12.0. EXPERIMENT-8

Curative effect of different doses of ECNPs against sodium arsenite mediated female repro-toxicity by *in-vivo*.

12.1. Objective of the study

1. In this experiment different doses of ECNPs 0.5 mg/ Kg BW, 1.0 mg/ Kg BW and 1.5 mg/ Kg BW have been used.

2. The dose dependent experiment has been conducted to find out the most effective dose of ECNPs and describe its therapeutic effect against the arsenic mediated toxicity in the reproductive organs of the female albino rats by curative mode.

12.2. Experimental design

Female Wistar rats (103-105 grams) were selected for this analysis. All these rats were put in five different groups. The experimental scheduled of this experiment has shown below:

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

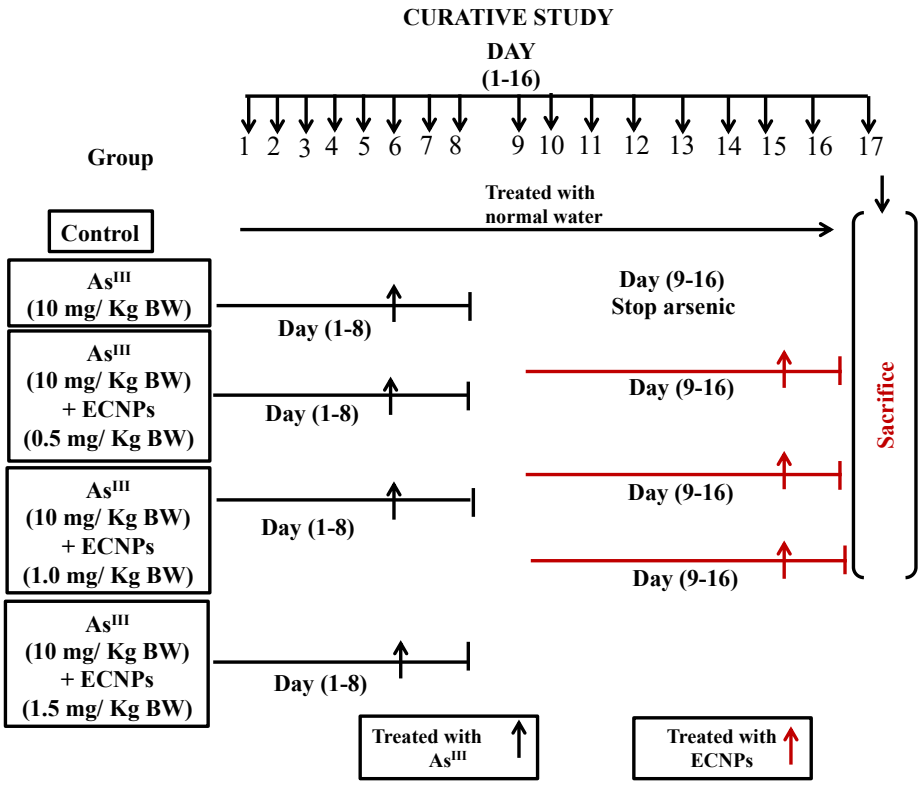
Group-II: Rats were treated with sodium arsenite (10 mg /Kg BW),

Group-III: As^{III} (10 mg /Kg BW) plus ECNPs (0.5 mg/ Kg BW),

Group-IV: As^{III} (10 mg /Kg BW) plus ECNPs (1.0 mg/ Kg BW),

Group-V: As^{III} (10 mg /Kg BW) plus ECNPs (1.5 mg/ Kg BW).

The all rats were treated via oral gavage in curative mode for sixteen days. First 8 days rats were treated with arsenic and from day 9 to day 16 rats were treated with different doses of ECNPs.



The body masses and feeding behaviours of all the rats were documented during the experiment. After completing 16 days treatment rats were anaesthetized by Hcl ketamine. The collected reproductive organs and blood samples from the rats were stored at temperature-20^{0}C. Rats were finally euthanized after organs collection using the over doses barbiturate.

12.3. Results

12.3.1. Body growth and organ weights

It has been noticed that arsenic treated rats significantly gained their body weight than that of the control rats (Table 12.1). The reproductive organs were also decreased in size appreciably. Though the body weight remained normal for arsenic treated group and ECNPs treatment also showed similar effect (Table 12.1). ECNPs at the dose of 0.5 mg/Kg BW didn't exhibit any significant change. Whereas, at the doses of 1.0 mg and 1.5 mg ECNPs /Kg BW have extensively maintained the rats body weight. It is observed that, ECNPs at doses have significantly changed the uterine and ovarian weight (Table 12.1).

12.3.2. Pattern of estrous cycle

After 3-4 days the arsenicated group showed an asynchronized pattern of the estrous cycle. A consistant diestrous phase was noticed in arsenic ingestion group (Fig 12.1). The control groups maintained the regular pattern of the estrous cycle (Fig 12.1). Three different doses of ECNPs on the arsenicated group maintained the regularity of estrous cycle pattern (Fig 12.1). It was observed that 1.0 mg and 1.5 mg doses of ECNPs effectively reverted the cycle towards regularity than the dose of 0.5 mg/ Kg BW (Fig 12.1).

12.3.3. Effect of ECNPs of lipid peroxidation and CD levels

Uterine MDA and CD level noticeably increased in the arsenic administered group as compared to the control (Table 12.2). This type of elevation was counteracted with different doses of ECNPs in arsenicated rats (Table 12.2). In case of uterine MDA, ECNPs at the dose of 1.0 and 1.5 mg/ Kg BW exhibited more significant effect than that of the dose of 0.5 mg (Table 12.2). In case of uterine CD 0.5 mg and 1.0 mg ECNPs did not show any significant recovery. Whereas, 1.5 mg of ECNPs significantly reverted back the uterine CD level (Table 12.2).

12.3.4. Effect of ECNPs on uterine SOD and catalase activities

The spectrophotometric study revealed that the arsenic administrated group has shown a significant diminution in uterine SOD and catalase activities (Fig 12.2A and 2B). The activities of uterine enzymes antioxidant significantly and increased due to the administration of dose dependent ECNPs in the arsenicated rats (Fig 12.2A and 2B). ECNPs at 1.0 and 1.5 mg doses which are more appreciably elevated the activities of uterine enzymes level than that of 0.5 mg dose (Fig 12.2A and 2B).

Electrozymographic study documented that arsenic induced group significantly reduced the uterine enzymes expression of SOD as compared to the control group (Fig 12.2C). In case of uterine catalase activity no significant changes were established (Fig 12.2D). It is also

observed that the ECNPs with 1.0 mg and 1.5 mg/ Kg BW doses in arsenic induced group have notably re-established the SOD enzyme antioxidant activity except the catalase expression (Fig 12.2C and 12.2D).

12.3.5. Effect of ECNPs on ovarian steroidogenesis, gonadotrophin and estradiol

A significant diminution of the ovarian steroidogenesis in terms of Δ^5, 3β-HSD and 17β – HSD activities in arsenic treated group as compared to the control (Fig 12.3A and 12.3B). ECNPs 0.5 mg/ Kg BW did not establish beneficial effect towards steroidogenic disturbances in arsenic treated rats (Fig 12.3A and 12.3B). (Fig 12.3A and 12.3B). Arsenication in rats were reduced the gonadotrophins LH and FSH along with estradiol significantly (Fig 12.3C-12.3E). ECNPs at the dose of 0.5 mg/ Kg BW did not show any significant variation in the LH, FSH and estradiol levels (Fig 12.3C-12.3E). However arsenic treated rats with 1.0 and 1.5 mg doses of ECNPs showed a significant enhancement in the level of LH, FSH and estradiol (Fig 12.3C-12.3E).

Table 12.1.

Groups	Body Weight (g)		Organo-somatic indices (g%)	
	Final	**Initial**	**Ovary in pair**	**Uterus**
Control	110.2±0.95	103.6±1.54	0.07±0.0019	0.169±0.001
As^{III} (10 mg /Kg BW)	117.56±1.66*	105.4±1.4	0.05±0.001***	0.123±0.001***
As^{III} + ECNPs (10+0.5 mg/ Kg BW)	111.6±2.48	104.2±0.86	0.058± 0.001***###	0.129±0.0006 ***#
As^{III} + ECNPs (10+1.0 mg/ Kg BW)	100.8±1.11*###	103.2±0.89	0.077±0.0006 ###	0.161±0.001 ***###
As^{III} + ECNPs (10+1.5 kg/ Kg BW)	98+2.06***###	105.4±1.46	0.081± 0.0008**###	0.168±0.001###

Table 12.1. Effect of different doses of ECNPs on somatic weight and reproductive organs' weight. Table represent mean ± SE, N = 6. Presented values here are expressed using one way ANOVA in association with Dunnett's post Hoc t-test. *,**,*** indicate $p<0.05$, $p<0.01$and $p<0.001$ versus the control with vehicle group, whereas # and ### indicate $p<0.05$, $p<0.001$ versus As^{III} treatment.

Figure 12.1.

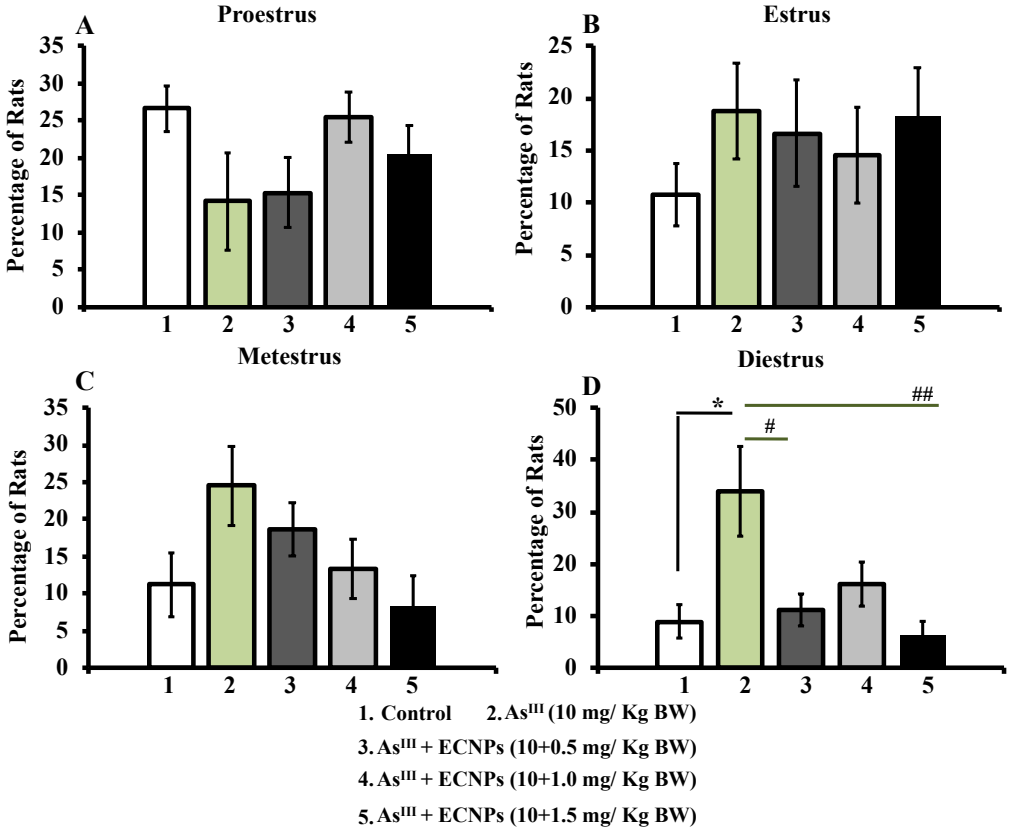


Fig 12.1. Effect of different doses of ECNPs on the pattern of estrous cycle against arsenicated mediated different group of rats. Data represent mean ± SE, N = 6. Presented values here are expressed using one way ANOVA in association with Dunnett's post Hoc t-test. * indicates $p<0.05$ versus the control with vehicle group, whereas # and ## indicate $p<0.05$ and $p<0.01$ versus As^{III} treatment.

Table 12.2.

	Control	**As^{III}**	**As^{III} + ECNPs (10+0.5 mg/ kg BW)**	**As^{III} + ECNPs (10+1.0 mg/ kg BW)**	**As^{III} + ECNPs (10+1.5 mg/ kg BW)**
Free radical Profile of Uterine tissue					
MDA (nmol/mg of tissue)	15.74± 0.39	26.94± 1.05***	22.46± 0.76***#	16.6±0.52 ###	13.5±1.33 ###
CD (nmol/mg of tissue)	12±1.67	17.98± 3.14*	14.8±1.61	11.56±1.55	9.72±1.06#

Table 12.2. Effects of different doses of ECNPs on lipid peroxidation in uterine tissue. Table represent mean ± SE, N = 6. Presented values here are expressed using one way ANOVA in association with Dunnett's post Hoc t-test. *, *** indicates $p<0.05$ and $p<0.001$ versus the control with vehicle group, whereas #, ### indicates $p<0.05$ and $p<0.001$ versus As^{III} treatment.

Figure 12.2.

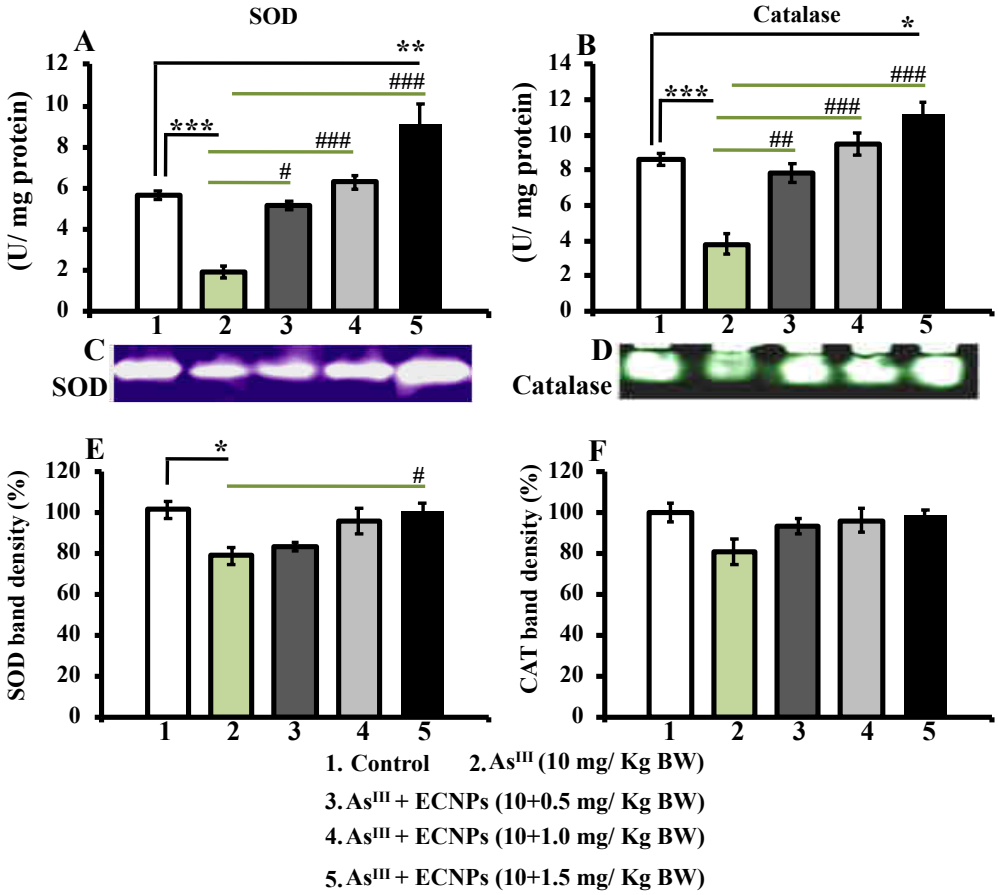


Fig 12.2. The spectrophotometric (A & B) data showing the effects of different doses of ECNPs on uterine endogenous antioxidant enzymes activities. Electrozymogram (C & D) images showing the effects of different doses of ECNPs on uterine endogenous antioxidant enzymes expression. E & F shows the band density (%) of SOD and catalase respectively. Data represent mean ± SE, N = 6. Presented values here are expressed using one way ANOVA in association with Dunnett's post Hoc t-test. *, *** indicate $p<0.05$, $p<0.001$ versus the control with vehicle group, whereas #, ##, ### indicate $p<0.05$, $p<0.01$ and $p<0.001$versus As^{III} treatment.

Figure 12.3.

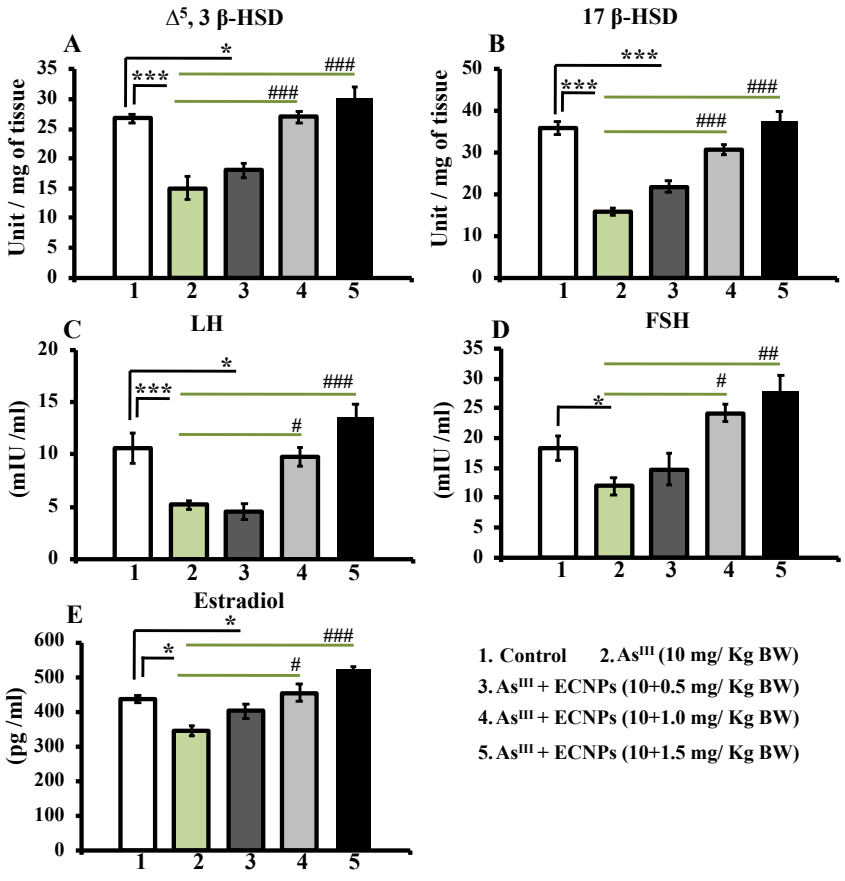


Fig 12.3. A & B denote the effects of different doses of ECNPs on ovarian key regulatory steroidogenic enzyme activities. C, D & E show the effects of different doses of ECNPs on gonadotrophin and estradiol hormones. Data represent mean ± SE, N = 6. Presented values here are expressed using one way ANOVA in association with Dunnett's post Hoc t-test. *, *** indicates $p<0.05$, $p<0.001$ versus the control with vehicle group, whereas #, ##, ### indicate $p<0.05$, $p<0.01$ and $p<0.001$ versus As^{III} treatment.

12.1.4. Discussion

In this experiment, the therapeutic role of encapsulated curcumin chitosan nanoparticles (ECNPs) has evaluated against the arsenic mediated female reproductive toxicity in the animal model. The curcumin has anti-oxidant property and previously it was reported that curcumin can be used to reduce the arsenic toxicity in human and other animals (Agarwal et al., 2010; Biswas et al., 2010; Roy et al., 2011). One of our current findings elaborated that curcumin could mitigate arsenic mediated uterine and ovarian toxicity in the Wistar rat (Perveen et al., 2019). Actually curcumin has poor bioavailability in aqueous solution and it is hydrophobic in nature. Nowadays, the important advantages of nano technology are being used as the drug carriers (Rizvi and Saleh, 2018). Nanoparticles generally have various properties such as higher stability in biological systems with higher carrier capacity with better high, incorporation capabilities for hydrophilic as well as hydrophobic substances (Gelperina et al., 2005; Bala et al., 2004; Vauthier et al., 2003; Soppimath et al., 2001). Curcumin is possibly associated to chitosan via involving the hydroxide groups of curcumin and ammonium groups of chitosan (Das et al., 2010). These properties of nanoparticles improve the drug bioavailability. Curcumin can be used through nanoparticles-based drug delivery system which has extensive possibility in the protection of cells against the toxicant-induced necrosis or apoptosis (Sun et al., 2010). In this study, the encapsulated forms of these nanoparticles have been prepared using the chitosan, the range between 8-40 nm and it was used in the amelioration of arsenic toxicity in rats. Yadav et al., 2012 reported that a lower dose of encapsulated curcumin in chitosan nanoparticles (1.5 mg/ Kg BW) was more effective than that of the higher dose of free curcumin (15 mg/ Kg BW) in the prevention of arsenic toxicity at the dose of 2 mg/ kg BW in male rats. Sankar et al., 2013 also reported that the encapsulated curcumin nanoparticles have (CUR-NP) a better protection than the free curcumin at the higher and the same doses (100 mg/ Kg BW) in the arsenic mediated (25

ppm, at the lower dose) renal and neural oxidative damage in the rats. It was found that encapsulated curcumin nanoparticles also have a better protection against than free curcumin of 100 mg/ Kg BW (Sankar et al., 2016). Several doses of encapsulated curcumin nanoparticles with 1.5 mg/ Kg body weight and low dose of 2 mg/ Kg BW sodium arsenite for 4 weeks were used to remove the toxic effect from different organs of male rats (Yadav et al, 2012). The encapsulated curcumin nanoparticles at the higher dose of 100 mg/ Kg BW low dose of 25 ppm sodium arsenite treatment for 42 days reported the reduced arsenic toxicity in several organs (Sankar et al, 2013; Sankar et al., 2016). However, in this present study we used comparatively lower dose of ECNPs against higher dose of sodium arsenite for short duration to investigate out the mitigation of arsenic toxicity in female reproductive organs. Three different doses of ECNPs including 0.5 mg/ kg BW, 1.0 mg/ kg BW and 1.5 mg/ kg BW were chosen in our experiment. Our results provided a new interesting and important eplanations that are in agreement with the earlier studies corelating the antioxidant and metal-chelating properties of the curcumin. Yadav et al., 2012 been reported that ECNPs in arsenicated rats ameliorated the alteration of oxidative stress parameters in hepatic organ. We also found that ECNPs in different doses could effectively reduce the uterine free radical generation as evident from reduced level of MDA and CD (Table 12.2). The electron donating phenol-hydroxyl groups and a beta-diketone are responsible for its ability to scavenge free radical and inhibit lipid peroxidation (Dinkova-Kostova and Talalay, 1999; Osawa et al., 1995). Curcumin has distinct structural motifs that is responsible for its antioxidant property. In addition ECNPs contributes to increase the uterine intracellular antioxidant enzymes expression (SOD and catalase) significantly (Fig 12.2). It is consistent with the information served by as Yadav et al., 2012 who had also illustrated that encapsulated curcumin nanoparticles improves the SOD and CAT actions in liver (Yadav et al., 2012). ECNPs in the arsenic ingested rats could restore the ovarian steroidogenic levels

followed by maintaining the normal estrous cycle pattern of rats (Fig 12.3). These results indicate enhanced antioxidant and chelating potential of ECNPs in comparison with free curcumin (Perveen et al., 2019) at a much lower dose that could be beneficial in the clinical recovery of rats for arsenic mediate female reproductive ailments.

Our study revealed that ECNPs might be worked at different doses showed more or less similar activites against arsenic induced repro-toxicity. But, it is confirmed that the dose 1.0 mg and 1.5 mg ECNPs per Kg BW is more significantly effective than that of 0.5 mg ECNPs dose and ameliorate the sodium arsenite induced alteration of uterine oxidative stress by the restoration of lipid peroxidation. Here it is hypothesized that possible mechanistic may be involved in the mitigation of arsenic induced uterine and ovarian disorders. Curcumin encapsulated chitosan nanoparticle (ECNPs) has extremely nano sized (8-40 nm) those are highly stable with possible higher bioavailability and capability to interect with sodium arsenite via involvement of hydroxide groups of curcumin and ammonium groups of chitosan which may possibly chelate the arsenic and thereby eliminating of arsenic via methylation process and finally improves the uterine and ovarian dysfunction against arsenic mediated toxicity (Fig 12.4).

Figure 12.4

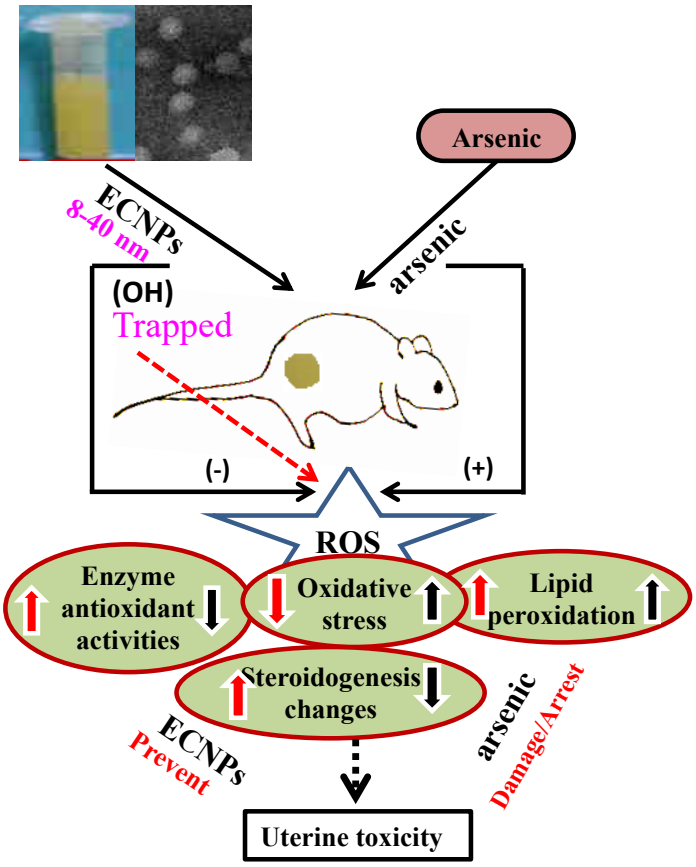


Fig 12.4. Schematic diagram showing the hypothetical mechanism action of curcumin against arsenic induced uterine toxicity. Black colour **(+)**, **(-)** sign showing stimulatory and inhibitory effect of arsenic respectively. Red colour (+), (-) sign showing stimulatory and inhibitory effects of ECNPs respectively.