10.0. EXPERIMENT-6

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

10.1. Objective of the investigation

1. This present investigation was deliberated to search out curative effect of CCPS against sodium arsenite ailments female repro-toxicity by post treatment mode.

2. To investigate the effect of dietary CCPS against arsenic mediated infertility and pregnancy outcome.

10.2. Experimental design

The female Wistar rats (85±10g) were accounted for this study. These rats were alloted into five groups. The detailed procedure of this experiment has shown below:

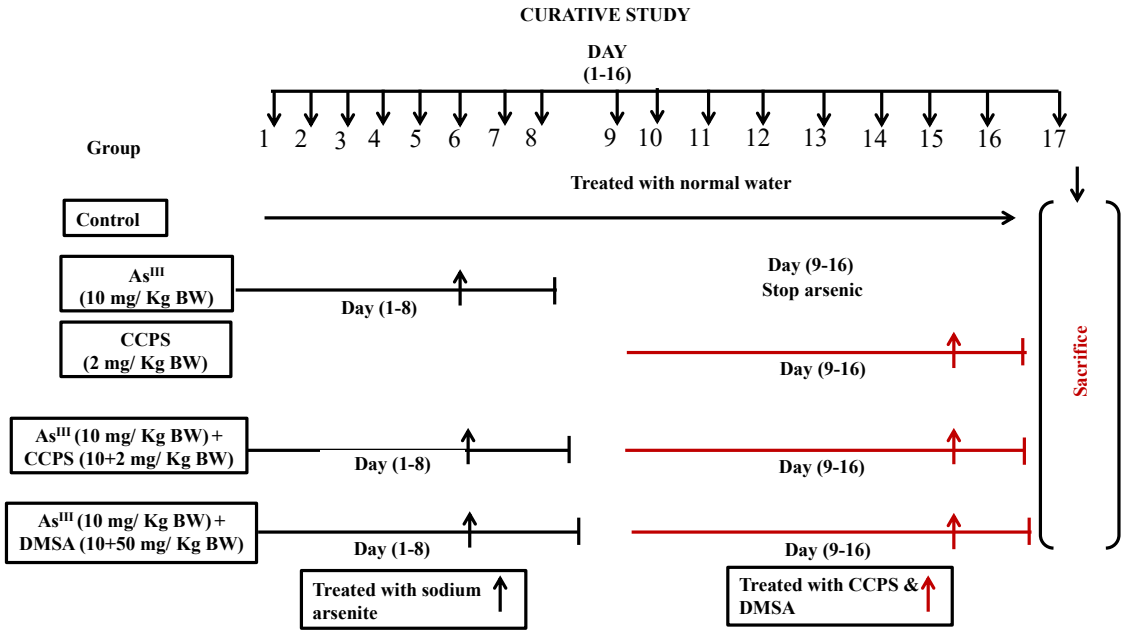
Group-I: This group was control group with vehicle,

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

Group-III: CCPS (2.0 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 DMSA (50 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 CCPS. Food habits and body weights of all these rats were checked during this experiment. The final body masses of all the rats were documented on 17th days. These rats were anaesthetized by the ketamine HCl. The blood samples, liver and sex organs were finally stored at -20^{0}C. All rats were euthanized using the overdoses barbiturate.

10.3. Results

10.3.1 Effect of CCPS on Body Growth and Organ Weights

Table 10.1 shows that the final body weights of these rats of all groups were not significantly changed. Weight of the ovaries significantly reduced followed by mild but insignificant uterine weight loss. In contrast post treatment with CCPS on arsenic ingested rats were appreciably maintained the uterine-ovarian weight in arsenic challanged rats (Table 10.1).

10.3.2. Effect of CCPS on estrous cycle pattern

After 3-4 days the arsenicated group showed an asynchronized pattern of the estrous cycle. It was observed that the estrous phase is substituted by a continue diestrous phase in the arsenic treated group (Fig 10.1). The control groups maintained the regular pattern of the estrous

cycle. Post treatment with CCPS on arsenicated group shown more regular pattern of the estrous phase (Fig 10.1).

10.3.3. Liver and kidney function

Figure 10.2Aa-2Ad shows that there was a significant degredation in the functional profile of liver followed by increased in serum SGPT, SGOT, serum urea and serum creatinine in arsenic fed rats when compared with the control rats. Similarly the treatment on arsenicated group with CCPS shown a significant considerable liver functional profile towatds normalcy (Fig 10.2Aa-2Ad). The lipid profile parameters of serum i.e cholesterol triglyceride and LDL significantly increased with the continuation of low level of HDL in arsenic fed rats as compared to the control (Fig 10.2Ba-2Bd). Post treatment of CCPS significantly reduced the elevated lipid profile with improved HDL (Fig 10.2Ba-2Bd).

10.3.4. Effect of post treatment of CCPS on sex organ's lipid peroxidation

A remarkable elevation in the uterine and ovarian MDA and CD levels were observed in the arsenic ingested group (Fig10.3A-3D). A significant reduction of NPSH level in the reproductive organs was also noted in arsenic treated rats (Fig10.3E-3F). MDA and CD levels were reduced due to the post treatment with CCPS on arsenic treated rats (Fig10.3A-3D). A noticeable restoration of the NPSH level was observed in the CCPS post treated group arsenic challanged rats (Fig10.3E-3F).

10.3.5. Effect of CCPS post treatment on sex organ's antioxidant activities

The spectrophotometric studies revealed that endogenous antioxidant i.e uterine and ovarian SOD, catalase and GPx activities were significantly diminished as compared to the respective control group (Fig 10.4Aa-4Af). CCPS exhibited a curative role by keeping the antioxidant enzymes activities towards normalcy (Fig 10.4Aa-4Af).

The electrozymographic study revealed that arsenic treated rats significantly diminished the uterine and ovarian SOD, catalase and GPx expression (Fig 10.4Ba-4Af). Uterine and ovarian

enzymatic expression were successfully improved due to the reappearance of distnict band of these enzymes due to CCPS post treatment in the arsenic ingested rats (Fig 10.4Ba-4Af).

10.3.6. Effect of CCPS on serum LDH

As shown in Fig 10.5A, there was a significant increase in the serum LDH level in arsenic fed group when compared with the control group. But serum LDH was significantly restored following the treatment of CCPS. Arsenic fed group also exhibited a prominent and strong band appearance of the serum LDH (Fig 10.5B). However, a significantly weak expression with a faint band density of serum LDH activity was observed by CCPS post treatment in arsenic fed rat (Fig 10.5B).

10.3.7. Effect of CCPS on circulating vitamins and Hcy

Arsenic induced rats suffered with a significant depletion of serum vitamins B_{12} ($p<0.05$) and folic acid with a surge of vitamin-C along with increased Hcy level in serum when compared with control group (Fig 10.6A-6D). The circulating level of vitamins B_{12} , folic acid as well as vitamin-C were significantly cured by the CCPS on arsenic fed group (Fig 10.6A-6C). CCPS treatment on arsenic fed rat was noticeably lifted the circulating level of serum Hcy (Fig 10.6D).

10.3.8. Effect of CCPS on gonadotrophin hormones, ovarian steroidogenesis and Esr-1

Arsenic fed group established significant inhibition of the ovarian key enzymes activities of 17β-HSD and Δ^5, 3β-HSD in comparison with the control group (Fig 10.7A-7B). The serum level of LH, FSH, estradiol was decreased considerable with notable suppression of Esr-1 receptor arsenic challanged rats (Fig 10.7C-7F). However, CCPS post treatment in arsenic fed group significantly retrived these suppressed activities of regulatory enzymes (Fig 10.7A-7B). On the other hand, CCPS in arsenic fed group significantly attenuated the key monitoring markers LH, FSH, and estradiol of the estrous cycle with an improved uterine receotor Esr-1 (Fig 10.7C-7F).

10.3.9. Effect of CCPS on inflammatory markers and MT-1 level

There was a significant elevation in the serum TNF-α, hepatic MT-1 uterine IL-6 and NF-κB in arsenic fed group in comparison with respective control group (Fig 10.8A-8D). CCPS played a key role on limiting the level of inflammatory markers. CCPS remarkably revived the elevation of inflammatory markers and the hepatic MT-1 level (Fig 10.8A-8D).

10.3.10. Effect of CCPS on apoptotic expression in uterus

There was a noteworthy over expression of uterine Bax, phospho p53, caspase-3, PARP and PCNA followed by suppression of the Bcl-2 and AKT in arsenic fed group when compare the respective control group (Fig 10.9). CCPS fairly displayed a crucial role in restoring with the above protein expression of uterine tissue. The expression of Bax, phospho p53, caspase-3, PARP and PCNA at protein level was significantly down-regulated whereas the Bcl-2 and AKT expression were up-regulated following the treatment with CCPS in arsenic treated group (Fig 10.9).

10.3.11. Effect of CCPS on apoptotic and necrotic factor at gene level in uterus

Fig 10.10 is showing that the gene expression of the uterine Bax, p53, TNF-α, and NF-κB significantly up-regulated with the exposure to arsenic when compared to the control group. CCPS in arsenic-treated group finally down-regulated the expression of above uterine gene (Fig 10.10).

10.3.12. Effect of CCPS on histo-morphology

Impairment of uterine layers and uterine secretory gland's loss were observed in arsenic fed group when compared with the control (Fig 10.11B). CCPS in arsenic fed group everted back the arsenic-induced degenerating uterine layers (Fig 10.11). CCPS exhibited its control over the loss of secretory glands in comparison with the arsenic fed group. Fig.10.11G shows that numbers of growing follicles were reduced and replaced by extensive follicular atresia in the arsenic challanged group in contrast to the control group. CCPS in the arsenic fed group

retrived the above condition with increasing number of growing follicles in comparison with the arsenic fed group (Fig 10.11).

10.3.13. Effect of dietary CCPS on infertility

Arsenic fed pregnant rats had notably reduced body weight in contrast to control rat (Fig 10.12C). CCPS in arsenic ingested group maintained the pregnant rat's body weight towatds control. Moreover, arsenic-treated rats delivered increasing numbers of dead pups with deformities (Fig 10.12D, 12E, 12F) low birth weight of the pup's. A higher mortality rate was also observed in pups following arsenication. Dietary CCPS in arsenic-treated pregnant rats was able to deliver healthy pups with reduced mortality in arsenic challanged rats (Fig 10.12D, 12E, 12F).

Table10.1.

Groups	Initial body weight (g)	Final body weight (g)	Ovary in pair (g%)	Uterus in pair (g%)
Control	83.1±4.93	98.1±3.06	0.058±0.005	0.187±0.021
As^{III} (10 mg/ Kg BW)	86.6±4.21	93.5±5.09	0.041±0.003*	0.141±0.047
CCPS (2.0 mg/ Kg BW)	90.4±6.83	88.9±6.98	0.057±0.002#	0.216±0.012
As^{III} + CCPS (10+2.0 mg/ Kg BW)	87.4±7	89.5±8.06	0.056±0.002#	0.198±0.014
As^{III} + DMSA (10 + 50 mg/ Kg BW)	84.3±5.02	92.6±5.45	0.054±0.003#	0.177±0.016

Table 10.1. Represents the effects of curative mode of arsenic and CCPS on body weight, organo-somatic indices (uterus & ovary). These data were represented as mean ± SE, N=6 using one way ANOVA in association with Dunnett's post Hoc t-test. Significant variations of data were expressed at *$p<0.05$ with the control group with vehicle and at #$p<0.05$, with the arsenic challenged group.

Figure 10.1.

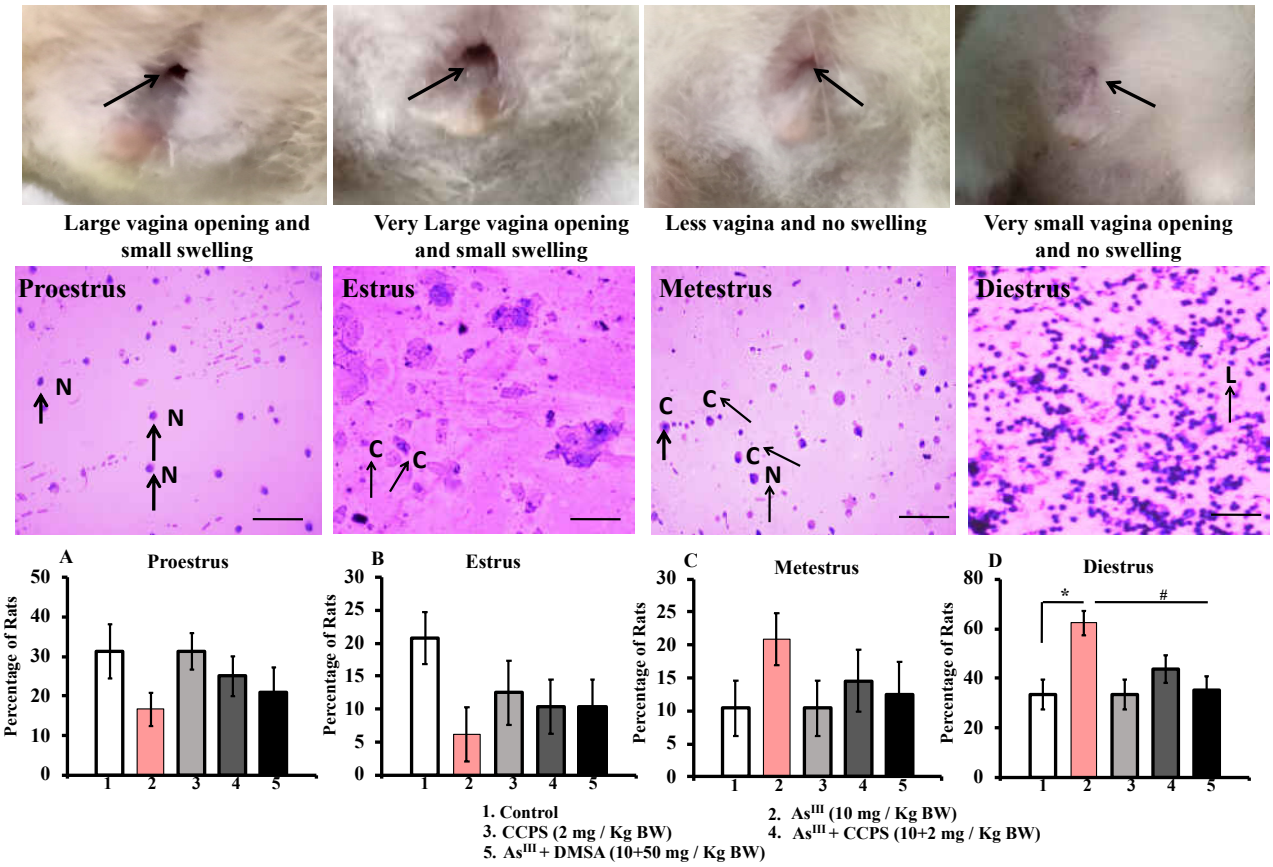


Fig 10.1. Represents the curative effects of CCPS on estrous cycle patterns. Scale bar in the cytological assessment is represented at 50 mm.

Figure 10.2A.

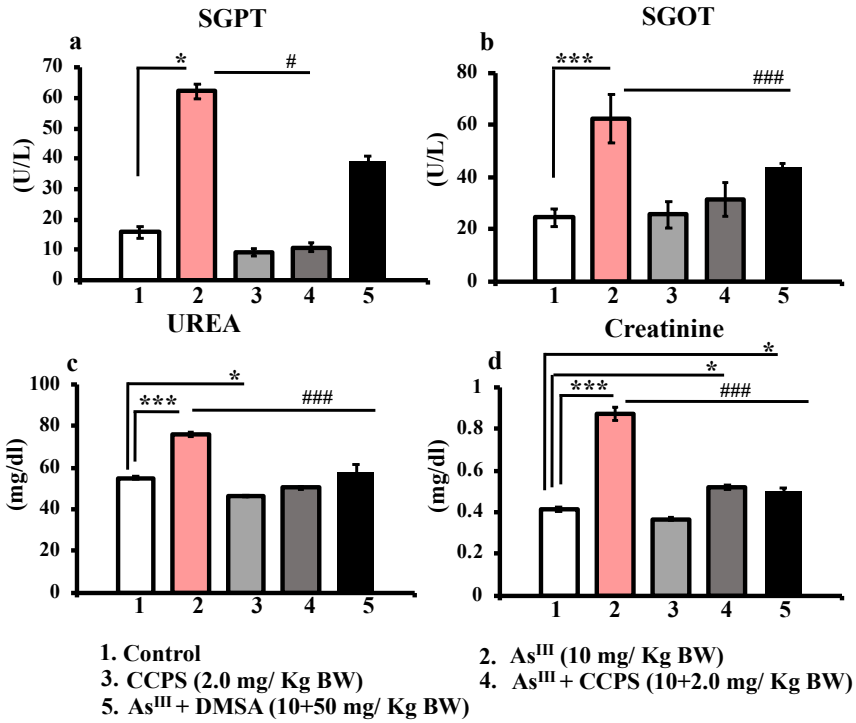


Fig 10.2A. Represents the curative effect of CCPS in arsenicated rats on liver markers. These data were expressed as means ± SE, N=6 using one way ANOVA in association with Dunnett's post Hoc t-test. Significant variations of data were expressed at *p<0.05, and ***p<0.001 control group with vehicle and at #p<0.05, and ###p<0.001 with the arsenic challenged group.

Figure 10.2B.

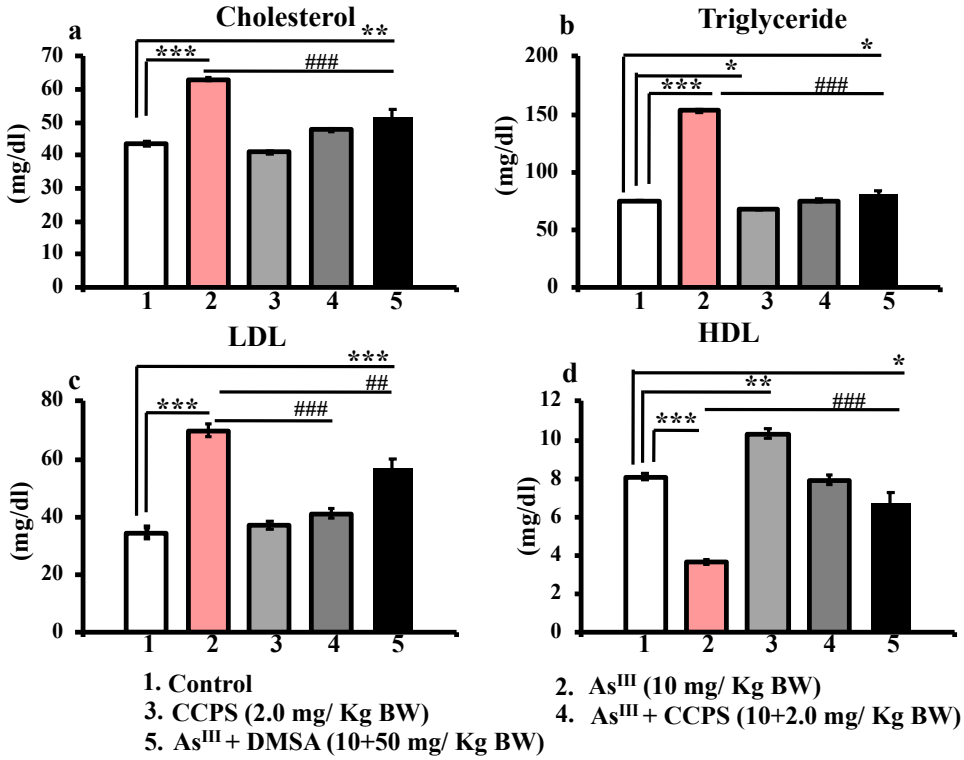


Fig 10.2B. Represents the curative effect of CCPS in arsenicated rats on the lipid profiles. These deta were represented as means ± SE, N=6 using one way ANOVA in association with Dunnett's post Hoc t-test. Significant variations of data were expressed at *p<0.05, **p<0.01 and ***p<0.001 control group with vehicle and at ##p<0.01, and ###p<0.001 with the arsenic challenged group.

Figure 10.3.

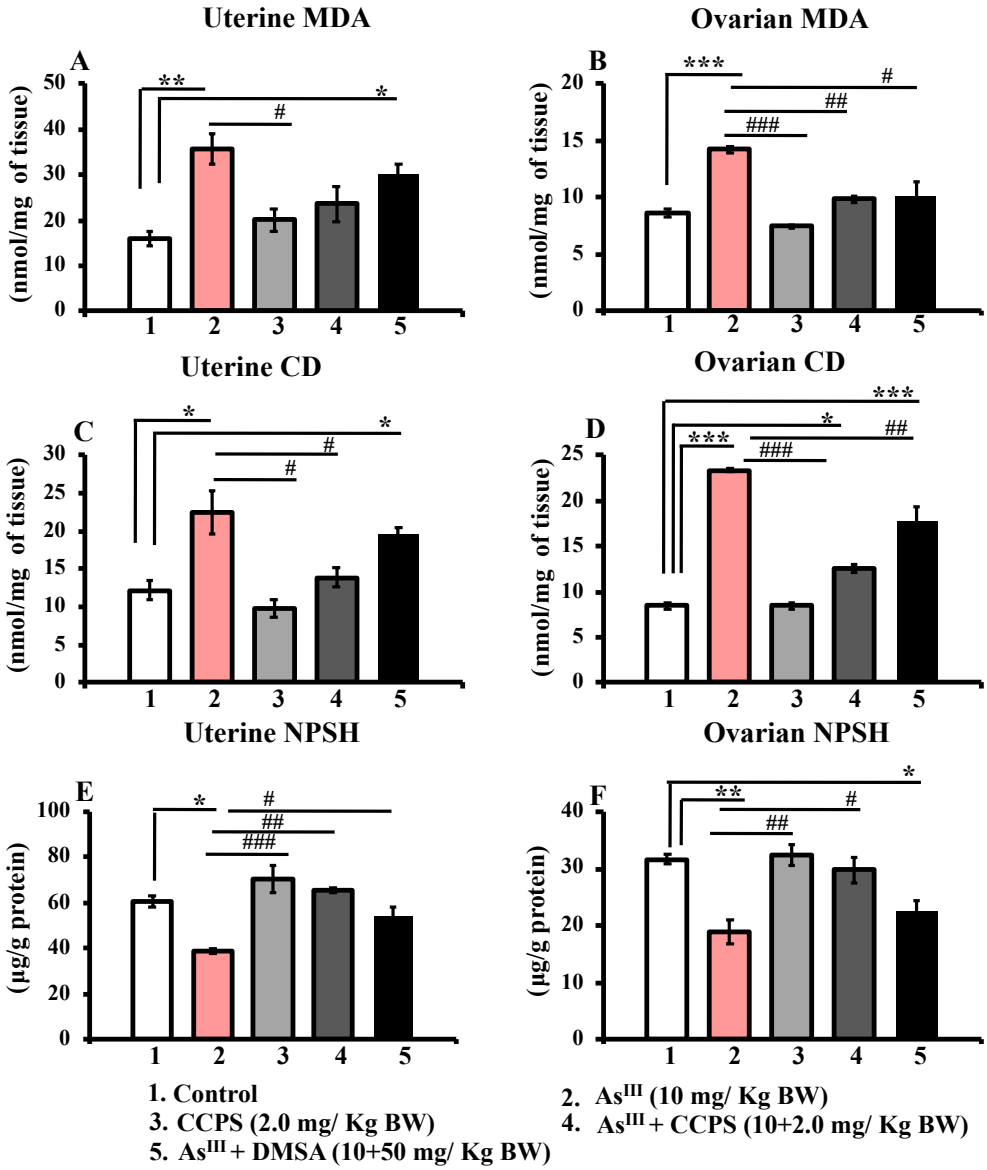


Fig 10.3. Represents the curative effects of CCPS on the uterine and ovarian oxidative stress markers against arsenic-treated rats by spectrophotometric evaluation. These results were represented as mean ± SE, N=6 using one way ANOVA in association with Dunnett's post Hoc t-test. Significant variations of data were expressed at *p<0.05, **p<0.01 and ***p<0.001 when control group with vehicle and at #p<0.05, ##p<0.01 and ###p<0.001 with the arsenic challenged group.

Figure 10.4A.

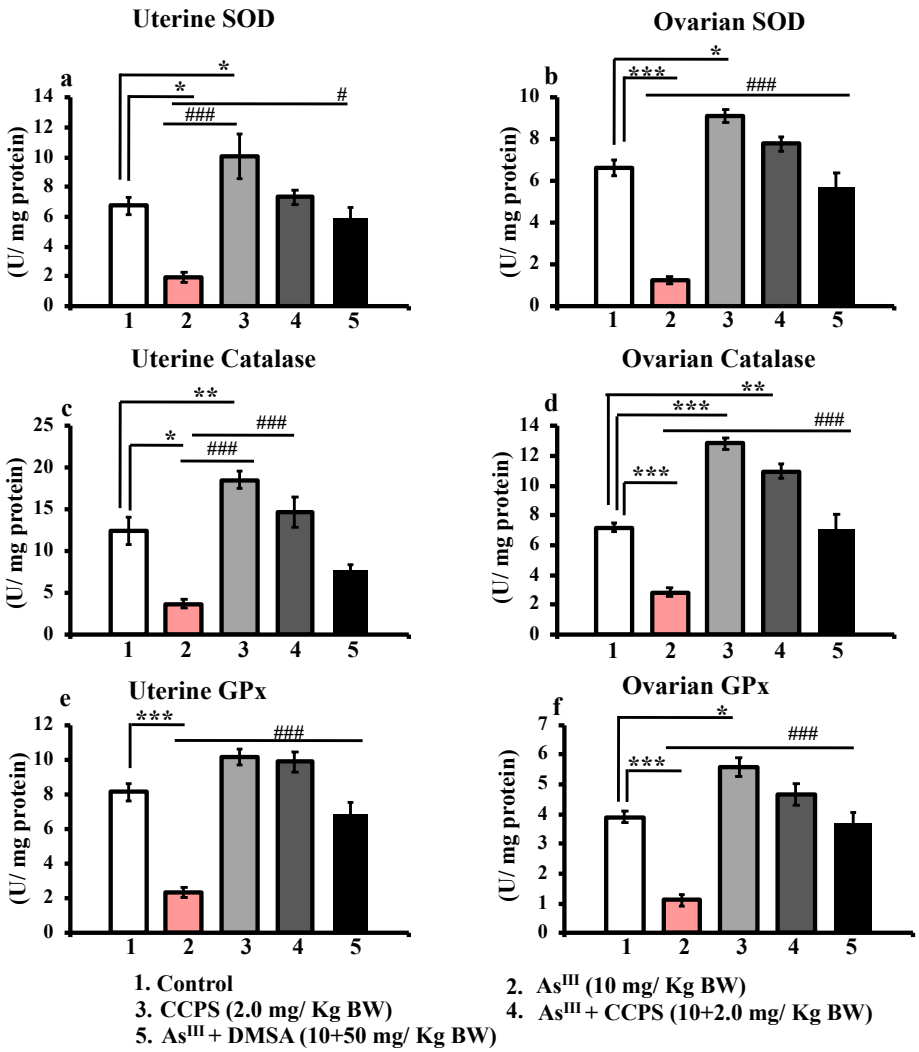


Fig 10.4A. Represents the curative effects of CCPS on the uterine and ovarian antioxidant enzymes activity against arsenic-treated rats when evaluated spectrophotometrically. These results were represented as means ± SE, N=6 using one way ANOVA in association with Dunnett's post Hoc t-test. Significant variations of data were expressed at the level of *p<0.05, **p<0.01 and ***p<0.001 when compared control with vehicle group and #p<0.05, and ###p<0.001 when compared with arsenic challenged group.

Figure 10.4B.

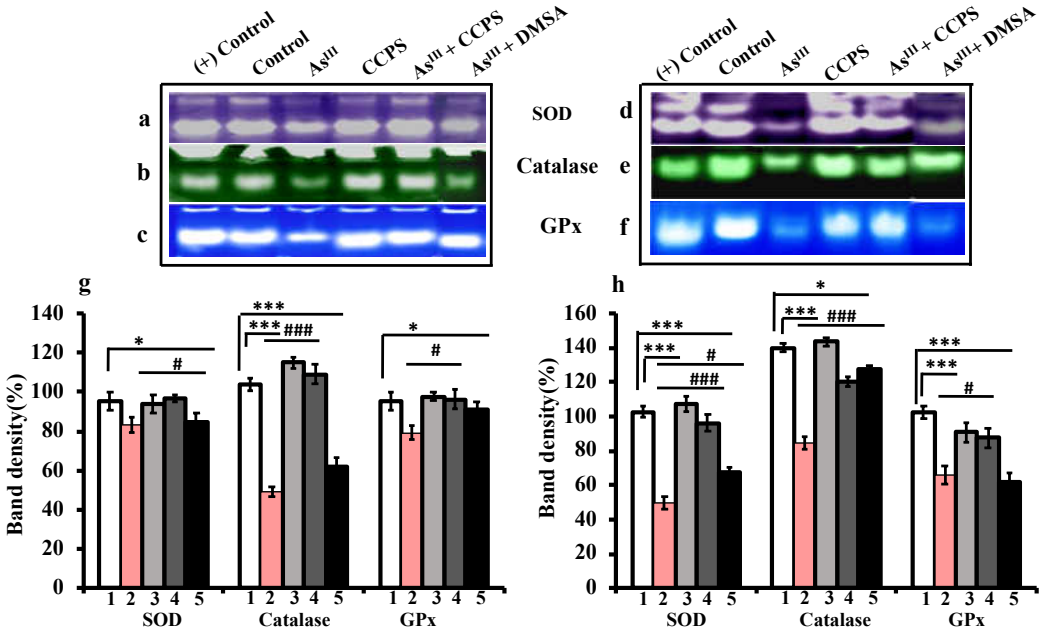


Fig 10.4B. CCPS showed a remedial effect on uterine and ovarian antioxidant enzymes (SOD, catalase and GPx) activities against arsenic treated rats. These results were represented as mean ± SE, N=6 using one way ANOVA in association with Dunnett's post Hoc t-test. Significant variations of data were expressed at the level of *p<0.05, and ***p<0.001 when compared control group with vehicle whereas at #p<0.05, and ###p<0.001 with arsenic challenged group.

Figure 10.5.

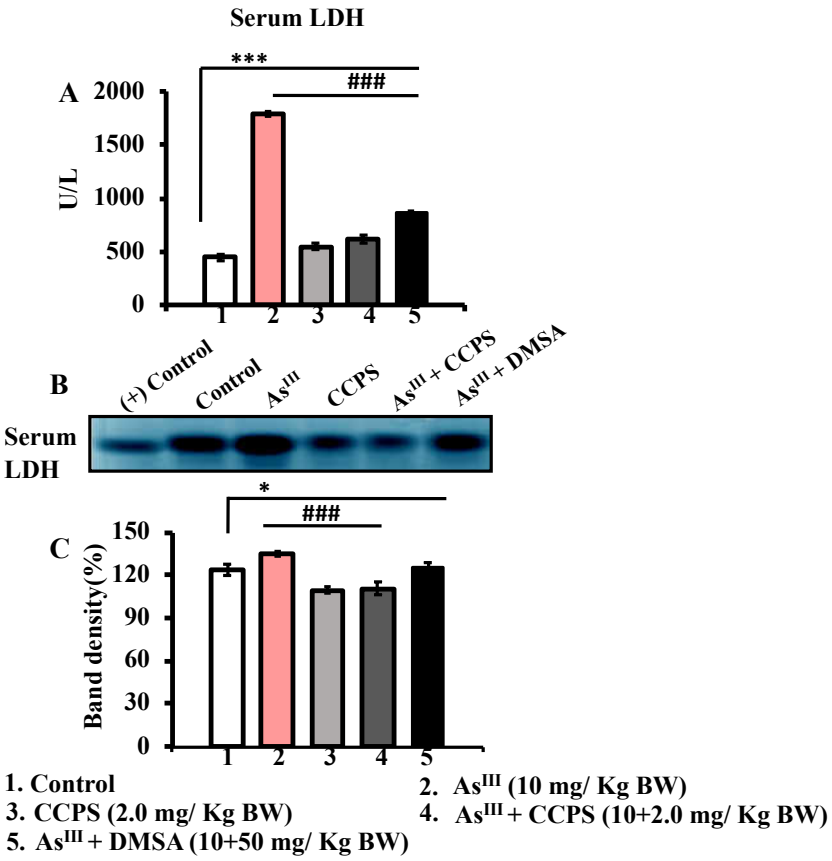


Fig 10.5. Spectrophotometric analysis denoted that CCPS improved the LDH status (5A) in arsenic treated rats. Zymographic data also focused that CCPS restored the serum LDH towards normalcy (5B). Deta here represented as mean ± SE, N=6 using one way ANOVA in association with Dunnett's post Hoc t-test. Significant variations of data were expressed at the level of *p<0.05 and ***p<0.001 compared control group with vehicle and at ###p<0.001 with the arsenic challenged group.

Figure 10.6.

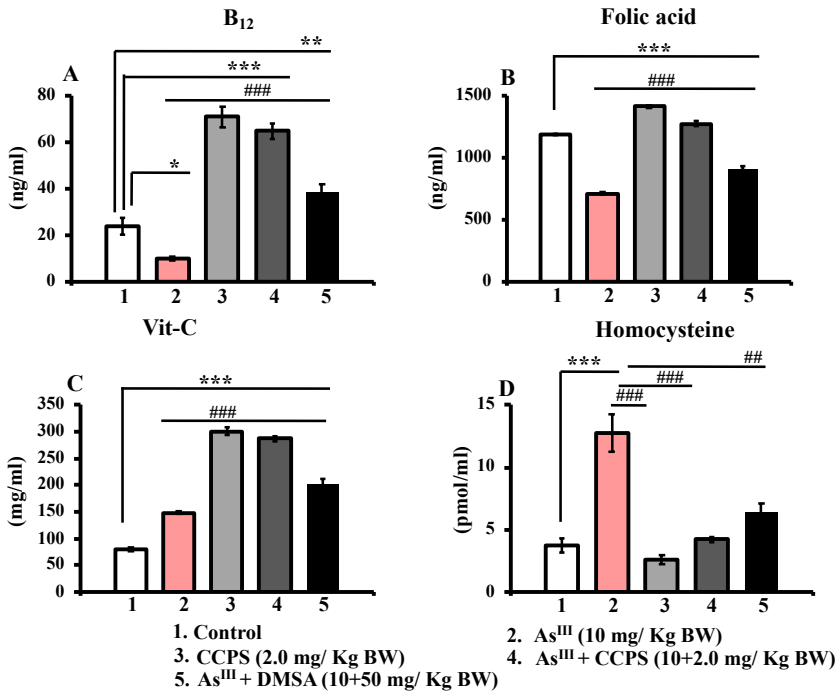


Fig 10.6. Represents the remedial effect of CCPS on circulating level of vitamins and Hcy in arsenic-treated rats. These results were represented as mean ± SE, N=6 using one way ANOVA in association with Dunnett's post Hoc t-test. Significant variations of data were expressed at **p<0.01 and ***p<0.001 which compared the control with vehicle and at ###p<0.001 with arsenic challenged group.

Figure 10.7.

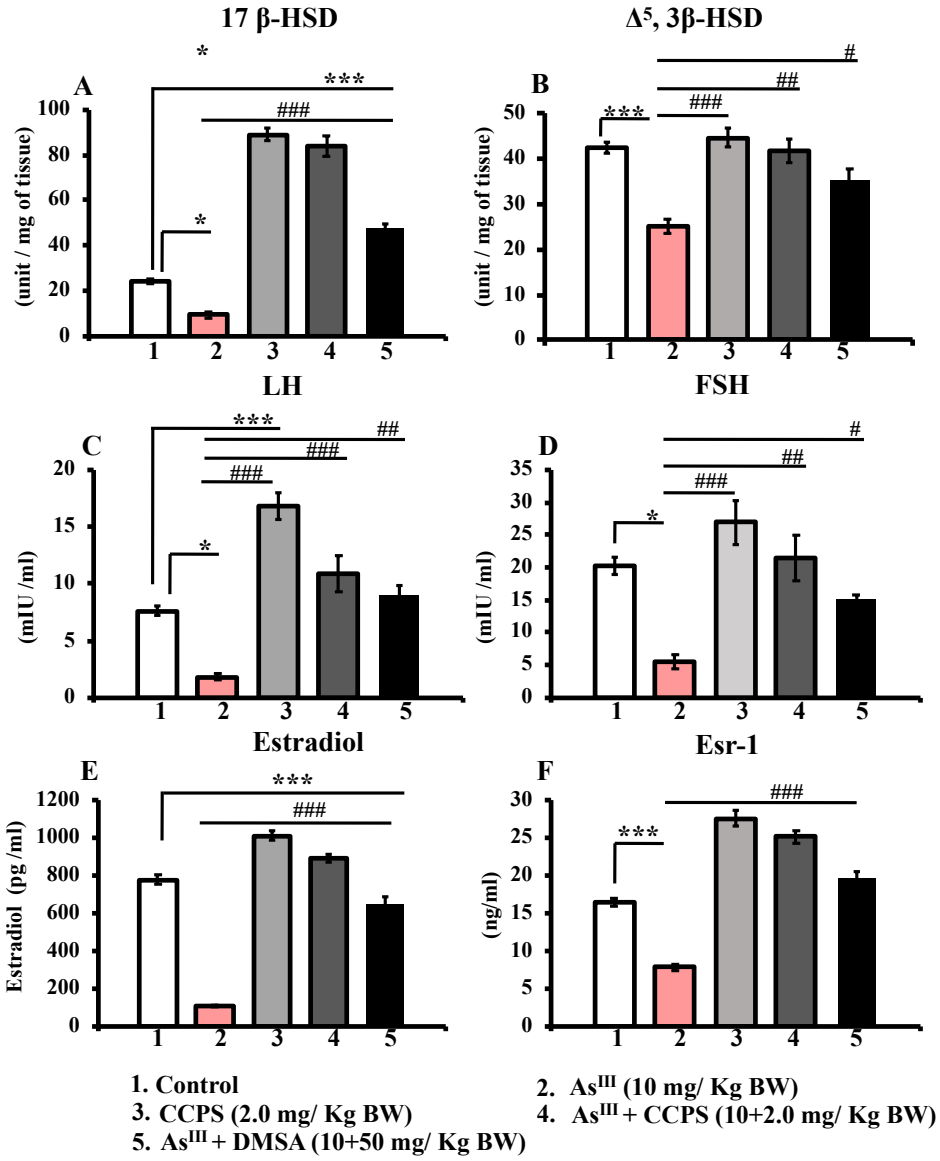


Fig 10.7. Represents the corrective effects of CCPS on the hormonal level, ovarian steroidogenic key enzymatic activities, and the uterine Esr-1 in arsenicated rats. These results were represented as mean ± SE, N=6 using one way ANOVA in association with Dunnett's post Hoc t-test. Significant variations of data were expressed at *p<0.05, and ***p<0.001 level, compared the control with vehicle and at #p<0.05, ##p<0.01 and ###p<0.001 with arsenic challenged group.

Figure 10.8.

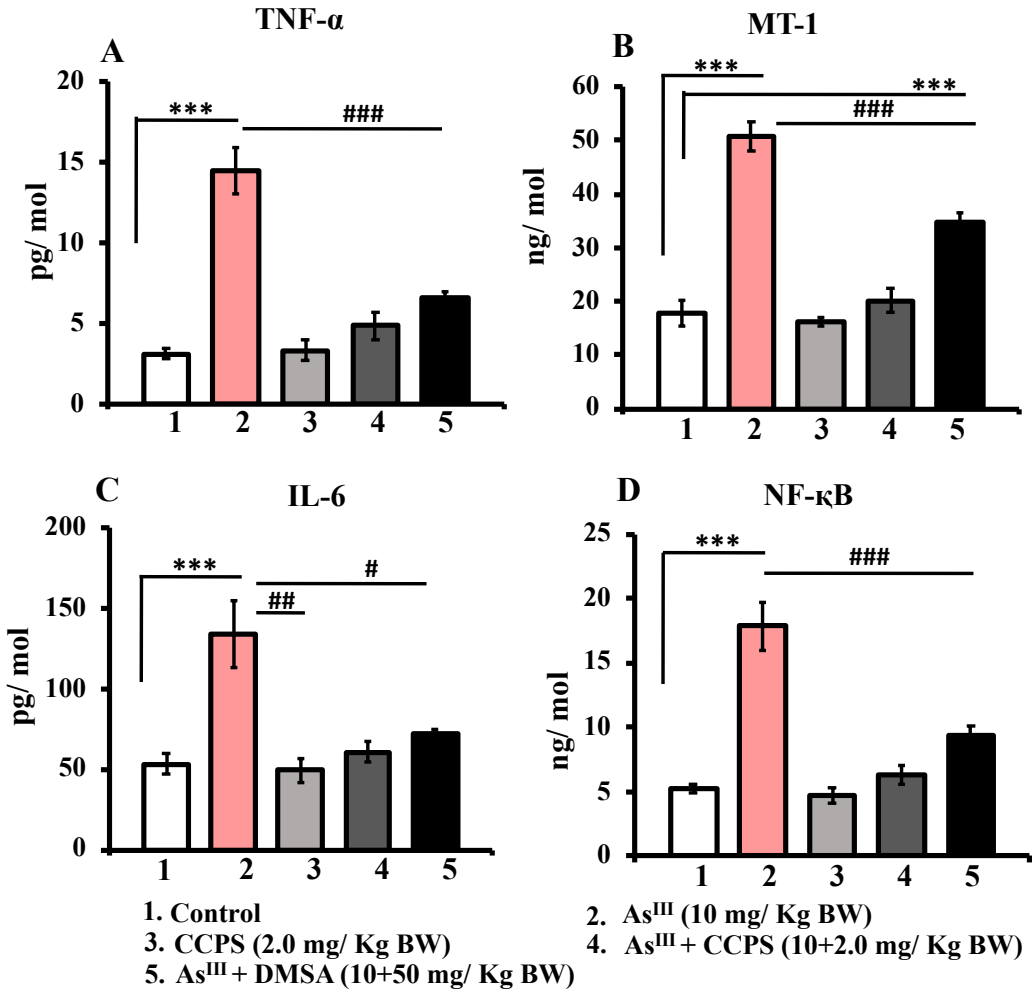


Fig 10.8. Shows the therapeutic efficacy curative of CCPS on the status inflammatory markers against arsenication in rats. These results were represented as mean ± SE, N=6 using one way ANOVA in association with Dunnett's post Hoc t-test. Significant variations of data were expressed at the level of ***$p<0.001$ compared the control with vehicle and #$p<0.05$, ##$p<0.01$ and ###$p<0.001$ with arsenic challenged group.

Figure 10.9.

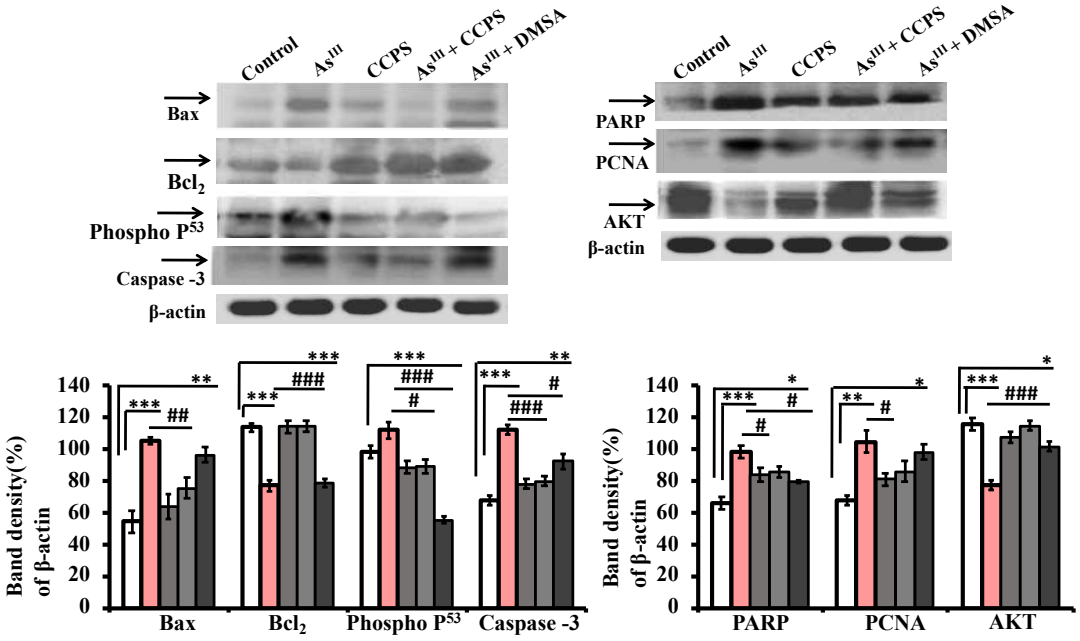


Fig 10.9. The revival mode CCPS action successfully improved the apoptotic protein expression on arsenic- affected rats. These results were represented as mean ± SE, N=6 using one way ANOVA in association with Dunnett's post Hoc t-test. Significant variations of data were expressed at the level of *p<0.05, **p<0.01 and ***p<0.001 compared the control with vehicle and at #p<0.05, ##p<0.01, and ###p<0.001 with arsenic challenged group.

Figure 10.10.

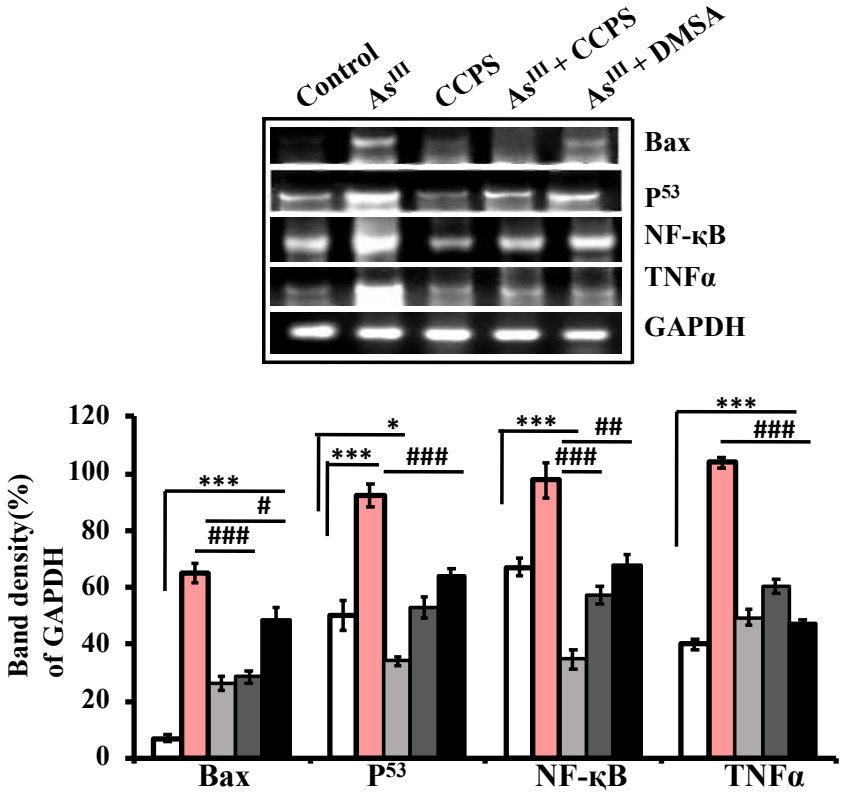


Fig 10.10. The curative mode of CCPS successfully recovered the gene expression in the arsenic- affected rats. These results were represented as mean ± SE, N=6 using one way ANOVA in association with Dunnett's post Hoc t-test. Significant variations of data were expressed at the level of *p<0.05 and ***p<0.001 compared control group with vehicle and at #p<0.05, ##p<0.01, and ###p<0.001 with the arsenic challenged group.

Figure 10.11.

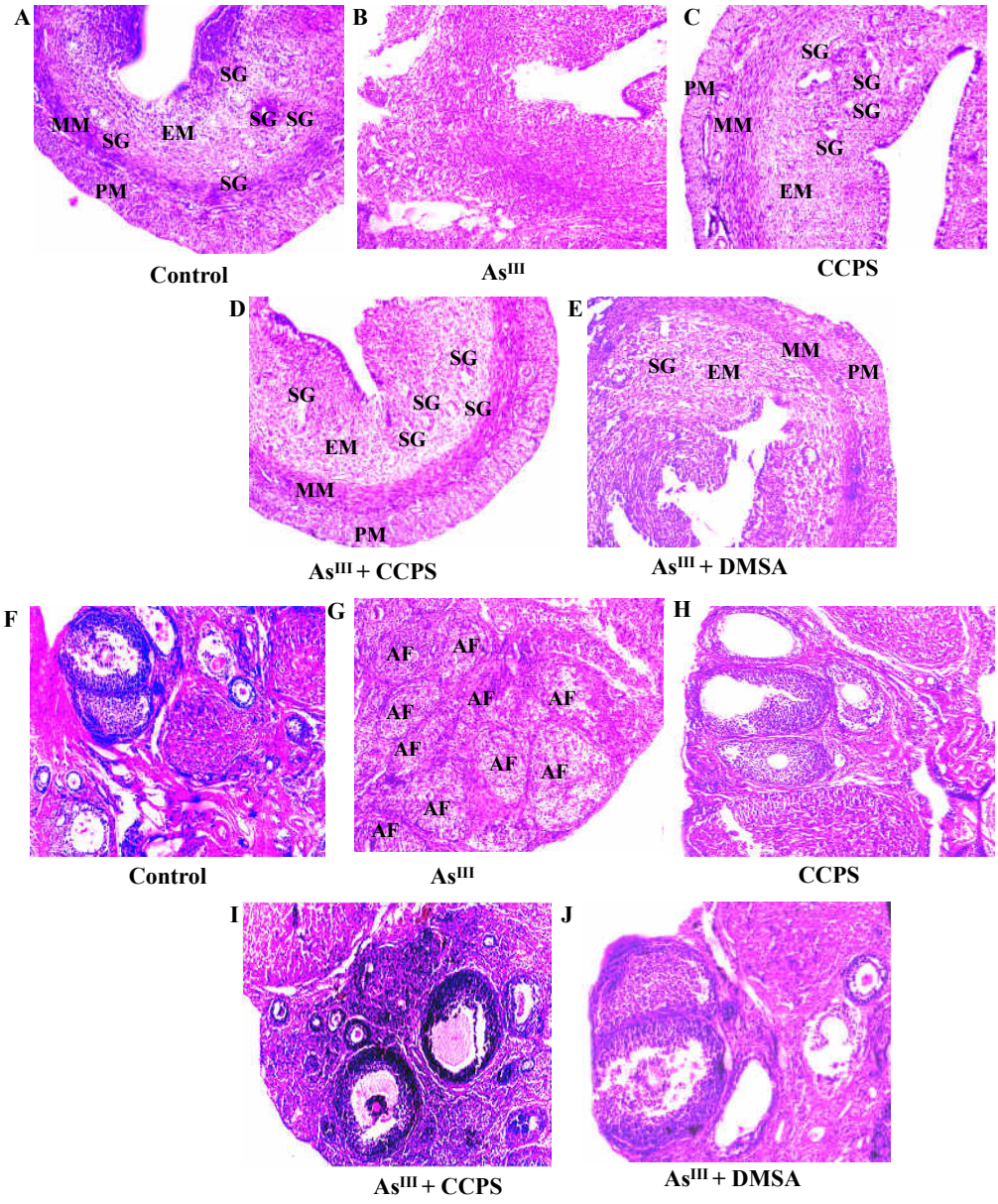


Fig 10.11. Represents the therapeutic potential of CCPS on the sex organs histo morphology in arsenic-ingested rats. Uterine cell morphology revealed the loss of uterine layers (PM- Perimetrium, MM- Myometrium and, EM- Endometrium) along with the loss of secretary glands (SG). Dietary CCPS played a crucial role in rejuvating the uterine layers along with the secretory glands. In ovarian cell morphology showed that, the numbers of atretic follicles were increased following arsenication. CCPS treatment diminished the numbers of ovarian follicular artesia at a considerable level.

Figure 10.12.

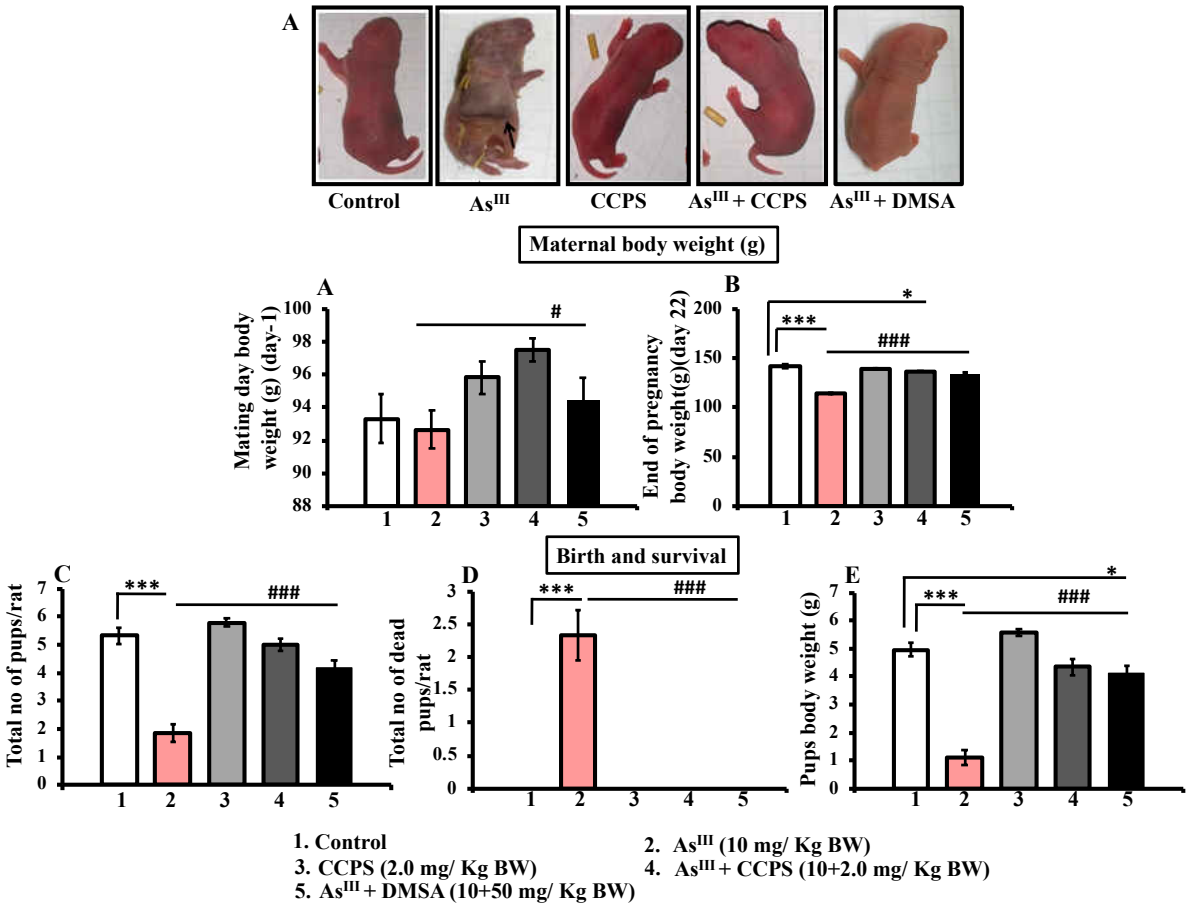


Fig 10.12. CCPS successfully alleviates the arsenic affected female infertility status. CCPS also improved pregnancy outcome. These results were represented as mean ± SE, N=6 using one way ANOVA in association with Dunnett's post Hoc t-test. Significant variations of data were expressed at the level of *p<0.05 and ***p<0.001 compared control group with vehicle and at #p<0.05 and ###p<0.001 with the arsenic challanged group.

10.4. Discussion

In this experiment, it was planned to explore the curative role of the extracted pectic polysaccharide (CCPS) from the bitter gourd against the arsenic mediated toxicity in the female reproductive organs of rats. The different biological systems are interfered due to arsenic exposure and produced oxidative stress in different organs (Agrawal et al., 2014). Earlier study reported that the arsenic ingestion caused inflammation in the peripheral zone of the liver (Liu et al., 1999). These findings have also been noticed in our present investigation. It was observed in this experiment that the liver and lipid function markers were increased in arsenic-treated rats (Fig 10.2A and 2B). It was reported that the hepatocellular apoptosis and necrosis cause an increase in the activities of the serum SGPT and SGOT (Karimov et al., 2002). Present study also revealed that arsenic ingestion caused hyperlipidemia with increased cholesterol, triglyceride, LDL and decreased HDL levels. The present experiment explored that CCPS successfully re-established the liver and kidney functions status (Fig 10. 2A and 2B). CCPS restored the urea and creatinine towards the level of control (Fig 10. 2A and 2B). However, the present study the important role of CCPS in healing the hepato-renal functions. It was established its ability to from protect the liver apoptosis cum necrosis (Mohammad, 2017) with sustaining the hyperlipidemia due to the arsenic toxicity. The intracellular antioxidant enzymes SOD and catalase exerted a positive action on increased of ROS generation due to arsenic exposure. These enzymes also eliminate excessive ROS from different organs. SOD also catalyzes the superoxide-dismutase to H_2O_2 and finally, is eliminated by catalase (Usoh et al., 2005). MDA is used as marker of the oxidative stress generation in the uterus and ovary. In this study it was also observed that uterine and ovarian lipid peroxidation and CD levels were increased in arsenicated rats (Fig 10.3). In addition a significant interaption of uterine and ovarian SOD, catalase and GPx activities were noted in sodium arsenite fed rats. These above results are in agreement with our earlier findings

(Perveen et al., 2019). *Momordica charantia* contains phenols and flavonoids. These phenolic groups and its antioxidant activities have potential interaction with the free radicals. These could be used to terminate the free radical from the body (Kumar et al., 2010). Other investigator reported that *Momordica charantia* could inhibit the stress-mediated lipid peroxidation state followed by reduced activities of SOD, catalase and GPx (Chaturvedi, 2009). Tan et al 2016 reported that polysaccharides are strong scavenger of hydroxyl radicals and superoxide anions in cells. Numerous studies reported that extracted polysaccharide from *Momordica charantia* has an antioxidant property (Gong et al., 2015; Mohammad, 2017). Interestingly, our results also established that the polysaccharides of Momordica exert its action on the interapted antioxidant enzymes. CCPS post-treated rats have been shown low level of uterine and ovarian MDA and CD (Fig 10.3A-3D) along an amplified uterine and ovarian SOD, catalase and GPx expression activity (Fig 10.4A & 4B). Both these results are similar with the investigation done by the group Mohammad et al 2016 where they established CCPS mediated increase the SOD, catalase and GPx activity followed by low levels of MDA and CD in the liver tissue (Mohammad et al., 2016). Arsenic is responsible for an argumated expression which of LDH and it has already been reported by us earlier (Maity et al., 2018). However CCPS in arsenic-treated rats successfully maintained the LDH circulating. It is to be noted that CCPS protects the tissue from necrotic progression by lowering serum LDH (Fig 10.5). Above observations confirmed that oxidative stress is diminshed and thereby limits necrosis by CCPS in arsenic fed rats. This present investigation has be shown to develop diminished activities of steroidogenesis Δ^5, 3β-HSD and 17β-HSD in the arsenic-treated rats (Fig 10.7). Similar findings were achieved by Chattopadhyay et al, 1999 in this regard. The level of serum Estradiol (Fig 10.7E) declined rats possibly by the suppressed activities of the ovarian steroidogenic enzymes (Dash et al., 2018). These steroidogenic enzymes levels are further regulated by the gonadotrophin hormones (Odell et

al, 1963). This investigation also confirmed the existence of low gonadotrophin hormones LH and FSH in arsenic-treated group (Fig 10.7C & 7D). These hormones further reppressed the activity of the ovarian enzymes (Ghersevich et al, 1994). Generally circulating level of estradiol regulates the uterine weight (Kulin and Reiter, 1973) whereas gonadotrophin hormones (LH and FSH) maintain the ovarian weight (Kulin and Reiter, 1973). This experimental outcome revealed that reproductive organs weight was worsen in the arsenic-treated rats (Table 10.1). This type of improvement may involve in two ways direct or indirect action on uterine and ovarian tissues via estradiol. One is that CCPS treatment may improve the dysfunction of the hypothalamic-pituitary–ovarian axis and maintain the ovarian steroidogenesis towards normalcy may be by restoring the gonadotrophin hormones levels near control during the arsenic toxicity (Fig 10.7). The gonadotrophin hormones level increased by CCPS treatment which could successfully stimulate the normal uterine and ovarian growth followed by recovering of synchronized estrous cycle pattern (Fig 10.1). On the other hand CCPS in arsenic treated rats renovation of better ovarian key steroidogenic activities (Fig 10.7A & 7B) may be due to the direct action on the modulation of the Esr-1 receptor (Fig 10.7F). CCPS also played a critical role in recovering the uterine tissue from degeneration of its different layers and by improving the numbers of secretory glands in arsenic intoxicated rats. CCPS acts as a safeguard for the ovary and improves the ovarian folliculogenesis in arsenic challanged rats (Fig 10.11). However findings revealed that CCPS has a critical action on estradiol synthesis resulted in maintenance of uterine and ovarian cell morphology. Transcription factor NF-κB plays a critical role in different inflammatory diseases. NF-κB helps to normalize the development of the pro-inflammatory cytokines IL-6 as well as TNF-α. TNF-α is one of the major inflammatory cytokine which contributes the up-regulattion and activation of NF-κB expression (Ji et al., 2011). Oliveira-Marques et al 2009 reported that NF-κB is the main regulator of oxidative stress which regulates the several

inflammatory genes. NF-κB controls different inflammatory genes those interacting with several inhibitory proteins. Arsenic exposure influenced NF-κB signaling pathway by altering different inflammatory markers (Peters et al., 2015). This finding is also in compliance with our present investigation. In the present study uterine NF-κB, serum IL-6 and TNF-α were up-regulated in arsenic treated rats (Fig 10.8A, 8C and 8D).

Maity et al 2018 and Peng et al 2007 also reported that hepatic MT-1 level pauses ROS generation through the apoptosis via IKK- NF-κB signaling pathway. Here it was noticed that a higher MT-1 level is produced in liver cell due to the arsenic toxicity (Fig 10.8B) as that of previous findings observed by Bhattacharya and Bhattacharya, 2007. Pectic Polysaccharide from bitter gourd has an effective action on different inflammatory markers (Mohammad, 2017). Present result explored that treatment with CCPS on arsenic ingested rats successfully down-regulates uterine NF-κB, serum IL-6 and TNF-α levels (Fig 10.8A, 8C and 8D). In addition, CCPS treatment suppressed the elevation level of hepatic MT-1 level sodium arsenite changed rats (Fig 10.8B). Above information postulates that CCPS can balance the above inflammatory cytokines and pro-inflammatory markers by halting the ROS generation via the NF-κB signaling pathway. This study also endorsed the arsenic feeding in rats regulates the expression of Bax at the protein and gene levels (Fig 10.9 and 10.10). In addition a lower expression of Bcl-2 protein was noted in arsenic exposed group (Fig 10.9). Normally at the physiological condition the anti-apoptotic protein Bcl-2 and pro-apoptotic protein Bax are regulated during arsenic induced apoptosis (Firdaus et al., 2018). Actually, in a normal physiological condition both these can counteract the activity of apoptosis promoting proteins to maintain a balance between the suppression and promotion of apoptosis (Heath-Engel et al., 2008). Generally the over expression of Bcl-2 protein protects the releasing of the cytochrome-c from the damaging cells. ROS generation during arsenic toxicity alters mitochondrial function. Arsenic toxicity also inhibits the cell proliferation and

arrests the cell cycle, and its growth via inhibiting of the caspase-3 signaling pathway (Person et al., 2015; Roussel and Barchowsky, 2000). Here it was found that CCPS treatment effectively reverted back the pro-apoptotic (Bax) and anti-apoptotic protein (Bcl-2) expression status in arsenicated rats (Fig 10.9). From this information it may be illustrated that CCPS treatment successfully reduces the arsenic mediated apoptotic development in uterus. It contributes in suppressing the release of cytochrome-c from the mitochondrial membrane into the cytoplasm via the regulation of the caspase cascade activation. Our study has established a down regulated Akt along with up-regulated p53 protein expression in arsenic-treated group (Fig 10.9). CCPS post treatment effectively inhibits the uterine apoptosis by maintaining the estradiol level via PKB/Akt signaling pathway (Kazi et al., 2009). CCPS consequently balance and downstream expression of Akt in arsenicated rats modulating a reverse antagonistic association between the p53 and PKB/Akt. A higher expression of PCNA protein was observed in arsenic-treated group (Fig 10.9). During DNA replication PCNA contribute as a link between the cellular genotoxicity (Strzalka and Ziemienowicz, 2011). Higher protein expression of PCNA slows down the Akt signaling pathway (Olaisen et al., 2015). CCPS post treatment in arsenic fed rats reduced the elevated trend of PCNA expression (Fig 10.9). Reducing trend of PCNA expression may control the Akt signaling pathway following the treatment with CCPS (Fig 10.9). PARP is also linked with the inflammatory responses in cells. Higher expression of PARP was observed in arsenic fed group (Fig 10.9). PARP encurages TNF-α gene expression via the NF-κB pathway activation (Ba et al., 2011). CCPS in arsenicated rats limits the extent of PARP expression which in turn minimizes inflammatory responses in cells. The methionine cycle is the most important way for the removal of arsenic toxicity by modulating the methylation process with S-adenosyl methionine (SAM) (Rydberg and Lindahl, 1982). Generally SAM acts as a methyl donar. This methyl donar has an important role to eliminate the arsenic toxicity from

the mammals (Tice et al., 1997). A previous study reported that level of SAM is diminished as well as the methylation process is altered due to the arsenic exposure (Ramirez et al., 2005). Our data showed that circulating level of vitamin $B_{12}$and folic acid were diminished along with increased serum Hcy level in arsenic challenged rats (Fig 10.6A and 10.6B). These findings are similar with the results of Maity et al., 2018. Vitamin B_{12} and also folic acid have important role in the methylation process. B_{12} exerts its action on methionine synthase which contributes to add methyl group in the process of methylation. Folate also acts as a co-factor of methionine synthase in methylation process (Henning et al., 1997). Vitamins have important contribution for the prevention of the tissue necrosis in uterine and ovarian cells exposed to arsenic (Mukherjee et al., 2006). The higher level of Hcy due to the arsenic ingestion may induce follicular artesia (Kanakkaparambil et al., 2009). From the above observation it may be enumerated that the follicular development and the oocytes maturation might be suppressed by the hyperhomocysteinemic condition. This hyperhomocysteinemic condition also caused to decrease the estradiol production that may lead to the infertility. This study substantiated that dietary CCPS in arsenic fed rats might be reverted back estradiol production and improved the follicular development following the surge of vitamin B_{12} and folic acid. Those further contributes in the methylation process (Fig 10.6A and 6B). On the other hand here we have tested the female fertility status. CCPS supplementation with diet successfully delivered the healthy pups with no deformities in arsenic challanged rats (Fig10.12). However, dietary CCPS improves pregnancy outcome in arsenic treated rats.

It may be concluded that oral application of CCPS played a crucial and curative as well as therapeutic action against arsenic induced female repro-toxicity. We assumed that presence of galacturonic acid residue in CCPS may interact with the arsenic. CCPS treatment protected the uterine and ovarian damages following the suppression of the arsenic mediated oxidative

stress, inflammation responses and apoptosis via the regulation of Akt signaling pathway. CCPS also improved the fertility status in arsenicated rats improving normal estrous cycle pattern and ovarian steroidogenic activity (Fig 10.13).

Figure 10.13.

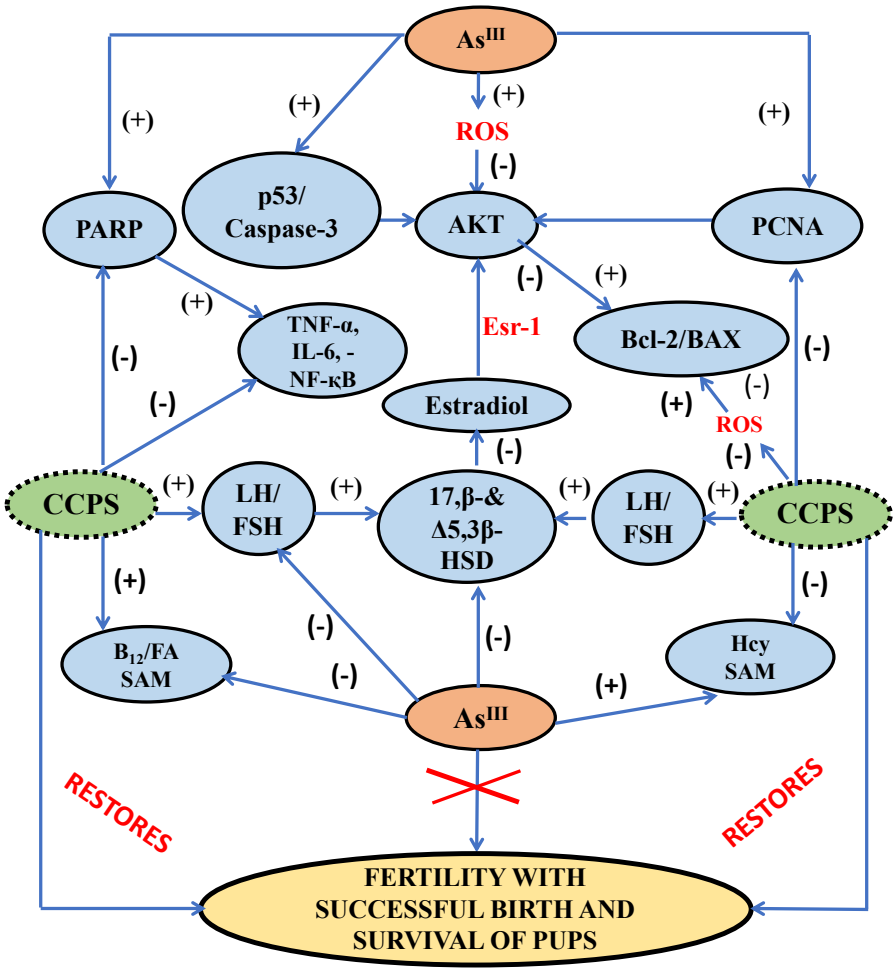


Fig 10.13. Represents mechanism of actions of CCPS against arsenic mediated female repro-toxicity. Here, (+) sign indicate the stimulatory effect (-) sign also indicate inhibitory effect.