CHAPTER-4

EXPERIMENTAL DESIGN

4.0. Experimental Design

Entire experimental work has been carried out on female reproductive organs (uterus and ovary) to delineate the therapeutic and remedial efficiency of NAC to resist sodium arsenite influenced repro-toxicity. All experimental protocols were executed via *in vivo* and *in vitro* mode wherein *in vitro* study was followed in hepatic plus ovarian-uterine tissues. However, only reproductive tissues were selected for *in vivo* investigation. During *in vivo* trial, protective (co-administration), preventive (pre-treatment) and curative (post-treatment via oral gavage and dietary post-treatment) mode of investigations were permitted. The group allocation and study design in various mode of experiment are given below.

Figure 4.1

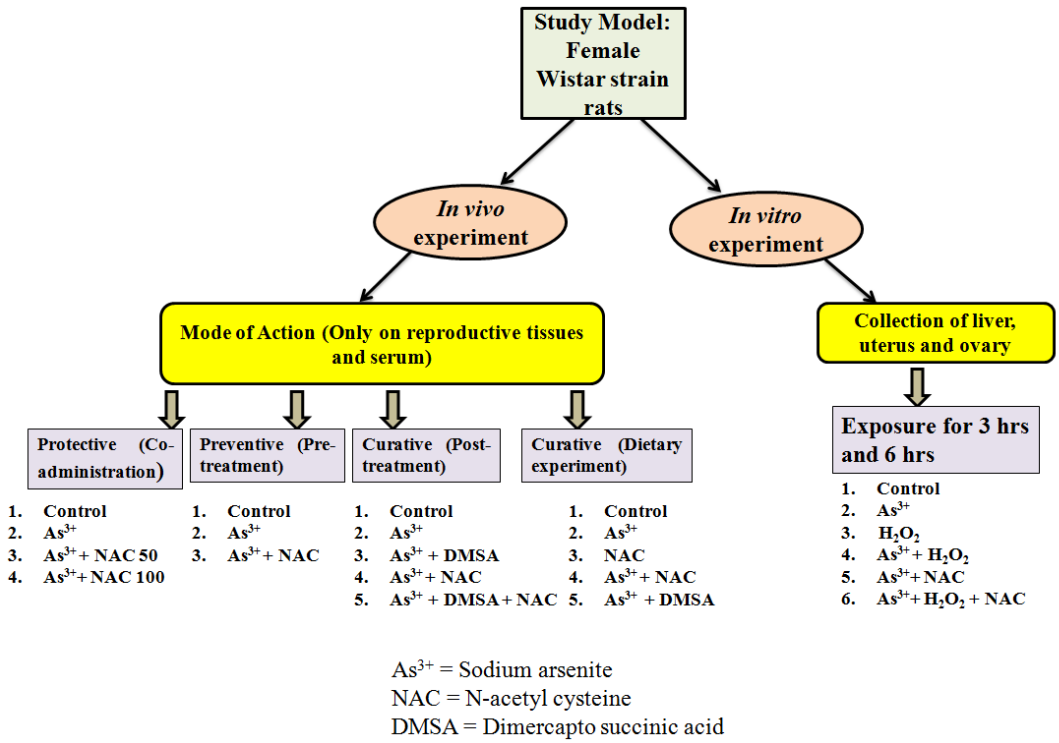

Fig. 4.1. Graphical representation of experimental design of this research work.

Experiment-I

In experiment-I six (6) groups were there; group 1 was vehicled control where no treatment was conducted; group 2 was sodium arsenite administered group (0.6 ppm/2.0 g of liver slices and 0.6 ppm/0.09 g of ovary and uterus tissue slices); group 3 was H_2O_2 treated group (100 mM/2.0 g of liver slices and 0.6 ppm/0.09 g of ovary and uterus tissue slices); group 4 was sodium arsenite plus H_2O_2 treated group; group 5 was sodium arsenite plus NAC (100 mg/2.0 g of liver slices and 100 mg/0.09 g of ovary and uterus tissue slices) treated group; and group 6 was sodium arsenite plus H_2O_2 plus NAC treated group. Here 2.0 g of hepatic slices and 0.09 g of ovary and uterus tissue slices were incubated in 20 ml of Kreb's solution for 3 hrs and 6 hrs respectively. The tissue slices were treated as per the above stated dose of sodium arsenite, H_2O_2 and NAC. This experiment was executed to evaluate the straight forward function of NAC on sodium arsenite guided hepatic and ovarian-uterine malfunction.

Experiment-II

Experiment-II was carried out to notify the protective role of NAC against uterine delinquencies developed by sodium arsenite. Here four (4) groups have been allocated; group 1 was vehicled control where only fresh distilled water was given as vehicle to point out similar physical stress; group 2 was sodium arsenite administered (10 mg/kg body weight) group; group 3 was sodium arsenite plus NAC (50 mg/kg body weight) challenged group; and group 4 was sodium arsenite plus comparatively higher dose of NAC (100 mg/kg body weight) challenged group. To fulfill the dose of 10 mg/kg body weight of sodium arsenite, rats were provided with 1 mg of sodium arsenite per 100 g body weight. A stock solution was prepared with

400 mg of sodium arsenite in 100 ml of distilled water and aliquoted separately in eppendorf tube and stored in refrigerator. Adult rat of 100 g body weight was provided with 250 µl of stock aliquoted solution containing 1 mg of sodium arsenite orally via gavage which is equivalent to 10 mg/kg body weight of sodium arsenite. Considering the dose of NAC, 100 g of rat was provided with 5 mg and 10 mg of NAC respectively which are equivalent to 50 mg/kg body weight and 100 mg/kg body weight accordingly. 2000 mg of NAC was dissolved in 100 ml of distilled water and this stock solution was aliquoted and 100 g of rats were provided with 250 µl stock aliquoted solution containing 5 mg of NAC orally which is equivalent to 50 mg/kg body weight of NAC. On the other hand, 100 ml of stock solution was prepared with 4000 mg of NAC which is equivalent to 100 mg/kg body weight and aliquoted separately. Adult rat of 100 g body weight was given 250 µl of stock aliquoted solution containing 10 mg of NAC and that is equivalent to 100 mg/kg body weight of NAC.

Experiment-III

Experiment-III was pursued to focused the preventive role of NAC wherein three (3) groups were there; group 1 was vehicled control where only fresh distilled water was given as vehicle to consider same physical stress; group 2 was sodium arsenite challenged group (10 mg/kg body weight); and group 3 was sodium arsenite plus NAC (100 mg/kg body weight) challenged group. Here NAC and sodium arsenite were given orally with gavage. The preparation of sodium arsenite and NAC solution were same as experiment-II.

Experiment-IV

Experiment-IV dealt with curative action of NAC and DMSA against sodium arsenite associated repro-anarchy. Here, arsenite was introduced via oral gavage and five (5) groups were assigned; group 1 was vehicled control where only distilled water was given to measure same physical stress; group 2 was sodium arsenite treated (10 mg/kg body weight) group; group 3 was sodium arsenite plus DMSA (100 mg/kg body weight) challenged group; group 4 was sodium arsenite plus NAC (100 mg/kg body weight) challenged group; and group 5 was sodium arsenite plus NAC plus DMSA treated group. NAC and DMSA were treated orally by gavage and the procedure for the preparation of sodium arsenite, NAC and DMSA solution were same as experiment-II.

Experiment-V

Experiment-V corroborated with curative role of NAC against arsenite but here NAC was introduced via basal diet and five (5) groups were considered; group 1 was vehicled control where only fresh distilled water was given as vehicle; group 2 was sodium arsenite ingested group orally (10 mg/kg body weight) wherein sodium arsenite was freshly dissolved in distilled water just prior to treatment and given orally via gavage; group 3 was NAC treated group (250 mg/kg body weight); group 4 was sodium arsenite plus NAC (250 mg/kg body weight) treated group; and group 5 was sodium arsenite plus DMSA (250 mg/kg body weight) treated group. Here NAC and DMSA were separately mixed with the diet and provided. Approximately 5.0 ± 0.5 g of food (dry weight) (Krishnakumari et al., 1979) was allotted for 100 g of animal and 25 mg of NAC and DMSA (powder form) were added separately with food prior to treatment which was equivalent to the dose of 250 mg/kg body weight.