SUMMARY

Essential oils extracted from the aromatic grasses belonging to the genera *Cymbopogon* and *Vetiveria* are of enormous commercial value for environmental, agricultural, food and medical applications as well as in perfumery and aromatherapy. The karyomorphological data of *Cymbopogon martini*, *Cymbopogon winterianus*, *Cymbopogon citratus* and *Vetiveria zizanioides* revealed that the somatic chromosome number is 2n=20. Detailed analysis showed that the karyotypes are nearly symmetrical with chromosomes having median to sub-median centromeres. The total chromatin length is largest in *Vetiveria zizanioides* whereas it is smallest in *Cymbopogon winterianus*.

The essential oils palmarosa, citronella, lemongrass and vetiver acetate were studied for safety/toxicity evaluation. Treatment of the essential oils on human lymphocytes demonstrated that they were toxic at concentrations much higher than used for human consumption. Palmarosa and citronella oils were found to be cytotoxic at concentrations of 1000 µg/ml whereas lemongrass and vetiver acetate oils were cytotoxic at 100 µg/ml. The mode of cell death was investigated at concentrations that resulted in significant cytotoxicity. Lymphocytes were incubated with palmarosa and citronella oils at concentrations 1000, 1500 and 2000 µg/ml whereas treatment concentrations of lemongrass and vetiver acetate oils were 200, 400 and 800 µg/ml. Cell death was found to be primarily due induction of early and late apoptosis as revealed by the flow cytometry analysis.

Similarly, palmarosa and citronella oils induced significant DNA damage at concentration 1000 µg/ml whereas lemongrass and vetiver acetate oils were genotoxic at concentrations 100 µg/ml as revealed by results of alkaline comet assay and DNA diffusion assay. Reactive oxygen species (ROS) are considered to play an important role in genotoxicity and cell death. Lymphocytes were incubated with palmarosa and citronella oils at concentrations 1000, 1500 and 2000 µg/ml whereas treatment concentrations of lemongrass and vetiver acetate oils were 200, 400 and 800 µg/ml.
Treatment with the essential oils except palmarosa oil significantly increased the level of fluorescent dichlorfluorescein (DCF) in lymphocytes which is an index of ROS level.

The antigenotoxic properties of the four essential oils were tested at the non genotoxic concentrations against an alkylating agent methyl methanesulphonate (MMS) and a strong oxidant hydrogen peroxide (H$_2$O$_2$) and that induces DNA strand-breaks (~65-70 % tail DNA) in human lymphocytes. The four essential oils tested could reduce the genotoxicity of MMS and H$_2$O$_2$ as DNA damage decreased significantly in all the treated sets. Our findings showed that the essential oils confer higher protective effect against an oxidizing agent (hydrogen peroxide) than an alkylating agent (methyl methanesulphonate), indicating their protective efficacy against cellular oxidative stress.

It is well known that the antioxidants protect cells against the deleterious effects of reactive oxygen species either by scavenging them or converting them to non toxic compounds or chelating the ions required for their activation. Among the cellular molecules, lipids that contain unsaturated fatty acids with more than one double bond are particularly susceptible to the action of free radicals. Since inhibition of lipid peroxidation and free radical scavenging activities are attributed to the reduction in damaging effects of ROS, further studies were done to study their antioxidant activity. Significant inhibition of lipid peroxidation was exhibited by the essential oils with the exception of vetiver acetate oil (10 µg/ml). The free radical scavenging capacity of the essential oils was evaluated by means of DPPH assay. Methanolic solutions of the essential oils significantly quenched DPPH free radical in a dose-dependent manner. In conclusion we can say that palmarosa, citronella, lemongrass and vetiver acetate oils are safe. They can be used as antioxidant in therapeutic uses for human being.

The protective activity of the essential oils was further validated in Swiss albino mice against cisplatin-induced toxicities in kidney and bone marrow cells. Cisplatin is a widely used anticancer drug whose clinical application at high dosage is limited due to its deleterious side effects mediated by generation of reactive oxygen species. The major side effects include nephrotoxicity, hepatotoxicity, ototoxicity, myelosuppression and spermatotoxicity.
The essential oils (palmarosa/citronella/lemongrass/vetiver acetate) administered orally to mice at concentrations 5, 10 and 20 mg/kg body weight for 7 days were found to be non toxic. Cisplatin treatment lead to significant reduction in the body weight of mice and organo-somatic indices of kidneys were elevated compared to the control group. Cisplatin-induced nephrotoxicity was indicated by histopathological examination of kidney tissues and serum biomarkers such as increased levels of blood urea nitrogen (BUN) and serum creatinine. Renal oxidative stress was evident from increased levels of protein oxidation and lipid peroxidation with simultaneous decrease in reduced glutathione content and glutathione-S-transferase activity.

Priming animals with the essential oils could improve the effect of cisplatin on the body weight of mice. With the exception of citronella oil (20 mg/kg body weight), the organo-somatic index of kidney was significantly reduced in all the treatment concentrations of the essential oils. The essential oils prevented cisplatin-induced necrosis of epithelial renal cells as observed in the histopathology of renal tissues. BUN was reduced significantly at all the treatment concentrations of the four essential oils with the exception of lemongrass oil (20 mg/kg body weight). Serum creatinine levels were significantly decreased by administration of the essential oils at concentrations 5 and 10 mg/kg body weight. Oxidative stress in kidney was mitigated by the administration of the essential oils. Protein oxidation was reduced significantly by the essential oils except that by vetiver acetate oil (20 mg/kg body weight) whereas lipid peroxidation was reduced significantly at all the concentrations (5, 10 and 20 mg/kg body weight). GSH content was increased significantly at the lowest concentration of 5 mg/kg body weight of the essential oils. The activity of the antioxidant enzyme GST was restored to the control levels by priming animals with the essential oils prior to cisplatin treatment.

Cisplatin induces DNA damage in the target organs kidney and bone marrow cells of mice. The clastogenic effect of cisplatin in bone marrow cells was assessed by chromosome aberration test and the rate of cell division (mitotic index) was also recorded. There was significant clastogenicity and reduction in the mitotic division of
the bone marrow cells. Cisplatin treatment also leads to cell cycle arrest at the G1 phase of cell division. Cisplatin-induced myelosuppression was reflected by alterations in the hematological parameters. Parameters such as total white blood cells (WBC), red blood cell (RBC) count, hemoglobin (Hb), hematocrit value (HCT) and platelet (PLT) count were reduced by the administration of cisplatin.

Oral administration of mice with the essential oils (palmarosa/ citronella/ lemongrass/ vetiver acetate) prior to cisplatin treatment significantly reduced DNA strand breaks in kidney tissue at the lowest dose (5 mg/kg body weight) and bone marrow cells at all the doses (5, 10 and 20 mg/kg body weight). Suppression of mitotic index and clastogenicity of bone marrow cells was restored to the normal values at the lowest concentration of the essential oils (5 mg/kg body weight). Normal distribution of the cells in the divisional phases was also restored. The hematological parameters such as total WBC, RBC and platelet count increased due to gavage of the essential oils with simultaneous increase in hemoglobin content and hematocrit values. Thus, the four essential oils could significantly ameliorate kidney and bone marrow toxicity by their free radical scavenging activities.

To conclude we can say that the essential oils palmarosa, citronella, lemongrass and vetiver acetate are safe for human use at low concentrations.