

Combined Dietary Action of Spirulina and Probiotics Mitigates Female Reproductive Ailments in Arsenicated Rats

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Abstract

Heavy metals contaminations in food and water are increased due to the environmental pollution. Managing arsenic toxicosis by dietary therapy is yet to be explored, although the conventional therapeutic strategy emphasizes the invasive chelating therapy. In this present study we elucidated the ameliorative effect of Spirulina and probiotics against arsenic-mediated female gonadal injury. The treatment was continued for 8 days (2 estrus cycles) on rats with sodium arsenite (1.0 mg/ 100g body weight) orally, but spirulina (40 mg/100g body weight) and commercially available probiotics mixture (2 mg/ 100g body weight) were added in rat chow, fresh daily. Uterine and ovarian tissue experienced a significant impairment of antioxidant status, while a pronounced ovarian follicular degeneration was apparent from the increased number of follicular atresia in arsenic treated rats. All these deleterious effects of sodium arsenite were diminished significantly by spirulina and probiotics in arsenic fed rat. Moreover, an increase in the serum levels of homocysteine (Hcy) in association with reduced serum levels of vitamin B₁₂ and folic acid were mitigated in arsenic fed rats following spirulina and probiotics dietary co-administration. However, the outcome of this study may indicate that spirulina and probiotics may be incorporated in the meal as nutraceuticals in limiting arsenic-mediated health hazards.

Keywords: Anti-oxidant, Arsenic, Oxidative stress, Uterus, Vitamin B₁₂.

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(Received: 25 January 2019; accepted: 05 March 2019)

Citation: Shamima Khatun, Sudipta Chatterjee and Sandip Chattopadhyay, Combined Dietary Action of Spirulina and Probiotics Mitigates Female Reproductive Ailments in Arsenicated Rats, *J Pure Appl Microbiol.*, 2019; **13(2)**:1175-1184. doi: 10.22207/JPAM.13.2.57

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INTRODUCTION

Arsenic exposure causes noticeable damage in various organs, including male reproductive malfunction as manifested by reduced androgenesis, inhibition of spermatogenesis, and diminution in the weight of testes and other sex organs¹. Several epidemiologic studies have been reported that arsenic exposure can cause the reproductive and developmental toxicity in animals and increased spontaneous abortion and stillbirth and loss of birth weight². Moreover, arsenic exposure also has been associated with the inhibition of ovarian steroidogenesis and gonadotrophins secretion³. This experiment has been performed to search out the protective effect of dietary administered spirulina and probiotics in the mitigation of the arsenic mediated toxicity on female reproductive organs. Phycocyanin (Pc) is a biliprotein of this blue-green alga spirulina. This protein contains a tetrapyrrole phycocyanobilin, which is responsible for antioxidant properties of Pc⁴. It has been reported that Pc has significant antioxidant and radical scavenging properties, offering protection against oxidative stress⁵. In addition, spirulina plays a protective role against arsenic toxicity in human⁶.

Probiotics are live microorganisms which contribute many health benefits to the host when administered in adequate amounts⁷. Lactic acid bacteria could prevent ROS production, including peroxide radicals⁸, superoxide anions, and hydroxyl radicals⁹. Considering the above background, this study was proposed towards the evaluation of the non-invasive dietary application of spirulina and probiotics against arsenic-induced uterine and ovarian toxicity.

MATERIALS AND METHODS

Experimental design

Forty two female albino Wistar strain rats 60 days of age, weighing 110 ± 5 g were selected in this experiment. All the rats were maintained in a controlled environment for 8 days for acclimatization in a room with a 12-hour light-dark cycle, with $32 \pm 2^\circ\text{C}$ temperatures and 50–70% humidity and were fed with standard pellet and water.

Treatment, study of estrous cycle and sample collection

Total 42 rats were equally distributed

into seven groups. This experiment was continued for 8 days, and rats were treated with spirulina and probiotics with normal rat chow following the institutional ethical guidelines. Rats were distributed in seven groups. Each group contains six rats. Group I was vehicle-treated control, group II was sodium arsenite treated (1.0 mg/100 g body weight), group III was considered as spirulina placebo group (40 mg/100g body weight) (West-Coast Pharmaceutical Ahmedabad, Gujrat), group IV was given the same dose of sodium arsenite, along with spirulina, group V was considered as probiotics placebo group (2mg/100g body weight), group VI was treated with arsenic (1.0 mg / 100gm body weight) + Probiotics and group VII was administered with same dose of arsenic along with same dose of spirulina and probiotic supplement.

In this experiment we used commercially available probiotics powder. A 100 mg of probiotics cocktail contains following composition:

Lactobacillus acidophilus - 13.714×10^7 CFU,
Lactobacillus rhamnosus - 13.714×10^7 CFU,
Bifidobacterium longum - 13.714×10^7 CFU,
Bifidobacterium bifidum - 13.714×10^7 CFU,
Saccharomyces boulardii - 2.857×10^7 CFU,

Above probiotics were mixed in normal diet, fresh daily. In this experimental study arsenic was fed to the rats via oral gavage of (group II, IV, VI and VII) for eight 8 days. The *Spirulina platensis* tablet (500 mg, West-Coast Pharmaceutical, Ahmedabad, Gujarat) was crushed to produce dust. Spirulina (40 mg/100g body weight) and commercially available probiotics mixture (2 mg/ 100g body weight) were mixed in rat chow, fresh daily. The body weights and feeding habits of animals were recorded during the entire treatment schedule. The pattern of estrous cycle was observed regularly. On day 9, the rats were anesthetized to collect blood and female organs.

Determination of uterine oxidative stress markers and antioxidant enzymes

By standard technique of Devasagayam et al. 2003 MDA was determined¹⁰. The CD was measured as per the method where lipids were extracted by chloroform and methanol mixture¹¹. Uterine SOD, catalase and GPx activities were measured following the previous method¹²⁻¹⁴.

Assessment of antioxidant enzymes and LDH by native gel electrophoresis

Based on the principle of inhibition of the reduction of Nitro blue Tetrazolium chloride (NBT), the activity of SOD on 12% gel was assessed. To evaluate catalase activity 60 µg proteins were electrophoresed on 8% PAGE using uterine tissue extract¹⁵. 8.0 % native gel was used for the determination of GPx activity. The activity of GPx was determined by the removal of peroxide that was essential for the transformation of potassium ferricyanide to ferrocyanide between samples¹⁶. For the detection of serum lactate LDH 8.0 % agarose gel was used¹⁷.

Vitamin B₁₂, Folic acid, Homocysteine analysis

Serum levels of B₁₂ (Cat no.ER1579) Folic acid (Cat no.EU0381) Homocysteine (Cat no.ER1509) were measured by ELISA kits according to the procedures recommended by the manufacturers (Wunhan Fine test, China).

Serum ES and uterine ER-α analysis and Ovarian and Uterine Histopathology

ES was determined based on the technology of Competitive-ELISA and ER-α was determined based on the technology of sandwich ELISA according to the procedures recommended by the Wunhan Fine test, China. The concentration of ES and ER-α in the samples was measured at a wavelength of 450 nm. The ovarian and uterine section (5.0µm thick paraffin embedded) were stained using hematoxylin (Harris) and eosin and observed under microscope (Olympus, CX21i, magnification x400).

Statistical analysis

In this experiment data were expressed in terms of mean ± SE, (N=6) of different groups. Differences in these variables between treated and control groups were evaluated using ANOVA followed by post hoc Dunnett t-test.

RESULTS

General Observations

No significant difference was found between the groups considering the animals' water intake (Table1). No significant changes of body weight among the experimental animals in seven groups were observed throughout the experiment. A significant weight loss of ovaries and uterus was observed in arsenic treated group (Table 1).

Vaginal smear study

Estrous cycles were noted regularly for 8 days. In due course of arsenic treatment of arsenic rats were remained in a consistent diestrus or metestrus after 4 days of treatment. The estrous phase was synchronized in control, spirulina and probiotics group.

Status of oxidative stress markers

There was a significant elevation in the uterine MDA and CD level in the sodium arsenite treated group in comparison to the control group (Table 2). A significant restoration of soluble thiol was observed in spirulina and probiotics supplemented group (Table 2).

Table 1. Body weight and organo-somatic indices (female reproductive organs)

	Body Weight (g)		Organo-somatic indices (g)		Water Intake (ml)
	Initial	Final	Ovary in pair	Uterus	
Control	85.2±3.7	102±3.1	0.05 ±0.002	0.16±0.003	9.8±1.3
Arsenic	91.4±5.8	108.4±6.7	0.04±0.002***	0.1±0.004***	14.9±0.6***
Spirulina	93.8±6.9	105±6.4	0.06±0.001###	0.14±0.003**###	8.7±0.4###
Arsenic + Spirulina	91.4±3.3	107±4.6	0.05±0.009###	0.15 ±0.001###	9.05±0.6###
Probiotics	91.2±3.8	105.4±4.8	0.05±0.008###	0.16±0.001###	8.9±0.3###
Arsenic+Probiotics	87.6±2.2	101±4.0	0.05±0.001###	0.15±0.002###	9.9±0.7###
Arsenic + Spirulina+ Probiotics	87.4±2.8	102.8±2.2	0.05±0.001###	0.13±0.003*###	9.2±0.1###

The changes of body growth and organo-somatic indices in response to sodium arsenite ingestion and the protection of the changes by spirulina and probiotics were shown. The data are mean±SE, N=6, ANOVA followed by Post Hoc Dunnett test to find out statistical significance at *p<0.05. In addition, data were compared with As3+ and As3+ + Spirulina, As3+ + Probiotics, As3+ + Spirulina + Probiotics, by two tailed paired t-test to find out statistical significance at ###p<0.05, **p<0.01, *p<0.001.

Table 2. Effect of Spirulina and probiotics on arsenic-induced oxidative stress and biochemical changes

	Control	Arsenic	Spirulina	Arsenic + Spirulina	Probiotics	Arsenic +Probiotics	Arsenic + Spirulina +Probiotics
Uterine MDA Level (nmole/mg of tissue)	20.35± 1.75	34.97± 1.88***	22.06 ±1.84###	27.94± 1.42*#	24.91 ±1.77##	29.15 ±1.29**#	28.89 ± 1.03**#
Uterine CD Level (nmole/ mg of tissue)	12.61 ±1.56	32.99 ±1.96***	15.73 ±0.71###	21.73 ±1.12###	18.28 ± 1.24###	21.89 ± 1.31###	22.59 ± 1.12**###
Uterine SOD Level (Unit/gm of protein)	5.95± 0.36	1.89± 0.32***	5.77± 0.23###	4.81 ±0.44###	5.64± 0.38###	5.19 ±0.39###	5.68± 0.27###
Uterine Catalase Level (Unit//gm of protein)	10.04 ± 1.02	2.48 ±0.48***	9.34 ± 0.38###	7.21± 0.34*###	9.52± 0.56###	8.63± 0.22###	9.21 ±0.99###
Uterine GPX Level (Unit/ mg protein)	6.21±0.30	2.14±0.28***	5.26±0.25###	4.78±0.15**###	5.15±0.23###	4.67 ±0.39**###	5.32± 0.37###
Estradiol(ng/ml)	640.8± 56.8	214.1 ± 22.23***	553.6 ± 20.1###	500 ±29.12###	518.16 ± 43.47###	440 ±59.69**##	512± 34.17###
ER-α (ng/ml)	18.43 ± 0.63	7.82 ± 0.17***	16.09± 0.56**###	13.96± 0.71###	15.68 ±0.29**###	12.37± 0.29**###	14.83± 0.42**###
Hcy (pmol/ml)	4.16± 0.45	14.57± 1.03***	5.67 ±0.85###	8.41 ± 0.58**###	4.18± 0.34###	8.13± 0.82**###	6.61± 0.55###
Folic Acid (ng/ml)	1367 ± 17.73	832.83 ±31.73***	1224.83± 31.68**###	1148 ±35.34**###	1254.83 ±22.64*###	1061.6 ±22.01**###	1083.8 ±21.3**###
Vitamin B ₁₂ (ng/ml)	30.05± 0.81	13.63 ± 2.35***	28.4± 2.47###	25.5± 1.93###	26.73 ±1.42###	21 ±0.31**#	24.98 ±0.63###

The colorimetric data of above enzymes, vitamins, homocysteine, estradiol and ER-α represent Mean ± SE, N=6, ANOVA followed by post hoc Dunnett t-test to find out statistical significance comparing with control group at *p < 0.05, **p < 0.01, ***p < 0.001. In addition, data were compared with As3+ and As3+ + Spirulina, As3+ + Probiotics, As3+ + Spirulina + Probiotics by two tailed paired t-test to find out statistical significance at ###p<0.05, ##p<0.01, #p<0.001.

Table 3. Effect of spirulina and probiotics on arsenic induced different follicle count in Wistar rats

	Control	Arsenic	Spirulina	Arsenic + Spirulina	Probiotics	Arsenic +Probiotics	Arsenic + Spirulina +Probiotics
Uterine Diameter (µm)	4913.61±68.2	3987.27±4730.4***	4730.4±65.1***##	4120.2±65.5***#	4592.9±26.8***##	4399.6±18.9***##	4554.7±22.8***##
Endometrium(µm)	392.7±7.48	80.2±3.1***	254.6±9.3***##	215.8±4.2***##	248.07±4.6***##	219.6±1.7***##	234.2±2.3***##
Mvometrium(vm)	88.8±2.3	48.57±1.9***	72.4±1.3***##	65.8±0.8***##	74.6±0.7***##	64.86±0.9***##	70.6±0.6***##
Perimetrium(vm)	122.5±2.5	79.7±0.9***	112.8±1.2***##	101.2±2***##	110.55±2.5***##	96.08±2.5***##	103.7±1.5***##
SPAF	13.6±0.5	3±0.7***	11.5±0.4***	10.3±0.7***##	11.5±0.4***	9±0.6***##	10.5±0.4***##
LPAF	7.1±0.3	1.16±0.3***	6±0.4***	4.1±0.2***##	5.6±0.2***	3.3±0.2***##	4±0.4***##
SAF	4.5±0.2	1.5±0.2***	4±0.3##	3.16±0.2***##	3.83±0.3##	2.83±0.2***##	3.16±0.2***##
MAF	3±0.2	0.33±0.2***	2.16±0.2***##	1.3±0.2***##	2±0.2***	1.3±0.2***##	1.16±0.2***#
LAF	2.66±0.2	0.5±0.2***	1.83±0.2***##	1.16±0.2***	1.5±0.2***	1±0.2***	1.3±0.2***#
GF	1.6±0.2	0.16±0.1***	1.16±0.1##	1±0.2#	1.3±0.2##	0.83±0.1*	0.66±0.2**
RF	0.9±0.3	3.7±0.3***	1.38±0.2###	1.87±0.2***##	1.65±0.3*##	2.31±0.3***##	1.72±0.2*##

SPAF indicates small preantral follicles, LPAF means large preantral follicles, SAF stands for small antral follicles, MAF as medium antral follicles, LAF as large antral follicles, GF stands for Graafian follicles and RF directed as regressing follicles. Significant changes occur in these parameters in the arsenic-fed rats group and were improved by the dietary co-treatment of spirulina and probiotics when compared with the control group. Data represents mean±SE, N=6, ANOVA followed by Post Hoc and Dunnett test, *p<?0.05, **p<?0.01, ***p<?0.001. In addition, data were compared with As3+ and As3++ + Spirulina, As3+ + Probiotics, As3+ + Spirulina + Probiotics, by two tailed paired t-test to find out statistical significance at ###p<0.05, ###p<0.01, ###p<0.001.

Activities of antioxidant enzymes in uterus

The activities of SOD, CAT and GPx in uterus were decreased significantly in arsenic treated group as compared to control group. Spirulina, probiotics and vehicle treated groups showed no significant differences in the activities of antioxidant enzymes as compared to the control group (Table 2).

We further evaluated uterine and ovarian SOD, catalase and GPx expression electrozymographically and it was observed a significantly reduced uterine and ovarian SOD, catalase and GPx expression in arsenic-exposed group compared to the control group (Fig. 1A₁ and 1A₂ A, B, C).

Furthermore, the combined mode of dietary co-treatment with spirulina and probiotics had shown more intense recovery of uterine and ovarian catalase and GPx expression and nature of bands intensity was more prominent. Whereas in case of uterine and ovarian SOD, the band intensity was decreased in combined mode of dietary co-treatment with spirulina and probiotics but in case of individual treatment with spirulina and probiotics the band intensity was effectively increased.

Serum LDH status

Electrozymogram of serum LDH was used

to focus the necrotic nature of the tissue (Fig. 1 A₁D). This release of the enzyme was significantly reduced by spirulina and probiotics alone or in combination with dietary mode in arsenic ingested rats as manifested from the weak expression of this necrotic marker.

Status of measured vitamins

Present study revealed that hyper-homocysteinemia (p<0.001) followed by significantly decreased (p<0.001) serum levels of folic acid and B₁₂ after arsenication. When arsenic combined with spirulina and probiotics resulted in the correction of the levels of Hcy, B₁₂ and folic acid along with lipid profile closed to the level of control groups (Table 2).

Ovarian and Uterine histopathology

Haematoxylin-Eosin stained ovarian tissue sections showed the significant degradation in the folliculogenesis with reduced numbers of growing follicles replaced by increasing numbers of regressing follicle in arsenic fed rats (Table-3; Fig. 2B₂) in comparison with control and placebo groups (Table 3; Fig. 2B₁, B₃, B₅). Uterine histomorphology revealed loss of secretory glands in arsenicated animals (Fig. 2A₂) along with the thinning of uterine diameter (Table 3; Fig. 2A₂) with respect to control and placebo groups (Fig. 2A₁, A₃, A₅). Arsenicated rats when

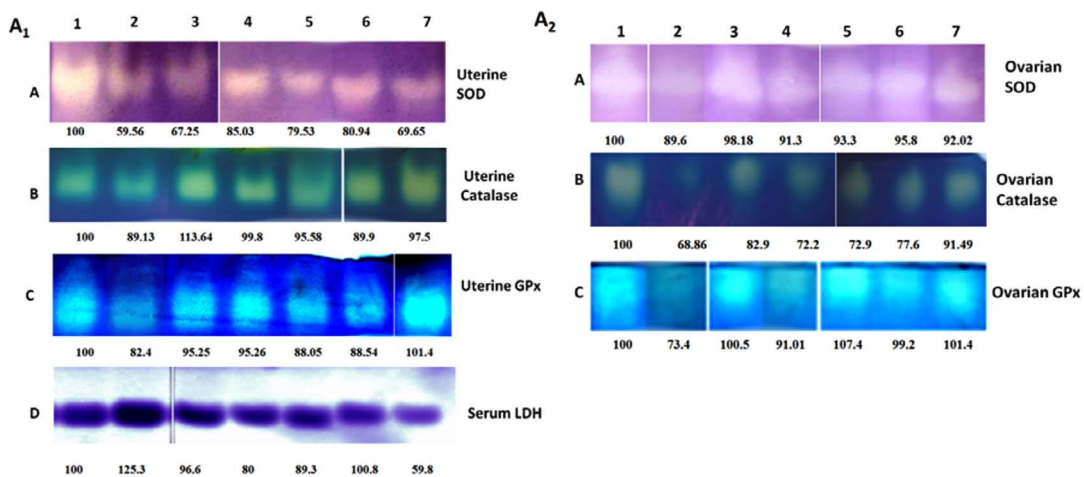


Fig. 1. Protective effect of spirulina and probiotics on uterine and ovarian tissue SOD, Catalase, GPx and serum LDH in As³⁺-intoxicated rats. (A, B and C) SOD, CAT and GPx expression in uterine tissue on native gel and (D) Serum profiles of LDH activity was performed on agarose gel electrophoresis followed by substrate specific development of SOD, CAT, GPx and LDH bands. Lane distribution Lane 1: Control; Lane 2: Arsenic; Lane 3: Probiotics; Lane 4: Arsenic + Probiotics; Lane 5: Spirulina; Lane 6: Arsenic + Spirulina; Lane 7: Arsenic + Spirulina + Probiotics.

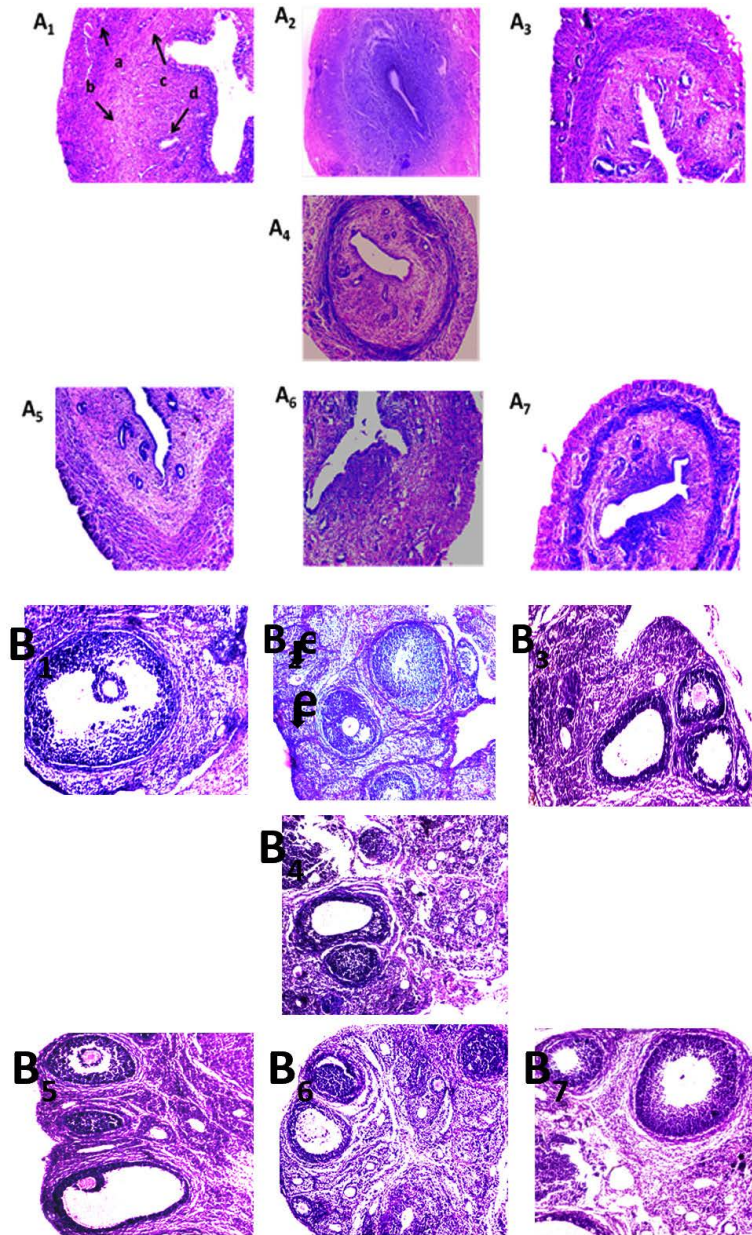


Fig. 2. Effect of spirulina and probiotics on histology of female reproductive organs. (A and B) Uterine and ovarian tissue were implanted in paraffin, serially sectioned laterally at 5 nm, stained with eosin and haematoxylin (Harris) and observed under a microscope (magnification x100) to study the uterine (A) and ovarian (B) histo-architecture. Arrow indicates in slide "d" the secretory glands and pictures showed in A₂ the remarkable loss of secretory cells of uterine tissue along with distortion of endometrial layer. The numbers of regressing follicles were increased in slide B₂ in the arsenic exposed group where arrows indicate follicular atresia. Spirulina and probiotics dietay co-treatment significantly protected such arsenic induced ovarian (Figure 4B₄, B₆, B₇) and uterine (Figure 4A₄, A₆, A₇) disorders. Panel distribution; A₁: vehicle treated control, A₂: arsenic, A₃: *S. platensis*, A₄: arsenic + *S. platensis*., A₅: probiotics, A₆: arsenia+probiotics, A₇: arsenic + *S. platensis*+ probiotics , B₁: vehicle treated control, B₂: arsenic, B₃: *S. platensis*, B₄: arsenic + *S. platensis*. B₅: probiotics, B₆: arsenia+probiotics, B₇: arsenic + *S. platensis* + probiotics (a- perimetrium, b- myometrium, c- endometrium, d- secretory cells, e- atretic follicles).

co-treated with spirulina and probiotics alone or in combination partially or moderately restored the number of growing follicles in the ovary with reducing number of regressing follicles (Table 3; Fig. 2B₄, B₆, B₇) compared to the vehicle treated control and the placebo groups. In uterus, dietary co-treatment of spirulina and probiotics alone or in combination were capable of maintaining the normal morphology of uterine tissue with an increasing number of uterine secretory glands (Table-3, Fig. . 2A). The thickness and diameter of uterine tissue were also restored towards normalcy following spirulina and probiotics dietary co-administration in arsenic-fed rats (Table-3).

DISCUSSION

This study demonstrates that strategic use of spirulina and probiotics may help in preventing arsenic-induced female reproductive toxicity to a significant extent. In this experiment our results showed a low-level expression of uterine and ovarian SOD, catalase and GPx in arsenic fed rats compared to the control (Fig. 1). These enzymes were considered as the first line of defense against oxidative injury at the cellular level. It was noted in the current study that spirulina was able to increase the activity of CAT and SOD after dietary intervention with spirulina in As³⁺ fed rats. Spirulina has some positive action on these enzymes activity¹⁸ and the result is consistent with our previous report. Increased uterine MDA and CD levels in arsenic fed rats (Table 2) directed an increase in lipid peroxidation. The inhibitory effects of spirulina on lipid peroxidation were in conformity with the previous result¹⁹. Other investigators explored the therapeutic effects of spirulina on arsenic-induced male reproductive toxicity²⁰. In this experiment electrozomographic evaluation of uterine and ovarian revealed an increased expression of SOD, catalase and GPx by following spirulina and probiotics dietary supplement in As³⁺ fed rats also indicated the attenuation of lipid peroxidation due to the decreased level of MDA and CD in this group.

Our results have been shown that suppression of E₂ (Fig. 3) and deterioration of follicular maturation (Fig. 2B₂) in response to As^{III} projected towards cellular degeneration in uterine layers (Fig. 2A₂). It has been reported that ovotoxic

effects of arsenic also resulted in a loss of balance between oxidant and antioxidant systems leading to oxidative stress²¹. Reactive oxygen species play a critical role in the alteration of physiological and reproductive functions like oocyte maturation and luteolysis. Arsenic probably binds to estrogen receptor (ER) and inhibits E₂ binding to the estrogen receptor alpha (ER α)²². Exposure to arsenic-caused a degradation of circulating levels of vitamin B₁₂ and folate, (Table 2). Dietary co-treatment with spirulina and probiotics to arsenic exposed rats exhibited a significant increase in ER α and E₂ signaling. The treated rats lost estrous cyclicity and focused persistent diestrus. Here in this study we predicted that consumption of healthy live microorganisms (lactic acid bacteria) with prebiotics (*Spirulina platensis*), may enhance ovarian and uterine histomorphology (Table 3 and Fig. 2) as well as hormonal status (Table 2).

Vitamin B₁₂ plays a key role as a cofactor for methionine synthase during the remethylation of Hcy to form methionine²³. These two vitamins are indispensable to protect the reproductive organs from necrosis and have a protective role by diminishing serum LDH level (Fig. 1A₁D) as a consequence of an inverse relationship between the serum LDH activities and serum vitamin B₁₂-folate levels²⁴. Our observations indicated that the treatment with spirulina and probiotics restored ovarian and uterine weight by improving ES level. Dietary co-treatment of spirulina significantly mitigated arsenic-induced uterine oxidative stress and ovarian disorders. This overall defensive mechanism of spirulina might have terminated arsenic-primed protection of uterine and ovarian tissues from necrotic damage, as there was an improvement in the ovarian-uterine histo-architecture (Fig. 2A and 2B) and this is further connected with the upregulation of gonadotrophin release and ovarian steroidogenesis²⁵. In-addition spirulina has the capacity to bind with heavy metals; spirulina may demonstrate a chelating property to trap arsenic²⁶. Methylation of arsenic in association with S-adenosylmethionine involves methylcobalamin (CH₃B₁₂) and reduced glutathione for arsenic clearance and detoxification²⁷. Arsenite S-adenosyl methionine methyl-transferase was isolated and characterized from spirulina²⁸. An *In vitro* assay system demonstrated the arsenic methylation activity of spirulina²⁸. This methylation

process is the essential prerequisite for the removal of arsenic. Therefore, spirulina eventually promotes arsenic methylation and detoxification. In addition, previous observation suggested that probiotics are reported for its increased activity of antioxidative enzymes in the protection of cells against carcinogen-induced damage²⁹. It has been shown that probiotics employ their self-protective effects against oxidative stress by re-establishment of the gut flora either by scavenging of oxidant compounds or by preventing intestinal free radical generation³⁰. Moreover, earlier studies have shown that lactic acid bacteria were shown to lengthen the resistance of the lipoprotein fractions to oxidation, reduce peroxidized lipoproteins, and improve total anti-oxidative activity³¹. The crucial mechanisms of probiotic anti-oxidant activity might be due to its metal-chelating properties. Lactic acid bacteria has been documented for its capability in the binding and removal of arsenic³². In-addition it has been reported that *Spirulina platensis* extract was regarded as the best algal source for prebiotic as it has a greater stimulatory effect on the growth of probiotic bacteria³³. Moreover, previous study revealed that *Spirulina platensis* consumes nitrogen from the growth medium and releases extracellular carbohydrates and other growth substances which may be responsible for stimulating the growth of Lactobacilli³³. A combination of probiotics and prebiotics constitutes a synbiotic, which can further stimulate and increase the survival of probiotic in the intestinal tract³⁴. However, our study revealed that spirulina and probiotics might be a good choice in limiting arsenic mediated reproductive toxicity.

CONCLUSION

From above points we conclude that spirulina and probiotics have a challenging action against arsenic induced red-ox imbalance. This might be possible by reducing the level of lipid peroxidation, improving the antioxidant enzymes activities of the uterus, and by regulating ES signaling. Spirulina and probiotics might be regulating these activities by influencing the SAM pool especially by modulating B vitamins and Hcy in this pool and by influencing ER- α expression and sensitivity. Based on these results, it is explored that spirulina and probiotics at 40 mg/ 100g body

weight and 2 mg/ 100g body weight reflected a better protective effect against reproductive damages in arsenic fed Wistar rats (1 mg/100g body weight). Dietary co-treatment of spirulina and probiotics have a potential role to improve uterine and ovarian anti-oxidant defense system and ovarian-uterine histoarchitecture either by their chelating action with arsenic or by sustaining B vitamins.

ACKNOWLEDGMENTS

None

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

SK, SC and SC conceived and designed the study. SK and SC performed the experiments. SK and SC analyzed and interpreted the data. SK and SC contributed resources. SK and SC supervised and revised the manuscript.

FUNDING

None

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was carried out in accordance with the following the standard protocol of Vidyasagar University's ethical guidelines (ethical clearance No. IEC/7-2/C-2/16, dated 26 August 2016).

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