Embryotoxicity and oxidative stress induced by arsenic in albino mice and protective role of *Moringa oleifera*

Kashif Ali  
University of Veterinary and Animal Sciences

Asia Iqbal  
University of Veterinary and Animal Sciences

Talha Omer (✉ talha.omer@ju.se)  
Jönköping University: Jonkoping University  
https://orcid.org/0000-0003-4793-9683

Maham Saleem  
Virtual University of Pakistan

Research Article

**Keywords:** Antioxidant, Glutathione, Spina bifida, Anophthalmia

**Posted Date:** August 3rd, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-774070/v1

**License:** © This work is licensed under a Creative Commons Attribution 4.0 International License.  
Read Full License
Abstract

Chronic exposure to arsenic causes the abnormal development of embryo during pregnancy. So the study is designed to investigate the antioxidant and tissue protective properties of *Moringa oleifera* extracts against sodium arsenate induced embryo toxicity. *Moringa oleifera* extracts (leaf and flower) were prepared using soxhelt apparatus. Forty-four pregnant females were equally divided in 11 experimental groups (A-K). Group A was of control while B and C were sodium arsenate treated groups as group A (0.00), B (6.00, 0.00), C (12.00, 0.00). Group D to G were of sodium arsenate + *Moringa oleifera* flower extract treated groups with doses D (6.00, 150.00), E (6.00, 300.00), F (12.00, 150.00), G (12.00, 300.00) and groups H to K were sodium arsenate + *Moringa oleifera* leaf extracts treated groups H (6.00, 150.00), I (6.00, 300.00), J (12.00, 150.00) and K (12.00, 300.00) mg/kg B.W. Significant (p < 0.05) increased in MDA (malanodialdehyde) values 41.75 ± 3.40 and decreased GSH (glutathione) values 7.75 ± 0.95 in sodium arsenate treated groups were observed as compared to control 25 ± 0.81(MDA) and 18.5 ± 0.57 (GSH). *Moringa oleifera* extracts treated groups especially *Moringa oleifera* leaf extract at dose of 300mg/kg B.W normalized the MDA (25.25 ± 0.50) and GSH (18.25 ± 1.70) values. Sodium arsenate induced the histopathological changes like malformed heart, meningocoel, spina bifida, anopathalmia, cavitation, degenerated kidney and intestine. Whereas *Moringa oleifera* leaf extract as ameliorant minimized these histopathological abnormalities. It is concluded that *Moringa oleifera* leaf extract is mixture of biomolecules as it has antioxidant activity, so can be used as ameliorant against sodium arsenate.

1. Introduction

Anthropogenic activities are the reason for the change of biodiversity, environmental changes, desertification, pollution and contamination of ocean and fresh water. These activities are threat to human survival and living organisms (Landrigan et al. 2018). Metals are required for the proper functioning of human body and growth. These are the building blocks of body and required in certain concentrations for normal body functions. These essentials metals are iron, copper, cobalt, sodium, zinc, magnesium, calcium whereas nonessentials metals also exist in the environment and are toxic to human beings as arsenic, cadmium, nickel, and lead (Muhammad et al. 2011). Heavy metals are included in non biodegradable substances so concentrations of heavy metals in our environment are increasing day by day (Wu et al. 2016). Drinking water is the common pathway of entry for heavy metals in humans while oral ingestion via fruits, vegetables and foods is also a route of entry for heavy metals in humans. Toxicity of heavy metals includes cardiovascular diseases, gastro intestinal pain, kidney damage, hepatic failure, skeletal disorders, apoptotic actions, ulcer, cancer and nervous system damage (Khan et al. 2013). Reactive oxygen production is the basic reason of heavy metals toxicity which causes destruction of DNA, changes in structure of proteins and oxidative stress through lipid peroxidation. These reactive oxygen species are dangerous to living beings. Different studies were carried out to investigate the toxicity mechanism of heavy metals which includes bioinformatics, biogenomics, genetics and biogenomics (Wu et al. 2016). Arsenicals (inorganic and organic) are increasing in the environment so
increasing the pollution which had serious teratogenic and cancerous threat to humans (Liu et al. 2015). Nearly 200 million humans are under threat of arsenic due to increasing level of arsenic than the prescribed limits of World health organization 10 microgram per litter (Chen et al. 2017). Dose concentrations, duration, species, age of individuals, gender (male or female) and genetics are the factors on which toxicity of arsenic depends (Tchounwou et al. 2003). Trivalent arsenic (As $^3+$) induces diseases like cardiovascular, nervous disorder, reproductive disorders, cancer of skin and bladder, genotoxicity and cytotoxicity as compared to pentavalent arsenic (Santra et al. 2007). Arsenic considered as teratogen which can decrease the antioxidant enzymes activities like glutathione (GSH) and malanodialdehyde (MDA) by generating reactive oxygen species (Mittal et al. 2007). This ROS and free radicals production due to arsenic results in DNA damage and hepatotoxicity (Chattopadhyay et al. 2011). Reproductive abnormalities and toxicity in mice and rats were observed due to arsenates when it was administered orally to mice and rats (Hong et al. 2004). Arsenic is a teratogen for animals causing embryo toxicity like, alters the growth, causing death and malformations (Robinson et al. 2011).

Treatment of patients intoxicated with arsenic is the challenge for world though, different medicines are available to control health related problem due to arsenic, but these medicines have shown some side effects (Flora et al. 2007). Plants are rich source of antioxidants so were used in medicine to control the cellular damage induced by free radicals. Dietary antioxidants are now prime focus of the world to reduce oxidative stress and related problems induced by ROS production by different hazardous substances like heavy metals (Gupta et al. 2007). Various plant extracts were used against arsenic induced oxidative stress and lipid peroxidation. These plants include Moringa, Terminalia and Phyllanthus. Among all Moringa oleifera found to possess antioxidant and anti-inflammatory properties and protected the mouse against arsenic induced liver abnormalities, fibrosis and necrosis (Verma et al. 2007).

Moringa oleifera of family Moringaceae is distributed all over the Pakistan including Srilanka, Bangladesh, India, Cuba, Myanmar, Singapore, Thailand, Malaysia, Nigeria and China (Okuda et al. 2001). In Pakistan, two species of family Moringaceae are present which includes Moringa oleifera and Moringa concanences. Moringa oleifera is commonly found in Province Punjab and KPK (Khaiber Pukhtunkhawa) while Moringa concanences is only available in Province Sindh of Pakistan (Qaiser et al. 1973). Moringa oleifera pod, leaf, flower, and seeds are rich source of antioxidants like phenols, polyphenols, vitamins A, C, E and minerals (Khatun et al. 2003). In different studies on albino mice the ameliorative role of Moringa oleifera flowers, seed and leaf against arsenic has been reported (Gupta et al. 2005). However, the role of Moringa oleifera against sodium arsenate induced embryo toxicity (histopathological changes and oxidative stress) reduction is not well established. Therefore, current study was planned to reveal the protective role of M. oleifera leaves and flower extracts against sodium arsenate induced oxidative stress and histopathological changes in embryo of mice. To the best of our knowledge against arsenic embryo toxicity this was the first study in Pakistan that described the beneficial (medicinal and therapeutic) effects of M. oleifera against environmental teratogens.

2. Materials And Methods
2.1. Animal handling and feeding

For this purpose 7–8 week old Albino mice (male and female), *Mus musculus* with initial body weight of 20–25g were kept under standard conditions i.e. temperature 25°C with 12 hours, dark and light cycle, at University of Veterinary and Animal sciences (UVAS) Ravi campus Pattoki, Pakistan. Animals before use were kept for seven days to acclimatize. Mice were kept in cages, in different groups, and treated with normal standard diet (National feed No. 14) which had Energy: 2700 Kcal/Kg ± 100, Crude Protein: 16.5% ±1, with ingredients like cereals and corn. To obtain pregnant female mice, timed mating was induced by placing 1 male and 2 female together and presence of vaginal plug was the indication of mating. The observation of a vaginal plug determined to be gestation day zero (Robinson et al. 2011). Ethical permission was obtained from the ethical committee of University of Veterinary and Animal sciences Lahore, Pakistan via Ref. N0.161. Dated 6–2-2020.

2.2. Collection and extraction of plant samples

Leaves and flowers of *Moringa oleifera* were collected from the botanical garden of C-Block, Ravi campus Pattoki, University of Veterinary and Animal sciences Lahore, Punjab, Pakistan. Plant samples were identified by (Plant taxonomist) Department of Botany, Government College University, Lahore (Voucher Specimen No. GC. Herb. Bot. 3725). Plant material was washed and sun dried. After drying plant material was grounded to form powder. Powder (400gm/litter) was extracted through the soxhelt apparatus using methanol (95%) as solvent. When extraction completed, solvent was evaporated using rotary evaporator apparatus, obtained residues approximately 25gm were stored at 4°C and dissolved in distilled water just before use (Tabidi et al. 2018).

2.3. Phytochemistry of *Moringa oleifera* extracts

Qualitative and quantitative phytochemical studies of *Moringa oleifera* leaf and flower crude extracts for the confirmation of its phytoconstituents were carried out following the standard methods Santhi and Sengottuvel (2016).

2.4. Sodium arsenate and *Moringa oleifera* extracts

Administration to animals

Chronic doses of sodium arsenate as toxicant were administered to pregnant mice to induce embryonic abnormalities and test extracts of *Moringa oleifera* (flower and leaves) as ameliorant were administered orally to experimental groups from GD8 to GD12 while control group received the standard diet. Dose concentrations for arsenic and *Moringa* were set according to previously published reports (Kaise et al. 1985 and Hill et al. 2008).

Given doses are as follows,

A (0.00), B (6.00, 0.00), C (12.00, 0.00) mg/kg B.W.

Where, groups D to G were sodium arsenate and *M. oleifera* flower extract treated groups.
D (6.00, 150.00), E (6.00, 300.00), F (12.00, 150.00) and G (12.00, 300.00) mg/kg B.W.

Where, groups H to K were sodium arsenate and *M. oleifera* leaf extract treated groups.

H (6.00, 150.00), I (6.00, 300.00), J (12.00, 150.00) and K (12.00, 300.00) mg/kg B.W.

All animals were observed daily to count mortality and morbidity. Gross maternal body weights were measured daily from GD0 to GD18.

### 2.5. Measurement of oxidative stress

Oxidative damage induced by sodium arsenate and ameliorative role of *Moringa* was investigated by measuring GSH (glutathione) and MDA (malanodialdehyde) level in embryonic tissues. Embryonic tissues were homogenized in 0.1 M Tris-HCl buffer (pH 7.4) at 4°C, and were centrifuged at 9000rpm for 15 minutes. Supernatants were kept at -20°C for MDA and GSH measurements. The concentration of malanodialdehyde (MDA) in the embryonic tissue homogenate was measured by the thiobarbituric acid (TBA) test (Ohkawa et al. 1979). The absorbance of the sample was read at 530 nm using spectrophotometer. Glutathione (GSH) content of embryonic tissues homogenate was measured by the method of Sedlak and Lindsay (1968) and absorbance was read at 412nm on the spectrophotometer.

### 2.6. Histopathological analysis

Fixed Fetuses were given repeated washes in 70% ethanol to decolorize. To dehydrate the fetuses were treated with different percentages of alcohol which were 90% and 100%. Xyline was used for clarity. The infiltration was done through overnight by molten wax in incubator at 60°C then fetuses were embedded in blocks in fresh molten wax. Molds of steel were used, which were set in blocks made up of plastic. Molten wax was added in bottom of molds then fetuses were placed in it. After solidification, microtome was used to obtain 4.5–5.5µm sections. Ehrlich’s stain Hematoxylin stain was used to stain the sections. Canada balsum was used for mounting which protected the slides sections from the microbial activity. Cover slips dipped in xylene were placed over these sections. Histological sections were micro photographed using microscope with digital camera (Canon, HD model A-2300). Adobe photoshop software was used for the modification, clearance and labeling of these microphotographs (Carleon et al. 1980).  

### 2.7. Statistical analysis

Significant differences between the control and treatment groups were analyzed using one way analysis of variance (ANOVA). In case of significant results, we used Duncan multiple range test (DMRT) for the multiple mean comparison (Duncan 1955). For all the analysis we considered 5% significance level, whereas p < 0.05 was considered significant. SAS (version 9.1) software was used as a statistical tool for the analysis.

### 3. Results
Qualitative Phytochemical analysis of *Moringa oleifera* leaf and flower extract revealed that alkaloids, flavanoids, terpenoids, phenols, tannins, glycosides, proteins and carbohydrates were present in both extracts. Saponins were present only in *Moringa oleifera* leaf extract. Results of quantitative analysis are shown in Table 1.

### Table 1

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Extract of Leaves</th>
<th>Extract of Flowers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tanins (mg/mL)</td>
<td>8.4</td>
<td>8.1</td>
</tr>
<tr>
<td>Flavonoids (mg/mL)</td>
<td>14.2</td>
<td>11.9</td>
</tr>
<tr>
<td>Phenolics (mg/mL)</td>
<td>38.5</td>
<td>25.9</td>
</tr>
<tr>
<td>Alkaloids %</td>
<td>6.1</td>
<td>5.4</td>
</tr>
<tr>
<td>Saponins %</td>
<td>9.3</td>
<td>0.00</td>
</tr>
</tbody>
</table>

#### 3.1. Maternal body weight analysis

Body weight analysis of all pregnant females was carried out in terms of initial body weight (start of experiment at GD8) and final body weight (dissection day at GD18). Females of control group A exhibit normal body weight while significant ($p \leq 0.05$) difference in weights of sodium arsenate treated groups C and D were observed as compared to control. Tendency to increase in body weight was observed in arsenic + *Moringa oleifera* extracts (leaf and flower) administered groups. *Moringa oleifera* leaf extract specifically, at dose of 300mg/kg B.W found effective against sodium arsenate induced embryo toxicity. It reduced the teratogenic effects of sodium arsenate which indicate that *Moringa oleifera* leaf extract has protective bioactive compounds (Fig. 1).

#### 3.2. Gross fetus analysis

The Table 2 illustrates the gross fetus analysis of fetuses recovered at gestation day 18. Fetuses recovered from control group at GD18 were greater in number than all the experimental groups. All fetuses were found normal and did not show abnormalities. Whereas sodium arsenate administered groups (B and C) showed less number of fetuses and most of these were abnormal (Table 2). Sodium arsenate + *Moringa oleifera* extracts (leaf and flower) showed increased litters size with less number of abnormal fetuses suggesting that *Moringa oleifera* has such phytoconstituents which have ameliorative properties against arsenic (Table 2).
### Table 2
Gross fetus analysis of fetuses recovered at GD-18

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>No. of females used</th>
<th>Total fetuses obtained</th>
<th>Normal fetus obtained</th>
<th>Abnormal fetus obtained</th>
<th>Resorbed</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA(^1) (mg/kg/BW)</td>
<td>MOFE(^2)/MOLE(^3) (mg/kg/BW)</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>39</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>4</td>
<td>28</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>4</td>
<td>25</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>6</td>
<td>150(^2)</td>
<td>4</td>
<td>29</td>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>300(^2)</td>
<td>4</td>
<td>31</td>
<td>23</td>
<td>8</td>
</tr>
<tr>
<td>12</td>
<td>150(^2)</td>
<td>4</td>
<td>30</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td>12</td>
<td>300(^2)</td>
<td>4</td>
<td>32</td>
<td>22</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>150(^3)</td>
<td>4</td>
<td>32</td>
<td>26</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>300(^3)</td>
<td>4</td>
<td>33</td>
<td>31</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>150(^3)</td>
<td>4</td>
<td>32</td>
<td>28</td>
<td>4</td>
</tr>
<tr>
<td>12</td>
<td>300(^3)</td>
<td>4</td>
<td>34</td>
<td>32</td>
<td>2</td>
</tr>
</tbody>
</table>

\(^1\) Sodium arsenate, \(^2\) Moringa oleifera flower extract and \(^3\) Moringa oleifera leaf extract

### 3.3. Oxidative stress analysis

Significant (p < 0.05) rise in MDA concentrations were observed in sodium arsenate treated groups B and C (6mg/kg B.W & 12mg/kg B.W) as compared to control group A (Table 3). While all other groups treated with sodium arsenate + Moringa oleifera extracts (leaf and flower) as antidote showed significant (p < 0.05) antioxidant activity and normalized the MDA values. Glutathione (GSH) values were decreased due to sodium arsenate produced oxidative stress in groups (B and C) as compared to control group. Whereas Moringa oleifera leaf and flower extract treated groups with sodium arsenate showed significant (p < 0.05) amelioration against arsenic toxicity. Normal GSH values were observed. Amelioration of Moringa oleifera leaf extract at dose of 300mg/kg/ B.W was found more significant as compared to Moringa oleifera flower extract against inorganic arsenicals (Table 3).
Table 3
Oxidative stress induced by sodium arsenate and ameliorative effects of *Moringa oleifera* extracts (leaf and flower) in mice embryo at gestation day-8 only

<table>
<thead>
<tr>
<th>Treatments</th>
<th>MDA (nmol/gm) (Mean ± SD)</th>
<th>GSH (nmol/gm) (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA&lt;sup&gt;1&lt;/sup&gt; (mg/kg/BW)</td>
<td>MOFE&lt;sup&gt;2&lt;/sup&gt;/MOLE&lt;sup&gt;3&lt;/sup&gt; (mg/kg/BW)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>22.5 ± 0.57&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>31.25 ± 0.95&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>36 ± 0.81&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>150&lt;sup&gt;2&lt;/sup&gt;</td>
<td>29.5 ± 1.29&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>300&lt;sup&gt;2&lt;/sup&gt;</td>
<td>25 ± 0.81&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>12</td>
<td>150&lt;sup&gt;2&lt;/sup&gt;</td>
<td>29.25 ± 1.70&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>12</td>
<td>300&lt;sup&gt;2&lt;/sup&gt;</td>
<td>26.50 ± 1.29&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>150&lt;sup&gt;3&lt;/sup&gt;</td>
<td>24.75 ± 0.95&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>300&lt;sup&gt;3&lt;/sup&gt;</td>
<td>23 ± 0.81&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>12</td>
<td>150&lt;sup&gt;3&lt;/sup&gt;</td>
<td>24.25 ± 0.50&lt;sup&gt;ef&lt;/sup&gt;</td>
</tr>
<tr>
<td>12</td>
<td>300&lt;sup&gt;3&lt;/sup&gt;</td>
<td>24 ± 0.81&lt;sup&gt;ef&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a−f</sup> Means within columns with different superscripts differ significantly (p < 0.05).

1 Sodium arsenate, 2 *Moringa oleifera* flower extract and
3 *Moringa oleifera* leaf extract

3.4. Histopathological analysis

Histological studies were carried out to analyze the internal structures of the fetuses. Normally developed organs were found internally in control group. Brain was with normal ventricles (lateral, 3rd and 4th ventricles) and pons and diencephalon. Eyes were well developed with cornea and lens. Hearts were with normal superior vena cava, atrium and ventricles. Kidneys with normal cortical and medullary regions were observed. Normally developed lungs, liver, intestine (small, large) and urinary bladder was found. Spinal cord, olfactory bulb, esophagus and tracheal system was clearly observed and found normally developed at 18th day of gestation (Fig. 2A, B, C).
Sodium arsenate as toxicant in chronic doses at GD8-GD12 was administered to Group B (6mg) and C (12mg) to induce fetus abnormalities. Histological transverse sections (head, heart and abdomen) of these groups showed poor development. Malformed and degenerated structures were observed due to increased sodium arsenate doses (12mg/kg). Meningocele and anophthalmia were observed clearly (Fig. 3D). Other abnormalities include spinal bifida, cavitation (gaps), large ventricles, under developed ventricles, mishappen heart, malformed lungs, degenerated large and small intestine. Abdominal region with all its vital organelles was degenerated (omphocoel) and not able to study (Fig. 3. D, E, F).

Histological sections obtained from toxicant + *Moringa oleifera* flower extract treated groups showed little amelioration against sodium arsenate. *Moringa oleifera* flower extract treated group displayed abnormalities in heart development, intestine and kidney where as *Moringa oleifera* leaf extract (as ameliorant) + sodium arsenate treated groups displayed normal growth and development of fetuses in all vital organs hence significantly (p < 0.05) decreased the teratogenic effects of sodium arsenate. MOLE at dose of 300mg/kg significantly detoxify the teratogenic effects of sodium arsenate and give well developed brain, pons, eyes, spinal cord, heart, lungs, stomach, duodenum, bladder and kidneys (Fig. 4. G, H, I). Different doses of antidote MOLE showed normal development.

**4. Discussion**

In this study ameliorative potential of *Moringa oleifera* leaf and flower extracts against sodium arsenate induced oxidative stress and histopathological changes was studied. Dose dependent embryo toxic response of arsenic was observed during the critical period of development. The prime objective of this study was to check the antioxidant property of *Moringa oleifera* against arsenic due to its bioactive compounds. GSH and MDA make the first defense line of immune system. Increased MDA (malondialdehyde) and decreased GSH (glutathione) values were observed in sodium arsenate administered groups which indicate the production of ROS (reactive oxygen species) by arsenic. It is in agreement with previous studies (Yang et al. 2009). When ZnO and SiO2 nanoparticles were applied to mouse embryo, glutathione and SOD activities were decreased while increased activity of MDA reflected the oxidative stress induced by these nanoparticles. These alterations in enzymes were dose and size dependent to nanoparticles. Various results of our study depicted that *Moringa oleifera* extracts reduced/removed all the oxidative stress induced anomalies by sodium arsenate. Specifically, *Moringa oleifera* leaf extract at dose of 300mg/kg B.W found to be more effective than the *Moringa oleifera* flower extract in our study. *Moringa oleifera* leaf extract significantly (p < 0.05) reduced the elevated level of MDA and increased the concentration of GSH in embryonic tissues which is an agreement with Faiza (2013), she confirmed the antioxidant activity of *Moringa oleifera* leaf extract against nicotine (as neurotoxin) in females during gestation. Antioxidant property of *Moringa oleifera* leaf extract suggest that it is a mixture of biomolecules which contain hydroxyl groups that protected the MDA and GSH molecules by retarding the release of hydrogen ions. This protected the lipid layer and increased the antioxidant mechanism against free radicals.
Meningocele, spinal bifida, diencephalon, omphocoel (degenerated kidney and intestine) were observed when pregnant females were treated with sodium arsenate. In the previous studies of Arshad and Asmatullah (2017) same embryo toxic effects of arsenic were studied. Meningocele, spinal bifida, degenerated kidneys and intestine were observed when pregnant females were treated with sodium arsenate at gestation day 6 with different doses. In this research malformed heart and cardiac malformations were commonly observed in sodium arsenate administered groups which are an agreement with studies of Spiegelstien et al., (2005). They studied when pregnant female mice were exposed to sodium arsenate deficiency of folate transportation occurred which induce cardiac malformations and neural tube defects. Poorly developed air passageway was finding of our work and is in accordance to the previous observations (Petric et al. 2009). When pregnant rats were treated with inorganic arsenic orally, poorly developed lungs were found and it was due to mutation in expression of β-catenin gene, which play important role in morphogenesis of lungs. Whereas histological sections of embryos obtained from arsenic + Antidote *Moringa oliefera* extracts (leaf and flower) treated groups showed normal development of fetuses specifically of *Moringa oliefera* leaf extract at dose of 300mg/kg showed similarity with control group in all its histological section which confirmed the ameliorative properties of *Moringa oliefera* leaf extract.

5. Conclusion

Arsenic is considered as potent environmental teratogen which can significantly decreased the growth of developing embryo during the critical period of development in pregnancy. It induces the oxidative stress by generating ROS and altered the normal concentrations of GSH (glutathione) and Malondialdehyde (MDA) in the embryonic tissues when orally administered to pregnant females. Sodium arsenate also reduced the growth of vital organs in embryo. Whereas *Moringa oliefera* extracts especially leaf extract when used as ameliorant against arsenic, significantly ameliorated all these changes induced by sodium arsenate in embryo. So it may be concluded that *Moringa oliefera* have some bioactive compounds (phenols, saponins) which have antioxidant properties. So can be used as therapeutics in future to protect the human beings from the hazardous effects of environmental teratogens like arsenic, especially for protection of pregnant females.

Declarations

7. CONFLICT OF INTEREST:

The authors declare no conflict of interest.

6. ACKNOWLEDGEMENT:

We would like to thank Dr. Muhammad Muddassir Ali (Assist. Professor) Institute of Biochemistry and Biotechnology, University of veterinary and animal sciences Lahore, Pakistan. A part of this study
(Oxidative stress analysis) was completed under his guidance in his lab (laboratory of Biogenomics).

References


**Figures**

![Graph showing Mean Maternal IBW and Mean Maternal FBW](image)

**Figure 1**

Mean maternal body weight ± SD analysis. Where, SA = Sodium arsenate, MOFE = Moringa oleifera flower extract, MOLE = Moringa oleifera leaf extract, IBW= initial body weight and FBW= Final body weight
Figure 2

Macro photographs of transverse sections of Head (A), heart (B) and abdomen (C) of Control group. Labels: Po: pons, 3v: 3rd ventricle, 4v: 4th ventricle, Le: lens, Sp: spinal cord, Lu: lung, At: atrium, Ve: ventricle, svc: superior vena cava, Oe: oesophagus, Ob: olfactory bulb, Bd: bladder, Ck: cortex of kidney, Mk: medullary region of kidney, St: stomach, Li: large intestine.

Figure 3

Macro photographs of transverse sections of Head (D), heart (E) and abdomen (F) of Sodium arsenate treated groups at 12mg/kg B.W. Labels: Me: meningocoel, Sb: spinal bifida, Po: pons, 3v: 3rd ventricle, Lv: large ventricle, An: anopthalmia, Dk: degenerated kidney, Di: diencephalon, Om: omphocoel, Di: degenerated stomach, Dh: degenerated heart.
Figure 4

Macrophotographs of transverse sections of Head (G), heart (H) and abdomen (I) of Moringa oleifera leaf extract treated group of dose arsenic 12mg + MOLE 300mg/kg B.W. Labels: Sp: spinal cord, Po: pons, 3v: 3rd ventricle, Le: lens, Lu: lung, At: atrium, Ve: ventricle, svc: superior vena cava, Oe: oesophagus, Ck: cortex of kidney, Mk: medullary region of kidney, St: stomach, Li: large intestine, Ob: olfactory bulb, Co: cornea