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DUAL ROLE OF ARJUNOLIC ACID AND B₁₂ IN MITIGATING SODIUM ARSENITE INDUCED MALFUNCTION OF FEMALE REPRODUCTIVE ORGANS

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ABSTRACT: The present study was proposed to delineate the curative role of arjunolic acid and vitamin B₁₂ alone or in combination against arsenic induced oxidative stress and female reproductive dysfunction. The Wistar strain adult female rats were treated with sodium arsenite at the dose of 1.0 mg/100 gm body weight orally for first eight days. Next eight days rats were treated with arjunolic acid (1.0 mg/100 gm body weight) and vitamin B₁₂ (0.09 µg/100 gm body weight) orally. In ovarian and uterine tissue showed a recovery in arsenic induced ROS-production following the post-treatment of arjunolic acid and vitamin B₁₂. Antioxidant status was also improved by arjunolic acid and vitamin B₁₂ as established from the electrozymographic analysis of SOD, catalase and GPx. Post-treatment of these two significantly retrieved ovarian estradiol receptor 1 (ESR-1) from arsenic induced down regulation. Arjunolic acid and vitamin B₁₂ also showed its curative ability in improving the level of inflammatory markers such as NF-κB, metallothioneine-1 (MT-1), TNF-α, IL-6. Thus these two bioactive substances might have the curative role against arsenic induced female reproductive dysfunction.

INTRODUCTION: The trioxide form of arsenic has the ability to react with cellular thiols to exert its toxicity and during the metabolism of thiols in cells reactive oxygen species generates which thereby promotes tissue damage¹. Arsenic is the cause of several reproductive hazards. It impairs spermatogenesis process and decreases the spermatogenic enzymes in male reproductive system. The spermatogenic meiosis and the post-meiotic stages were altered by arsenic. Gonadal dysfunctions like declined testosterone synthesis, apoptotic and necrotic changes due to arsenic mediated oxidative damage² may cause male infertility.

It may exert its effect on the brain or pituitary or directly on the germ cells. Interference of ovarian steroidogenesis with prolonged diestrous, degeneration of ovarian follicles and uterine tissue and suppressed levels of plasma estradiol and progesterone are the outcome of arsenic ingestion. Arsenic enters into the developing fetus through the placenta and exerts its effect on fetal brain development and postnatal behaviors³. Fetal mortality and preterm birth elevated due to chronic exposure of arsenic. During pregnancy constant arsenic exposure can alter the urine excretion and metabolites distribution⁴. In zebra fish, arsenic develops embryo toxicity. It down regulates Dvr1, the key regulator of establishing left-right embryo asymmetry⁵.

Few herbal formulations are in trial phase to cope arsenic induced health hazards. *Emblica officinalis* (amla) can diminish the arsenic induced DNA and hepatic damage⁶. Sodium selenite can ameliorate arsenic induced female reproductive toxicity⁷.

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Arsenic induced toxicity in hepatic tissue can be protected by polysaccharides of *Momordica charantia*⁸. Arjunolic acid, a triterpenoid saponin is a cardiac tonic which is isolated from the extract of *Terminalia arjuna*. It has cardioprotective effect by showing the effects on platelet aggregation, coagulation and myocardial necrosis⁹. The extracts of *T. arjuna* and arjunolic acid has protective role against arsenic induced renal toxicity by increasing the antioxidative defense activities¹⁰. Arjunolic acid has the ability to improve the oxidative stress in murine brain and liver tissue under arsenic intoxication¹¹. Cancerous growth can be protected by arjunolic acid on the cell lines of human ovary (PA 1) and liver (HepG-2 & WRL-68)¹². The chemo-preventive role of arjunolic acid and its antioxidant property eliminate the toxicity in testis¹³.

Vitamin B₁₂ (cyanocobalamin) plays an important role to maintain methylation process that controls biotransformation involving methionine synthase enzyme and thereby repairs DNA and prevents cancer¹⁴. Vitamin B₁₂ prevents atherosclerosis and other cardiovascular diseases by lowering the level of homocysteine¹⁵. Separately or in combination vitamin B₁₂ and folic acid diminish the arsenic induced hepatic damage¹⁶ and hepatic mitochondrial oxidative stress¹⁷, pancreatic islet cell mitochondrial dysfunction¹⁸ and DNA damage¹⁷.

Hence, the present investigation focused a therapeutic approach towards the cure of arsenic induced oxidative damage of ovarian and uterine tissue by arjunolic acid and vitamin B₁₂.

MATERIALS AND METHODS:

Animal Selection and Treatment: For the experiment female Wistar rats (150 ± 10 g) were selected. According to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Indian Council of Medical Research (ICMR, India) the rats were kept in polycarbonate cages in the Central Animal House, Vidyasagar University (Midnapore, India) (IAEC/3/C-8/14 dated 03.11.14). With a 12-hour light-dark cycle, with 32 ± 2°C temperatures and 50–70% humidity the rats were acclimatized for 10 days in a room. Animals were fed with a standard pellet diet and water *ad libitum*.

The rats were distributed in seven groups which contains six rats in each group. The experimental scheduled was as followed: Vehicle treated control group intake only water, Arsenic treated group intake only As (III) (1.0 mg/100 gm body weight), arjunolic acid placebo group intake only arjunolic acid (1.0 mg/100gm body weight) (Sigma-Aldrich, St. Louis, MO), arsenic + arjunolic acid group intake As (III) and arjunolic acid at dose of 1.0 mg/100gm body weight, vitamin B₁₂ placebo group ingest only B₁₂ (0.09µg/100gm body weight), Arsenic + Vitamin B₁₂ group intake As (III) at the dose of 1.0 mg/100gm body weight and B₁₂ at the dose of 0.09µg/100gm body weight, Arsenic + Arjunolic acid, vitamin B₁₂ combination group ingest As (III) and arjunolic acid (1.0 mg/100gm body weight) and B₁₂ (0.09µg/100gm body weight).

The rats are treated *via* oral gavage for sixteen days. First eight days arsenic was fed and from day nine to day sixteen the rats were treated with arjunolic acid and B₁₂. For the collection of the blood, uterine horns and ovaries the rats are anesthisized by following the standard protocol of institutional ethical guideline (IAEC/3/C-8/14 dated 03.11.14) on the day seventeen. At -20°C temperature of insulated ice unit the samples were kept into a separate sterile bag.

Assessment of Super Oxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx) by Native Gel Electrophoresis: SOD was separated by the uses of protein of ovarian and uterine supernatant on 12% native PAGE and the achromatic bands of SOD were visible against a dark blue background upon fluorescent light by the incubation of gels with TEMED, NBT and riboflavin for 20 min in dark¹⁹.

The proteins were electrophoresed on 8% PAGE for the detection of catalase. By using potassium ferricyanide and ferric chloride the gels were stained after incubation with H₂O₂ solution for 10 min. Bluish yellow bands were appearing against a blue, green background²⁰. To determine the GPx level peroxide was removed for the transformation of potassium ferricyanide to ferrocyanide between samples and where the GPx was present the acromatic clearing band was seen on the blue green background of 8% native gel.

GPx eliminate peroxide which inhibited the interaction with ferric chloride²¹. The densitometric analysis of electrozymographic image was analyzed by using Image J software where the density of control was taken as 100% and the data was presented as percentage of density²².

Determination of Serum Total Lactate Dehydrogenase (LDH): Using serum, 1.2% agarose gel was used to determine the LDH activity in Tris-HCl buffer (pH 8.2). With slight modification, agarose gel was incubated at 37 °C in the presence of H₂O, Tris, tetrazolium-blue, phenazine-methosulphate, Na-lactate and NAD to develop color reaction following the rinsing of the gels with water under light²³.

Determination of Esr 1, NF-κB, Metallothionein 1, TNF-alpha and IL-6: According to the procedures recommended by the manufacturers (Wunhan Fine test, China and RayBio) the level of ovarian Er-1 (Cat no. ER0424), uterine NF-κB (Cat no. ER1186), liver metallothionein (Cat no. ER0447), serum TNF-α (Cat no. ELR-TNFα) and serum IL-6 (Cat no. ELR-IL6) were measured by using ELISA kits.

Ovarian and Uterine Histopathology: The ovarian and uterine tissues were embedded with paraffin. The tissues were sectioned at 5 μm. The staining procedure was performed with the help of hematoxylin (Harris) and eosin and observed under a microscope (Olympus, CX21i, magnification x400).

To identify the nature of the follicles the diameter of each follicle was measured and quantifies the number of follicles in different stages of folliculogenesis in each ovary²⁴. The follicles were categorized as small preantral follicles (SPAF), large preantral follicles (LPAF), small antral follicles (SAF), medium antral follicles (MAF), large antral follicles (LAF), graafian follicles on the basis of their morphology and diameter and the numbers of regressive follicles (RF) were counted. The uterine diameter and the thickness of different layers (μm) of uterus (*i.e.* endometrium, myometrium and perimetrium) were also measured on microscope.

Statistical Analysis: The statistical significance of the differences in these variables between treated

cases and controls was evaluated using ANOVA followed by post hoc Dunnett t test. Differences of data (Mean ± SE, N= 6), p<0.05 were considered significant statistically.

RESULTS:

Effect of Arjunolic Acid and Vitamin B₁₂ on SOD, Catalase and GPx: Electrozymogram revealed diminished activity of SOD, catalase and GPx in arsenic ingested rats in comparison with control group **Fig. 1** in ovarian and uterine tissue. Modification of cysteine in SOD in response to redox imbalance might be plausible in the inactivation of SOD²⁵. Arsenic also decreases the mRNA expression of the SOD gene in the way of generation of oxidative stress in response to decrease nullification of superoxide anion radicals (O₂^{•-})²⁶. The superoxide anions were converted into H₂O₂. The activity of the catalase **Fig. 1C** and **1D** was diminished in arsenic group. Progressive accumulation of H₂O₂ in ovarian and uterine tissue is indicative of decreased activity of catalase. In arsenic treated group faint band of GPx **Fig. 1E** and **1F** was shown due to enhanced H₂O₂ deposits in ovarian and uterine tissue during the programmed cell death²⁷. However, the post treatment of arjunolic acid and vitamin B₁₂ in arsenic treated animals alone or co-jointly have been shown its ability to restore the activity of SOD, catalase and GPx **Fig. 1**.

Serum LDH Status: In necrotic cells the level of serum LDH is generally higher than normal cells²⁸. We have considered the measuring of serum LDH in arsenic treated rat's electrozymographically judge the necrotic risk factors in these reproductive tissues **Fig. 1G**. Serum LDH was high which was seen as a distinct intense band of LDH was found in arsenic treated group **Fig. 1G**. During elevation of the serum LDH the apoptotic tissue lesions were found in uterine histological tissue sections. Nevertheless, this leakage of serum LDH was significantly reduced by co-treatment of arjunolic acid and vitamin B₁₂ alone or together.

Esr 1, NF-κB, Metallothionein-1, TNF alpha and IL-6: A low level of expression of ovarian estradiol receptor-1 (Esr-1) might be indicated by ELISA test showed significantly decreased level of this receptor **Table 1**. Uterine NF-κB, liver metallothionein, serum TNF-alpha and IL-6 was

noticeably amplified in arsenic treated group. But, arjunolic acid and vitamin B₁₂ alone or in combination counteracted the arsenic induced

changes of these above parameters significantly **Table 1**.

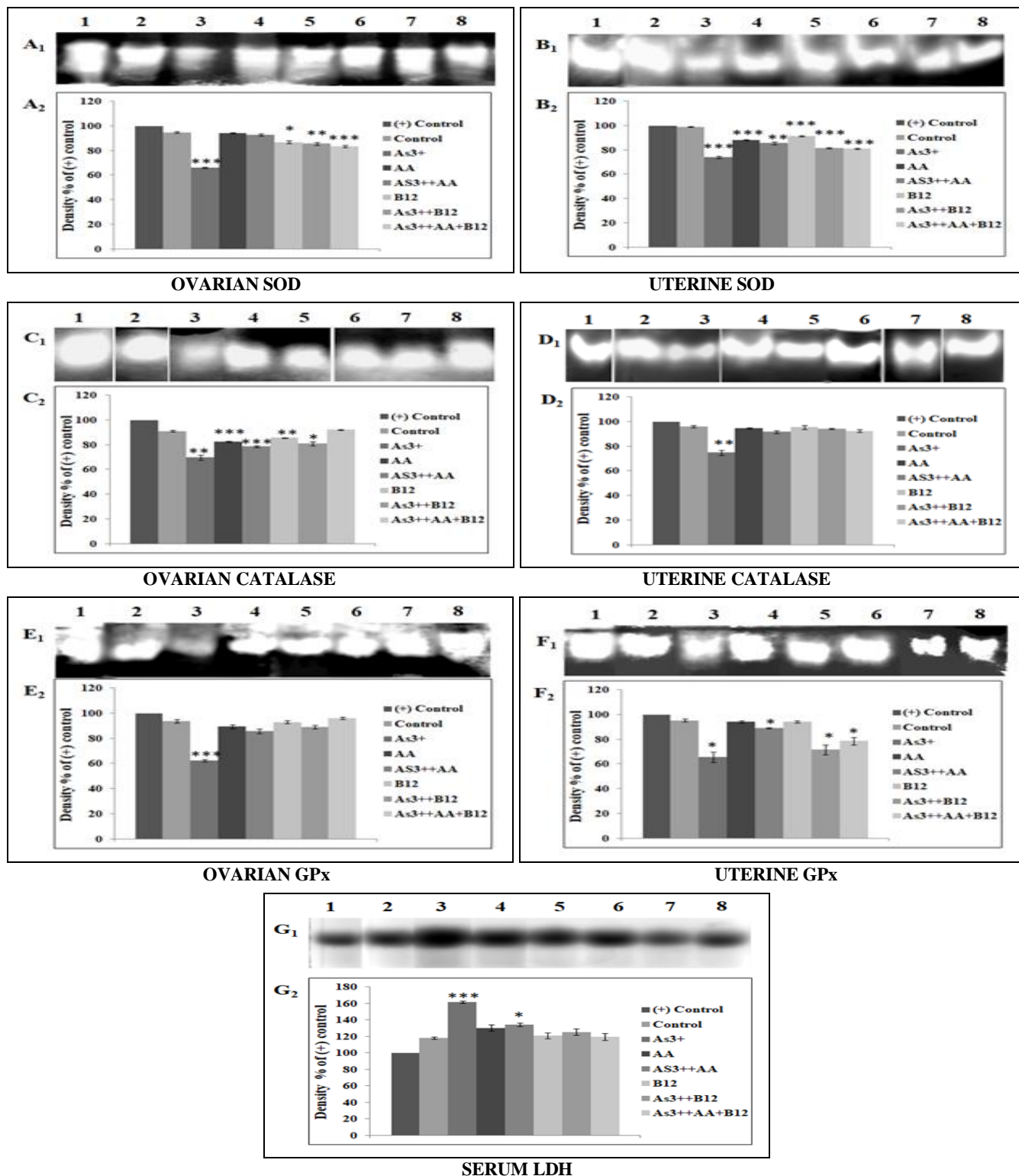


FIG. 1: EFFECT OF ARJUNOLIC ACID AND VITAMIN B₁₂ ON SOD, CATALASE, GPx AND SERUM LDH

Fig. 1: (A, B, C, D, E, F and G). The activity of SOD, catalase and GPx on native gel in ovarian and uterine tissue shown. To the extent of cellular damage serum LDH activity was performed on agarose gel. Lane distribution Lane 1: (+) control; Lane 2: control; Lane 3: arsenic; Lane 4: arjunolic acid; Lane 5: arsenic+arjunolic acid, Lane 6: B₁₂, Lane 7: arsenic+B₁₂, Lane 8: arsenic+arjunolic acid+B₁₂. The same protein was electrophoresed on 12% and 8% native gel in each lane. After staining the achromatic bands of SOD and GPx was shown. On blue-green background of native gel the yellow bands of catalase were shown. Densitometric analysis represents mean ± SE, N=6, ANOVA followed by post hoc Dunnett t test. *p<0.05, **p<0.01, ***p<0.001.

Ovarian and Uterine Histopathology: A significant level of normal ovarian follicular regression followed by increased numbers of atretic follicles was observed in arsenic treated group in comparison with the control group **Fig. 2A** and **Table 2**. A significant numbers of primary classes of preantral and antral follicles were reduced in ovaries of arsenic group. However, arjunolic acid and B₁₂ alone or in combination successfully achieved an increase in the numbers of growing

and matured follicles in contrast to arsenic treated rats **Fig. 2A** and **Table 2**. By the degeneration of a noteworthy thinning of uterine layers perimetrium, myometrium and endometrium were noticed in arsenic intoxicated rats when compared with the vehicle treated control group. Arjunolic acid and B₁₂ alone or in combination in arsenicated rats could modify the breadth of these layers with a significant restoration of the secretory glands **Fig. 2B** and **Table 2**.

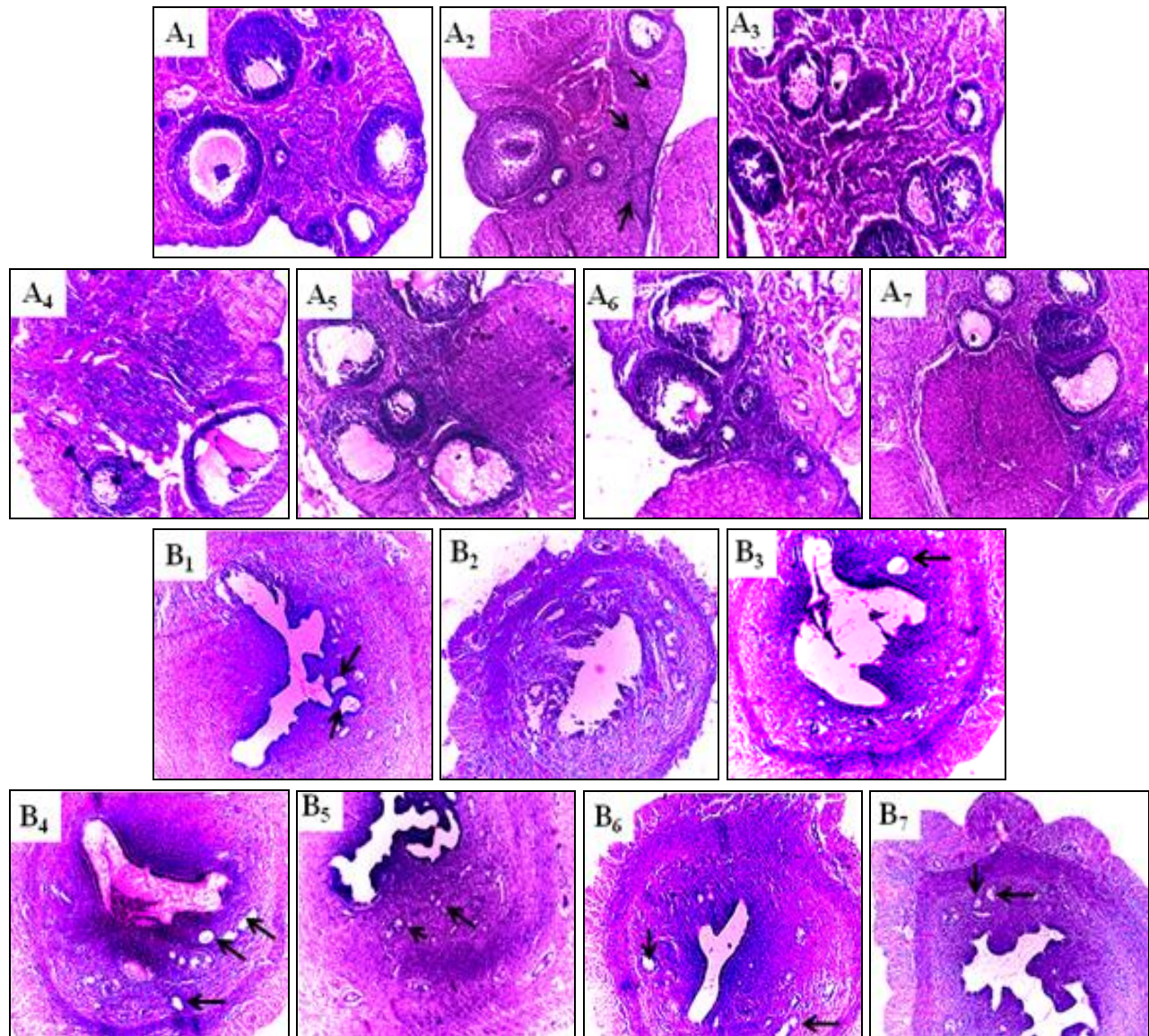


FIG. 2: HISTOPATHOLOGY OF OVARY AND UTERUS

Fig. 2: (A and B) the ovarian and uterine tissue was sectioned at 5 μ M, stained with eosin and hematoxylin (Harris) and observed under a microscope. Arjunolic acid and vitamin B₁₂ recover the As(III) induced follicular atresia and reappearance of number of follicles alone or in combination in ovarian histoarchitecture (A). Panel distribution; A₁: control, A₂: As(III), A₃: arjunolic acid, A₄: As(III)+ arjunolic acid, A₅: B₁₂, A₆: As(III)+B₁₂, A₇: As(III)+arjunolic acid+B₁₂. Arrows indicate follicular atresia. In uterine histoarchitecture (B) the uterine secretory glands were lost by arsenic and recovered in response to Arjunolic acid and vitamin B₁₂ alone or combination. Panel distribution; B₁: control, B₂: As(III), B₃: arjunolic acid, B₄: As(III)+arjunolic acid, B₅: B₁₂, B₆: As(III)+B₁₂, B₇: As(III)+arjunolic acid+B₁₂. Arrows indicate secretory glands.

TABLE 1: NF-κB, METALLOTHIONEIN-1, TNF ALPHA, IL-6 AND ESR 1

	NF-κB (ng/ml)	Metallothionein MT-1 (ng/ml)	TNF-α (pg/ml)	IL-6 (pg/ml)	Er-1 (ng/ml)
Control	5.70 ± 0.16	24.99 ± 0.35	16.99 ± 0.29	39.32 ± 0.87	23.24 ± 0.18
As ³⁺	13.20 ± 0.09***	43.52 ± 0.52***	42.26 ± 0.14***	110.99 ± 0.63**	11.88 ± 0.21***
AA	9.32 ± 0.23***†	24.25 ± 0.51†††	17.78 ± 0.13†††	29.55 ± 0.39††	14.72 ± 0.32***†††
As ³⁺ +AA	10.06 ± 0.04***††	25.13 ± 0.59†††	18.10 ± 0.13†††	34.13 ± 2.29†	12.31 ± 0.18***
B ₁₂	7.03 ± 0.35†	24.47 ± 0.30††	14.83 ± 0.13†††	17.90 ± 1.72†	14.87 ± 0.24***†††
As ³⁺ +B ₁₂	7.51 ± 0.21††	24.87 ± 0.80††	15.61 ± 0.21†††	17.95 ± 5.45	13.65 ± 0.23***†††
As ³⁺ +AA+B ₁₂	10.32 ± 0.27***††	26.07 ± 2.36	18.22 ± 0.10†††	22.79 ± 8.87	17.37 ± 0.21***†††

The data represents mean ± SE, N=6. ANOVA followed by post hoc Dunnett t test. * indicates the comparison between control and rest of the groups while † indicates comparison with arsenic treated groups. †p<0.05, **/††p<0.01, ***/†††p<0.001.

TABLE 2: CHANGES IN DIFERENT GENERATIONS OF OVARIAN FOLLICLES AND UTERINE HISTOMETRY

	SPAF	LPAF	SAF	MAF	LAF	GF	RF
Control	14.66 ± 0.90	7.16 ± 0.28	3.33 ± 0.30	3.66 ± 0.61	2.66 ± 0.30	1.33 ± 0.30	1.16 ± 0.28
As ³⁺	2.83 ± 0.72***	1.83 ± 0.54**	1.66 ± 0.51	0.83 ± 0.36	0.83 ± 0.28*	0.50 ± 0.20	7.50 ± 0.90*
AA	10.33 ± 0.45††	8.16 ± 0.54†††	4.16 ± 0.28	3.66 ± 0.30††	1.50 ± 0.31	1.16 ± 0.28	1.33 ± 0.30†
As ³⁺ +AA	8.83 ± 0.54***††	7.16 ± 0.79†	3.50 ± 0.39	2.83 ± 0.28†	0.83 ± 0.28*	0.83 ± 0.15	2.16 ± 0.28†
B ₁₂	11.66 ± 0.50†††	7.16 ± 0.43††	5.16 ± 0.28*†	1.83 ± 0.28	1.33 ± 0.30	1.33 ± 0.30	2.16 ± 0.28†
As ³⁺ +B ₁₂	10.16 ± 0.43***††	6.33 ± 0.69†	4.16 ± 0.54	1.83 ± 0.28	1.16 ± 0.15	1.16 ± 0.28	3.33 ± 0.30*
As ³⁺ + AA+B ₁₂	10.66 ± 0.45†††	5.66 ± 0.45†	3.50 ± 0.39	2.50 ± 0.51	1.50 ± 0.20	0.66 ± 0.38	3.50 ± 0.39*

	Uterine diameter (µm)	Thickness of different layers in uterus (µm)		
		Endometrium	Myometrium	Perimetrium
Control	6086.33 ± 149.55	261.62 ± 23.27	88.41 ± 6.95	138.28 ± 10.59
As ³⁺	5445.24 ± 195.35	83.93 ± 4.91**	52.05 ± 9.9	71.33 ± 7.51*
AA	6017.56 ± 27.39	219.89 ± 15.85††	61.39 ± 9.58	119.48 ± 10.59
As ³⁺ +AA	5922.21 ± 63.51	206.62 ± 15.84††	55.05 ± 9.06	112.18 ± 10.14
B ₁₂	5805.94 ± 115.23	246.26 ± 15.41††	72.43 ± 2.37	176.86 ± 17.85†
As ³⁺ +B ₁₂	5782.00 ± 111.14	236.81 ± 14.84††	60.61 ± 2.35	163.57 ± 16.64†
As ³⁺ +AA+B ₁₂	6140.53 ± 136.71	198.79 ± 12.44††	94.06 ± 10.6	101.54 ± 10.46

Treatment with arjunolic acid and vitamin B₁₂ alone or jointly change the effects of ovarian follicle counting and uterine diameter, thickness of different uterine layers in arsenic group. The follicles were categorized as small preantral follicles (SPAF) (< 94 µm), large preantral follicles (LPAF) (94–260 µm), small antral follicles (SAF) (261–350 µm), medium antral follicles (MAF) (351–430 µm), large antral follicles (LAF) (431–490 µm), graafian follicles (> 491 µm) on the basis of their morphology and diameter. Data represents mean ± SE, N=6, ANOVA followed by post hoc Dunnett t test. *indicates the comparison between control and rest of the groups while † indicates comparison with arsenic treated groups. */†p<0.05, **/††p<0.01, ***/†††p<0.001.

DISCUSSION: Arjunolic acid and vitamin B₁₂ exhibit its antioxidant activity with different biological actions. Several reports indicate that arjunolic acid and vitamin B₁₂ protect the hepatic organ¹⁶ and uterus²⁹ in female rats due to arsenic toxicity. Previously, we reported that treatment of arjunolic acid inhibits the uterine dysfunction with oxidative stress against the arsenic-induced female rats³⁰. In this study, we intended to focus that how the arjunolic acid and vitamin B₁₂ alone or jointly protect the uterine and ovarian disorders in curative mode against arsenic-induced female rats.

The intracellular antioxidant enzymatic activity in female reproductive organs was reduced by the excessive ROS production caused by the arsenic

intoxication. These results consistent with previous findings²⁴. Electrozymographic impression revealed that, the treatment of AA (1.0 mg / 100gm body weight) and vitamin B₁₂ (0.09 µg / 100gm body weight) alone or jointly in arsenic ingested rats protected the uterine and ovarian antioxidant enzymatic activities (SOD, catalase, and GPx) due to its probable own intrinsic antioxidant properties **Fig. 1**. These results are similar with the other investigator, where the arjunolic acid³¹ and vitamin B₁₂³² could prevent the inhibition in the activities of SOD, catalase, and GPx in arsenicated rats.

Bhattacharya *et al.*, investigated that arsenic ingestion caused an increase level of MT-1 level in liver cells³³. This finding is also validated by the

findings of present study. Our results also similar to this report **Table 1**. The liver is the most sensitive organ for the elevation of MT. MT-1 has a key role for the primary detoxification of heavy metal and that is crucial for metal homeostasis³⁴, however MT-1 act as a cellular defense component to protect the cells against arsenic-induced toxicity³⁵. Treatment of AA and vitamin B₁₂ alone or jointly in arsenic ingestion rats reduces the level of MT-1 **Table 1**. Normally, MT is cysteine rich metalloprotein and acts as neutralizing nucleophilic equivalent to promote heavy metals binding³⁴. So, we predicted that arjunolic acid and B₁₂ could minimize the oxidative stress by sustaining the normal cellular level of MT. In chemical structure of AA have hydroxyl groups (one primary and two secondaries) and have one carboxylic hydrogen atom which may important for the arsenic chelation and it can easily interact with ROS. The curative mechanism of AA against the arsenic-induced female reproductive toxicity may possibly remove the toxin by the five-member chelate complex formation between the sodium arsenite and hydroxyl groups of AA. So, we hypothesized that the chelate formation possibly inhibits the oxidative damage of uterus-ovary and altered the different cell signaling pathway *via* chelation therapy of arjunolic acid. In addition, the AA may inhibit the free radical scavenging activity because arjunolic acid has one carboxylic hydrogen atom.

The supplementation of AA and B₁₂ alone or jointly in arsenic ingested rats elevated the estradiol signaling receptor (ER-1) favouring the growth of uterus and ovary and maintaining the normal histo-architecture of these organs. AA and or B₁₂ improved the deterioration of uterine layers and elevated the reducing number of secretory glands. In ovarian tissue, these findings are consistent with the findings of other investigators where AA and or B₁₂ recovered the different ovarian folliculogenesis and reduced the follicular-atresia ER-1³⁰. B₁₂ could enhance the ovulation process by improved the development of ovum in arsenic-treated rats ER-1³⁶. In zymogram, our results revealed that arjunolic acid, vitamin B₁₂ alone or together successfully supports to renovate the higher expression of LDH activity in arsenic fed rats.

Our earlier study confirms that arjunolic acid has a reducing effect on serum LDH activity and which

may protect the necrotic progression through the limiting the serum leakage by the tissue³⁰. In this study, we investigated the inflammatory marker (NF-κB) and pro-inflammatory cytokines (TNF-α, and IL-6). A noticeably elevation in the uterine NF-κB and serum TNF-α, or IL-6 was observed following the increasing of LDH in arsenic ingested rats **Table 1**. These results are also corroborated with Das et al where a higher dose of arsenic enhances TNF-α and IL-6 which is associated with the increased collagen deposition in liver³⁷.

However, this study revealed that supplementation of arjunolic acid or B₁₂ successfully inhibit the elevating level of uterine NF-κB and serum TNF-α, or IL-6 in arsenic ingested rats **Table 1**. It has been reported where arjunolic acid could protect the oxidative stress through protecting the antioxidant status, apoptotic and necrotic damage of uterus³⁰. Arjunolic acid has the ability to suppress the activation of NF-κB and TNF-α by decreasing the production of ROS which leads to apoptotic cell death³⁸. Vitamin B₁₂ has an anti-inflammation property. B₁₂ could down-regulate the transcription factor of NF-κB³⁹. It has the ability to down-regulate the level of NF-κB by decreasing the TNF-α level⁴⁰. However, from this information we predicted that the both of arjunolic acid and B₁₂ may maintain the increased collagen deposition, as well as protect the necrotic and apoptotic uterine and ovarian tissues degeneration.

On the other hand, vitamin B₁₂ has an important role in the detoxification of heavy metal by the SAM pool methylation where vitamin B₁₂ acts as a co-factor to synthase- the methionine enzyme and catalyzes the endogenous methionine synthesis from the S-adenosyl homocysteine⁴¹. As well as, it maintains the intracellular folate pools and protect the cells from toxicity¹⁴. Vitamin B₁₂ has a direct reaction with reactive oxygen and nitrogen species that can stimulate the activity of methionine synthase⁴⁰.

CONCLUSION: From these above results, we conclude that the arsenic chelation by arjunolic acid might have better advantageous curative effects in restoration of antioxidant in ovarian and uterine tissue. Vitamin B₁₂ and arjunolic acid act as a safeguard to mitigate the inflammatory response

of the arsenicated cells. The necrotic and apoptotic tissue degeneration were protected possibly by the elimination of arsenic from organs where arsenic detoxification takes place by the involvement of the SAM pool. Arsenic perhaps removed from the system *via* methylation process where methionine plays a significant role.

However, the above study will be helpful for further research related to the implementation of noninvasive treatment strategy in arsenic affected people.

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CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

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