7.0. EXPERIMENT-3

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

7.1. Objective of the investigation

1. In this experiment, three different 15 mg/ Kg BW, 20 mg/ Kg BW and 25 mg/ Kg BW doses of curcumin have been used.

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

7.2. Experimental design

The female Wistar rats of weights 122-124 grams were chosen in this experiment. All these rats were put in five different groups. The detailed procedure of this experiment has shown below:

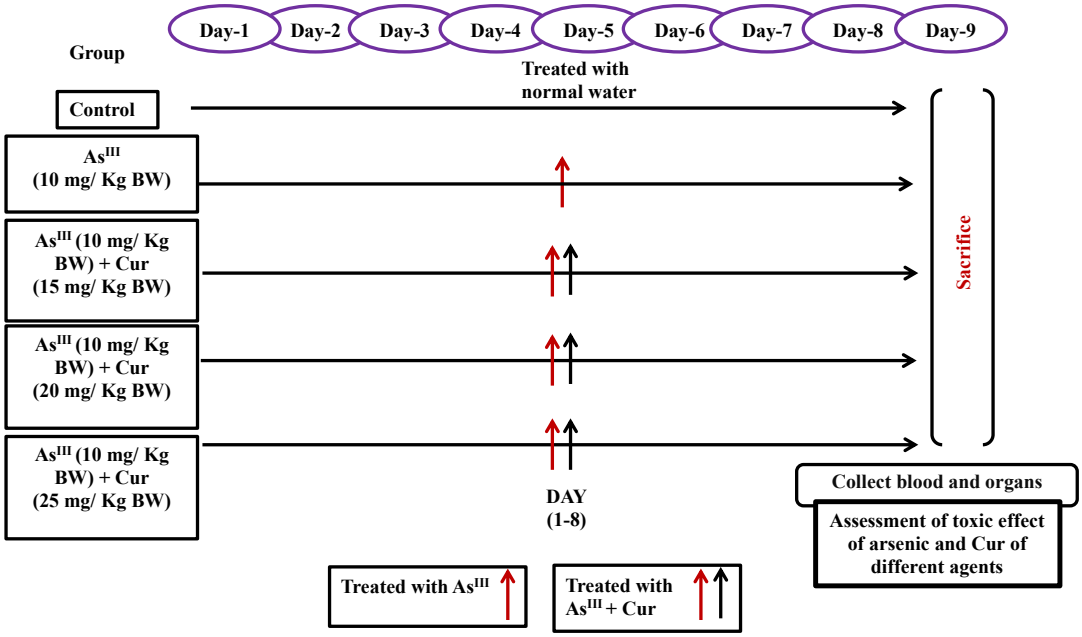
Group-I: This group was control group with vehicle,

Group-II: Rats were given As^{III} (10 mg/Kg BW),

Group-III: As^{III} (10 mg/Kg BW) plus Cur (15 mg/ Kg BW),

Group-IV: As^{III} (10 mg/ Kg BW) plus Cur (20 mg/ Kg BW),

Group-V: As^{III} (10 mg/ Kg BW) plus Cur (25 mg/ Kg BW), All rats were treated via oral gavage in co-administration fashion for eight (8) days.



All experimental rats were given normal pellet diet before sacrifice. Food habit as well as rat's body weights were remarked during the experiment. The final body masses of all the rats were computed at the last days of experiment. After 8 days these rats were anaesthetized by Hcl ketamine. Blood samples, liver and sex organs were collected following the institutional standard protocol. Sample was stored at -20^{0}C temperature. Finally all the operated rats were euthanized with barbiturate.

7.3. Results

7.3.1. Body Growth and Organ Weights

Arsenic treatment exhibited a significant deterioration body masses of the animals along with reproductive organs' weight loss in contrast towards control groups (Table 7.1). Then again treatment with various curcumin doses on the arsenicated rats showed significantly maintained body weight as well as reproductive organs towards normalcy. Curcumin at the dose of 15 mg/Kg BW regained the reproductive organs' (uterus & ovary) weight but did not show any changes in rat's body weight (Table 7.1). Whereas, at the doses of 20 and 25 mg/ Kg BW curcumin showed more extensive alteration of the above condition (Table 7.1).

7.3.2 Effect of curcumin on estrous cycle pattern

Regular estrous phase in the control rats was observed between 1 to 8 days **(**Fig 7.1). A significant interruption of estrous cycle was exhibited in arsenic ingested group when compared with the control group (Fig 7.1). After 3-4 days of arsenication a consistent diestrous phase was noticed. Curcumin treatment at the dose of 15 mg/ Kg BW for 8 days was not helpful to prevent arsenic mediated interruption of estrous cycle (Fig 7.1). However, at the doses of 20 and 25 mg/ Kg of curcumin on the arsenic ingested group noticeably replaced the interrupted pattern of estrous cycle towards uninterrupted pattern **(**Fig 7.1).

7.3.3. Uterine lipid peroxidation and CD levels

Eight days of As^{III} treatment revealed a significant increase in uterine lipid peroxidation followed by higher CD level in comparison control group with vehicle rat (Table 7.2). Different doses of curcumin co-administration in arsenic challenged rats restored the uterine levels of MDA and CD (Table 7.2). Different doses of curcumin have shown to be inactive in restoring CD (Table 7.2). Whereas, maximum diminution in the lipid peroxidation (MDA) was observed in rats treated with curcumin at the two doses of 20 and 25 mg/ kg BW (Table 7.2).

7.3.4. Uterine enzymatic antioxidant expression

SOD and catalase uterine activities significantly reduced when these rats were treated with As^{III} for 8 days in contrast to control rats (Fig 7.2A and 2B). Curcumin post-treated rats with 15 mg/ kg BW dose did not establish any changes in uterine SOD and catalase activities. Whereas, co-administration at 20 and 25 mg/ kg of curcumin in these As^{III} treated rats for same duration exhibited a significant correction in these uterine enzymes activity.

Electrozymographic detection confirmed that ingestion of As^{III} by these rats significantly reduced uterine expression of SOD and catalase as compared to the control rats (Fig 2C and 2D). Though different doses of curcumin (15, 20 and 25 mg/ kg respectively) in arsenic

treated rats elevated the uterine expression of these enzymes. It was observed that curcumin at the dose of 20 mg/ kg and 25 mg/ kg BW elevates the SOD and catalase expression maximally than that of the dose 15 mg/ kg BW (Fig 7.2C-2F).

7.3.5. Ovarian steroidogenesis, gonadotropins and estradiol

A significant reduction of ovarian Δ^5, 3β-HSD and 17 β –HSD activities were observed in the rats challenged with arsenic (Fig 7.3A-3B). Curcumin at the dose of 15 mg /kg BW did not show any significant variation in the ovarian steroidogenic enzymatic status (Fig 7.3A-3B). However, arsenicated rats treated with 20 and 25 mg/ kg BW of curcumin for 8 days resulted in a significant enhancement in ovarian key enzyme activities (Fig 7.3A-3B).

Eight days of As^{III} treatment also caused a significant diminution in LH, FSH and estradiol level in comparison with control (Fig 7.3C-3E). Curcumin at the dose of 15 mg/ kg did not correct these changes of gonadotropin and estradiol significantly. However, curcumin at 20 mg/ kg and 25 mg/ kg showed better remission in circulating LH, FSH and estradiol level (Fig 7.3C-3E).

Table 7.1.

Groups	Body Weight (g)		Organo-somatic indices (g%)	
	Final	Initial	Ovary in pair	Uterus
Control	128.56±0.44	123±0.76	0.091+0.002	0.187+0.001
As^{III} (10 mg/ Kg BW)	145±2.3***	124.84±0.25	0.0625±0.001***	0.134+0.0007***
As^{III} + Cur (10+15 mg/ Kg BW)	124.96±0.57	123.84±0.59	0.0660±0.0004***	0.138+0.0004***
As^{III} Cur (10+20 mg/ Kg BW)	128.2±0.33###	122.28±0.39#	0.075±0.0008***###	0.163+0.0021***###
As^{III} (Cur (10+25 mg/ Kg BW)	130.4±1.08##	122.72±0.48#	0.077±0.0007***###	0.173+0.0005***###

Table 7.1. Effect of different doses of the curcumin on body mass and reproductive organs. Table represents mean ± SE, N = 6. Presented values here are expressed by one-way ANOVA with Dunnett's post Hoc t-test *** indicate $p<0.001$ versus control with vehicle, whereas #, ## and ### indicate $p<0.05$, $p<0.01$ and $p<0.001$ versus As^{III} treatment.

Figure 7.1.

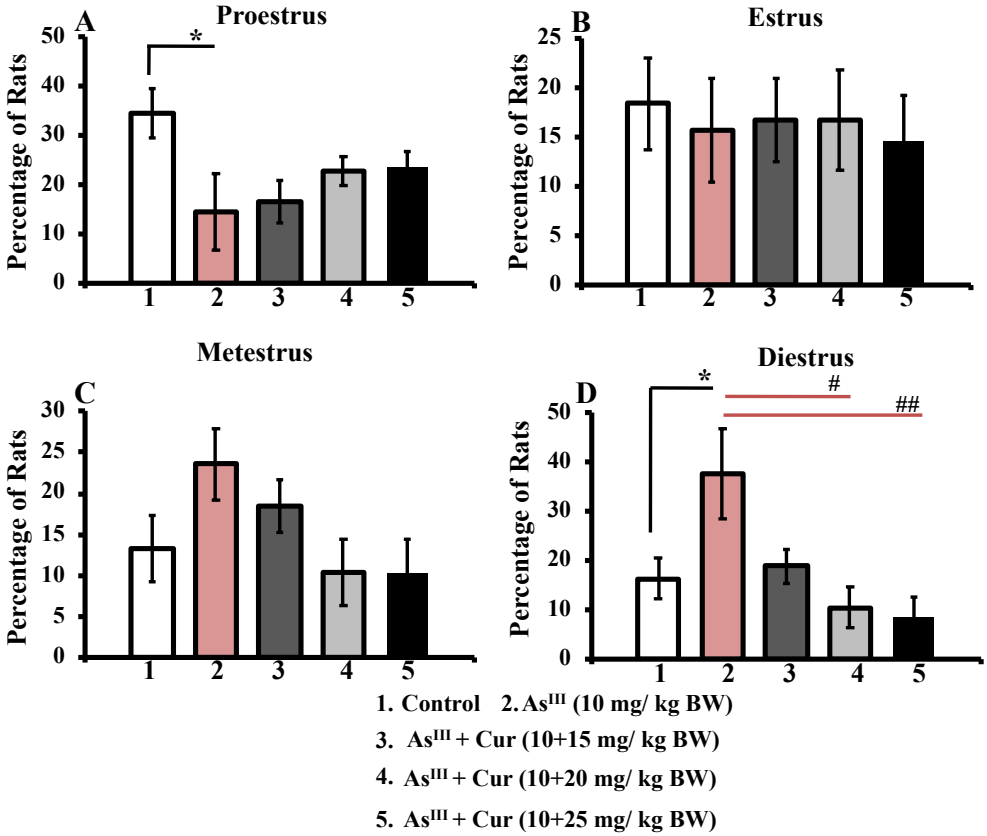


Fig 7.1. Effect of three doses of Cur on the pattern of estrous cycle against arsenic mediated different groups. Data represent mean ± SE, N = 6, Presented values here are expressed using one-way ANOVA with Dunnett's post Hoc t-test * indicate $p<0.05$ versus the control with vehicle group, whereas # and ## indicate $p<0.05$ and $p<0.01$ versus As^{III} treatment.

Table 7.2.

	Control	**As^{III} (10 mg/ kg BW)**	**As^{III} + Cur (10+15 mg/ Kg BW)**	**As^{III} + Cur (10+20 mg/ Kg BW)**	**As^{III} + Cur (10+25 mg/ Kg BW)**
Free radical Profile of Uterine tissue					
MDA(nmol/ mg of tissue)	27.70± 0.95	34.28± 1.73***	33±1.83***###	26±2.49***###	23.29±1.38 ***###
CD(nmol/mg of tissue)	11.5±0.75	19.76± 1.42*	18.16±1.43*	16.02±1.89	15.06±0.72

Table 7.2. Effects of three doses of curcumin on uterine oxidative related stress markers against arsenic challenged group. Table represent mean ± SE, N = 6, Presented values here are expressed using one-way ANOVA with Dunnett's post Hoc t-test *, *** indicate $p<0.05$ and $p<0.001$ against control with vehicle, whereas ### indicate $p<0.001$ against As^{III} treatment.

Figure 7.2.

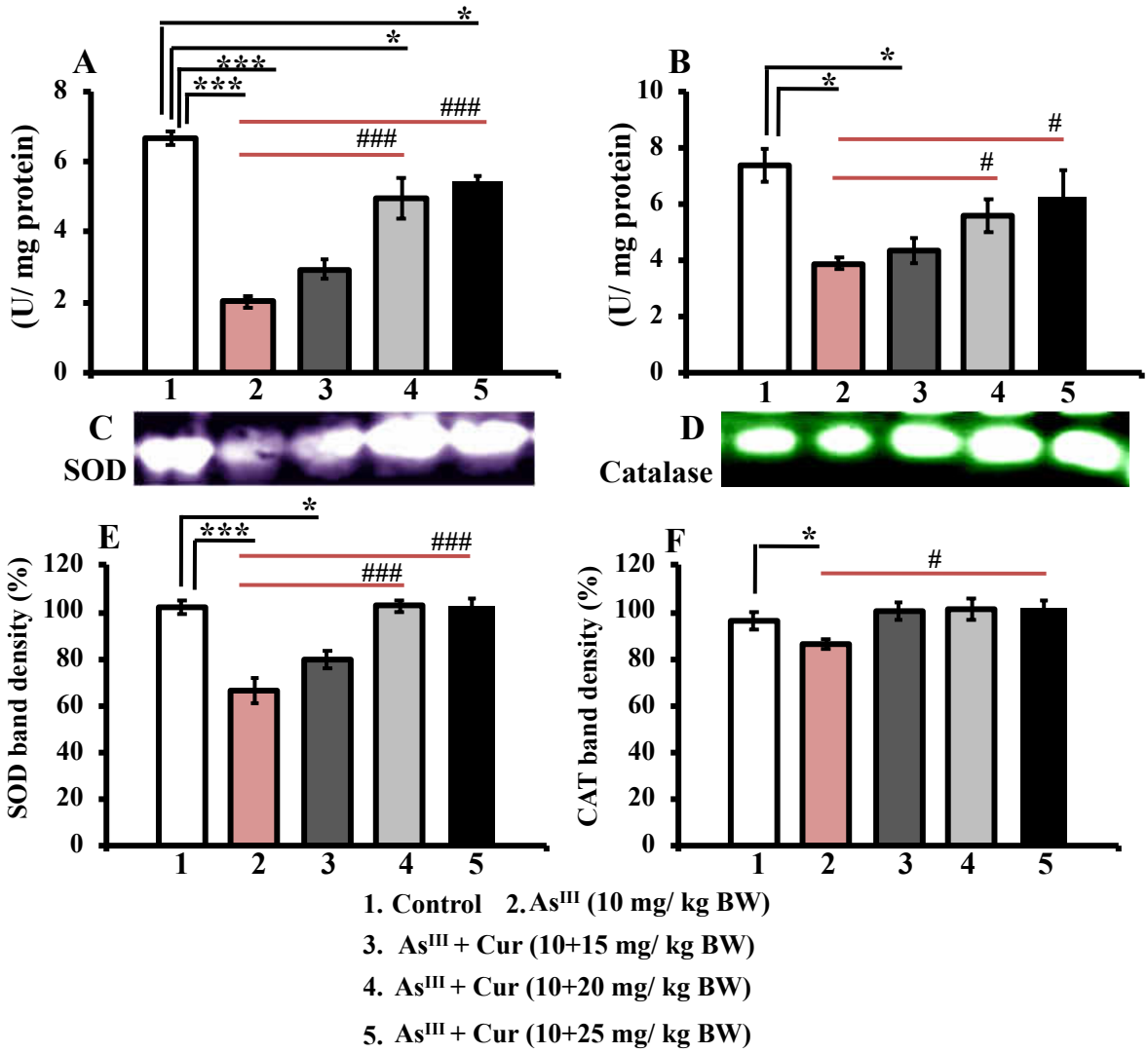


Fig 7.2. The spectrophotometry (A & B) data showing the effects of three doses of curcumin on uterine endogenous antioxidants (SOD & catalase) levels. Electrozymogram (C & D) images also showing the uterine endogenous enzymatic antioxidant expression. E & F data shows the band density (%) of SOD and catalase expression respectively. Data represent mean ± SE, N = 6, Presented values here are expressed using one-way ANOVA with Dunnett's post Hoc t-test *, *** indicate $p<0.05$, $p<0.001$ versus vehicle with control, whereas #, ### indicate $p<0.05$, $p<0.001$versus As^{III} treatment.

Figure 7.3.

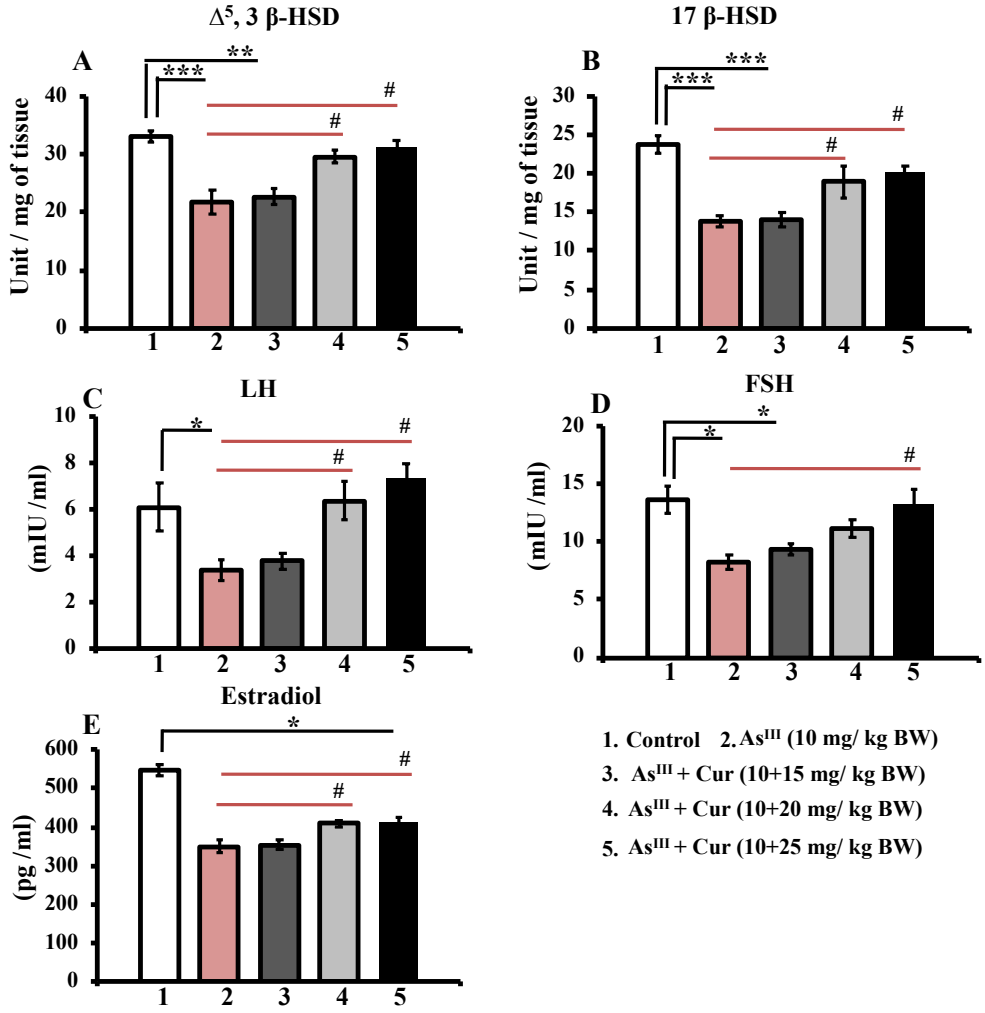


Fig 7.3. A & B denotes the effects of different doses of curcumin on ovarian key regulatory steroidogenic enzyme activities in arsenic treated animals. C, D & E data shows that the effects of different doses of curcumin on gonadotropin and estradiol hormones in arsenicated rats. All data represent mean ± SE, N = 6, Presented values here are expressed using one-way ANOVA with Dunnett's post Hoc t-test *, *** indicate $p<0.05$, $p<0.001$ versus vehicle with control, whereas # indicate $p<0.05$ versus As^{III} treatment.

7.4. Discussion

Exposures to arsenic increased the risk of the reproductive system in animal model (Perveen et al., 2019). Several studies confirmed that, the excess of oxidative stress is directly coupled to the female reproductive function (Agarwal et al., 2012). Researchers also reported that, trivalent form of the arsenic is responsible for generation of ROS including the superoxide anion, hydroxyl radicals, hydrogen peroxide and especially peroxyl radicals which are also associated with H_2O_2 (Flora, 2016). Arsenic poisoning has distorted different biological systems by elevating ROS generation that resulted in the higher level of lipid peroxidation (Flora et al., 2005). Both SOD and catalase are equally supportive of anti-oxidant enzyme activity that provides the protective defence against the ROS (Shalaby and Mouneir, 2010). Arsenic executes its toxic effects on uterine endogenous antioxidant enzymes activities by suppressing SOD and catalase (Fig 7.2). Arsenic treatment developed a faint and weakly dose band of SOD and catalase in the uterine tissue as compared with control (Fig 7.2). Here, the uterine end products MDA and CD of lipid peroxidation were elevated in arsenic intoxicated group as compared to vehicle with control (Table 7.2). These results were also supported with the other researcher's findings (Maity et al., 2018). It has revealed that, diminution of these antioxidant enzymes activities resulted in reduced H_2O_2 detoxification from the uterine tissue. It is also to be noted that, the programmed cells died when H_2O_2 were accumulated in the uterine tissue (Miller et al., 2010). Previous investigation confirmed that, arsenic could reduce the activity of key enzymes like Δ^5, 3β-HSD, and 17β-HSD ovarian steroidogenesis. This finding is also consistence with our present investigation. Actually the ovarian steroidogenic enzymes are regulated by the gonadotropins LH and FSH which further elevates estradiol signaling (Odell et al., 1963; Hinshelwood et al., 1994). The low level of gonadotropins LH and FSH are closely associated to the glucocorticoids as well as related with hypersecretion of ACTH (Ghosh et al., 1999). Chattopadhyay et al 2012 reported that,

higher level of plasma glucocorticoids counteracted with the gonadotropin releasing hormone (GnRH). This present study explored that, low levels of gonadotropin hormones and estradiol following arsenication in rats diminished the uterine and ovarian weight (Table 7.1) as gonadotropin hormones are prime regulators for maintaining ovarian weight whereas estradiol hormones help to maintain the uterine weight. It is assumed that, the weight of the uterine tissue is reduced by the down regulation of the estradiol signal due to the arsenic toxicity (Edman, 1983). It has been reported that, the arsenic treatment at the lower dose leads to the toxic effects in different organs (Chattopadhyay et al., 2012; Acharyya et al., 2015). The lower dose of arsenic also accumulates in the uterine tissue (Chattopadhyay and Ghosh, 2010). The curcumin has a strong antioxidant property and it is accepted widely. Curcumin molecule contains a phenolic group, 1, 3- diketone and methoxy group of phenyl ring which probably eliminate the single oxygen, hydroxyl radical and also reactive nitrogen species from the tissue (Reddy and Lokesh, 1994; Rao et al., 1995; Priyadarsini et al., 2003; Ohara et al., 2005). This existing structure of curcumin is also responsible for the free radical catching (Masuda et al., 2001) since, it is a chain breaking antioxidant. Daniel et al 2004 reported the ability of curcumin in the chelation of the toxic metals. The metal binding property of curcumin diminishes the repro-toxicity against the sodium arsenite (Dairam et al., 2007). Studies demonstrated that, curcumin was used to eliminate the toxic effect from the various parts of organs (Yousef et al., 2008; El-Demerdash et al., 2009). It was informed that, curcumin treatment restrained the lipid peroxidation level of the brain, maintained the other biological system and elevated the antioxidant enzyme activities (Younes et al., 1981).

Actually curcumin has poor bioavailability while feed orally and has high metabolism rate and rapid systemic elimination property from the body. The maximum plasma concentration testified 0.051µg / ml from 12g curcumin in human and 1.35µg/ml from 2g/kg in rat (Lao et al., 2006). Another investigation also revealed that, 2.0 g curcumin /kg of oral dose to the rats

could reach a highest level of serum concentration of 1.35±0.23 µg/ml within few hours whereas same dose of curcumin in the humans resulted in negligibly small (0.006±0.005 µg/ml at 1 hour) (Shoba et al., 1998) serum levels. Another report explored that, 1.0 gm/ kg curcumin in rats have shown a concentration of 15 ng/ml plasma within 50 minutes (Chang et al., 2013). Researchers demonstrated that, reproductive disorders could be minimized by the application of curcumin with variable doses. Previous studies have been used the lower dose of curcumin 15 mg/ kg BW and lower dose of sodium arsenite 5 mg/ kg body weight for 30 days used to remove the toxic effect from different parts of the body (El-Demerdash et al., 2009). In this investigation therapeutic role of curcumin has evaluated in arsenic-induced female rats. Here the dose-dependent actions of curcumin have been used on female reproductive dysfunction against arsenic-induced toxicity. Three different doses i.e 15 mg/ kg BW, 20 mg/ kg BW and 25 mg/ kg BW of curcumin were used in this study. It was observed that, the application of different doses of the curcumin on arsenic ingested rats diminished the lipid peroxidation and maintained the uterine enzymes antioxidant level (Table 7.2 and Fig 7.2). It was also observed that, after the application of three curcumin doses on different groups of rats the body mass significantly changed as compared to the arsenicated group (Table 7.1). A significant alteration of the uterine and ovarian somatic indices was noted in curcumin treated group as compared to the arsenicated group (Table 7.1). Investigator reported that, use of curcumin could protect the animal from ovarian injury via managing oxidative stress markers (Sak et al., 2013). Here we observed that, curcumin could restore the ovarian steroidogenic function (Fig 7.3) and thereby maintains the normal estrous cycle pattern of rats (Fig 7.1). Hence from the above information it is confirmed that, curcumin has an extensively remedial effect against the arsenic-induced oxidative stress that ultimately causes uterine and ovarian damages. Considering this observation we assumed that, the chelated complex of curcumin with arsenic could be mostly responsible for reducing

oxidative stress. It is also justified that, curcumin in different doses successfully renovates the arsenic-induced reproductive dysfunctions.

Our study revealed that, curcumin might be worked equally and better at the dose of 20 and 25 mg per Kg BW than that of 15 mg curcumin/ Kg BW. We conclude that, above mentioned dose 20 mg is the critical dose of curcumin and that, could effectively exert its critical effect on the arsenic mediated repro-toxicity followed by maintaining normal estrous phase. Curcumin by its antioxidant property contributes in the scavenging of free radicals from the body thererby limits the extent of oxidative stress as well as various adverse effects. Other notion is that, curcumin with its metal chelating property may chelate arsenic in organs thereby improves the methylation process. The possible mechanism action of curcumin against arsenic-induced repro-toxicity is described schematically in Fig 7.4.

Figure 7.4.

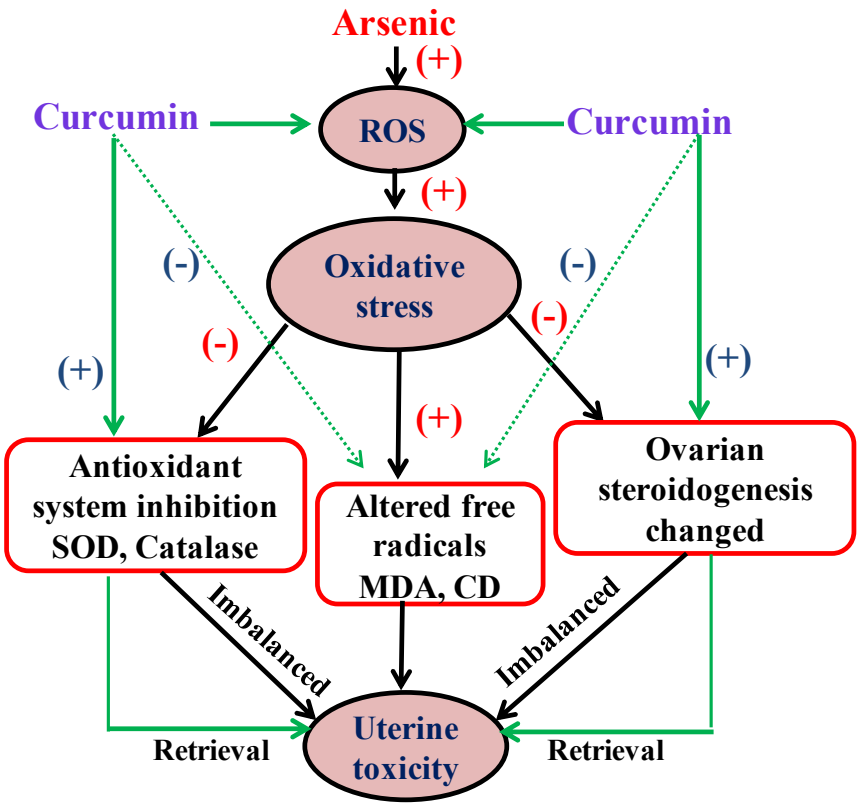


Fig 7.4. Schematic diagram showing the hypothetical mechanism action of curcumin against arsenic-induced uterine toxicity. Red colour (+), (-) sign and black line expresses the stimulatory and as well as the inhibitory effect of arsenic respectively. Black colour **(+)**, **(-)** sign and green line also expresses the stimulatory and inhibitory effects of curcumin respectively