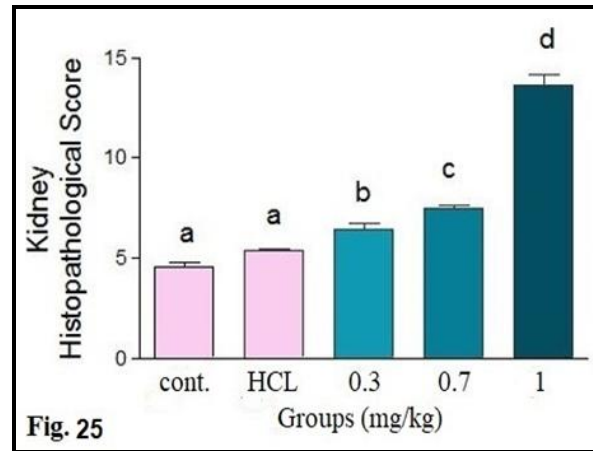
Fig. 25: Kidney histophathological score, data are mean ± S.E. from independent experiments. Values with unlike superscript letters in the same column are significantly different (P < 0.05).

2- Liver:

The histopathological examination in liver of 21 day mice in the control groups and the negative control group treated with HCL revealed normal architecture of liver with central veins. Also, polygonal hepatocytes, blood sinusoids with kupffer cells (arrows) were observed (Figs. 26 & 27).

However, the histopathological examination of treated group with As III low dose (0.3 mg/Kg) showed a degenerated and abnormality of central vein and the architecture of hepatic plates is disrepute. Pyknotic nuclei and hydropic degeneration (HD) were also observed (Fig. 28).

In the As III middle dose group (0.7 mg/Kg), the histopathological examination showed a disorganized structure architecture of hepatic plates, dilated sinusoid (Si), vacuolated (arrow heads) and giant nuclei (GN) (Fig. 29). In the As III high dose (1 mg/Kg) group, the histopathological examination showed a severe degenerated hepatic tissues, hemolysis (Hy), hemorrhage (H), necrosis (N), severe inflammation (IN), thick walled vein and there were numerous of pyknotic and vacuolated nuclei (Figs. 30 & 31). Liver histophathological score was assessed by Heijnen's score, As III high group recorded highest scores than As III low and middle groups compared to control groups (Fig. 32).
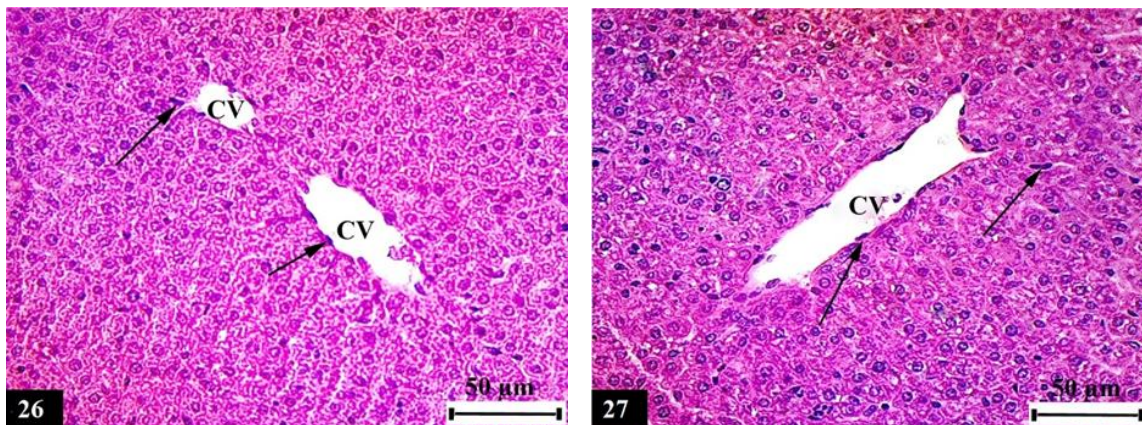
Figs. 26 & 27: Photomicrographs of liver section of 21 day mice of control and negative control groups treated with HCL, showing central vein (CV) and kupffer cells (arrows).
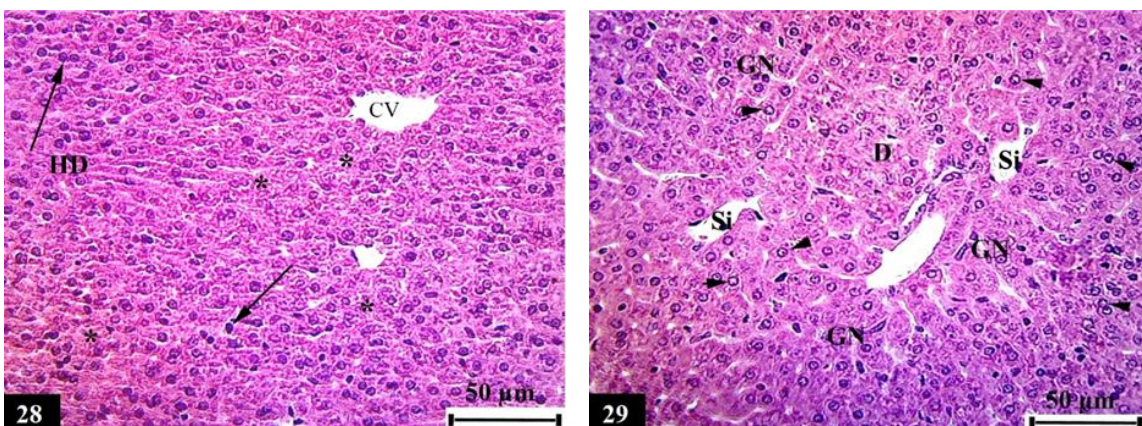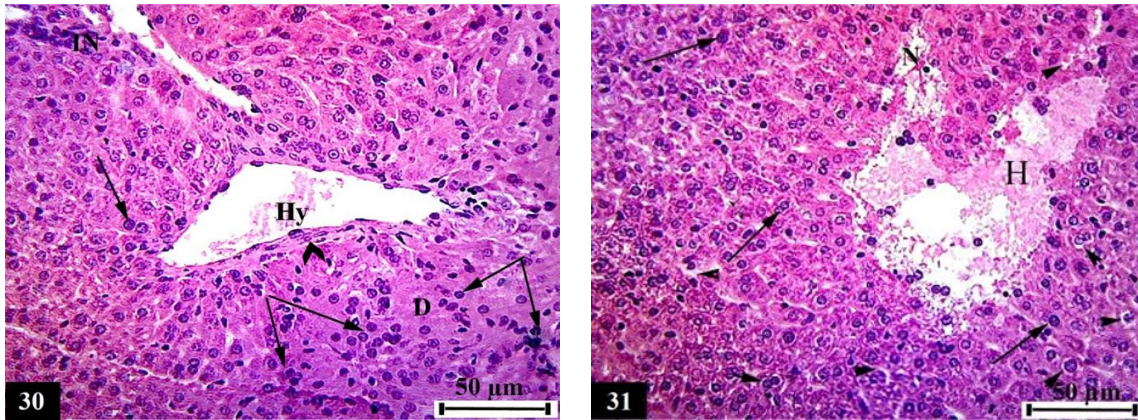
Fig. 28: A photomicrograph of liver section of 21 day mice of As III low dose (0.3 mg/kg) showing degenerated areas (*) hydropic degeneration (HD) and pyknotic nuclei (arrows).

Fig. 29: A photomicrograph of liver section of 21 day mice of As III middle dose (0.7 mg/kg) showing degeneration (D), dilated sinusoid (Si), giant (GN) and vacuolated nuclei (arrow heads).

Figs. 30 & 31: Photomicrograph**s** of liver section of 21 day mice of As III high dose (1 mg/kg) showing degeneration (D), thick walled vein (chevron arrow), hemorrhage (H), hemolysis (Hy), necrosis (N), pyknotic nuclei (arrows), vacuolated nuclei (arrow heads) and inflammation (IN).
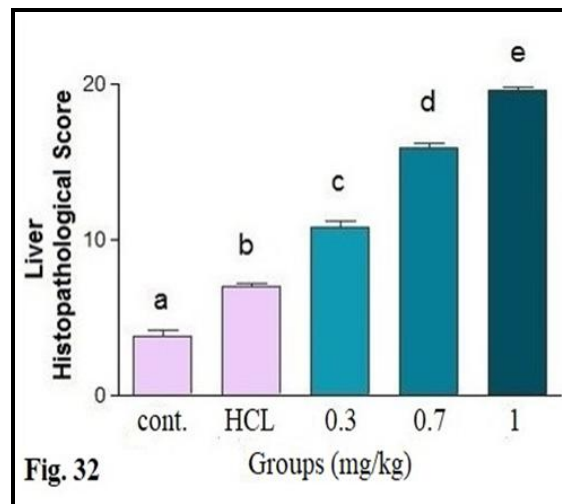

Fig. 32: Liver histophathological score, data are mean ± S.E. from independent experiments. Values with unlike superscript letters in the same column are significantly different ($P < 0.05$).

DISCUSSION

The present study is a trial to evaluate the developmental toxicity of arsenic trioxide (As_2O_3) by oral gavage on the mice embryo model. Arsenic trioxide was chosen as the test chemical because it is the most important arsenic in terms of occupational exposure. The mice were chosen as the test species because it is a frequently used and appropriate model for both developmental and toxicity studies.

In fact, arsenic toxicity is dependent on some factors including its chemical species, uptake, valance state, accumulation and efflux, etc [17]. Among all arsenic species, trivalent arsenicals are more toxic than pentavalent arsenicals [18]. More importantly, trivalent arsenicals have a high binding affinity to cysteine residues of proteins (e.g., enzymes) and could alter the conformation of the proteins, resulting in the loss of its function.

The current study demonstrated that there are some toxicity levels in the groups treated with arsenic trioxide on morphological and histological measurements compared to the control group.

There is a study stated that during the time of pregnancy, arsenic exposure (<50 μg/L) via drinking water affects the placental growth and uterus resulting in progeny birth weight defects [19]. A cross- sectional study Stated that, arsenic exposure through drinking water (range 0-1710 μg/L) has shown a strong association with spontaneous abortion, still birth, and neonatal death in Bangladesh [20].

However, some studies stated that arsenic exposure had no strong association with birth complications such as childhood stunting/under-weight, low birth-weight and still birth weight [21]. In addition, a study conducted in Inner Mongolia, China has also found no relationship between maternal arsenic exposure and still birth [22]. In utero arsenic exposure and birth defects are warranted. At any level of arsenic exposure, the risks of birth defects are subsequently high [23]. Moreover, chronic exposure to arsenic increases the risk of late fetal and infant mortality [24]. It has been shown that arsenic was actively transported to the fetus and induced developmental toxicity (i.e. malformation, growth retardation and death).

The changes in morphology that were noticed in this study such as the increase of foot and tail measurements in some cases might be explained as a result of increased proliferation rate and mutation in developing systems.

In the present study, the severe damage levels in the kidney coincide with the findings of Madden and Fowler [25] who demonstrated that during elimination process of arsenic through the renal system, the accumulation of arsenic in kidneys could leads to cytotoxicity in renal tissue.

It is suggested that the inhibitory effects of arsenic trioxide on embryonic development and body measurement might be attributed to elevating proapoptotic and autophagy properties and decreasing antiapoptotic gene activity. There are known mechanisms on how arsenic trioxide can cause DNA and cell damage. It is commonly

believed that As_2O_3 mediated production of ROS initiates changes in structure of DNA, such as base-pair mutations, translocation, deletions, insertions, sequences amplifications, sister chromatid exchange, as well as DNA hypermethylation and hypomethylation. Hence, these alterations in the DNA structures induced by As_2O_3 may lead to cellular transformation [26].

Some of the clinical manifestations such as hypourea, and elevated levels of serum creatinine led to renal injury (acute tubular necrosis) due to arsenic toxicity [13]. The degeneration of hepatocytes might be due to liver dysfunction and disturbance in the biosynthesis of alanine aminotransferase and aspartate transaminase enzymes with alteration in the permeability of liver membrane resulting from hepatocyte membrane damage.

In the present study, light microscopic assessment of liver and kidney in mice of the control group showed ordinary development of liver and kidney tissues. Severe histopathological changes of liver and kidney resulted by oral administration of As III in mice as in As III Low, medium & high groups of such organs. Hepatic and renal tissues had many damages compared to the control. These damages include congestion, hydropic degeneration, vacuolated cytoplasm and inflammation of many livers and kidney tissues. It was previously reported that in rat hepatocellular carcinoma cells and testis, As III is toxic and causes apoptosis and oxidative stress. Furthermore, At the G2/M stage, As III will inhibit the cell cycle. It can also trigger apoptosis in cells at various stages of the cell cycle causes severe damage [27, 28 & 29].

CONCLUSION

In conclusion, the result of the current study revealed that oral administration of arsenic trioxide in different doses induces severe damage in the liver and kidney. The morphological and histopathological changes that were found in this study are suggested to be as a result of altering effects of arsenic trioxide that were explained in earlier works of literature.

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