ABSTRACT

Tea is cultivated on a large scale in northern part of West Bengal, India. To keep pests populations under control mixed pesticide formulations are regularly applied in the tea gardens. Pesticides are known to cause serious hazards to environment as well as non-target organisms including human beings. The genotoxic effects of pesticides on the tea workers of North Bengal have not yet been thoroughly studied. Therefore, a multidimensional approach has been conducted to determine the extent of toxic effects induced by pesticides on the worker population. A total of 225 individuals (95 pesticide-exposed individuals, 60 controls, 39 smokers and 31 alcohol consumers) were sampled for the study. Since all males and few females of pesticide-exposed worker group smoked cigarettes and all consumed alcohol, smokers and alcohol consumers were included in the study for comparison. The results based on enzyme, micronuclei, comet assay and CYP2C9 gene polymorphism are summarized below.

Acetylcholinesterase and Butyrylcholinesterase activity

Results showed acetylcholinesterase activity of 11.81±3.40 and 6.43±1.85 µmoles/min/ml in controls and tea garden workers (p<0.001), respectively. The activities in smokers and alcohol consumers were 11.04±2.48 and 12.45±2.58, µmoles/min/ml, respectively. The difference with control was not significant.

Butyrylcholinesterase activities were 4.73±1.84 and 3.50±1.89 µmoles/min/ml in control and exposed groups, respectively. The difference in the activity was significant at p<0.001. Butyrylcholinesterase activities of 5.06±1.87 and 5.02±1.41 µmoles/min/ml

observed in smokers and alcoholics, respectively were not significantly different than controls.

In contrast to a slight increase of acetylcholinesterase activity in the females (6.78±2.21 µmoles/min/ml) than males (6.23±1.59 µmoles/min/ml) of the exposed group, the females had significantly higher activity than males in control group (13.47±4.18 versus 11.15±2.83 µmoles/min/ml, $p \leq 0.05$).

The butyrylcholinesterase activities were 4.08±1.66 and 3.15±1.95 µmoles/min/ml in exposed females and males. The activity was significantly high in females ($p \leq 0.05$), whereas, control females and males did not show significant difference (5.14±1.78 versus 4.57±1.86 µmoles/min/ml) in activity.

The effect of gender appears to have very little influence on the acetylcholinesterase and butyrylcholinesterase activity.

Though not significant but reduced acetylcholinesterase and butyrylcholinesterase activities were observed in the age group 15-30 years than age groups 31-45 and 46-62.

Results showed that acetylcholinesterase and butyrylcholinesterase activities were markedly inhibited in the tea garden workers.

Micronucleus Assay

The micronucleus assay showed a significant increase of micronuclei (9.77±2.66, $p \leq 0.001$), nuclear bud (4.39±1.42, $p \leq 0.001$), binucleate (6.12±2.92, $p \leq 0.001$), karyorrhectic (8.01±2.29, $p \leq 0.001$), pyknotic (5.74±1.81, $p \leq 0.05$) and karyolytic (6.89±2.98, $p \leq 0.001$) nuclei in the exposed group than control. Compared to control, the smokers revealed a higher frequency of micronuclei (6.15±2.47, $p \leq 0.001$), nuclear bud

(4.13±2.35, $p \leq 0.05$), binucleate (4.38±2.24, $p \leq 0.01$), karyorrhectic (6.67±2.30, $p \leq 0.001$) and karyolytic (6.18±2.19, $p \leq 0.01$) nuclei except pyknotic cell. Frequency of binucleate (3.97±1.99, $p \leq 0.05$), karyorrhectic (5.55±1.84, $p \leq 0.05$) and karyolytic (6.29±2.64, $p \leq 0.01$) nuclei was higher in the alcoholics than control. An analysis between smokers, smoking <10 cigarettes per day (group I) and those smoking >10 cigarettes per day (group II) showed higher frequency of micronuclei (7.72±1.60, $p \leq 0.001$), nuclear bud (5.61±2.28, $p \leq 0.001$), binucleate (5.83±1.72, $p \leq 0.001$), karyorrhectic (8.00±1.33, $p \leq 0.01$), pyknotic (6.28±2.05, $p \leq 0.01$) and karyolytic (7.72±1.32, $p \leq 0.001$) cells in group II. A higher proportion of micronuclei (10.87±2.38, $p \leq 0.001$), nuclear bud (4.90±1.35, $p \leq 0.001$) and binucleate nuclei (6.98±3.04, $p \leq 0.001$) were detected in the pesticide exposed males than females. The results indicated a synergistic effect of pesticide, smoking and alcohol on cellular damage. Age and duration of exposure have no influence on the micronucleus and other cell death parameters.

Comet assay

The comet assay showed a mean tail length of 45.98±4.25 and 15.14±2.99 μm in the tea garden workers (exposed) and the control individuals, respectively which was about 3 times longer in the worker ($p \leq 0.001$). The smokers and alcohol consumers revealed tail length of 16.83±2.60 and 14.24±1.32 μm, almost similar to that of controls. The mean tail moment and olive tail moment were 8.86±1.18 and 6.41±0.78 in exposed workers and 2.89±0.48 and 2.32±0.36 in controls, respectively indicating significantly higher damage in the workers ($p \leq 0.001$). The smokers and the alcoholics showed a mean tail moment and olive tail moment of 3.96±0.89 and 2.69±0.48 and 3.19±0.55 and 2.60±0.42, respectively which were statistically not different than control.

Age, gender and duration of exposure had no effect on DNA damage.

CYP2C9 polymorphism

The polymorphism of CYP2C9 gene was studied in 94 pesticide-exposed tea garden workers and 130 non-tea garden workers. All the individuals were analysed by PCR-RFLP for CYP2C9 allelic variants compared to wild type.

CYP2C9*1 allele was most predominant both in tea garden workers and non-tea garden workers, present at a frequency of 0.87235 (77.66% of individuals) and 0.87695 (78.46% of individuals), respectively. 2.13% tea garden workers and 2.30% non-tea garden workers were homozygous for CYP2C9*3 allele showing frequency of 0.10105 and 0.09615. No homozygotes for CYP2C9*2 allele were detected in any groups. The CYP2C9*2 allele was least frequent with a frequency of 0.0266 and 0.0269 in the tea garden and the non-tea garden worker population, respectively.

Genotyping revealed that one individual was heterozygous for CYP2C9*2/*3 genotype in each of the worker and non-tea garden worker population with a frequency of 1.06% and 0.77%, respectively. In tea garden worker population, 4.26% and 14.89% were heterozygous for CYP2C9*1/*2 and CYP2C9*1/*3 alleles, while 4.62% and 13.85% were heterozygous for CYP2C9*1/*2 and CYP2C9*1/*3 alleles, respectively in non-tea garden worker population. The allele frequency in pesticide exposed worker population is comparable to those of non-tea garden workers. The observed and expected frequency distribution indicated that the results are in good agreement with that of the expected as calculated by the Hardy-Weinberg law.