Summary
CHAPTER VI

SUMMARY

- The present study included 187 experimentals (exposed to fireworks chemicals) subjects and 187 unexposed control subjects, matched with age, sex, tobacco and alcohol habits.

- Venous blood samples were collected from the controls and experimentals in two separate blood collection vials, one containing heparin and the other containing EDTA. The heparin tubes with blood samples were utilized for the analyses of the cytogenetic variables like chromosomal aberrations, micronucleus and comet assay. The EDTA tubes with blood samples were utilized for genotypic assays.

- In the present study, experimental subjects were grouped into group I, group II and group III, in accordance with their duration of exposure. Further in each group they were divided into different categories namely tobacco and alcohol user category, only tobacco user category, only alcohol user category and non habit category in order to know the influence of confounding factors on the cytogenetic and genetic variables analyzed. In each group an equal number of age, sex and habit matched controls were included in order to compare the results obtained from experimentals.

- The mean±SD of duration of subjects exposed to fireworks in experimentals was 6.26±0.38 Yrs, 15.44±0.40 Yrs and 29.42±0.8 yrs for the group I, group II and group III respectively. The controls had no previous exposure of fireworks chemicals, but they lived in around and Sivakasi area for more or less similar duration as the exposure duration of experimentals studied.

- The percent of tobacco users in controls were 55.88% (38), alcohol users 4.41% (03), tobacco and alcohol users 8.82% (6) and normal subjects without habits 30.88% (21) in the group I, in the group II tobacco users were 57.35%(39), alcohol user 1.47% (01), tobacco and alcohol user 20.58% (14) and normal subjects 20.58%(14) and in group III only tobacco users 56.25% (24), alcohol user subjects was nil, tobacco and alcohol habitats 41.17% (20) and non tobacco and non alcoholics subjects 13.72%(07).
Likewise in the group I experimentals, percent of tobacco users were 55.88% (38), alcohol user 4.41% (03), tobacco and alcohol users were 8.82% (6) and non tobacco and non alcohol but only chemicals exposure 30.88% (21), in the group II experimentals tobacco users were 57.35% (39), alcohol users 1.47% (01), tobacco and alcohol users 20.58% (14) and only chemicals exposure subjects were 20.58% (14) and in group III only tobacco users were 56.25% (24), alcohol user subjects was nil, tobacco and alcohol habits were 41.17% (20) and non tobacco and non alcoholic workers with only firework chemical exposure was 13.72% (07)

Cytogenetic variables CAs, MN and DNA damage analyses were scored manually for each subject following criteria specified for each. The scored results were subjected to statistical analysis using standard software (SPSS 20.0).

In the present investigation the recruited firework chemical exposed experimental and unexposed control subjects were subjected for the analyses of chromosomal aberrations (Chromatid gaps, breaks, acentric fragment, dicentrics) and micronuclei. Eventually the number of chromosomal aberrations was scored and also the number of micronuclei was scored and micronucleus cell rate (MCR) was calculated and compared between control and experimental groups.

The number of chromosomal aberrations was found to be increased in all experimental groups with certain duration of exposure to chemicals as compared to control groups who had no exposure.

The chromosomal aberration and micronuclei frequency were found to be increased in exposure and habit combined categories of experimentals as compared to exposure alone category of experimental subjects.

Group III firework chemical exposed experimentals showed the maximum aberrations and maximum micronuclei frequency in tobacco and alcohol habit subjects, in tobacco habit subjects, only alcohol subjects, and non habit subjects as compared to other groups I and II.

For comparison within groups of experimentals and controls for the different cytogenetic parameters, Group III was found to have greater amount of
chromosomal aberrations (6.37±2.26 in experimentals and 3.76±1.35 in controls) as compared to group II and group I. The values were significant at $p < 0.05$. Also group II was found to have increased number of aberrations as compared to group I in both experimentals and controls.

- The micronuclei number was found to be higher in all the experimental groups as compared to control groups. Group III experimentals firework chemical exposed showed maximum micronuclei frequency in tobacco and alcohol habit subjects, in tobacco habit subjects, only alcohol subjects, and non habit subjects as compared to other groups I and II.

- For the comparison within groups of experimentals and controls for the different cytogenetic parameters, both in experimentals and controls, Group III was found to have greater amount of MN frequency (39.86±10.17 in experimentals and 24.27±9.05 in controls) as compared to group II (19.75±8.73 in experimentals and 9.66±6.11 in controls) and group I (10.30±7.20 in experimentals and 4.48±4.03 in controls). The values were significant at $P < 0.05$.

- DNA damage (comet assay - SCGE) analyses were also carried out for the subjects of experimentals and controls. The mean values were tested for significance using the $t$ - test. Statistical significance was set at $P < 0.05$.

- In this assay too, all the experimental groups had increased DNA damage as compared to their respective control groups. The group III experimentals were found to have greater damage compared to other experimental groups I and II.

- For the comparison within groups of experimentals and controls for the assessment of DNA damage by Comet Assay, both in experimentals and controls, group III was found to have increased DNA damage (3.082±0.286) in experimentals as compared to group II (2.552±0.403) and group I(2.134 ±0.480) experimentals. The values were significant at $P < 0.05$.

- In controls the values were more or less similar with 0.512±0.114, 0.473±0.057, 0.475±0.039 in group I, II and III respectively.
• The above experiments proved that genotoxicity was found in all experimental groups as compared to controls which can be linked to occupational exposure of firework chemicals at work place of the experimentals. Confounding factors like smoke tobacco, smokeless tobacco, alcohol habits, and age were also found to play a role in the damage.

• In genotyping assay, PCR-RFLP analysis of XRCC1 Arg399Gln and p53 Arg72Pro gene variants were carried out after PCR amplification of genomic DNA.

• In the group I (XRCC1 Arg399Gln) Subjects the Arg/Arg genotypes were prevalent in 39.70% of the controls and 42.64% of the experimental studied. About 52.94% of subjects showed Arg/Gln genotype in control group and 47.05% showed in the experimental group. Only 7.35% of subjects belonging to control and 10.29% of subjects belonging to experimentals had Gln/Gln genotype.

• For p53 Arg72Pro typing in group I, Arg/Arg genotype was observed in 33.82% of control subjects and 35.39% of experimental subjects of group I. Arg/Pro genotype was shown by 52.94% of controls and 47.05% experimentals. About 13.23% subjects in the controls and 17.64% of subjects in the experimentals showed Pro/Pro genotype.

• In the group II for XRCC1 Arg399Gln typing, Arg/Arg genotype was shown by 41.17% of controls and 38.23% of experimentals. The Arg/Gln genotype was shown by 50.0% of controls and 48.52% of experimentals. Regarding the Gln/Gln genotype about 8.82% of controls showed Gln/Gln genotype and 13.23% of individuals showed Gln/Gln genotype.

• For p53 Arg72Pro typing, Arg/Arg genotype was exhibited by 27.94% and 30.88% of controls and experimentals subjects respectively. About 63.23% of control subjects showed Arg/Pro genotype and 58.82% of experimental subjects showed the Arg/Pro genotype. About 8.82% of control subjects and 10.29% of experimental subjects were Pro/Pro genotypes.
In the group III XRCC1 Arg399Gln typing, Arg/Arg genotype was shown by 43.13% of controls and 41.17% of experimentals. The Arg/Gln genotype was shown by 49.01% of controls and 45.09% of experimentals. Regarding the Gln/Gln genotype about 7.84% of controls showed Gln/Gln genotype and 13.72% of individuals showed Gln/Gln genotype.

For p53 Arg72Pro typing, Arg/Arg genotype was exhibited by 19.60% and 23.52% of controls and experimentals subjects respectively. About 74.50% of control subjects showed Arg/Pro genotype and 62.74% of experimental subjects showed the Arg/Pro genotype. About 5.88% of control subjects and 13.72% of experimental subjects were Pro/Pro genotypes.

But due to similarity of genotype distribution in experimental and control groups, lack of any risk to disease conditions like cancer was considered to be absent.