doi.org/10.36721/PJPS.2020.33.6.SUP.2721-2728.1

Ameliorative effects of *Moringa oleifera* leaf and flower extracts on sodium arsenate induced oxidative stress and histopathological changes in mice embryo

Kashif Ali[1]*, Asia Iqbal[1], Syed Mohsin Bukhari[1] and Athar Mahmud[1]
[1]Department of Wildlife & Ecology, Faculty of Wildlife and Fisheries, University of Veterinary and Animal Science, Lahore, Pakistan
[2]Department of Poultry Production, University of Veterinary and Animal Sciences, Lahore, Pakistan

Abstract: The study is aimed to investigate the protective role of *Moringa oleifera* extracts against sodium arsenate induced embryo toxicity in albino mice. Forty four pregnant mice were divided into 11groups (A-K). Group A was control while B and C were sodium arsenate treated groups with dose, 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 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. *Moringa oleifera* leaf extract treated groups showed significant ($p<0.05$) amelioration against sodium arsenate induced histopathological changes as malformed heart, spina bifida, enlarged ventricles, poorly developed kidneys, anopthalmia and cavitation in brain. Significant ($p<0.05$) increased in malondialdehyde 36±0.81 and decreased glutathione 8.25±0.95 values in sodium arsenate treated groups were observed as compared to control 22.5±0.57 and 19±0.81.Whereas *Moringa oleifera* leaf extract at dose of 300mg/kg B.W normalizesd the malondialdehyde 23±0.81 and glutathione 17.75±3.20 values. So concluded that *Moringa oleifera* leaf extract has ameliorative effects against sodium arsenate induced embryotoxicity.

Keywords: *Moringa*, Spina bifida, anopthalmia, glutathione.

INTRODUCTION

Birth defects are structural, metabolic and functional disorders in developing embryo (Zhang *et al*., 2012) and these defects are due to genetic factors or adverse effects of the environment. Metals, different types of drugs and pestochemicals are the most common teratogens of environment. Heavy metals are such pollutants which cannot be broken down by microorganisms like other organic pollutants. These heavy metals are toxicant of environment in individual or in combine form such as mercury, cadmium, lead and arsenic. These heavy metals are percolating speedily which is dangerous to food crops grown in affected areas, absorbed by the plants resulting in threats to humans (Known *et al*., 2017). Metals are classified according to their density, atomic mass and atomic number (Ali and khan, 2018).These metals are accumulating in our ecosystems through Erosion of soil, weathering of rocks, industrial waste, urban sewage, pesticides and mining are common sources of heavy metals (Chen *et al*., 2019).

Arsenic is present in more than 200 minerals naturally, decomposition and adsorption of these arsenic containing minerals is the major cause of drinking water arsenic contamination (Shakoor *et al*. 2016). Arsenic is one of the dangerous heavy metal and abundantly found in earth crust. It has valency of (III) or (V) and found in inorganic and organic forms. Foods contaminated with arsenic especially meats, sea foods and drinking water effects human health. Chile, Argentina, India, Nevada, California, China, Taiwan, Thailand, Mongolia, Mexico, North Dakota, Nepal and Central Oklahoma in USA are hotspots of arsenic in world for surface or groundwater (Abdul *et al.*, 2015). 100 million peoples are at risk of arsenic according to WHO safe limits of 10µg/L while in developing countries of Asia more than 45 million peoples are at risk of 50µg/L exposure of arsenic (Khalid *et al*. 2017). Arsenic (As) and its derivatives are ranked first in its toxicity out of twenty toxic and dangerous heavy metals. It has been reported that over 200 million peoples are at risk of As poisoning in 105 countries and about 100 million peoples from India, Bangladesh, Pakistan and China are at health risk, due to toxicity of underground drinking water (Shakoor *et al*., 2018). Drinking water contamination with inorganic arsenic is globally found to be major health concern. WHO has given maximum arsenic limit which is 10 ppb in ground drinking water while in Pakistan arsenic level is above this safe limit (Rasool *et al*., 2016). 20% peoples living in Punjab and 36% of Sindh province are drinking ground water containing >10µg/L of As (Kazi *et al*., 2009).

Arsenic induces the neurotoxicity, retards the development of neurons in cortex and effects cortical tissues of mice brain (Aung *et al*., 2016). Individuals when exposed to arsenic showed liver dysfunction with increased level of aspartate aminotransferase and glutamic transpeptides (Li *et al.,* 2016). Hepatic damage, dermal infections, pulmonary and pancreatic cancer is linked with

**Corresponding author:* e-mail: 2017-phd-1038@uvas.edu.pk

exposure to higher arsenic which leads to cardiovascular and nervous disorders (Rao *et al.,* 2017). When different doses of arsenicals were given to mice of CD1 strain, fetuses showed reproductive and physical defects (Rudriguez *et al*., 2016). Glutathione, glutahionoe peroxidase and SOD (superoxidase dismutase) are antioxidants compounds which play protective role against the reactive oxygen species (ROS) and arsenic decreased the activities of these enzymes due to increased oxidative stress (Han *et al*., 2010). Natural products contain bioactive compounds which can be used as ameliorants against arsenic induced toxicity as Biochanin A showed significant protection against arsenic induced liver toxicity (Jalaludeen *et al.* 2016). Olive oil has antioxidant property and can be used against arsenic induced oxidative stress and hepatotoxicity (Mohammadian *et al*. 2018). Lentils were used as antioxidant by Kalantari *et al*. (2017) against sodium arsenate induced oxidative stress in rats. Salma *et al*. (2016) visualized the effects of *Aloe vera*, *Azadirachta indica* and *Moringa oleifera*. Aquatic mixture of neem, *Moringa* and *Aloe vera* was found to be protective against liver damage due to carbon tetrachloride.

In Pakistan, *Moringa concanensis* and *Moringa oleifera* are present and widely cultured in moderate areas like, Punjab Plains, Sindh, NWFP and Balochistan. It has been under study because of its bactericidal and numerous uses like hepatoprotective, antioxidant, hypotensive, antimicrobial, anticarcinogenic and antidiabetic activity (Dilawar *et al.*, 2018). *Moringa oleifera* roots, gum, leaf, bark, fruit, flower, seed and seed oil had medicinal property can be used for treatment of menstrual disorders, as cardioprotective, antioxidant, to reduce abortion and fertility enhancer (Nwamarah *et al*., 2015). Neutraceutical products can be prepared to overcome malnutrition by using the edible parts of the *Moringa oleifera* (Aprioku *et al.* 2018). *Moringa oleifera* tree has significant value due to its valuable nutrients (micro and macro) for human beings. Different parts of the *Moringa oleifera* has different chemical properties depending upon the cultivation and source (Oyeyinka *et al.* 2018). Its economic and dietary value can be estimated through its enormous use in African continent (Matic *et al.,* 2018).When leaves were added into diet of male mice showed aphrodisiac activity, increased mating ability and fertility (Dafaalla *et al*., 2017). Seed and flower of *Moringa* have been used against arsenic induced hepatotoxicity in female albino rats (Chattopadhyay *et al*., 2011). Clear Data regarding the ameliorative effects of natural products against sodium arsenate induced embryo toxicity is still not available. So the present study was conducted to evaluate the antioxidant and ameliorative properties of *Moringa oleifera* extracts on mice embryo against sodium arsenate induced embryotoxicity and oxidative stress.

MATERIALS AND METHODS

Experimental animals handling

In the present study all the procedures and protocols were adopted under the guidelines of National Bioethics Committee (NBC) of Pakistan and 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. The mice were kept under standard conditions i.e., Relative humidity 45-50%, temperature 27±2^{o}C with 12 hours, dark and light cycle, at UVAS Ravi campus Pattoki, Pakistan. Room temperature was maintained by electric heater in winter and by air conditioner in summer. Animals before use were kept for seven days to acclimatize. Mice were kept in plastic cages. To absorb urine and excreta of animal, proper bedding of woody material was maintained. Mice were treated with normal diet and water. Commercially prepared feed No. 14 of National Feeds Ltd containing natural ingredients i.e. corn and cereals was given in form of pellets as standard feed. Water was given to animals in glass bottles. To remember the date of mating, dissection and dose, cage tags were used.

Induction of Mating and estimation of gestation period

7-8 week old Albino mice male and female, *Mus musculus* with initial body weight of 20-25g were collected from University of Veterinary and Animal sciences, Lahore. To obtain pregnant female mice, timed mating was induced by placing 1healthy male and 2 females together and presence of vaginal plugs were the indication of mating. The observation of a plug was determined to be gestation day '0' zero (Rodriguez *et al.*, 2016).

Collection and identification of plant samples

Leaves and flowers of *Moringa oleifera* were collected from the Botanical garden of University of Veterinary and Animal sciences Lahore, C-Block, Ravi campus Pattoki, Pakistan and herbarium specimens were subjected to analysis in Government College University, Lahore Pakistan. Plant samples were identified by (Plant taxonomist) Department of Botany, Government College University, Lahore (Voucher Specimen No.GC. Herb. Bot. 3725).

Preparation of Extracts

The method of extraction for leaves was given in report of Tabidi *et al*. (2018). Briefly the leaves were sun dried for 7 days and grounded to form powder which was extracted through methanol with soxhlet extractor apparatus to obtain the extract. While the extract of flowers was obtained following the method of Velaga *et al*., (2017). Obtained extracts were stored at 4^{o}C and administered to animals with distilled water.

Phytochemical analysis of Moringa oleifera leaves and flower extracts

Qualitative Phytochemical analysis of *Moringa oleifera* leaf and flower extracts for the presence or absence of phytochemicals was carried using standard procedures given in report of Santhi and Sengottuvel (2016).

Ferric chloride test for phenols = 4 drops of $FeCl_3$ + Test extract

- H_2SO_4Test for flavonoids = Few drops ofhydro sulfuric acid (H_2SO_4) + Test extract
- Liebermann Burchard test for steroids = 5 ml extract +2 ml chloroform+ 3 ml H_2SO_4 and CH_3COOH
- Salkowski test for terpenoids =Extract +2 ml chloroform + 3 ml of H_2SO_4
- Frothing test for saponins = 0.4mg extract in 4 ml of dH_2O
- Ferric Chloride test for tannins = 2 ml extract +2 ml dH_2O+ $FeCl_3$ drops
- Nitroprusside test for glycoside = 2 ml extract+ HCl+ nitroprusside in pyridine + NH_4OH solution
- Molisch test for carbohydrates = 2drops molisch reagent + 5mg extract+ 5ml Aq.sol +1 ml H_2SO_4

Administration of toxicant and Test extract to Animals on GD8

Different doses of sodium arsenate 6mg/kg and 12mg/kg B.W were selected according to previously published report (Kaise *et al*., 1985). Fourty four pregnant females were divided into 11 groups for different experimental doses of sodium arsenate and *Moringa* extracts. Ameliorent *Moringa* extracts as antidote were given 1hour before sodium arsenate administration in all experimental groups with 0.1ml of distilled water as given below. The dose concentrations for *Moringa* extracts were chosen according to previously published report of Onyewuchi *et al*., (2018).

Whereas, Group A was of control (treated only with distilled water) while B and C were sodium arsenate treated groups.
A (0.00), B (6.00, 0.00), C (12.00, 0.00) mg/kg B.W.
Whereas, 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.
Whereas, groups H to K were sodium arsenate and *M. oleifera* leafextract 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 in all experiments.

Dissection and Fetuses recovery

At gestation day 18 (GD18) pregnant females were weighed and anaesthetized using ether. The Fetuses were obtained by giving surgical incision to anaesthetized females. Live, dead and resorbed fetuses were numbered and recorded. Weight of fetuses was measured at digital weight balance (Schimadzu Ltd., Japan). Some fetuses were fixed in Formalin 10% for 48hrs; after this kept in 70% alcohol for histological studies (Carson and Hladik, 1997).

Histopathology

Fixed Fetuses were given repeated washes in 70%, 90% and 100% ethanol to decolorize and dehydrate. Then xylene was used for clarity. The infiltration was done through molten wax, and fetuses were embedded in blocks. Molds of steel were used, which were set in blocks made up of plastic. Firstly molten wax was added in bottom then fetuses were placed in such a way that head was in upward direction, wax was allowed for solidification. Serial sections of 4.5-5.5µm size were made using microtome. To avoid wrinkle formation, obtained sections were spread on water bath with water of temperature 37-40^oC. These sections were stained with Ehrlich's Hematoxylin, and then canada balsam was used to mount these sections. Xylene dipped cover slips were placed at each section of slide (Carleton *et al*., 1980). Stereoscopic Microscope was used for histological studies and selected histological section were micro photographed using microscope with digital camera (Canon, HD model A-2300). These photographs were resized, cleared and background was set and cropped with adobe Photoshop software.

Oxidative stress measurements

Oxidative damage induced by sodium arsenate and ameliorative role of *Moringa* was investigated by measuring GSH (Glutathione) and MDA (Malondialdehyde) level in embryonic tissues. For this purpose embryonic tissues were homogenized in 0.1 M Tris-Hcl buffer 4^oC, and then centrifuged at 1000rpm for 15 minutes. Upper layer of sample (supernatant) were stored at -20^oC for MDA and GSH measurements. To measure the MDA concentrations from the embryonic tissue homogenate, thiobarbituric acid test (TBA) was used. Tissue homogenate 0.2ml of 10% (w/v) was taken in test tube and add 0.2ml of SDS (Sodium dodecylesulphate) + 1.5 ml CH_3COOH + 1.5 ml TBA aqueous solution. Then added distilled water and heated for 1hour at 90^0C in water bath. Mixture was cooled and added 1ml dH_2O + Pyridine+ n-butanol+ shaked at vortex mixer then centrifuged this mixture at 4000 rpm/10minutes. Using spectrophotometer absorbance was noted at 530nm.Values of MDA written in nM/g (Okahawa *et al*., 1979).

For measurement of GSH, homogenized tissues solution was treated with TBA (50%) to form the precipitates. This precipitation was then centrifuged for five minutes at 1000rpm. Then a mixture of solutions was made with

following concentrations of different compounds as, 0.5 ml supernatant (embryonic tissue homogenate) + 2ml Tris-EDTA buffer +0.1ml 5'5'-dithiobis-2-nitrobenzoic acid. This solution was kept for 5minutes at room temperature. Spectrophotometer was used to read absorbance of GSH at 412nm and its values were written in nM/g (Sedlak and Lindsay, 1968).

STATISTICAL ANALYSIS

One way analysis of variance (ANOVA) was used to compare the means between control group and experimental groups. 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 $p \leq 0.05$ was considered significant. SAS version 9.1 software was used as a statistical tool for the analysis.

RESULTS

Moringa oleifera is rich source of different phytochemicals. Phytoconstituents which includes alkaloids, steroids, phenols, terpenoids, tannins, saponins, flavonoids, glycosides, protein and carbohydrates were present in *Moringa* extracts. Out of these tannins were absent in *Moringa oleifera* leaf extract and saponins were absent in *Moringa oleifera* flower extract.

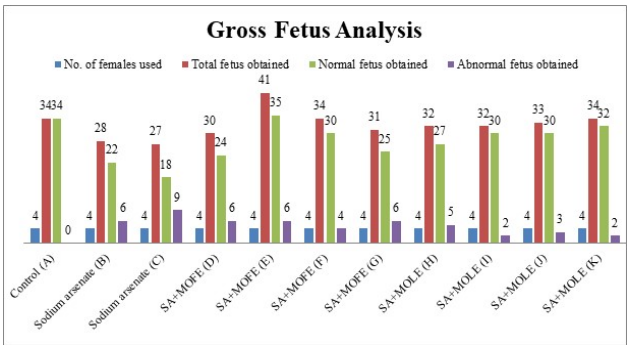
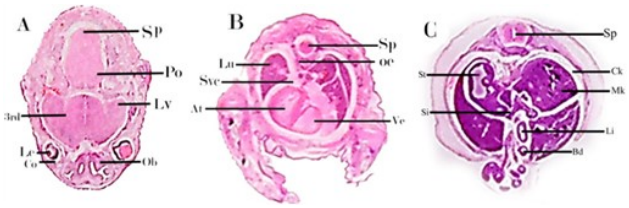


Fig. 1: Gross Fetus Analysis recovered at GD18

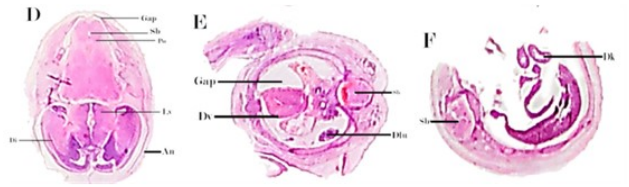
Gross fetus analysis

Gross fetus analysis was carried out for fetuses of each group which includes total female used, total fetuses recovered including normal and abnormal fetuses for all experimental groups and control. Significant decreased in litter size with greater number of abnormal fetuses were observed in sodium arsenate administered groups B and C (fig. 1) whereas SA + *Moringa* extracts treated all groups specifically *Moringa oleifera* flower extract (Group E) showed significant amelioration against sodium arsenate and increased number of normal fetuses were obtained as compared to arsenic treated groups B and C (fig. 1).



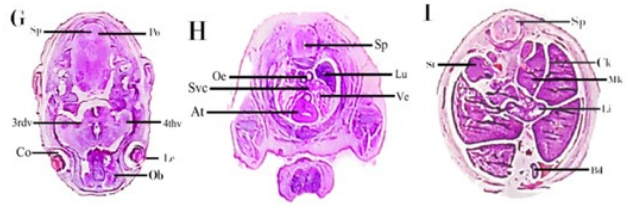
Labels: Po: pons, Lv: lateral ventricle, 3rd v: 3rd ventricle, Le: lens, Co: cornea, Sp: spinal cord, Lu: lung, At: atrium, Ve: ventricle, SVC: superior vena cava, Oe: oesophagous, Ob: olfactory bulb, Bd: bladder, Ck: cortex of kidney, Mk: medullary region of kidney, St: stomach, Li: large intestine, Si: small intestine.

Fig. 2: Macrophotographs of transverse sections of Head (A), Heart (B) and Abdomen (C) of Control group.



Labels: Sb: spina bifida, Po: pons, An: anopthalmia, Lv: lateral ventricle, Dk: degenerated kidney, Dv: degenerated ventricle, Dlu:degenerated lungs, Gap (cavitation), Di: diencephalan.

Fig. 3: Macrophotographs of transverse sections of Head (D), heart (E) and abdomen (F) of Sodium arsenate treated groups at 12mg/kg B.W



Labels: Po: pons,3rdv: 3rd ventricle, 4thv: 4thventricle, Le: lens, Sp: spinal cord, Lu: lung, At: atrium, Ve: ventricle, SVC: superior vena cava, Ob: olfactory bulb, Oe: oesophagous, Bd: bladder, Ck: cortex of kidney, Mk: medullary region of kidney, Co: cornea, St: stomach, Li: large intestine

Fig. 4: Macrophotographs of transverse sections of Head (G), heart (H) and abdomen (I) of arsenic+*Moringa oleifera* leaf extract treated group (I)of dose 6+300mg/kg B.W

Maternal body weights analysis

Maternal body weights as initial body weights and final body weights were calculated before the start of experiment and at the day of dissection (GD18) respectively. Mean body weight ± standard deviation was measured for each experimental and control group. Significant rise in final body weight of control group was observed as compared to all other groups including sodium arsenate and sodium arsenate + *Moringa oleifera* flower and leaf extract administered groups (table 1). Whereas increase in final body weights were observed in sodium arsenate + *Moringa oleifera* flower and leaf

Table 1: Maternal body weight (Mean ± SD) analysis

Treatment Groups	Doses (mg/kg/B.W)	Maternal IBW (Mean ± SD)	Maternal FBW (Mean ± SD)
Control (A)	-	23±0.81	42±0.81^{a}
Sodium arsenate (B)	6	24±0.81	30±0.81^{g}
Sodium arsenate (C)	12	24±0.81	29.7±0.95^{g}
SA+MOFE (D)	6+150	23.2±0.95	31.7±0.50^{f}
SA+MOFE (E)	6+300	25±0.81	36±0.81^{e}
SA+MOFE (F)	12+150	23.2±0.95	32±0.81^{f}
SA+MOFE (G)	12+300	23.2±0.95	38±0.81^{d}
SA+MOLE (H)	6+150	24.7±0.95	39±0.81^{cd}
SA+MOLE (I)	6+300	23±0.81	40.2±0.50^{b}
SA+MOLE (J)	12+150	24.2±0.50	38.2±0.95^{d}
SA+MOLE (K)	12+300	24±0.81	40±0.81^{bc}

Table 2: Oxidative stress induced by sodium arsenate and ameliorative effects of *Moringa oleifera* extracts (leaf and flower) in mice embryo at GD8

Treatment Groups	Doses (mg/kg/B.W)	MDA (nmol/gm) (Mean ± SD)	GSH (nmol/gm) (Mean ± SD)
Control (A)	-	22.5±0.57^{g}	19±0.81^{a}
Sodium arsenate (B)	6	31.25±0.95^{b}	11.25±0.95^{e}
Sodium arsenate (C)	12	36±0.81^{a}	8.25±0.95^{f}
SA+MOFE (D)	6+150	29.5±1.29^{c}	13.75±0.95^{cd}
SA+MOFE (E)	6+300	25±0.81^{e}	14.50±1.29^{c}
SA+MOFE (F)	12+150	29.25±1.70^{c}	12±0.81^{de}
SA+MOFE (G)	12+300	26.50±1.29^{d}	14.25±0.95^{c}
SA+MOLE (H)	6+150	24.75±0.95^{e}	14±0.81^{cd}
SA+MOLE (I)	6+300	23±0.81^{g}	17.75±3.20^{a}
SA+MOLE (J)	12+150	24.25±0.50^{ef}	15.75±1.70^{b}
SA+MOLE (K)	12+300	24±0.81^{ef}	15.75±0.50^{bc}

Values bearing the same letters are insignificant and vice versa. Whereas ($p<0.05$). Where, SA = Sodium arsenate, MOFE = *Moringa oleifera* flower extract and MOLE = *Moringa oleifera* leaf extract

extract administered groups as compared to only sodium arsenate treated groups B and C which showed the protective role of *Moringa oleifera* against arsenic toxicity.

Histopathological analysis

Normally developed internal structures were found in control group. Brain was normal with lateral, 3rd, 4th ventricles and Pons. Eyes were with cornea and lens. Normal structure of Heart with superior vena cava, atrium and ventricles was observed. All other vital body structures like lungs, liver, intestine (small, large), kidney and urinary bladder were normal. Spinal cord, olfactory bulb, esophagus and tracheal systems were clearly observed and found normally developed at day 18thof gestation (fig. 2 A, B, C).

Sodium arsenate administered groups (B & C) showed poorly developed ventricles of brain, diencephalon, malformed nasal septum, spina bifida, and cavitation (gaps) of brain, malformed heart with degenerated atrium and ventricles. Similarly anopthalmia, underdeveloped stomach, degenerated lungs, bladder and degenerated kidneys were observed due to increased dose of sodium arsenate upto12mg (fig. 3. D, E, F).

Histological sections obtained from toxicant + *Moringa oleifera* flower treated groups showed little amelioration against arsenic but displayed abnormalities in heart, intestine and kidneys While *Moringa oleifera* leaf extract as antidote treated groups exhibited normal growth and development of vital organs in fetuses hence significantly mitigated the teratogenic effects of sodium arsenate. *MOLE* at dose of 300mg/kg significantly detoxify the effects of sodium arsenate and well developed brain, Pons, eyes, spinal cord, heart, lungs, stomach, oesophagus, bladder and kidneys similar to control groups were observed (fig. 4. G, H, I)**.**

Oxidative stress analysis

Sodium arsenate generated the reactive oxygen species (ROS) when administered to pregnant females at gestation day8 with different doses of 6mg/kg B.W and 12mg/kg B.W. MDA values were significantly ($p<0.05$) increased in sodium arsenate treated groups (B & C) while reduced glutathione (GSH) values were observed as compared to control group (table 2) whereas *Moringa oleifera* extracts treated groups showed amelioration against sodium arsenate induced oxidative stress. Among extracts *Moringa oleifera* leaf extract at dose of 300mg/kg/B.W were found significant, it normalized

MDA and GSH values through its antioxidant activity (table 2).

DISCUSSION

Decrease in litter size and mean maternal body weights were commonly observed in this study in sodium arsenate treated groups which was in accordance with study of Arshad *et al*., (2017). They evaluated, when sodium arsenate as toxicant with different dose concentrations were administered to female pregnant mice at gestation day 6, decreased maternal body weight and less number of fetuses with large number of abnormal fetuses as compared to normal fetuses were obtained.

Spina bifida, diencephalan and cavitation (gaps) were observed in histological sections when higher concentrations of sodium arsenate were administered to the pregnant females which were in accordance with the findings of Hill *et al*., (2009). They studied oxidative stress increased which increased the neural tube defects in developing chick embryo. In this study misshapen heart ventricle and degenerated atrium were observed in sodium arsenate intoxicated groups which was an agreement with study of Li *et al*., (2012). He observed the cardiac abnormalities in zebra fish due to arsenic. He found that malformed heart and ventricular defects in embryo of zebra fish was due to Dvr1 genes which is homologous to GDF1 and originated all these cardiac abnormalities and same changes were found in mice due to toxicity of arsenic which causes genetic mutations. Degenerated kidney, stomach and intestine were also observed. Hypoplasia in lungs and degenerated lungs were findings of this study when pregnant mice were exposed to different doses of sodium arsenate during gestation days. These findings were similar to the observations of Hays *et al*., (2008) when 50ppb of sodium arsenate was given to female mice during pregnancy in drinking water it altered the functioning of ER-αβ protein of lungs and induces carcinoma. Arsenic effects the embryonic air passage way and produced malformed lungs.

Dose dependent increased in malondialdehyde (MDA) level and decreased in glutathione (GSH) values in embryonic tissues were observed due to sodium arsenate induced oxidative stress in developing mice embryo. Which is an agreement with Han *et al*., (2010) they demonstrated that glutathione, glutahionoe peroxidase and SOD (superoxidase dismutase) are antioxidant compounds which play protective role against the reactive oxygen species (ROS) and arsenic decreased the activities of these enzymes due to increased oxidative stress through ROS generation. Our results were in accordance with reports of Nagata *et al*., (2007).They also observed the significant decrease in glutathione activity due to inorganic arsenicals. While *Moringa oleifera* extracts specifically leaf extract reduced the toxic effects of arsenic by maintaining MDA and GSH values which was accordance with Luqman *et al*., (2012) they observed increased oxidative stress when inorganic arsenicals were administered to Swiss albino mice but *Moringa oleifera* leaf administration maintained the GSH and MDA values by increasing antioxidant activity due to its bioactive compounds which scavenge the free radicals and increased the antioxidant enzymes activity, resulting reduced oxidative stress.

Amelioration from the teratogenic effects of heavy metals by the natural products was the focus of this study. *Moringa oleifera* flower and leaf extracts were used against sodium arsenate as ameliorant and our findings showed *Moringa oleifera* flower and leaf extracts reduced teratogenic effects of arsenic. *Moringa oleifera* extracts reduced the histopathological anomalies of fetuses induced by sodium arsenate specifically *Moringa oleifera* leaf extracts at dose of 300mg/kg B.W found to be more effective than the *Moringa oleifera* flower extract. Similarly antioxidant property of *Moringa oleifera* extracts was confirmed, extracts reduces the elevated level of MDA and increased the concentration of GSH in embryonic tissues which were disturbed in sodium arsenate treated groups. *Moringa oleifera* leaf extract showed dose dependent hydroxyl scavenging ability and reduces the toxic effects of sodium arsenate.

CONCLUSION

Results of this study confirmed the sodium arsenate induced histopathologiacl abnormalities in internal organs of fetuses and oxidative stress (increased MDA values and decreased GSH activity). However ameliorative property of *Moringa oleifera* leaf and flower extracts significantly reduced these teratogenic effects of arsenic and its antioxidant activity normalized the MDA and GSH values specifically *Moringa oleifera* leaf extract which suggested that it could be used as ameliorant in pharmacological purposes and further studies are required for its clinical uses as ameliorant against teratogens like arsenic.

REFERENCES

Abdul KS, Jayasinghe SS, Chandana EP, Jayasumana C and De Silva PM (2015). Arsenic and human health effects: A review. *Environ. Toxicol. Pharmacol*., **40**(3): 828-846.

Ali H and Khan E (2018). What are heavy metals? Long-standing controversy over the scientific use of the term 'heavy metals' proposal of a comprehensive definition. *Toxicol. Environ. Chem*., **100**(1): 6-19.

Aprioku JS and Onyenaturuchi PO (2018). Comparative phytochemical analysis of *Moringa oleifera* (moringaceae) extracts and reproductive effects of the aqueous leaf, seed and bark extracts in rats. *World. J. Pharm. Res*., **7**(9): 42-54.

Arshad M and Asmatullah CA (2017). Embryotoxic effects of sodium arsenate in *Musmusculus*. *Punjab Univ. J. Zool*., **32**(2): 189-195.

Aung KH, Kyi-Tha-Thu C, Sano K, Nakamura K, Tanoue A, Nohara K, Kakeyama M, Tohyama C, Tsukahara S and Maekawa F (2016). Prenatal exposure to arsenic impairs behavioral flexibility and cortical structure in mice. *Front. Neur*., **10**: 137.

Carleton HM, Drury RA and Wallington EA (1980). Carleton's histological technique: *Oxford University Press*.

Carson FL and Hladik C (1997). Histotechnology: a self instructional text. Chicago: *Ascp*press.

Chattopadhyay S, Maiti S, Maji G, Deb B, Pan B and Ghosh D (2011). Protective role of *Moringa oleifera* (Sajina) seed on arsenic induced hepatocellular degeneration in female albino rats. *Biol. Trace Elem. Res*., **142**(2): 200-212.

Chen QY, DesMarais T and Costa M (2019). Metals and mechanisms of carcinogenesis.*Ann. Rev. Pharmacol. Toxicol*., **59**(1): 537-554.

Dafaalla MD, Abass S, Abdoun S, Hassan, AW and Idris F (2017). Effect of ethanol extract of *Moringa oleifera* Lam. seeds on fertility hormone and sperm quality of male Albino Rats. *Int. J. Multidiscip. Res. Develop*., **4**(4): 222- 226.

Dilawar S, Shah A, Khan HU and Imad S (2018). Antibacterial, antibiofilm, hemolytic activity and phytochemical study of various aerial parts of *Moringaoleifera* in Pakistan. *Asian J. Med. Bio. Res*., **4**(1): 77-85.

Duncan GB (1955). Duncan multiple range test. *Biometrics*, **11**(1): 42.

Han YH, Moon HJ, You BR, Kim SZ, Kim SH and Park WH (2010). Effects of arsenic trioxide on cell death, reactive oxygen species and glutathione levels in different cell types. *Int. J. of Mol. Med*., **25**(1): 121-128.

Hays AM, Lantz RC, Rodgers LS, Sollome JJ, Vaillancourt RR, Andrew AS and Camenisch TD (2008). Arsenic-induced decreases in the vascular matrix. *Toxicol. Pathol*., **36**(6): 805-817.

Hill DS, Wlodarczyk BJ, Mitchell LE and Finnell RH (2009).Arsenateinduced maternal glucose intolerance and neural tube defects in a mouse model. *Toxicol. Appl. Pharmacol*., **239**(1): 29-36.

Jalaludeen A, Ha W, Lee R, Kim J, Do J, Park C, Heo Y, Lee W and Song H (2016). Biochanin A ameliorates arsenic-induced hepato-and hematotoxicity in rats. *Molecules*. **21**(1): 69.

Kaise T, Watanabe S and Itoh K (1985).The acute toxicity of arsenobetaine. *Chemosphere*., **14**(9): 1327-32.

Kalantari H, Houshmand G, Hasanvand A, Kalantar M, Goudarzi M and Haghighian HK (2017). Ameliorative effects of red lentil extract on sodium arsenite-induced oxidative stress in rats. Jundishapur. *J. Natur. Pharmaceut. Prod*., **12**(3 (Supp): e64309.

Kazi TG, Arain MB, Jamali MK, Jalbani N, Afridi HI, Sarfraz RA, Baig JA and Shah AQ (2009). Assessment of water quality of polluted lake using multivariate statistical techniques: A case study. *Ecotoxicol. Environ. Saf*., **72**(2): 301-309.

Khalid S, Shahid M, Niazi NK, Murtaza B, Bibi I and Dumat C(2017). A comparison of technologies for remediation of heavy metal contaminated soils. *J. Geochem. Explor*., **182**(Part B): 247-268.

Kwon JC, Nejad ZD and Jung MC (2017). Arsenic and heavy metals in paddy soil and polished rice contaminated by mining activities in Korea. *Catena*., **148**(Part1): 92-100.

Li X, Ma Y, Li D, Gao X, Li P., Bai N, Lou M, Tan X, Lu C and Ma X (2012). Arsenic impairs embryo development via down-regulating Dvr1 expression in zebrafish. *Toxicol. Lett*., **212**(2): 161-168.

Li Y, Ye F, Wang A, Wang D, Yang B, Zheng Q, Sun G and Gao X (2016). Chronic arsenic poisoning probably caused by arsenic-based pesticides: findings from an investigation study of a household. *Int. J. Enviro. Res. Publ. Health*., **13**(1): 133.

Luqman S, Srivastava S, Kumar R, Maurya AK and Chanda D (2012). Experimental Assessment of Moringaoleifera Leaf and Fruit for its Antistress, Antioxidant and Scavenging Potential Using In vitro and In vivo Assays. *Evid. Based Compl. Alternat. Med*., (Art Id 519084): 12.

Matic I, Guidi A, Kenzo M, Mattei M and Galgani A (2018). Investigation of medicinal plants traditionally used as dietary supplements: A review on *Moringaoleifera*. *J. Publ. Health in Africa*, **9**(3): 841.

Mohammadian M, Mianabadi M, Zargari M, Karimpour A, Khalafi M and Amiri FT (2018). Effects of olive oil supplementation on sodium arsenate-induced hepatotoxicity in mice. *Int. J. Preve. Med*., 9.

Nagata K, Iwasaki Y, Yamad T, Yuba T, Kono K, Hosogi S and Marunaka Y (2007). Over expression of manganese superoxide dismutase by Nacetylcysteine in hyperoxic lung injury. *Respi. Med*., **101**(4): 800-807.

Nwamarah JU, Otitoju O and Otitoju GT (2015). Effects of *Moringaoleifera* Lam. aqueous leaf extracts on follicle stimulating hormone and serum cholesterol in Wistar rats. *Afr. J. Biotech*., **14**(3): 181-186.

Okahawa H, Ohishi N and Yagi K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem*., **95**(2): 351-358.

Onyewuchi CB, Aprioku JS and Siminialayi IM (2018). Evaluation of the Effect of Ethanol Bark Extract of *Moringaoleifera* on Reproductive Biology of Non pregnant Wistar Albino Rats. *J. Complem. Altern. Med. Res*., **29**(2): 1-7.

Oyeyinka AT and Oyeyinka SA (2018). *Moringaoleifera* as a food fortificant: Recent trends and prospects. *J. Saudi Soci. Agri. Sci*., **17**(2): 127-136.

Rao CV, Pal S, Mohammed A, Farooqui M, Doescher MP, Asch AS and Yamada HY (2017). Biological

effects and epidemiological consequences of arsenic exposure, and reagents that can ameliorate arsenic damage *in vivo*. *Oncotarget*. **8**(34): 57605.

Rasool A, Farooqi A, Masood S and Hussain K (2016). Arsenic in groundwater and its health risk assessment in drinking water of Mailsi, Punjab, Pakistan. Human and Ecological Risk Assessment: *An Int. J*., 22(1): 187-202.

Rodriguez KF, Ungewitter EK, Crespo-Mejias Y, Liu C, Nicol B, Kissling GE and Yao HH (2016). Effects of in utero exposure to arsenic during the second half of gestation on reproductive end points and metabolic parameters in female CD-1 mice. *Environ. Hlth. Perspect*., **124**(3): 336-343.

Salama RA, Mohamed MM, AbdElwahab MB and Shakweer MM (2016). Assessment effect of *Aloe vera*, *Azadirachta indica* and *Moringa oleifera* aqueous extracts on carbon tetrachloride-induced hepatotoxicity in rats. *Int. J. Pharm. Pharmceu. Sci.,* **8**(4): 83-89.

Santhi K and R Sengottuvel (2016). Qualitative and quantitative phytochemical analysis of *Moringa concanensis* Nimmo. *Int. J. Curr. Microbiol. Appl. Sci*., **5**(1): 633-640.

Sedlak J and Lindsay RH (1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal. Biochem*., **25**(1): 192-205.

Shakoor MB, Bibi I, Niazi NK, Shahid M, Nawaz MF, Farooqi A, Naidu R, Rahman MM, Murtaza G and Luttge A (2018). The evaluation of arsenic contamination potential, speciation and hydrogeochemical behaviour in aquifers of Punjab, Pakistan. *Chemosphere*., **199**(1): 737-46.

Shakoor MB, Niazi NK, Bibi I, Murtaza G, Kunhikrishnan A, Seshadri B, Shahid M, Ali S, Bolan NS and Ok YS (2016). Remediation of arsenic-contaminated water using agricultural wastes as biosorbents. *Crit. Rev. Environ. Sci. Technol*., **46**(5): 467-499.

Tabidi RH, Babeker EA and ELlaithi SO (2018). Phytochemical and antibacterial of *Moringa oleifera* leaves. *World J. Pharmaceu*. *Res.,* **8**(2): 212-224

Velaga VS, Suryadevara N, Chee L and Ismail NE (2017).Phytochemical analysis and immuno-modulatory effect of *Moringa oleifera* flowers. *Int. J. Pharm. Pharmaceut. Sci*., **9**(6): 24-28.

Zeng B, Luo J, Wang P, Yang L, Chen T, Sun J, Xie M, Li M, Zhang H, He J and Zhang Y (2019). The beneficial effects of *Moringa oleifera* leaf on reproductive performance in mice. *Food Sci. Nutr*., **7**(2): 738-746.

Zhang X, Li S, Wu S, Hao X, Guo S, Suzuki K, Yokomichi H and Yamagata Z (2012). Prevalence of birth defects and risk-factor analysis from a population-based survey in Inner Mongolia, China. *BMC Pediat*., **12**(1): 125.