Applied Biological Research 24(4): 494-502; (2022)
DOI: 10.5958/0974-4517.2022.00059.3

MONITORING OF HERBICIDE ALACHLOR-PLASMID DNA INTERACTION BY SPECTROSCOPY TECHNIQUES

Suraya Jabeen, Zarina Arif, Asim Badar and Khursheed Alam*

Department of Biochemistry, Jawaharlal Nehru Medical College, Faculty of Medicine, Aligarh Muslim University, Aligarh – 202 002, Uttar Pradesh (India)
*e-mail: *kalam786@rediffmail.com*

(Received 28 August, 2022; accepted 20 November, 2022)

ABSTRACT

Alachlor [2-chloro-2,6-diethyl-N-(methoxymethyl) acetamide] is a widely applied herbicide, mainly used to control weeds. It is a substance of eco-toxicological concern, labeled as suspected carcinogen. Alachlor is metabolized by human cytochrome P-450 monooxygenase system. Alachlor or its metabolites may cause cellular damage through oxidative modification of DNA and other macromolecules. In present study, alachlor-induced changes in plasmid DNA were studied by UV, fluorescence, circular dichorism, agarose gel electrophoresis, HPLC, and thermal denaturation. Of the four bases, guanine was preferred by alachlor for adduct formation. The results suggest that alachlor may cause impairment in the structure of DNA, affect its functions and enter into pathological phase. Further, the workers engaged in its production or handling are at high the risk of damaging macromolecules and organs (especially liver) vis-à-vis onset of diseases. Whether or not they may develop cancer will largely depend on the load of alachlor absorbed/inhaled, its interaction with DNA/gene accompanied or followed by activation of certain proto-oncogenes and defective or poor repair system.

Keywords: Alachlor, cancer, DNA, guanine-DNA adduct

INTRODUCTION

Herbicides are used in agriculture to get rid of weeds selectively without injuring crops (Das *et al*, 2016). The long-term and chronic exposure to different herbicides may accumulate in various tissues of the exposed organisms and cause adverse toxic effects including cancer (Tu *et al*., 2013). In aquatic animals, the herbicides have induced reproductive toxicity (Chang *et al.,* 2013), immunotoxicity (Wang *et al.,* 2017) and produced DNA strand breaks (Yang *et al.,* 2021). The published reports suggest a wide range of chemicals and/or herbicides may induce toxic effects via oxidative stress (Xiang *et al.,* 2018). The toxic effects of alachlor, butachlor and metachlor have been attributed to the induction of oxidative stress (Xu *et al.,* 2015). Pesticides, plasticizers, disinfectant's by-products, heavy metals, and organic pollutants have been associated with carcinogenesis, chronic diseases, endocrine disruptions, and congenital anomalies/malformations (Vieira *et al.,* 2016).

Alachlor [2-chloro-2,6-diethyl-N-(methoxymethyl) acetamide] is mainly used to control weeds in the fields of corn, soybean, peanut, etc. (Das *et al.,* 2016). It is amongst the thirty three priority substances listed in the European Water Framework Directive (2000/60/EC) for which environmental monitoring programs are in place (Gil *et al*., 2011). Alachlor is an ortho-disubstituted chloro-acetanilide herbicide and shares structural similarity with propachlor, acetochlor, metolachlor and several other organochloride herbicides. It is toxic to aquatic animals, and accumulation in fish species may represent a risk to predators and human consumers. In rats, alachlor and its metabolites have been

found to be hepatotoxic, nephrotoxic, and to cause damage to the nasal mucosa, the eye, and the thyroid. It is considered aa a probable human carcinogen (Gil *et al*., 2011). United States Environmental Protection Agency has categorized alachlor as a probable human carcinogen.

DNA is quite often the main molecular target of the chemical substances in the environment; thereby people facing increased risk of diseases and many types of cancer are partly attributed to the dramatic changes in DNA physiological functions (Campos-Carrillo *et al*., 2020). Pesticides are one class of the chemical matters of concern, due to their potential effects of carcinogenicity, teratogenicity, and mutagenicity. Studies have indicated that alachlor can induce DNA damage, mutagenicity, and cytotoxicity (Ojha *et al*., 2013). Alachlor-induced DNA single strand breaks have been reported *in vivo* in rat hepatocytes, brain tissue and human peripheral blood lymphocytes (Ojha *et al*., 2013). Mutagenicity testing in eukaryotes showed alachlor to be genotoxic as such or in the presence of various activation systems. *In vivo* treatment with alachlor induced chromosomal aberrations in bone marrow cells of Wistar rats (Soloneski and Larramendy, 2010) and in cultured Chinese hamster ovary cells (Soloneski and Larramendy, 2010). Increased thyroid, stomach and nasal tumors were observed in Long-Evans and Sprague-Dawley rats exposed to alachlor (Gadagbui *et al.*, 2010). Furthermore, alachlor also produces chromosomal damage in human lymphocytes *in vitro* (Dwivedi *et al.*, 2012 Alachlor and its metabolites 2-chloro-N-(2,6-diethylphenyl) acetamide [CDEPA] and 2,6-diethylaniline [DEA] form covalent derivatives with DNA and protein (Dwivedi *et al.*, 2012); its metabolites covalently bind to the cysteinyl moieties of hemoglobin and plasma proteins forming adduct (Dwivedi *et al.*, 2012). Boerth *et al.* (2008) synthesized and characterized adduct of alachlor and CDEPA with 2´-deoxyguanosine, 2´-deoxythymidine and their 3´-monophosphate derivatives. The formation of DNA adduct(s) and generation of single strand breaks by genotoxic chemicals are critical steps in the initiation of cancer. The present study was aimed to examine the interaction between alachlor and plasmid DNA (pUC 19) using techniques like UV, florescence, circular dichorism (CD), agarose gel electrophoresis, thermal denaturation, and HPLC.

MATERIALS AND METHODS

Chemicals

Alachlor, ethidium bromide, sodium azide, adenine, guanine, cytosine, and thymine bases and dialysis tubing were purchased from Sigma Chemical Company, USA. The remaining reagents and chemicals were of the highest analytical grade available.

Isolation of plasmid DNA

Plasmid DNA (pUC 18) was isolated in a highly purified form by gentle lysis of the bacterial cells (*E. coli*, TB1 strain) followed by centrifugation to remove bulk of chromosomal DNA using plasmid isolation kit purchased from Qiagen, USA (Soleimani *et al*., 2016).

Modification of plasmid DNA (pUC18) by alachlor

Plasmid DNA was modified with alachlor as described previously (Dong *et al*., 2015). Briefly, 30 μM plasmid DNA was mixed with varying concentrations of alachlor (30, 150 and 300 μM) in TE buffer (10 mM tris-HCl containing 1 mM EDTA, pH 7.5) and incubated for 72 hat 37°C. After incubation, the samples were extensively dialyzed against TE buffer to remove unbound alachlor.

UV spectroscopy

The ultraviolet absorption of plasmid DNA, alachlor and alachlor-modified plasmid DNA samples were studied in the wavelength range of 200-400 nm (Tantry *et al.*, 2018) on a spectrophotometer (Shimadzu, UV/Vis 240).

Fluorescence measurement

Fluorescence emission profiles of plasmid DNA and alachlor-modified plasmid DNA were recorded on a spectrofluorometer (Shimadzu RF-5301) in presence of ethidium bromide and at an excitation wavelength of 310 nm (Dixit *et al.,* 2014).

Circular dichroism (CD) spectroscopy

CD profiles of plasmid DNA and alachlor-modified plasmid DNA were recorded on spectropolarimeter (Jasco-J720). The measurements were taken in 1 mm path length cell at 25°C in the wavelength range of 220-350 nm. All scans were recorded at an interval of 0.2 nm. The base line was adjusted with PBS, pH 7.4 and the plasmid DNA was used at 0.03 mM concentration. Molar ellipticities [θ] were calculated in terms of base pair concentration according to the following equation (Charak *et al.,* 2011):

$$[\theta] = \frac{\theta}{10 \times c \times d}$$

Where, [θ] is measured ellipticity in mdeg, 'c' is the molar concentration of plasmid DNA per base pair and 'd' is the path length.

Agarose gel electrophoresis

Single strand breaks in plasmid DNA were analysed on agarose gel **(**Sakuma *et al.,* 2021). Native and alachlor-modified DNA samples were subjected to 0.8% agarose gel electrophoresis under alkaline conditions and migration pattern was noted. The gel was prepared in alkaline electrophoresis buffer (50 mM NaOH and 1 mM EDTA, pH 8.0). Samples were precipitated with ethanol and dissolved in alkaline loading buffer (50 mM NaOH, 1 mM EDTA, 5-10% glycerol, 0.025% bromophenol blue), loaded into the wells and electrophoresed for 8 h at 2 V cm^{-1}. DNA bands were visualized under UV light after staining with ethidium bromide (0.5 mg mL^{-1}) [Tantry *et al.,* 2018] and photographed.

High performance liquid chromatography (HPLC)

Alachlor-treated guanine, adenine, thymine, and cytosine were analyzed by HPLC on Biologic Duo Flow System equipped with UV-visible multiwavelength detector; absorbance was monitored at 254 nm. Fifty microlitres each of 50 µM guanine, adenine, thymine or cytosine or their respective modified counterparts was loaded onto C-18 reversed phase column (Supelco Discovery, 25 cm x 4.6 mm; pore size, 5 µM). The elution was carried out with 12.5 mM citric acid, 25 mM sodium acetate, 25 µM ethylene diamine tetraacetate (EDTA), pH 5.2 buffer at a flow rate of 1 mL min^{-1}. The formation of alachlor-base adduct was confirmed from changes in the retention time compared to the retention time of their respective controls (Brohi *et al*., 2016).

Thermal denaturation of alachlor-modified-plasmid DNA

Heat-induced denaturation of alachlor-modified plasmid DNA was carried out from 30 to 95°C on automated melting device attached to a spectrophotometer (Shimadzu, UV-240 equipped with temperature programmer and controller). The temperature was raised at a rate of 1.5°C min^{-1} after 10 min equilibration at 30°C. The changes in absorbance at 260 nm were recorded and percent denaturation was calculated using the following formula (Tantry *et al*., 2018):

$$\text{Percent denaturation} = \frac{A_T - A_{30}}{A_{max} - A_{30}} \times 100$$

Where, A_T is the absorbance at different temperatures, A_{max} is the maximum final absorbance at 95°C and A_{30} is the initial absorbance at 30°C.

RESULTS AND DISCUSSION

Chemical carcinogens as such or their metabolites may bind to nucleic acids and form covalent adducts. Occupational exposure to herbicides may cause chromosomal alterations (Langie *et al*., 2015). Alachlor is a pre- and early post-emergent chloroacetanilide herbicide widely used to control

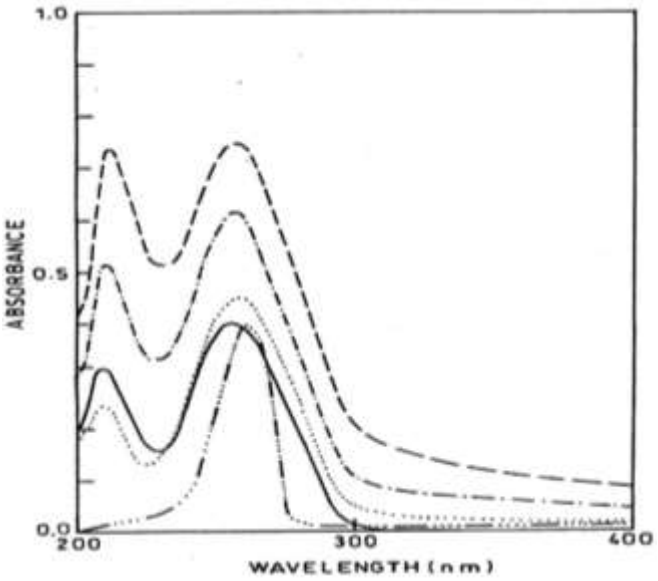

Fig. 1: UV absorption profile of 30 μM plasmid DNA (——), and alachlor (−·····−). Plasmid DNA modified with 30 μM alachlor (−−−), 150 μM alachlor (−·−·−), and 300 μM alachlor (·····)

annual grasses and broadleaf weeds in corn, soybean, peanut, etc. (Mohanty and Jena, 2019). It is metabolized by cytochrome P-450 monooxygenase system (Nykiel-Szymańska *et al.*, 2018). In human, CYP 3A4 isoform is most abundantly expressed and is responsible for metabolism of alachlor to 2-chloro-N-(2,6-diethylphenyl) acetamide [CDEPA] and 2,6-diethylaniline [DEA] which on further oxidation yields an unstable intermediate 3,5-diethyl benzoquinone-4-imine [DEBQI] capable of binding with the macromolecules (Li and Lampe, 2019). Herbicides, insecticides, and heavy metals continuously generate reactive oxygen species which can oxidize proteins, cause lipid peroxidation and DNA damage (Agrawal and Sharma, 2010). In present study several concentrations of alachlor (30, 150 and 300 μM) was mixed with 30 μM bp plasmid DNA and incubated for72 h at 37°C. After incubation, excess alachlor was removed by dialysis against TE buffer (10 mM Tris-HCl, 1 mM Na_2-EDTA, pH 7.5).

UV-spectroscopy of native and alachlor-modified plasmid DNA

Compared to the control plasmid, the 03 versions (30, 150 and 300 μM) of alachlor-treated plasmid DNA exhibited 46.6, 34.42, and 11.11% hyperchromicity, respectively, at 260 nm (Fig. 1). Moreover, the trend of hyperchromicity was inversely related to the alachlor amount. The λ_{max} for alachlor was observed at 270 nm compared to 260 nm λ_{max} for plasmid DNA. The hyperchromicity exhibited by alachlor modified plasmid DNA may be due to the consequence of single strand breaks, loosening of stacked bases, destabilization of hydrogen bonds and increased exposure of chromogenic bases due to unstacking (Tripathi *et al.*, 2014).

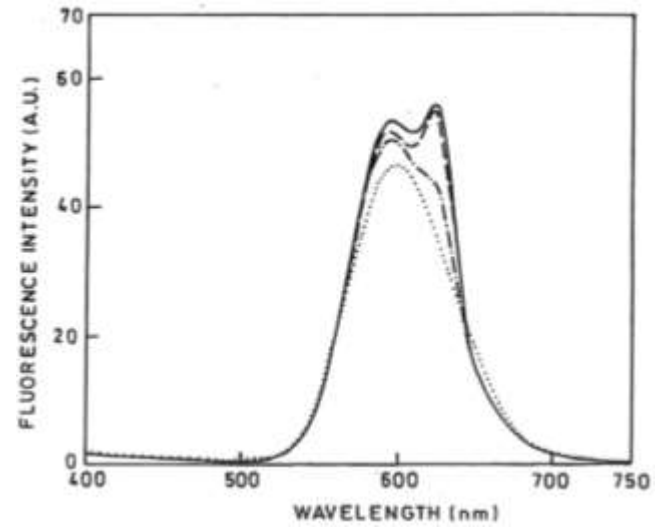

Fig. 2: Emission profile of ethidium bromide (·····), ethidium bromide mixed with plasmid DNA (—), plasmid DNA with 150 μM alachlor (−·−·−), and plasmid DNA with 300 μM alachlor (– – –).

Fluorescence analysis of native and alachlor-modified-plasmid DNA

Neither plasmid DNA nor alachlor has its own fluorescence; therefore, an extrinsic fluorophore, ethidium bromide, was used to peep into the structure of plasmid DNA and its alachlor-modified forms. Plasmid DNA and its alachlor counterpart were incubated with ethidium bromide for 30 min and emission profile were recorded using excitation wavelength of ethidium bromide (310 nm). The results are depicted in Fig. 2. Ethidium bromide showed an emission peak at around 585 nm. Its interaction with plasmid DNA

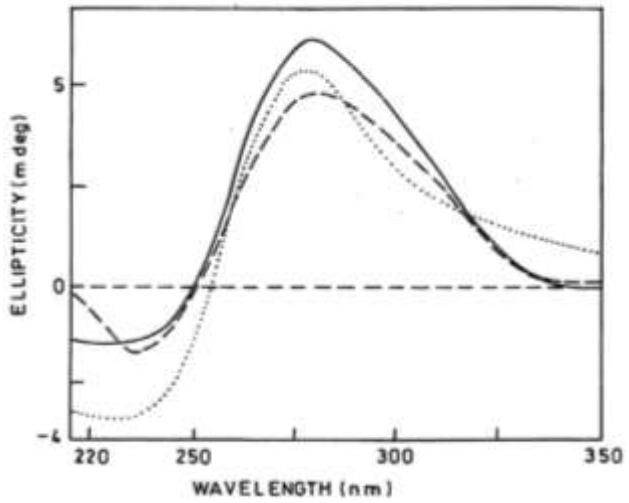

Fig. 3: Circular dichroic profile of 30 µM plasmid DNA (——), plasmid DNA with 150 µM alachlor (– – –), and plasmid DNA with 300 µM alachlor(·····)

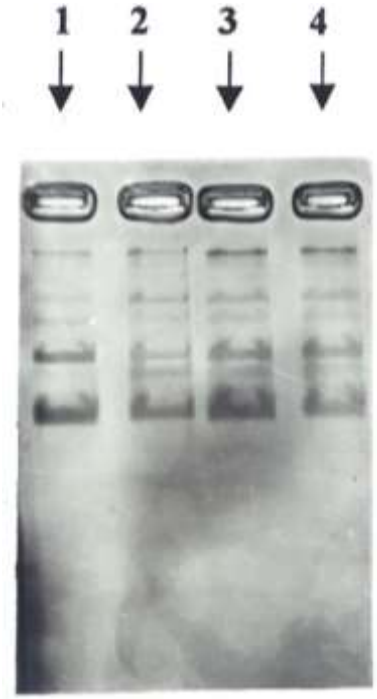

Fig. 4: Electroporetic pattern of plasmid DNA (lane 1). Lanes 2-4 show plasmid DNA treated with 30 µM, 150 µM, and 300 µM alachlor. Electrophoresis was carried out on 0.8% agarose gel for 2 h at 25 mA current.

caused enhancement in fluorescence intensity of 585 nm peak and birth of a new peak at 635 nm. The profile of plasmid DNA modified with 300 µM alachlor was almost over-lapping with control plasmid DNA spectrum, except a slight decrease in fluorescence intensity at 585 nm peak. Furthermore, the plasmid DNA modified with 150 µM alachlor showed significant decrease in fluorescence intensity of 585 nm peak; 635 nm peak completely vanished and only kink remained, signifying alachlor-induced changes in the plasmid DNA. The decrease in fluorescence intensity of 585 nm peak (for 300 and 150 µM alachlor-modified plasmid DNA) indicated perturbations in structural integrity of plasmid DNA vis-à-vis poor intercalation of ethidium bromide (Dixit *et al.,* 2014). Taken together, the UV and fluorescence results clearly suggested relaxations in plasmid DNA, produced by alachlor.

Circular dichroism (CD) profiles of native and alachlor-modified-plasmid DNA

The CD spectrum of plasmid DNA (30 µM bp) gave a positive ellipticity in the wavelength range of 220-350 nm with a maximum at 278 nm (Fig. 3). However, plasmid DNA modified with 150 and 300 µM alachlor showed ellipticity in the range of 220-350 nm but there was decrease in 278 nm positive ellipticity values to the extent of 9.25 and 5.55%, respectively. The circular dichroic studies of alachlor (150 µM) modified plasmid DNA showed 9.25% decrease in ellipticity; whereas its 300 µM modified analog showed 5.55% decrease as compared to the control plasmid. The change in the ellipticity after modification by alachlor could be due to unstacking of base pairs vis-a-vis helix destabilization (Ahmad *et al.,* 2016). This simply indicates that alachlor has produced perturbations in plasmid DNA without changing its overall conformation.

Agarose gel electrophoresis

The resolving power of agarose gels for topoisomers is excellent but more highly supercoiled species co-migrate as a broad band. Plasmid DNA and alachlor-modified plasmid DNA were subjected to electrophoresis in 0.8% agarose gel (Fig. 4). Plasmid DNA (lane 1) showed the usual electro-phoretic pattern. Banding pattern of plasmid DNA treated with 30 and 300 µM (lane 2 and 4) showed decrease in the fluorescence intensities of supercoiled form and appearance of additional band due to nicks introduced in plasmid DNA by alachlor. The additional band did appear in 150 µM alachlor-modified plasmid DNA (lane 3) but the change in fluorescence intensity of super-

coiled form was not electrophoretically identifiable in this case. The increased mobility and decreased EtBr fluorescence in the gels indicate the generation of strand breaks and opening of double stranded DNA (Hsieh *et al*., 2020).

HPLC analysis of 150 μM alachlor-modified DNA bases

Under identical experimental conditions, the HPLC chromatogram of guanine showed a retention time of ~5.2 min, whereas the retention time for alachlor-treated guanine was observed at ~7.0 min (Fig. 5i). Increased retention time for alachlor-treated guanine is suggestive of alachlor-guanine adduct(s)

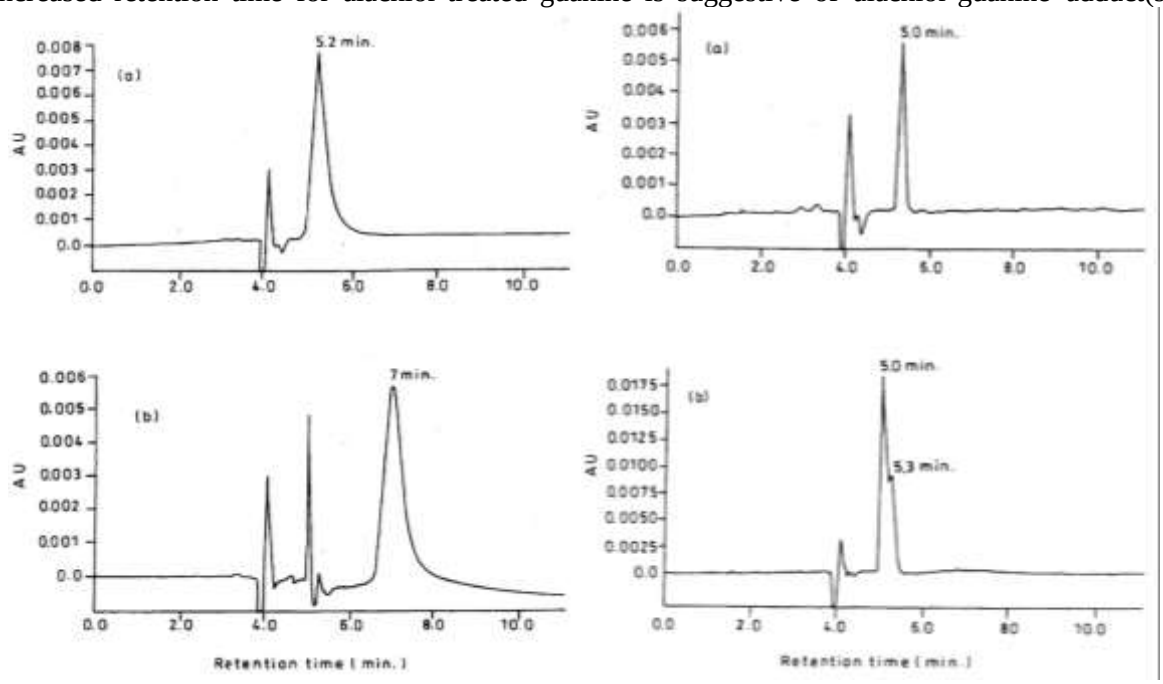

Fig. 5i: HPLC chromatogram of a) guanine and b) alachlor-treated guanine.

Fig. 5ii: HPLC chromatogram of a) thymine and (b) alachlor- treated thymine.

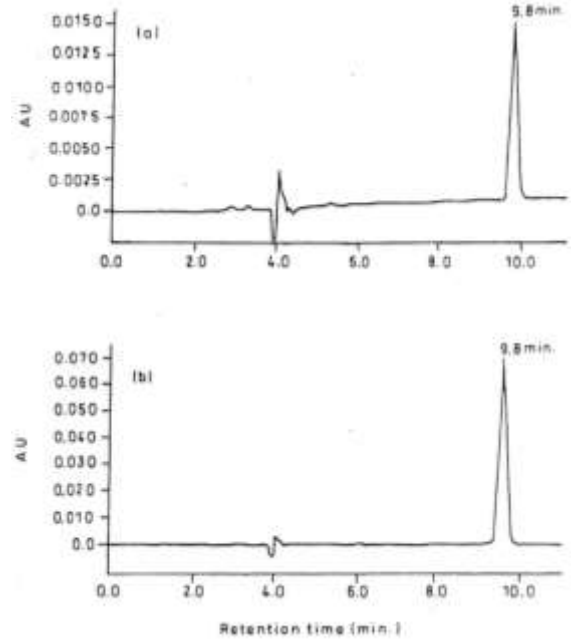

Fig. 5iii: HPLC chromatogram of (a) adenine and (b) alachlor-treated adenine.

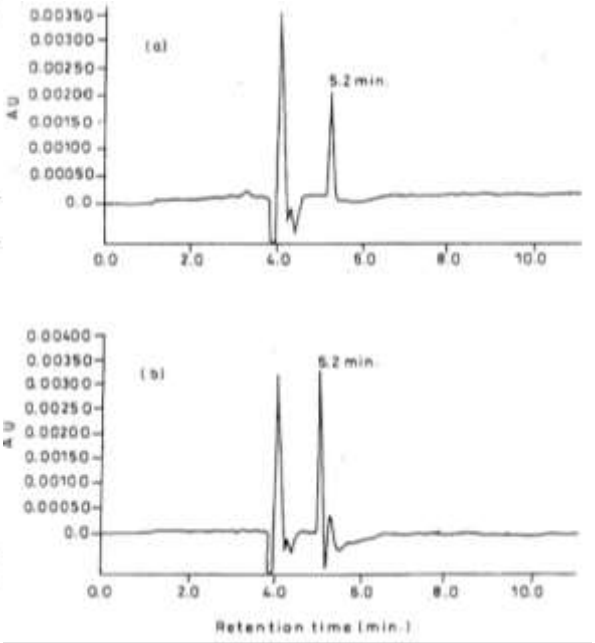

Fig. 5iv: HPLC chromatogram of a) cytosine and b) alachlor-treated cytosine.

formation. Both thymine and alachlor-treated thymine eluted at an identical retention time of ~5 min (Fig. 5ii), except for a kink at ~5.3 min in case of alachlor-treated thymine which could be due to poor interaction of alachlor with thymine. The retention time for cytosine and its alachlor-treated form was identical (Fig. 5iv). Adenine too did not show any change in the retention time upon alachlor-treatment (Fig. 5iii). The higher retention time of alachlor-treated guanine as shown by HPLC is suggestive of alachlor-guanine adduct formation. Adduct formation did not take place with other DNA bases. The potential of certain carcinogens and drugs to form *in vivo* DNA adducts have been studied previously (Stiborová *et al.,* 2017). DNA adducts are covalently modified bases resulting from the binding of electrophilic carcinogens or their metabolites (Barnes *et al.,* 2018). A variety of endogenous reactive electrophiles are formed because of normal cellular metabolism, many of which can react with DNA to form covalent adducts (Ross *et al*., 2012). Alachlor produces DNA single-strand break (Türkoğlu, 2012) cytogenetic effects (Dwivedi *et al.,* 2012) and has been found to be mutagenic (Szewczyk *et al.,* 2015). Alachlor and its metabolite CDEPA form covalent adducts with 2´-deoxyguanosine, 2´-deoxythymidine and their 3´-monophosphates with the displacement of chlorine atom of alachlor and CDEPA by nucleophiles (Ross *et al.,* 2012).

Melting behaviour of alachlor-modified-plasmid DNA

Effect of alachlor modification on the stability of plasmid DNA was ascertained from melting temperature (Tm). A plot of change in absorbance at 260 nm versus temperature indicated denaturation (Fig. 6). Under identical conditions we failed to record for the Tm of non-modified plasmid DNA. In this context Lim *et al*. (2022) in a study on plasmid DNA reported that plasmid DNA with good supercoiling density melt below physiological temperature. Further, the treatment of plasmid DNA with higher concentration of alachlor showed late melting. In our experimental conditions the unmodified plasmid DNA did not melt despite increasing the temperature up to 95˚C. If an analogy is drawn, then similar conclusion may be drawn for non-melting of un-modified plasmid DNA. Further, the Tm observed for alachlor-modified plasmid DNA is quasi-evidence of alachlor-induced structural changes in plasmid DNA (Habib *et al.,* 2013).

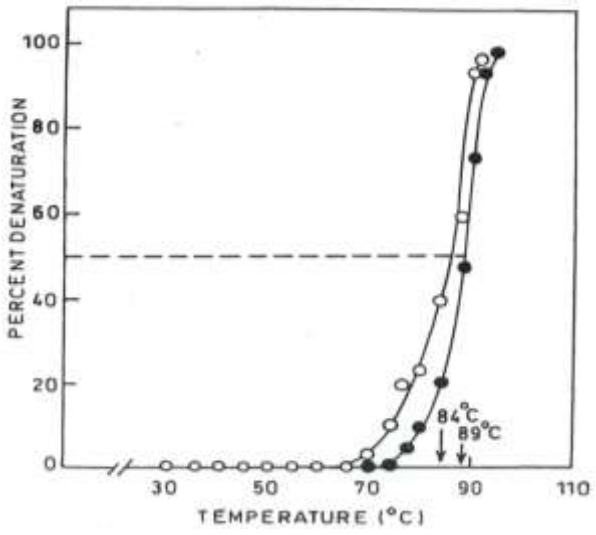

Fig. 6: Thermal melting profile of plasmid DNA modified with 150 μM alachlor (–○–), and 300 μM alachlor (–●–)

Conclusion: In conclusion, the adduct forming potential of alachlor suggests that its direct or indirect exposure to humans may cause genotoxicity. In an environment of poor DNA repair system or adduct-induced activation of pro-carcinogenic pathways, cancer may develop.

Disclosure statement: The authors declare that there are no competing interests.

REFERENCES

Agrawal, A.N.J.U. and Sharma, B. 2010. Pesticides induced oxidative stress in mammalian systems. *International Journal of Biological and Medical Research*, **1**: 90-104.

Ahmad, P., Rabbani, G., Dixit, K., Siddiqui, S.A. and Ali, A. 2016. Neo-epitopes on crotonaldehyde modified DNA preferably recognize circulating autoantibodies in cancer patients. *Tumor Biology*, **37**: 1817-1824.

Barnes, J.L., Zubair, M., John, K., Poirier, M.C. and Martin, F.L. 2018. Carcinogens and DNA damage. *Biochemical Society Transactions*, **46**: 1213-1224.

Boerth, D.W., Eder, E., Stanks, J.R., Wanek, P., Wacker, M., Gaulitz, S., Skypeck, D., Pandolfo, D. and Yashin, M. 2008. DNA adducts as biomarkers for oxidative and genotoxic stress from pesticides in crop plants. *Journal of Agricultural and Food Chemistry*, **56**: 6751-6760.

Brohi, R.O., Khuhawar, M.Y. and Khuhawar, T.M.J. 2016. GC-FID determination of nucleobases guanine, adenine, cytosine, and thymine from DNA by precolumn derivatization with isobutyl chloroformate. *Journal of Analytical Science and Technology*, **7**: 1-6.

Campos-Carrillo, A., Weitzel, J.N., Sahoo, P., Rockne, R., Mokhnatkin, J.V., Murtaza, M., Gray, S.W., Goetz, L., Goel, A., Schork, N. and Slavin, T.P. 2020. Circulating tumor DNA as an early cancer detection tool. *Pharmacology and Therapeutics*, **207**: 107458. [*DOI: 10.1016/j.pharmthera.2019.107458*].

Charak, S., Jangir, D.K., Tyagi, G. and Mehrotra, R. 2011. Interaction studies of Epirubicin with DNA using spectroscopic techniques. *Journal of Molecular Structure*, **1000**: 150-154.

Das, S.K., Mukherjee, I. and Roy, A. 2016. Alachlor and metribuzin herbicide on N_2-fixing bacteria in a sandy loam soil. *International Journal of Bio-resource and Stress Management*, **7**: 334-338.

Dixit, K., Ahmad, S., Shahab, U., Habib, S., Naim, M., Alam, K. and Ali, A. 2014. Human DNA damage by the synergistic action of 4-aminobiphenyl and nitric oxide: An immunochemical study. *Environmental Toxicology*, **29**: 568-576.

Dong, W., Chen, Q., Hou, Y., Li, S., Zhuang, K., Huang, F., Zhou, J., Li, Z., Wang, J., Fu, L. and Zhang, Z. 2015. Metabolic pathway involved in 2-methyl-6-ethylaniline degradation by *Sphingobium* sp. strain MEA3-1 and cloning of the novel flavin-dependent monooxygenase system meaBA. *Applied and Environmental Microbiology*, **81**: 8254-8264.

Dwivedi, S., Saquib, Q., Al-Khedhairy, A.A. and Musarrat, J. 2012. Butachlor induced dissipation of mitochondrial membrane potential, oxidative DNA damage and necrosis in human peripheral blood mononuclear cells. *Toxicology*, **302**: 77-87.

Gadagbui, B., Maier, A., Dourson, M., Parker, A., Willis, A., Christopher, J.P., Hicks, L., Ramasamy, S. and Roberts, S.M. 2010. Derived reference doses (RfDs) for the environmental degradates of the herbicides alachlor and acetochlor: Results of an independent expert panel deliberation. *Regulatory Toxicology and Pharmacology*, **57**: 220-234.

Gil, F.N., Gonçalves, A.C., Jacinto, M.J., Becker, J.D. and Viegas, C.A. 2011. Transcriptional profiling in Saccharomyces cerevisiae relevant for predicting alachlor mechanisms of toxicity. *Environmental Toxicology and Chemistry*, **30**: 2506-2518.

Habib, S., Ahmad, S., Dixit, K. and Ali, A. 2013. Peroxynitrite modified DNA may be an antigenic trigger for antibodies in various cancers of gynecologic origin. *Human Immunology*, **74**: 1239-1243.

Hsieh, T.C., Chao, H.H. and Wu, J.M. 2020. Control of DNA structure and function by phytochemicals/DNA interaction: Resveratrol/piceatannol induces Cu^{2+}-independent, cleavage of supercoiled plasmid DNA. *Free Radical Biology and Medicine*, **147**: 212-219.

Jarabek, A.M., Pottenger, L.H., Andrews, L.S., Casciano, D., Embry, M.R., Kim, J.H., Preston, R.J., Reddy, M.V., Schoeny, R., Shuker, D. and Skare, J. 2009. Creating context for the use of DNA adduct data in cancer risk assessment: I. Data organization. *Critical Reviews in Toxicology*, **39**: 659-678.

Langie, S.A., Koppen, G., Desaulniers, D., Al-Mulla, F., Al-Temaimi, R., Amedei, A., Azqueta, A., Bisson, W.H., Brown, D., Brunborg, G. and Charles, A.K. 2015. Causes of genome instability: The effect of low dose chemical exposures in modern society. *Carcinogenesis*, **36**: S61-S88.

Li, H. and Lampe, J.N. 2019. Neonatal cytochrome P450 CYP3A7: A comprehensive review of its role in development, disease, and xenobiotic metabolism. *Archives of Biochemistry and Biophysics*, **673**: 108078. [*DOI:10.1016/j.abb.2019.108078*].

Lim, W., Randisi, F., Doye, J.P. and Louis, A.A. 2022. The interplay of supercoiling and thymine dimers in DNA. *Nucleic Acids Research*, **50**: 2480-2492.

Mohanty, S.S. and Jena, H.M. 2019. A systemic assessment of the environmental impacts and remediation strategies for chloroacetanilide herbicides. *Journal of Water Process Engineering*, **31**: 100860. [*DOI:10.1016/j.jwpe.2019.100860*].

Nykiel-Szymańska, J., Bernat, P. and Słaba, M. 2018. Potential of *Trichoderma koningii* to eliminate alachlor in the presence of copper ions. *Ecotoxicology and Environmental Safety*, **162**: 1-9.

Ojha, A., Yaduvanshi, S.K., Pant, S.C., Lomash, V. and Srivastava, N. 2013. Evaluation of DNA damage and cytotoxicity induced by three commonly used organophosphate pesticides individually and in mixture, in rat tissues. *Environmental Toxicology*, **28**: 543-552.

Ross, J.A., Leavitt, S.A., Schmid, J.E. and Nelson, G.B. 2012. Quantitative changes in endogenous DNA adducts correlate with conazole *in vivo* mutagenicity and tumorigenicity. *Mutagenesis*, **27**: 541-549.

Sakuma, C., Tomioka, Y., Li, C., Shibata, T., Nakagawa, M., Kurosawa, Y., Arakawa, T. and Akuta, T. 2021. Analysis of protein denaturation, aggregation and post-translational modification by agarose native gel electrophoresis. *International Journal of Biological Macromolecules*, **172**: 589-596.

Soleimani, N., Derakhshan, S. and Memariani, M. 2016. Plasmid profile analysis of aminoglycoside-resistant *Escherichia coli* isolated from urinary tract infections. *International Journal of Enteric Pathogens*, **4**: 1-7.

Soloneski, S. and Larramendy, M.L. 2010. Sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary (CHO-K1) cells treated with the insecticide pirimicarb. *Journal of Hazardous Materials*, **174**: 410-415.

Stiborová, M., Arlt, V.M. and Schmeiser, H.H. 2017. DNA adducts formed by aristolochic acid are unique biomarkers of exposure and explain the initiation phase of upper urothelial cancer. *International Journal of Molecular Sciences*, **18**: 2144. [*DOI: 10.3390/ijms18102144*].

Szewczyk, R., Soboń, A., Słaba, M. and Długoński, J. 2015. Mechanism study of alachlor biodegradation by *Paecilomyces marquandii* with proteomic and metabolomic methods. *Journal of Hazardous Materials*, **291**: 52-64.

Tantry, I.Q., Waris, S., Habib, S., Khan, R.H., Mahmood, R. and Ali, A. 2018. Hypochlorous acid induced structural and conformational modifications in human DNA: A multi-spectroscopic study. *International Journal of Biological Macromolecules*, **106**: 551-558.

Tripathi, P., Dixit, K., Mir, A.R., Habib, S., Alam, K. and Ali, A. 2014. Peroxynitrite modified DNA presents better epitopes for anti-DNA autoantibodies in diabetes type 1 patients. *Cellular Immunology*, **290**: 30-38.

Tu, W., Niu, L., Liu, W. and Xu, C. 2013. Embryonic exposure to butachlor in zebrafish (*Daniorerio*): Endocrine disruption, developmental toxicity and immunotoxicity. *Ecotoxicology and Environmental Safety*, **89**: 189-195.

Türkoğlu, Ş. 2012. Determination of genotoxic effects of chlorfenvinphos and fenbuconazole in *Allium cepa* root cells by mitotic activity, chromosome aberration, DNA content, and comet assay. *Pesticide Biochemistry and Physiology*, **103**: 224-230.

Vieira, K.C.D.M.T., Couto, J.C., Zanetti, E., Junior, J.M.S. and Favareto, A.P.A. 2016. Maternal and fetal toxicity of Wistar rats exposed to herbicide metolachlor. *Acta Scientiarum Biological Sciences*, **38**: 91-98.

Wang, Y., Lv, L., Yu, Y., Yang, G., Xu, Z., Wang, Q. and Cai, L. 2017. Single and joint toxic effects of five selected pesticides on the early life stages of zebrafish (*Deniorerio*). *Chemosphere*, **170**: 61-67.

Xiang, Q., Xu, B., Ding, Y., Liu, X., Zhou, Y. and Ahmad, F. 2018. Oxidative stress response induced by butachlor in zebrafish embryo/larvae: The protective effect of vitamin C. *Bulletin of Environmental Contamination and Toxicology*, **100**: 208-215.

Xu, H.D., Wang, J.S., Li, M.H., Liu, Y., Chen, T. and Jia, A.Q. 2015. 1H NMR based metabolomics approach to study the toxic effects of herbicide butachlor on goldfish *(Carassius auratus). Aquatic Toxicology*, **159**: 69-80.