8.0. EXPERIMENT-4

Protective effect of CCPS with different doses against repro-toxicity ailments induced by sodium arsenite in female rats *in-vivo*.

8.1. Objective of the study

1. In this experiment different doses of CCPS 1.5 mg/ Kg BW, 2.0 mg/ Kg BW and 2.5 mg/ Kg BW have been used.

2. The dose-dependent experiment has been conducted to realize the most effective CCPS dose to highlight its therapeutic action against arsenicated female organs by co-administration mode.

8.2. Experimental design

Female Wistar rats weighted 94-97 grams were taken in this experiment. Total thirty (30) rats were equally distributed in each group. The detailed procedure of this experiment has shown below:

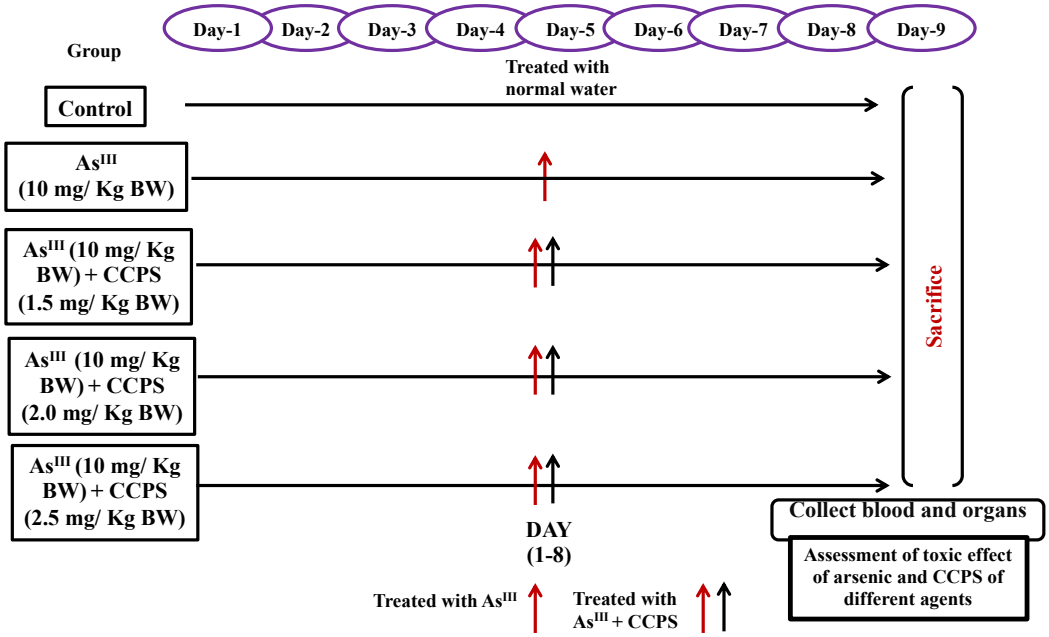
Group-I: This group was control group with vehicle,

Group-II: Rats were treated with sodium arsenite (10 mg /Kg BW),

Group-III: As^{III} (10 mg /Kg BW) plus CCPS (1.5 mg/ Kg BW),

Group-IV: As^{III} (10 mg /Kg BW) plus CCPS (2.0 mg/ Kg BW),

Group-V: As^{III} (10 mg /Kg BW) plus CCPS (2.5 mg/ Kg BW), All rats were treated via oral gavage in co-administration fashion for 8 day.



Body weights of these rats were examined throughout the experiment. Finally, after 8 days of the arsenic treatment and CCPS ingested rats were anaesthetized by HCl ketamine. Blood samples, liver and sex organs were collected from rats following institutional ethical guidelines. All the dissected samples were preserved at -20^{0}C temperature. All anaesthetized rats were finally euthanized using the overdoses barbiturate.

8.3. Results

8.3.1 General growth of the animals and reproductive organ weights

After treatment with sodium arsenite for 8 days, there was no significant variation in somatic growth when compared with control (Table 8.1). But after the treatment with sodium arsenite a significant diminution was noted in ovarian as well as uterine somatic indices when compared with that of vehicle with control group (Table 8.1). The doses of 1.5 mg 2.0 mg and 2.5 mg CCPS /Kg BW have extensively maintained the rats body weight (Table 8.1)

8.3.2. Pattern of estrous cycle

A consistent diestrous phase was noticed in arsenic ingestion group when compared to vehicle with control (Fig 8.1). The control rats have maintained the normal and regular estrous phases throughout experimental time. Three different doses of CCPS on the

arsenicated group maintained the regularity of estrous cycle pattern (Fig 8.1). For maintaining the estrous phase, 1.5 mg dose of CCPS has significantly effective. Though 2.0 and 2.5 mg doses of CCPS more significantly protective than 1.5 mg/ Kg BW (Fig 8.1).

8.3.3. Uterine lipid peroxidation and CD levels

A significant increment in uterine MDA and CD level was noticed in arsenic challenged group (Table 8.2) as compared to respective control. Same effect was also noted in supplementation with 1.5 mg/ Kg BW CCPS dose (Table 8.2). However, CCPS at 2 and 2.5 doses showed more significantly ptotective the uterine MDA and CD (level (Table 8.2).

8.3.4. Uterine enzymatic antioxidant activities

A noticeable reduction was observed in uterine SOD and catalase activities in As^{III} ingested group (Fig 8.2A and 2B). CCPS at the dose of 1.5 mg/ Kg BW has no significant effect in the uterine enzymatic activities (Fig 8.2A and 2B). Whereas, arsenic ingested groups along 2 and 2.5 mg doses exhibited more significant protection for the uterine SOD and catalase activities (Fig 8.2A and 2B).

Electrozymographic imaging revealed that arsenic ingested rats appreciably reduces the SOD and catalase band intensity expression as compared to the control (Fig 8.2C and 2D). Dose of 1.5 mg CCPS / Kg BW did not maintain the expression of uterine enzymatic. Various dosses especially at 2 and 2.5 mg CCPS dosses significantly gained the SOD and catalase and expression (Fig 8.2C and 2D).

8.3.5. Ovarian steroidogenesis, gonadotrophins and estradiol

Diminution of the ovarian key enzymes Δ^5, 3β-HSD and 17β–HSD activities were noticed in As^{III} ingested group (Fig 8.3A and 3B). Arsenicated rats were also reduced the LH, FSH and estradiol hormones levels significantly (Fig 8.3C-3E). The two different doses of CCPS (2 and 2.5 mg/Kg BW respectively) supplemented on the arsenic ingested group significantly maintained the ovarian Δ^5, 3β–HSD and 17β–HSD activities except for the dose of 1.5 mg/

Kg BW (Fig 8.3A and 3B). However, arsenicated rats with 2.0 and 2.5 mg doses of CCPS showed a significant enhancement in the level of LH, FSH and estradiol (Fig 8.3C-3E).

Table 8.1.

Groups	Body Weight (g)		Organs-somatic indices (g%)	
	Final	Initial	Ovary in pair	Uterus
Control	107.4±3.41	95.8±2.37	0.08±0.0008	0.194±0.0008
As^{III} (10 mg/ Kg BW)	116.8±1.58	97.8±2.42	0.07±0.002**	0.144±0.0012***
As^{III} + CCPS (10+1.5 mg/ kg BW)	114.8±3.52	94.4±2.05	0.087±0.0004* ###	0.185±0.001*** ###
As^{III} + CCPS (10+2.0 mg/ kg BW)	106.4±3.33	97.4±2.68	0.089±0.001* ###	0.185±0.001*** ###
As^{III} + CCPS (10+2.5 mg/ kg BW)	109.6±3.19	95.6±3.36	0.096± 0.0005*** ###	0.188±0.0008* ###

Table 8.1. Effects of three doses of CCPS on body mass and reproductive organs. Table represents mean ± SE, N = 6. Presented values here are expressed using one-way ANOVA ANOVA with Dunnett's post Hoc t-test *, **, *** indicate $p<0.05$, $p<0.01$, $p<0.001$ versus the control with vehicle, whereas ### indicate $p<0.001$ versus As^{III} treatment.

Figure 8.1.

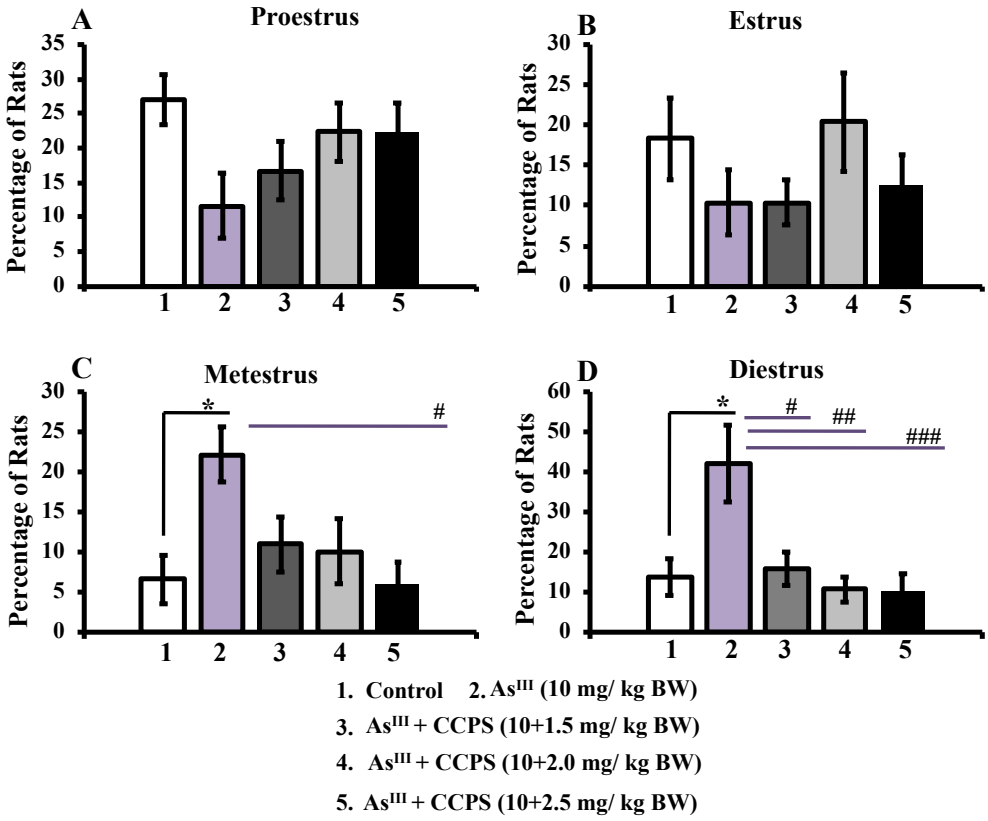


Fig 8.1. Effects of three doses of CCPS on the pattern of estrous cycle against arsenic ingested groups. The all data here represent mean ± SE, N = 6. Presented values here are expressed using one-way ANOVA with Dunnett's post Hoc t-test * indicate $p<0.05$ versus control with vehicle, whereas #, ## and ### indicate $p<0.05$, $p<0.01$ and $p<0.001$ versus As^{III} treatment.

Table 8.2.

	Control	**As^{III}**	**As^{III} + CCPS (10+1.5 mg/ kg BW)**	**As^{III} + CCPS (10+2.0 mg/ kg BW)**	**As^{III} + CCPS (10+2.5 mg/ kg BW)**
Free radical Profile of Uterine tissue					
MDA(nmol/mg of tissue)	19.37±1.85	24.83±1.44*	23.14±1.80	14.78±2.16#	14.32±1.19#
CD(nmol/mg of tissue)	16.22±1.06	26.48±1.82*	24.46±1.32*	17.2±02.09#	16.76±1.13#

Table 8.2. Effects of three doses of CCPS on oxidative stress markers in uterine organ. Table represents mean ± SE, N = 6. Presented values here are expressed using one-way ANOVA with Dunnett's post Hoc t-test *indicate $p<0.05$ versus the control with vehicle, whereas #indicate $p<0.05$ versus As^{III} treatment.

Figure 8.2.

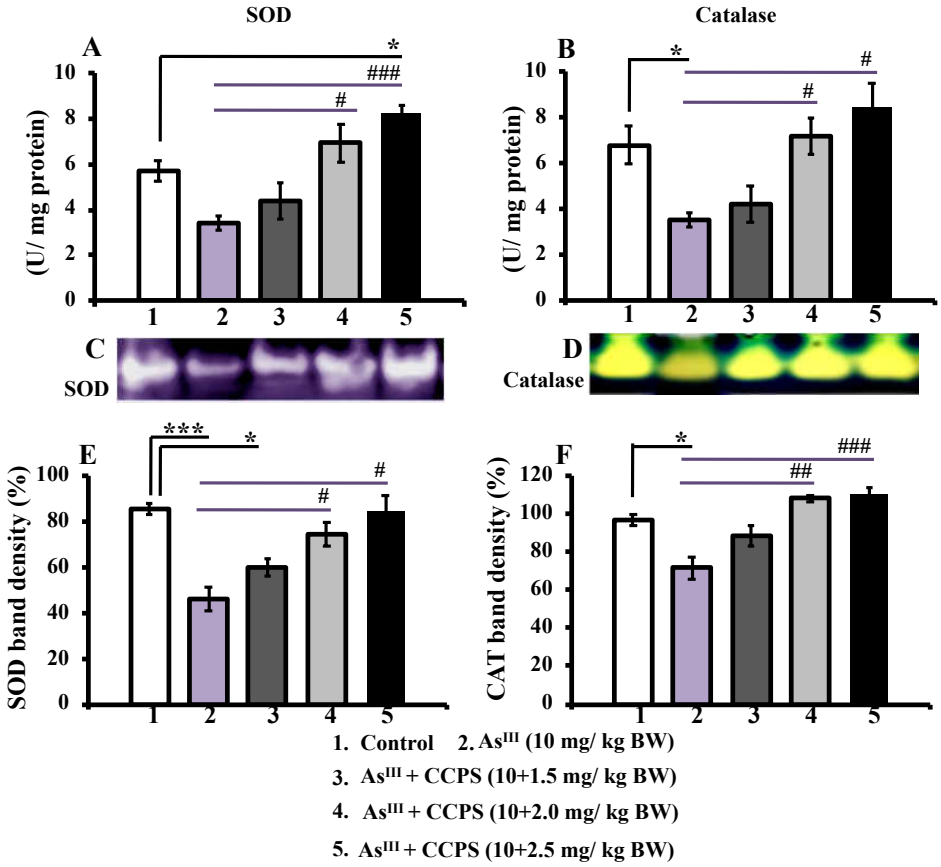


Fig 8.2. The spectrophotometry (A & B) data showing the effects of three doses of CCPS on uterine endogenous enzymes antioxidant (SOD & catalase) activities. Electrozymogram (C & D) images showing the effects of different doses of CCPS on uterine endogenous enzymes antioxidant expression. E & F data shows the band density (%) of SOD and catalase respectively. Here, data represent mean ± SE, N = 6, Presented values here are expressed using one-way ANOVA with Dunnett's post Hoc t-test *, *** indicates $p<0.05$, $p<0.001$ versus the control with vehicle, whereas #, ##, ### indicates, $p<0.05$, $p<0.01$, $p<0.001$versus As^{III} treatment.

Figure 8.3.

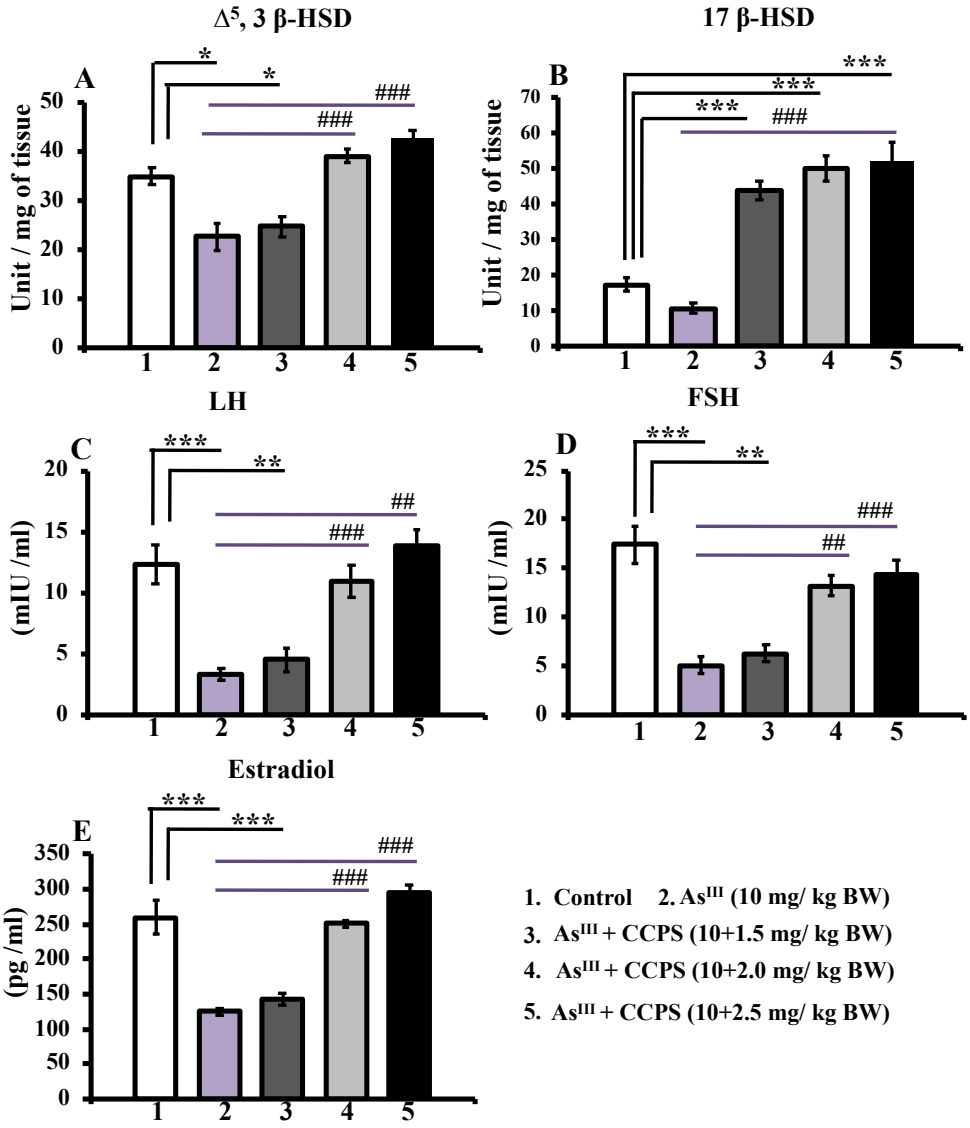


Fig 8.3. A & B denotes the effects of three doses of CCPS on ovarian key regulatory steroidogenic enzyme activity. C, D & E data shows that effects on gonadotrophin and estradiol hormones. Data are represent mean ± SE, N = 6, Presented values here are expressed using one-way ANOVA with Dunnett's post Hoc t-test *, **,*** indicate $p<0.05$, $p<0.01$, $p<0.001$ versus the vehicle with control, whereas ##, ### indicate $p<0.01$, $p<0.001$ versus As^{III}.

8.4. Discussion

The sodium arsenite is a well-known repro-toxicant. Investigators experimentally explored the management of arsenic-induced hepato-toxicity (Sharma et al., 2009) as well as repro-toxicity (Jana et al., 2018) by the treatment of several plant extracts.

From the previous study it was found that various doses of polysaccharide of *Momordica charantia* have been used for the treatment of streptozotocin (STZ)-mediated diabetic mice at the doses of 100, 200 and 300 mg/ Kg BW for 30 days (Zhang et al., 2018). High doses of polysaccharide also have been used including 100, 200 and 300 mg/ Kg BW for 25 days and remove the myocardial infarction against isoproterenol (ISP) induced toxicity (Mohammad, 2017). Our earlier observation confirmed that polysaccharide extracted from *Momordica charantia* mitigates arsenic-induced oxidative stress in liver *in-vitro* (Perveen et al., 2017). In this experiment first time, we intended to observe the effect three different low doses of CCPS against arsenic-induced toxicity. This dose-dependent response will be also helpful to find out the most effective dose of CCPS against arsenic mediated ailments of the reproductive organs of female Wistar rats. Three different doses of CCPS consisting of 1.5 mg, 2.0 mg, and 2.5 mg per kg BW respectively were chosen for this experiment. However, the uterine tissue showed free radical generation following the arsenic ingestion. ROS further interacts with different cellular macromolecules (Hultberg et al., 2001). Our study revealed that arsenic caused significant elevation of MDA and CD in uterine tissue (Table 8.2) followed by a considerable arrestation of the intracellular antioxidant enzyme activities (Fig 8.2). This result is also consistent with the statement of other investigators that explored similar fashion of results with same dose (Maity et al., 2018). Body weight of the animals showed insignificant changes followed by reproductive organs' weight loss significantly in arsenicated group (Table 8.1). Consistent diestrus was noted in arsenicated rats (Fig 8.1).

Consistent diestrus is the indication of the alteration of uterine physiological responses due to the low level of estradiol (Maity et al., 2018). This experiment of CCPS dose-response is also documented with the low estradiol signaling in arsenic-treated rats. This is again supported by the inhibited ovarian steroidogenic activities (Δ^5,3β-HSD and 17β-HSD) following arsenication in rats and (Fig 8.3) is also corroborated with the previous investigation of Dash et al., 2018. Moreover, our study first time indicates that CCPS with different-dose on arsenic fed rats significantly increased the size of the reproductive organs without significantly affecting body weight (Table 8.1). It was noticed that the extracted pectic polysaccharide of *Momordica charantia* has high radical scavenging activity. It scavenges nitric oxide through the involvement of the adequate intracellular antioxidant enzyme activities along with deprived lipid peroxidation (Gong et al., 2015). Our results illustrated that the various doses of CCPS on arsenic ingested rats were able to reduce the free radical products MDA and CD in uterine tissue (Table 8.2). It was earlier established that polysaccharide extract of *Momordica charantia* has a stimulatory action on intracellular antioxidant enzyme activities (Akila and Vennila, 2016). CCPS in arsenicated group in our experiment also contributed an increase of the intracellular antioxidant enzyme activities significantly (Fig 8.2). In addition, CCPS successfully protected ovarian steroidogenic response by inducing the activities of Δ^5, 3β-HSD and 17β-HSD (Fig 8.3). *Momordica charantia* has numerous pharmacological components such as saponins, momorcharins, glycosides, alkaloids, cryptoxanthin, etc (Grover and Yadav, 2004). CCPS extract of bitter gourd is composed of D-methyl galacturonate and D-galactose residue (4:1) (Panda et al., 2015). The structure has a series of negatively charged galacturonate acid. It also has excellent capability for the cation chelation (El-Zoghbi and Sitohy, 2001). Presence of hydroxyl groups in polysaccharide structure serve as hydrogen donators in contributing free radicals scavenging and neutralization of oxidative stress (Mohammad et al., 2016). It is confirmed that, arsenic mediated free radical generation

was effectively arrested by CCPS co-administration since it maintains the reproductive organs weight towards normalcy by including steroidogenic activities in arsenicated rats. However, from the above information, it is confirmed that CCPS successfully renovates the arsenic-induced distorted function of female reproductive organs.

Based on these results it could be revealed that CCPS at two doses (2.0 and 2.5 mg/ Kg BW) showed more or less similar activities against arsenic-induced reproductive toxicity. The critical dose 2.0 mg/ Kg BW of CCPS effectively ameliorated sodium arsenite mediated alterations of uterine oxidative stress and ovarian steroidogenic activities following the restoration of enzymatic antioxidant activities. Here it is speculated that two possible mechanistic may be involved in the mitigation of arsenic-mediated ovarian and uterine ailments. CCPS exerts its positive action like antioxidant activity by scavenging free radicals and thereby reducing the oxidative stress and as well as other effects. Another mechanism explains that galacturonate in CCPS structure contributes to chelate arsenic and thereby eliminating arsenic via methylation process and finally improves uterine function and ovarian steroidogenesis (Fig 8.4).

Figure 8.4.

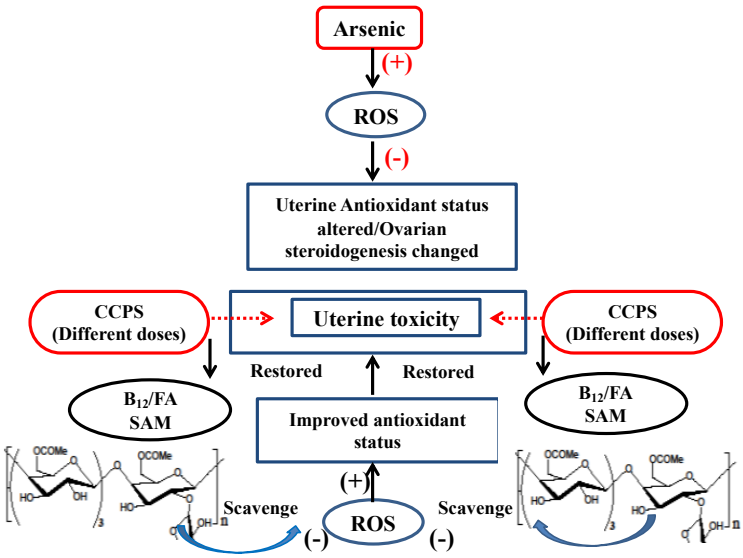


Fig 8.4. Schematic figure showing the hypothetical mechanism of action of CCPS against arsenic induced uterine toxicity. Red colour (+) and (-) sign expresses stimulatory and inhibitory effect of arsenic respectively. Black colour **(+)** and **(-)** sign expresses the stimulatory and also the inhibitory effect of CCPS respectively.