Discussion
Historically, aromatic amines are among the first chemical carcinogens that have been implicated in human cancer (Beland and Kadlubar, 1990). Epidemiological studies have shown an unambiguous association between urinary bladder cancer and exposure to 2-naphthylamine, benzidine and 4-ABP (Parkes and Evans, 1984). The International Agency for Research on Cancer (IARC) has classified 4-ABP as a group I carcinogen, resulting in the cessation of commercial production and usage of 4-ABP. However, the aromatic amine, 4-aminobiphenyl still continues to be an environmental and occupational contaminant since it is generated mainly from cigarette smoke. Other potential sources include, hair dye, combustion of fossil fuels, rubber, coal, textile and printing industries (Turesky et al., 2003, Akyüz and Ata, 2008; Steineck et al., 1990; Haugen et al., 1992). However, cigarette smoke is the most significant source of aromatic amines. Side-stream smoke (SS) emitted from the tobacco products constitutes the major source of environmental pollutants. Main-stream smoke (MS) which escapes to the environment from the mouth piece of the cigarette, cigar, or pipe can also contribute to indoor air pollution (Kuller et al., 1986). 4-ABP is found in particulate phase of both SS and MS. It is found in MS at a level of 1-5 ng per cigarette (PatrIanakos and Hoffman, 1979). In addition, the concentration of 4-ABP in sidestream smoke is over 10 times greater than in mainstream smoke (Hammond et al., 1993). Earlier works have reported an association between 4-ABP and bladder cancer and known levels of 4-ABP in side-stream smoke (Hammond et al., 1993; Vineis and Pirastu, 1997). The presence of 4-ABP in SS explains the role of 4-ABP adducts in case of non-smokers.

Estimation of systemic dose in smokers, via the haemoglobin adduct formed by 4-ABP metabolite which indicates a value of 5 pg adduct /g of haemoglobin per each cigarette smoke per day, suggests that a considerable fraction inhaled by a smoker is absorbed (Skipper and Tannenbaum, 1990). Epidemiological studies have indicated the relative risk ratio of bladder cancer to smokers in comparison to that of non-smokers to be in the range of 1.5-3.0; higher relative risks have also been cited (Bryant et al., 1987).

The non-bonding pair of electrons on the nitrogen of 4-ABP can be delocalized into the aromatic system by resonance and are thus relatively unreactive. The interaction of 4-ABP with biomolecules and its resulting toxicities require it to be
metabolically activated to a more electrophilic form. For this purpose, 4-ABP is oxygenated by a specific cytochrome P450, CYP1A2, to its more reactive hydroxylamine, \(N\)-OH-ABP, which, after phase II metabolism is transported to the kidney where it can form DNA adducts in human uroepithelial cells (Skipper and Tannenbaum, 1994). Further metabolism of \(N\)-OH-ABP occurs by assistance from the acetylation phase II enzymes, arylamine \(N\)-acetyltransferase 1 (NAT1) and \(N\)-acetyltransferase 2 (NAT2), which have been shown to play a major role in both the in situ metabolic activation as well as the detoxification of arylamine. Reactions of \(N\)-OH-ABP catalyzed by these enzymes forms \(N\)-hydroxy-\(N\)-acetyl-ABP (Minchin et al., 1992). Interaction of \(N\)-OH-AABP with DNA can induce both mutagenicity and cytotoxicity (Vineis, 1994). \(N\)-OH-AABP is considered to be the metabolite of 4-ABP most likely to form the nitrenium ion that reacts with DNA to yield mutagenic adducts in tissues outside, and perhaps even within, the uroepithelium.

DNA adducts formed by metabolically activated electrophilic derivatives of 4-ABP have been found to correlate well with bladder carcinogenesis in both experimental animals (al-Atrash et al., 1995; Poirier et al., 1995) and humans (al-Atrash et al., 1995; Curigliano et al., 1996; Romano et al., 1999). The predominant DNA adduct induced by 4-ABP in vivo is \(N\)-(2'-deoxyguanosin-8-yl)-4-ABP (dG-C8-4-ABP), contributing around 80% of the total adducts; minor amounts of \(N\)-(2'-deoxyadenosine-8-yl)-4-ABP (15%) and 3-(2'-deoxyguanosin-N2-yl)-4-ABP (5%) are also formed (Kadlubar et al., 1982; Beland et al., 1983). Formation of these DNA adducts is affected by liver enzymes such as cytochrome P450 1A2 (CYP1A2), \(N\)-acetyltransferase 2 (NAT2), and glutathione S-transferase M1 (GSTM1). Some of these enzymes are also present in the urothelial cells. CYP1A2 is an inducible enzyme that demethylates aromatic amines and thereby increases DNA adduct formation (Butler et al., 1989). NAT2 is a major acetylating enzyme that detoxifies amines and thus decreases DNA adduct formation (Risch et al., 1995). These enzymes are polymorphic in the general population; that is, there are variants of these enzymes in a population with slightly different molecular structures and biologic activities.

Apart from age, gender, and a few occupational groups (dye workers, rubber workers, leather workers, painters, truck drivers, and aluminium workers), smoking is
the only risk factor of bladder cancer for which clear epidemiologic evidence has been found (Silverman et al., 2006). Active cigarette smoking has been proposed to account for >50% of bladder cancers in men and 30% in women (Vineis and Caporaso, 1995; Kogevinas and Trichopoulos, 2002; Skipper et al., 2003). This association between active (cigarette) smoking and bladder cancer has been confirmed by more than 35 case-control studies and 10 cohort studies (Pitard et al., 2001; Bjerregaard et al., 2006; Samanic et al., 2006; Silverman et al., 2006). Moreover, the tobacco smoke constituent 4-aminobiphenyl (4-ABP) is a well-established risk factor for bladder cancer (Vineis and Caporaso, 1995; Landi et al., 1996; Vineis and Pirastu, 1997; Martone et al., 1998; Saletta et al., 2007). 50-60% of human cells treated with 4-ABP develop chromosomal instability (Saletta et al., 2007). Data also suggest that smokers of blond tobacco are at lower risk for bladder cancer than smokers of black tobacco (Bartsch et al., 1993; Martone et al., 1998). The latter type is richer in arylamines such as 4-ABP, which is thus known as the most potent human bladder carcinogen (Malaveille et al., 1989; Vineis, 1992; Vineis and Caporaso, 1995).

The etiologic involvement of aromatic amines in the genesis of bladder cancer is attributable to their ability to form DNA-adducts, which upon eluding repair and causing mispairing during replication, may initiate mutagenesis (Yoon et al., 2011). When the bladder and other tissues of smokers are compared to non-smokers, significantly higher levels of 4-ABP-DNA adduct formation has been detected by various methods (Skipper and Tannenbaum, 1994; Romano et al., 1997; Martone et al., 1998). Studies have been carried out to determine whether or not individual variation in amine-associated DNA adduct formation is due to a particular phenotype and correlates with risk. These studies indicate that cigarette smokers with slow NAT2/rapid CYP1A2 phenotypes were at higher risk for developing bladder cancer than those with rapid NAT2/slow CYP1A2 phenotypes (Kaderlik and Kadrubic, 1995). In addition to covalent adduct formation, these arylamines also cause oxidative DNA damage producing reactive oxygen species (Burger et al., 2001) that ultimately generate DNA strand breaks and base modifications such as 8-oxo-guanine and related products (Dizdaroglu et al., 1992; Marnett et al., 2000).

In the present study, human DNA was modified by varying concentration of N-OH-AABP (0.378 mM, 0.757 mM, 1.136 mM and 1.515 mM), which caused
extensive damage to human DNA, leading to the formation of single strand breaks and base modifications. Observed hyperchromicity (18.4%, 41.5%, 50.5% and 62%) was found directly proportional to \( N\)-OH-AABP concentration. This \( N\)-OH-AABP induced hyperchromicity could be attributed to the disruption of interactive forces holding the double helical structure of the DNA macromolecule. Therefore, stacking interactions, hydrogen bonds and hydrophobic effects between the complementary bases are disturbed. There might be the modification of nitrogenous bases which results in the exposure of chromophoric groups through attack on the sugar-phosphate back bone.

Appreciable evidence for generation of strand breaks (single and double) in the human DNA as a consequence of \( N\)-OH-AABP modification was gathered by agarose gel electrophoresis. The electrophoretic pattern of \( N\)-OH-AABP modified human DNA revealed an increasing mobility with increasing concentration of carcinogen. It was observed that at 1.515 mM concentration of \( N\)-OH-AABP the mobility of modified DNA was found to be maximum and further increase in concentration resulted in complete loss of double helical structure. The result clearly indicates generation of strand breaks in human DNA upon \( N\)-OH-AABP exposure.

The single-cell gel electrophoresis (SCGE) or comet assay is a very sensitive method for measuring DNA strand breaks, alkali labile sites, abasic sites and relaxed chromatin formation in individual cells. Damaged DNA migrates during electrophoresis from the nucleus towards the anode, forming a shape of a “comet” with a head (cell nucleus with intact DNA) and a tail (relaxed and broken DNA). Therefore, comet assay can be used to test the genotoxicity of carcinogenic agents in cultured cells including freshly isolated human lymphocytes. Using the comet-assay as an endpoint for DNA damage, the study results showed that \( N\)-OH-AABP was genotoxic to human lymphocytes. The results clearly establish that 1.136 mM \( N\)-OH-AABP is capable of causing DNA breakage in lymphocytes which is evident by formation of distinct tail from the diffused head. However, with further increase in \( N\)-OH-AABP concentration to 1.515 mM, the damage to lymphocyte DNA was found to be enhanced, suggesting concentration dependent genotoxic effect of \( N\)-OH-AABP. Furthermore, in the case of 1.515 mM \( N\)-OH-AABP treated lymphocytes, the increase
in OTM, % DNA in tail and tail length was found to be 94%, 89% and 72% respectively when compared with untreated lymphocytes.

Fluorescence spectra of both native and N-OH-AABP modified human DNA was taken using ethidium bromide as an external fluorophore. A decrease in fluorescence intensity of EtBr-N-OH-AABP modified human DNA compared to EtBr-DNA in the fluorescence emission spectra confirms the destruction of structural integrity of DNA and generation of single strand breaks which result in poor intercalation of EtBr in the modified form of DNA.

The CD spectrum of modified human DNA showed an increase of 39.9 % in the positive ellipticity at 275 nm as compared to the native form. The decrease in ellipticity with slight change in the peaks of DNA after N-OH-AABP modification may be due to unstacking of bases as a result of helix destabilization. The structural perturbations suggest unfolding of DNA which might be a result of single strand breaks. The data is compatible with an earlier report in this regard (Ahmad et al., 2011).

Upon modification by N-OH-AABP, native DNA showed susceptibility to rise in temperature when subjected to thermal denaturation studies. The melting temperature of the modified DNA was found to be approximately 10 ºC less as compared to the melting temperature of native human DNA. This could be attributed to the presence of single strand regions in the modified DNA and also a partial destruction in the secondary structure. Stacking of bases and hydrogen bonding stabilize DNA structure and their disruption by high temperature favours denaturation (Casperson and Voss, 1983; Thomas, 1993). The decrease in Tm, therefore, points towards the destabilization of base stacking and hydrogen bonding and consequent helix disruption.

Earlier studies have demonstrated that the structural alterations in DNA following damage by various agents result in the generation of single strands in the DNA molecule (Shishido and Ando, 1974; Yamasaki et al., 1997). Native and modified DNA were subjected to digestion by nuclease S1, in order to confirm the generation of single strand breaks. The results show substantial digestion of modified DNA by nuclease S1, while native DNA remained unaffected. These observations
clearly demonstrate that sufficient distortions are caused in DNA helix by \(N\text{-OH-AABP}\), rendering it susceptible to digestions by single strand specific nuclease S1.

DNA adduct measurement has played an important role in assessing the impacts of environmental factors in the etiology of cancer, and provides a useful parameter for cancer risk assessment. DNA adducts of 4-ABP have been reported \textit{in vitro}, in bacterial and mammalian cells, in experimental animals exposed to 4-ABP or its metabolites, and in human tissues. Most common site for stable adduct formation with aromatic amine is known to be C8 of guanine and for 4-ABP, or its metabolite, the major adduct is dG-C8-ABP. This implies that dG-C8-ABP might be a useful biomarker for monitoring \(N\text{-OH-AABP}\)-induced DNA damage in response to environmental exposure to 4-ABP. The preparative HPLC was employed for the synthesis of (deoxyguanosine-8-yl)-4-aminobiphenyl (dG-C8-4-ABP). The adduct, dG-C8-4-ABP formed by preparative HPLC was further proved by nuclear magnetic resonance analysis. The retention time of the synthesized dG-C8-4-ABP was found to be 22.23 min. The HPLC performed for native and modified DNA showed remarkable difference in peak retention times. The extra peak at a retention time of 22.029 min in the modified DNA is characteristic of \(N\text{-}(\text{deoxyguanosine-8-yl})\text{-}4\text{-aminobiphenyl}\) (dG-C8-4-ABP) adduct. This is in accordance with dG-C8-4-ABP results wherein also when deoxyguanosine was exposed to \(N\text{-acetoxy-4-trifluoroacetyl-aminobiphenyl}\), a distinct peak at retention time of 22.23 min was observed. However, native DNA did not show the peak at this retention time. The LC-MS was performed so as to detect the adduct, dG-C8-4-ABP formed with the double stranded human DNA. The acid hydrolysate of \(N\text{-OH-AABP}\) modified human DNA showed a m/z value of 435 in the negative ion mode. This is in conformity with the synthesised dG-C8-4-ABP wherein also we obtained the m/z value of 435, which is characteristic of dG-C8-4-ABP adduct formed with human DNA.

Mammalian DNA is generally inert and does not stimulate dendritic cells. Even when coupled to a protein carrier and presented in adjuvant, mammalian DNA fails to elicit significant antibody response (Madaio \textit{et al.}, 1984). This failure is in contrast to animal models of disease induced by immunization with protein auto-antigens (e.g., collagen-induced arthritis or experimental allergic
encephalomyelitis), suggesting that DNA differs from other biological macromolecules in its immunological capacity. However, studies from our laboratory (Ahmad et al., 2011) as well as from others (al Arfaj et al., 2007) suggest that upon modification, the DNA macromolecule undergoes structural perturbations which lead to the generation of a new form or neo-epitopes that are recognized as foreign by the immune system and are able to elicit antibody responses. Immunization with single stranded DNA can elicit a limited antibody response, while double stranded RNA, RNA-DNA hybrids, DNA modified with carcinogens, drugs etc. or DNA complexes with DNA binding proteins are effective immunogens (Stollar, 1975; Anderson et al., 1988; Desai et al., 1993; Moinuddin and Ali, 1994; Dixit et al., 2005; Habib et al., 2005; Khan et al., 2006). Indeed, bacterial DNA, by virtue of characteristic sequence motifs, can activate the immune system and drive the production of antibodies to sequential as opposed to backbone DNA determinants (Pisetsky, 1996 a&b). In its antigenic properties, foreign DNA resembles foreign proteins in that it has an epitope structure based on non-conserved sequences that are absent from the host and that are therefore not subject to tolerance.

Antibodies against N-OH-AABP modified human DNA were induced in rabbits by immunizing with N-OH-AABP modified human DNA complexed with methylated bovine serum albumin. The N-OH-AABP-DNA was a potent immunizing stimulus, inducing high titre antibodies. Antigenic specificity of anti-N-OH-AABP modified human DNA IgG was ascertained by competitive binding assay. A maximum of 89.3% inhibition in the antibody activity was obtained at inhibitor (immunogen) concentration of 20 µg/ml. Just 3 µg/ml of the inhibitor concentration was required to cause 50% inhibition, clearly indicating very high specificity and affinity of the induced antibodies towards the immunogen. Low inhibition with native human DNA (34%) suggests that majority of the antibodies are directed towards N-OH-AABP modified human DNA.

Visual detection of interaction between immune IgG and the immunogen was facilitated by the gel retardation assay. The result reflects high affinity of the induced antibodies for the immunogen. However, anti-N-OH-AABP-human DNA IgG did not show any appreciable binding with the native human DNA. These results further confirm that the induced antibodies are predominantly directed against the antigenic
determinants, or neo-epitopes, generated as a result of N-OH-AABP modification of human DNA.

Native calf thymus and lymphocyte DNA showed inhibition of 28% and 29.6% respectively, whereas their N-OH-AABP modified forms showed moderate inhibitions, 61.7% and 73.8% respectively. These results demonstrate the preferential recognition of N-OH-AABP modified epitopes by the immune IgG. However, ROS-modified form of calf thymus DNA as well as lymphocyte DNA inhibited antibody activity to a lesser extent possibly because the ROS modified epitopes are not so distinctly recognized by the induced antibodies. Amongst N-OH-AABP modified bases, guanine showed substantial inhibition (76.8%).

The occurrence of subclinical glomerular immune complex deposits among animals and humans with neoplasms had been a subject of interest (Oldstone et al., 1972; Pascal et al., 1973; Pascal et al., 1975). AKR leukemic mice have been shown to have immune complex deposits as well as C-type viral particles in their glomeruli by immunofluorescence and electron microscopy (Oldstone et al., 1972; Batzing et al., 1973; Pascal et al., 1973). It has been shown that laboratory animals may have a humoral immune response to spontaneously occurring neoplasms as evidenced by the deposition of immune complexes in their kidneys (Batzing et al., 1973; Pascal et al., 1973; Pascal et al., 1975). In the present study light microscopy detected glomerulonephritis whereas immunofluorescence studies showed deposition of immune complexes in the kidneys of rabbits immunized with N-OH-AABP-DNA. These observations points towards the possible involvement of N-OH-AABP in glomerulonephritis and opens up a new area of study for the carcinogen. Immune complex (IC) deposition in renal tissue is considered as a possible tumor marker which raised the possibility to detect some tumor markers in tissue samples also, apart from blood (Faria et al., 2010).

Biomarkers that show high sensitivity and specificity are needed for the early diagnosis and prognosis of cancer. An immune response to cancer is elicited in humans, as demonstrated, in part, by the identification of autoantibodies against a number of tumor-associated antigen (TAAs) in sera from patients with different types of cancer. Identification of TAAs and their cognate autoantibodies is a promising strategy for the discovery of relevant biomarkers (Desmetz et al., 2009). A number of
immunological studies have shown that certain neoplastic cells have associated tumor antigens of varying specificity which can under certain circumstances elicit cellular and humoral responses (Klein, 1966). The experimental methods used in the studies and the results obtained are summarized in a number of reviews (Tjalsma and Swinkels, 2008; Rauch and Gires, 2008; Caron et al., 2007, Hardouin et al., 2007; Gunawardana and Diamandis, 2007). Significant examples of such antigens are carcinoembryonic antigen (CEA) (a glycoprotein associated with colon cancer), alpha-fetoglobulin (a globulin associated with human hepatomas), gastric juice fetal sulfaglycoproteins (glycoproteins associated with gastric cancer), and other glycolipid antigens associated with gastrointestinal cancers. The specificity and clinical usefulness of detection of these antigens is quite variable depending on the antigen detected, the major limitation being a high proportion of false-positive and false-negative reactions.

A number of studies in animals and man have suggested that bladder tumors may have specific, associated antigens. But only a limited number of tumor antigens have been identified in bladder cancer cells, including cancer-testis antigens such as MAGE-1, -2, -3, -12 and NY-ESO-1 (Heidecker et al., 2000; Kurashige et al., 2001). Studies have established that bladder cancers in man share antigens specific to their tissue of origin in the bladder (Bubenik et al., 1970). Thus, peripheral blood leukocytes from bladder cancer patients are cytotoxic to primary cultures of bladder cancer cells from their own (autologous) tumor and from other patients (allogeneic) tumors. Cytotoxic complement-dependent antibodies and blocking antibodies which can prevent the cytotoxic action of leukocytes on bladder tumor target cells have also been demonstrated. These findings of a general immunological reaction by the host towards antigenic determinants of bladder tumors are similar to findings in several other forms of malignancy using similar immunological methods (Hellstrom and Hellstrom, 1969). Taken together, these studies suggest the possibility that specific antigens may be associated with human bladder carcinomas.

In view of this, the possible involvement of N-OH-AABP modified human DNA in bladder cancer patients was probed. Sera from bladder cancer patients were screened for the presence of autoantibodies reactive to native and N-OH-AABP modified human DNA. The binding of circulating autoantibodies from eighty bladder
cancer patients divided into two groups, smoker and non-smoker (forty each) and forty healthy normal subjects with and without history of smoking (twenty each) to native and N-OH-AABP modified human DNA was studied by direct binding ELISA. Of the 40 sera in smoker group of bladder cancer, 72.5% showed preferentially high binding to N-OH-AABP modified human DNA as compared to its native analogue. Serum antibodies from healthy normal subjects having smoking history did show binding with native and N-OH-AABP modified human DNA, but only to a little extent. In competition ELISA, native DNA caused 21.2 to 30.8% inhibition in the activity of antibodies from bladder cancer patients (smoker group), whereas 48.4 to 63.2% inhibition was observed with N-OH-AABP modified human DNA. The results indicate appreciable recognition of N-OH-AABP modified human DNA by the autoantibodies in the smoker group of bladder cancer patients. The binding specificity of isolated IgG, towards native and N-OH-AABP modified human DNA, was evaluated by competition ELISA. Immunoglobulin G (IgG) from bladder cancer patients (smoker group) recorded an inhibition of 58.4 to 74.4% with the N-OH-AABP modified human DNA, while with native human DNA it ranged from 27.6 to 33.8%. Appreciably high binding of affinity purified IgG towards N-OH-AABP modified human DNA, indicates specific recognition of N-OH-AABP modified epitopes on the DNA molecule by autoantibodies in bladder cancer patients.

Recognition of the modified epitopes was further confirmed by band shift assay through visual detection of the immune complexes on agarose gel, formed between N-OH-AABP modified DNA and IgG isolated from bladder cancer patients in smoker group. An increase in the amount of high molecular weight immune complexes having retarded mobility with progressive increase of IgG content clearly establish the N-OH-AABP modified human DNA as a preferred antigen for these autoantibodies as compared to the native analogue.

The experimentally induced antibodies against N-OH-AABP modified human DNA were used as an immunochemical probe to detect the N-OH-AABP or related arylamine induced lesions in the genomic DNA of bladder cancer patients, in the smoker group. The binding pattern of these DNA isolates was quite revealing. Our data has a direct correlation, as the lymphocyte DNA of bladder cancer patients, whose antibodies were extensively inhibited by the modified DNA, produced
appreciable inhibition in the binding of experimentally induced anti-N-OH-AABP-DNA antibodies. Inhibition of anti-N-OH-AABP-DNA IgG by lymphocyte DNA from bladder cancer patients (smoker group) was recorded in the range of 60.5% to 77.6%. While, inhibition of anti-N-OH-AABP-DNA IgG by lymphocyte DNA from normal healthy smokers was low. Significantly high recognition of the lymphocyte DNA from bladder cancer patients by the experimentally induced antibodies against the modified DNA is a clear indicator of epitope sharing between the human DNA modified in vitro by N-OH-AABP and the genomic DNA of bladder cancer patients. This leads to the conclusion that N-OH-AABP generates neo-epitopes on the DNA molecule that are recognized as ‘alien’ or non-self by the immune system resulting in autoantibody generation in bladder cancer patients. The strong binding of autoantibodies as well as significantly high level of recognition of the lymphocyte DNA from bladder cancer patients having history of smoking to N-OH-AABP modified human DNA is an evidence towards the involvement of modified bases and single strand regions in disease pathogenesis.

In bladder cancer patients belonging to non-smoker group (total 40), 40% serum samples showed moderate binding to N-OH-AABP modified human DNA as compared to its native analogue. The degree of binding was moderately higher than serum antibodies from normal healthy subjects. The recognition of N-OH-AABP modified human DNA by the auto-antibodies in non-smoker group of bladder cancer patients was further checked by competitive inhibition analysis. The inhibition in the antibody activity with N-OH-AABP modified human DNA was in the range of 36.1% to 52.8%, however for native human DNA it was found to be 22.2% to 29.4%. The IgG isolated from bladder cancer patients in non-smoker group recorded an inhibition in the range of 24.2% to 34.8% and 47.1% to 55.1%, when native and N-OH-AABP modified human DNA were respectively used as inhibitors. On the similar pattern, the lymphocyte DNA samples isolated from the blood of bladder cancer patients, in the non-smoker group, caused an inhibition in the range of 47.4%-57.4% in the activity of experimentally induced antibodies against N-OH-AABP modified DNA; which was a bit less as compared to the inhibition caused by the lymphocyte DNA from the smoker group of bladder cancer patients.
Based on above studies the following conclusions can be drawn:

1. *N*-OH-AABP modification of human DNA results in the formation of strand breaks and base modifications.

2. Comet assay shows the genotoxic effect of *N*-OH-AABP on freshly isolated human lymphocyte DNA.

3. Thermal denaturation studies show that modification has rendered the human DNA susceptible to rise in temperature. This shows the destabilization of helix.

4. Both HPLC and LC-MS support the formation of the adduct, *N*-(deoxyguanosine-8-yl)-4-aminobiphenyl (dG-C8-ABP) in modified human DNA. dG-C8-ABP is a useful biomarker for monitoring *N*-OH-AABP-induced DNA damage in response to environmental exposure to 4-ABP.

5. *N*-OH-AABP modification of human DNA renders it highly immunogenic inducing high titre antibodies in experimental animals. This shows the generation of neo-epitopes on the DNA molecule upon modification.

6. The induced antibodies, are highly specific for the immunogen. The antibodies also show binding with various nucleic acid conformers and nitrogenous bases significantly recognizing epitopes formed as a result of *N*-OH-AABP modification.

7. Animals immunized with *N*-OH-AABP-DNA show typical deposits of immune complex in glomeruli as revealed by simple and fluorescence microscopy.

8. Bladder cancer auto-antibodies in smoker group show preferential binding to *N*-OH-AABP modified human DNA as compared to the native form. This shows that *N*-OH-AABP modified DNA could be an antigenic stimulus for these auto-antibodies.

9. Antibodies from non-smoker bladder cancer patients exhibit low to moderate binding with *N*-OH-AABP modified human DNA.

10. Anti-*N*-OH-AABP-DNA-IgG can be used as a probe to detect the lesions, caused by *N*-OH-AABP or related aryl amines, in the DNA of bladder cancer patients.