5 DISCUSSION

The current investigation was taken up with the objective of studying the genotoxic and antigenotoxic properties of plant compounds apocynin and diosgenin. The chemotherapy drugs cyclophosphamide and cisplatin were employed as positive controls or drug controls to induce genotoxic and cytotoxic conditions in the experimental procedures. The genotoxic and cytotoxic effects of apocynin and diosgenin observed in the different *in vivo* as well as *in vitro* assays and the possible mechanisms responsible for the observed results are discussed in this chapter.

5.1 Genotoxic and Antigenotoxic Influences of Apocynin

The capacity of apocynin to amend the genotoxic damage inflicted by CP was investigated using the mouse bone marrow erythrocyte micro nucleation assay, assessment of haematological parameters, evaluation of the oxidative stress biomarkers namely lipid peroxidation and reduced glutathione and *Allium cepa* root meristem chromosomal aberrations assay. The cytotoxic and anticytotoxic abilities were assessed by performing cell line growth inhibition test *in vitro*.

The mouse erythropoietic system is a highly suitable *in vivo* system which allows the evaluation of antigenotoxicity and erythropoietic safety of any biomaterial that has therapeutic value, by interpreting the frequency of micronuclei in polychromatic erythrocytes of bone marrow [116]. Occurrence of micronuclei is a consequence of genotoxic insult to the chromosomes.

CP is recommended as a standard agent to induce genotoxicity as per OECD guide lines [27,54]. Cyclophosphamide is a nitrogen mustard alkylating agent from the oxazophorines group and is also known as cytophosphane. It alkylates the 7th nitrogen atom in the imidazole structure of guanine [63] and forms DNA crosslinks, within, as well as between the strands. Cells with cross-linked DNA are incapable of cell division and subsequently die. CP is used in the management of lymphomas, leukaemia, tumours of the brain and other tissues [39] and also to produce immunosuppressive effects [192]. It is bio activated in the liver by cytochrome P-450 oxidase system to its reactive electrophilic metabolites phosphoramidie mustard and acrolein. The slow accumulation
of these activated molecules in the plasma, leads to severe cytotoxic effects in non-target tissues also [171]. Phosphoramide mustard is the component which causes alkylation of DNA strands. Several analyses have shown CP to disrupt the redox homeostasis of normal tissues indicating that oxidative stress is the basis of physiological and biochemical damages caused by it [15,65]. In the current study, CP induced chromatin damage was evident by spiking of the frequency of MnPCE in mice bone marrow following an exposure to CP, at the rate of 50mg/kg of the experimental animals.

Apocynin showed marked reduction of micronucleation in the PCE’s of mice exposed to CP in the present investigation, when administered in combination treatments along with CP, in a dose dependent pattern. The higher dose of 200µg/kg body weight was more efficient. Available experimental literature by different authors indicates that CP induced geno- and cyto-toxicity may be countered by activity of quite a number of natural and synthetic antioxidants [115, 65, 27] deliver significant anticlastogenic effects against CP, as reflected by reduction in a array of biological indicators of genotoxic damage.

Plant polyphenols contribute several such agents capable of protecting DNA from genotoxic damage inflicted by CP and other chemotherapeutics also [13, 26, 28, 48, 64]. Apocynin, the molecule under investigation in the current study is a phenolic substance, already well known for its other potent therapeutic applications. The information related to its genotoxic and antigenotoxic nature was scarcely available. The antigenotoxic nature of apocynin was clearly evident from the results of the micronucleus test in our study. It is vital to evaluate the direct effect of these plant constituents, on the genetic material. In the present study apocynin was found to be non-genotoxic to the cells. Further, it clearly did not show any synergistic effects with CP in causing genotoxicity. Earlier literature also supports that apocynin lacked any genotoxicity in Ames test and SCE-test as well [193].

Several different mechanisms are said to be involved in the antigenotoxic nature of plant compounds. Phytochemicals can be incorporated in to chemoprevention systems, by virtue of their direct antioxidant mechanisms or indirect modulation of enzyme activities [143]. Grape fruit juice may function as a catalytic inhibitor of
topoisomerase II and also elicits phase II enzymes responsible detoxification [64]. A probable mode of antigenotoxic activity of cimetidine against benzene is reversible inhibition of cytochrome P-450 obstructing the conversion of toxins into their active metabolites [7]. Phenolic compounds are also capable of boosting the activity of detoxifying phase I enzymes besides suppressing the phase II enzymes implicated in the xenobiotic activation [13, 115]. They can even amend the DNA rescue and repair machinery along with hindering the formation of DNA adducts or methylation, by blocking the electrophilic site(s) in the DNA, which are labile to assault by reactive species [7, 114].

The major mechanism of action of apocynin in being a powerful antioxidant and anti inflammatory agent is blocking of NADPH oxidase [9]. Other mechanisms are also proposed for the anti-inflammatory action of apocynin by different authors [76]. Apocynin is a effective inhibitor of NAT enzymes related with a broad range of cancers and projected as possible drug targets [143]. It is also reported that in nonphagocytic cells apocynin principally acts as a reducer of ROS and subsequent ROS-induced signalling [9] rather than inhibiting NADPH oxidases. In osteoblasts, apocynin, by activation of factors PI3K and CREB, appreciably reduced oxidative mitochondrial dysfunction induced by Antimycin-A [62]. In other tissues like endothelium, it inhibits cytochrome P450 activity, and in PMN, affects polymerization of actin and on lipid metabolism [9]. In the current study, reduction in the frequency of micronuclei is an indicator of the ability of apocynin to shield the cells from DNA injury. The possible mechanism behind this action of apocynin to protect DNA is by scavenging of free radicals, whose production is known to be greatly spiked by CP. Another highly possible reason may be inhibition of cytochrome p-450 by apocynin, which in turn can prevent activation of CP into its toxic metabolite, which are actually responsible for generation of DNA breakage. Since there is a strong correlation between genomic damage and carcinogenesis, substances that prevent genotoxic insult to cells are potential chemopreventive agents [15, 26, 102].

Understanding the effect of potent therapeutic molecules on haematological parameters like leucocytes, erythrocytes and HB forms a key component in further making sure their compatibility with the biological system. Chemotherapy agents like
CP, CCNU and 5-FU are known to cause a significant reduction in the total lymphocyte as well as total WBC counts [114, 171, 194]. Cyclophosphamide weakens both the cell-mediated and humoral immune response in a dose-dependent manner, causing immunosuppression [192, 195]. Phosphoramide mustard is the major causative factor for this loss of immune function. This makes the individual susceptible to various types of secondary infections [171]. In the present study also, CP caused a significant depression in total WBC count where as no significant influence of CP and apocynin on HB content and RBC count could be observed in the current study. Apocynin could alleviate the leucopenia induced by CP in mice. Studies by other workers with natural extracts have shown comparable protective/ restorative consequence on CP-induced WBC depression [196,197]. The results indicate that apocynin could stimulate the haemopoetic system and have immunomodulatory effects. The exact mechanism of apocynin in the amelioration of CP induced WBC loss needs to be further explored. One possible explanation is non conversion of CP into phosphoramide mustard; Apocynin is a potent inhibitor of cytochrome system needed for this conversion.

We also evaluated the prophylactic effect of apocynin on the oxidative stress induced by CP. Measurement of lipid peroxidation is a vastly accepted indicator of oxidative turbulence. The positive association between degree of lipid peroxidation and genotoxicity as has been verified by many researchers [6, 198].

In the current study, the administration of CP enhanced LPO significantly compared to the normal control. Acrolein, one of the CP metabolites is responsible for the diminution of nucleophilic antioxidants, such as glutathione [199].

Exposure to apocynin alone did not enhance the LPO in the hepatic tissues in the current study. Further, priming of experimental animals with apocynin significantly reduced the CP induced lipid peroxidation in the liver. Several experimental results support this observation. Dietary inclusion of natural antioxidants like EPA [65] and Garcinia indica fruit extract [82] prevented lipid peroxidation in tissues of experimental animals. Apocynin also was shown to boost the antioxidative defence system by increasing the activity of enzymes like SOD, GSHpx and GSH [76]. Earlier studies have also shown apocynin to normalize oxidative DNA damage triggered by cisplatin in mice [79].
A major decline in the GSH content has frequently been observed in different tissues as a result of CP [80, 171, 199]. The results of our current study are in agreement with these, where in, the levels of GSH were lowered significantly by CP. Apocynin did not contribute the depletion of GSH; rather, we noticed a significant revival of hepatic GSH in the mice subjected to apocynin pretreatment. This enhancement of GSH by apocynin could be crucial in annulling the genotoxic effects of CP. GSH and GSH-inducing compounds were found important in the prevention of such peroxidative damage induced by CP [27, 171, 199]. Glutathione is supposed to have a dual role in protection against the cytotoxicity of CP. It minimizes formation of the toxic species phosphoramid mustard [199] and also can neutralize another of its metabolite, acrolein [200]. The exact system of action of apocynin warrants further examination.

The antigenotoxic capability of apocynin was also evidenced in another biological system namely the Allium cepa root tip meristems, in our current research. This test system has been widely used, either singly or as a part of a battery of tests [90, 93, 95] to assess the mutagenicity of several natural and synthetic compounds, for biological monitoring, investigation of environmental pollution, as well as evaluation of cytotoxicity and anti-proliferative potentials of medicinal compounds. Two parameters of importance namely the MI and fCA were calculated as markers of geno- and antigenotoxic influences of the compounds.

A good mitotic index (also called growth index) reveals normal progression of cell cycle. It is considered an indicator of adequate cell multiplication for evaluating the cytotoxic and genotoxic action of diverse environmental or therapeutic agents. We observed a mitotic depression in root tips in contact with CP. CP was found to be a significant mitodepressant in mice bone marrow and in root meristems as well, by other researchers [29, 90, 92]. The results of the onion root meristem analysis under the different treatments in the current study indicate that apocynin shows no substantial depression of MI. In onion bulbs treated simultaneously with apocynin and CP, apocynin notably restored the MI. CA also are one of the results of genomic damage by free radicals. The frequencies of CA in the root tips showed significant reduction in combination of apocynin and CP as opposed to CP alone, in this analysis. More than a few other plant compounds were also revealed to have moderating effects against
clastogenic substances using the *Allium* assay. Kumara *et al*, 2012 reported antigenotoxic effects of aqueous extract of seeds of broccoli on cell division in root tips against herbicide ciluron[89]. Curcumin revealed antimutagenic properties against sodium azide [92, 188].

One of the important reasons for the wide spread use of *Allium* assay to evaluate possible antimutagenic substances is the presence of cytochrome P450 in higher plants [201]. Apocynin has an inhibitory effect on cytochromeP-450 [143]. As such, suppression of cytochromeP-450 mediated activation of CP to its active metabolites in the roots treated with a combination of apocynin and CP might be the plausible mechanism for the protection afforded by apocynin, since formation of these metabolites is inevitable for CP- mediated nucleic acid strand breaks. Apocynin also showed a cell cycle stimulatory effect by restoring the mitotic index.

To further interpret the eligibility of apocynin as a possible chemopreventive or chemoprotective supplement by virtue of its antigenotoxic strength, we performed cell growth inhibition assays using the MTT method. This is an efficient and dependable colorimetric assay for viability of cells, employed by researchers to understand the cytotoxic and anticytotoxic properties of a wide range of substances which in turn can reveal their potential as chemotherapeutic or chemopreventive agents, as well as cytoprotective abilities against environmental carcinogens [98, 101, 102]. With the current emphasis towards research and development of natural molecules as medicines, it is important to generate information regarding the toxicity and efficacy of plants and plant compounds utilized to treat ailments in the ethno botanical systems.

In the present investigation, two cell lines namely CHO-K1, a normal cell line and HepG2, a hepatic cancer cell line, were chosen to study the cytotoxic and anticytotoxic effects of apocynin, against cisplatin induced cytotoxicity. The study aimed at understanding if apocynin had any inherent cytotoxicity and whether it had any synergistic/antagonistic effects with cisplatin. It also aimed to study whether apocynin had any selectivity towards a normal cell or towards a cancerous cell line, in its effects.

Apocynin did not exhibit any cytotoxic action on both the cell lines employed in our research. Cytotoxicity is the property of a substance to cause cell growth inhibition
or death. The current study uses cisplatin \([\text{cis-Diaminedichloroplatinum(II)}]\), a chemotherapeutic drug to induce cytotoxic conditions. Cisplatin is recommended for managing cancer of the testes, ovaries, cervix, brain, neck and the lungs in mono- or multi-drug regimen. It kills cancer cells by promoting DNA damage and inhibiting its synthesis. Interal and intral strand cross-links generated in this process provide adjacent deoxyguanosines, which are the target sites for platination \([86]\) by cisplatin. The use of cisplatin is compromised by severe nephrotoxicity and ototoxicity \([202]\).

Cells in culture, when exposed to cisplatin, as a part of this study, showed severe growth inhibition, whereas apocynin did not show any inhibitory effects indicating a lack of inherent cytotoxic influence, towards both the cell lines. Also, apocynin did not show synergistic effects with cisplatin.

Prior exposure of both the cell lines to apocynin, in our treatments, could reduce the cell inhibition caused by cisplatin. Several studies have shown that the administration of antioxidants can reduce the side effects associated with cisplatin \([99, 107, 129, 202]\). Cisplatin treatment is associated with increased NADPH oxidase activity \([203]\). Apocynin, being a strong inhibitor of NADPH oxidase could have lowered the cisplatin cytotoxicity observed in the current study. NADPH oxidase inhibitors were earlier found to be significant in protecting cells from cisplatin-induced toxicity mediated by a significant reduction in ROS generation \([202, 204]\). Another mechanism by which cisplatin induces nephrotoxicity is depletion of the intracellular GSH \([205]\). Supplementing with cysteine, a glutathione precursor, also reduced the cisplatin-induced genotoxicity \([86]\). Apocynin is also proposed to be an enhancer of intracellular GSH \([206]\), which was also observed in the hepatic tissues of experimental animals in the current study.

Agents which can offer selective protection to normal cells without interfering with the effectiveness of therapeutic drugs on cancer cells are best suited inclusion as adjuvants chemopreventive and chemoprotective strategies \([101, 207, 208]\). Natural substances of varied nature were shown to possess selective anticytotoxic efficiency towards normal cells rather than cancer cells, using the MTT assay. The methanolic extract of \(\text{Solanum nigrum}\) showed superior activity on \(\text{HeLa}\) cell line relatively low toxicity to \(\text{Vero}\) cells \([100]\). Studies by Senthilraja and Kathiresan \([2015]\) prove marine
yeast to have apoptotic effects on cancer cells and little activity on normal cells [101]. Emodin has synergistic effects on the cytotoxicity of four different drugs in prostate cancer cell lines, but poses no threat to normal fibroblasts [207]. Mangiferin in combination with low non cytotoxic concentrations of cisplatin and 5-fluorouracil increased the cytotoxicity of these chemotherapeutic agents in mouse colon carcinoma cell lines without significant increase of cell death in CHO-K1 cells [208]. In case of apocynin, we did not observe any selective cytoprotective activity, between the two studied cell lines. It showed similar effects towards both the cancer and normal cell lines. It is necessary to further elucidate this aspect by further studies, employing different cell lines and experimental conditions. Cytotoxic actions of antitumour drugs are in general facilitated by prominent increase of ROS generation; cells bearing a lower ROS status usually respond less to therapy. Fine and careful modulation of cellular redox status is a prospective strategy to enhance sensitivity of cancer cells to drugs. The mechanisms underlying the modulation of the level of ROS have to be carefully designed to ascertain the right redox-modulating agents which are efficient and at the same time, cancer cell-selective [207].

To evaluate haemocompatibility of apocynin haemolysis assay was performed as a component of this research work. Biomaterials are capable of structural destabilization of phospholipid bilayer of the membrane and ensuing lysis of RBCs or by damaging the cytoskeletal components. The cytoskeleton of RBC comprises of many proteins like spectrin and actin. The breakdown of erythrocytes impairs the ability of this fluid connective tissue to carry oxygen to various tissues. Free radicals are capable of lysing erythrocytes by oxidative injury to the membranes and certain antioxidants are potent inhibitors of such damage [209]. Cisplatin induces negligible haemolysis, confirming that chromatin is its biological target [110]. The same was also observed in the haemolysis study carried out currently. Treatment with apocynin or a combination of apocynin and cisplatin also had minimal effect on haemolysis. Apocynin and its analogs like ethyl vanillin could inhibit oxidative haemolysis as reported in an earlier study [138]. Apocynin does not show any aggravation of haemolytic properties of cisplatin. Our results point to the haemocompatibility of apocynin, and may be safely integrated into chemotherapy regimen.
We also evaluated DNA protecting strength of apocynin by *in vitro* DNA fragmentation assay. Substances with strong antioxidant nature are known to be capable of protecting DNA from ROS induced damage [105]. This, in turn, is a hindrance for carcinogenic and genotoxic substances [2]. Many natural substances have been shown act as potent antioxidants that prevented genotoxicity in bone marrow cells as well as DNA fragmentation [107, 210]. The current DNA fragmentation study indicated that apocynin offered protection against cisplatin imposed threat to DNA in cultured CHO-K1 cells, indicated by the absence of a smear in the DNA extracted from the cells treated with a combination of apocynin and cisplatin, where as cisplatin alone caused smearing. In an earlier report, apocynin had caused a profound drop in caspase-3 activity and fragmentation of nuclear DNA in cisplatin-treated group [79]. This was attributed to the capability of apocynin to ease the severity of the intracellular stress favouring apoptotic activation. Apocynin was also found to be efficient in protecting DNA of oesophageal cells from damage, by inhibition of ROS generation and increasing the DNA repair capacity [211, 212]. The ability of apocynin in protecting the cultured CHO-K1 cell DNA may be due to its ability to inhibit cisplatin evoked NADPH oxidase activity and the concomitant ROS production [203].

The results of this study indicate the antigenotoxic and anticytotoxic efficiency of apocynin under the studied experimental conditions. Apocynin *per se* did not demonstrate any genotoxicity towards any of the investigated endpoints. These findings also warrant further studies to get deeper insight into the prospects of apocynin as an antigenotoxic compound. Apocynin could be a potential antigenotoxic, anticytotoxic and chemopreventive agent against the effect of different environmental, industrial and chemotherapeutic genotoxic substances. It also shows prospective application as an adjuvant in chemotherapeutic interventions.

### 5.2 Genotoxic and Antigenotoxic Influences of Diosgenin

Diosgenin is evident in its applications against various metabolic disorders including dyslipidemia, diabetes and liver disorders, different types of tumours occurring in colon, breast, cervix, prostate, liver and other organs and other human ailments like dementia [32, 34, 173]. Diosgenin and other saponins have been shown to
target multiple stages of cancer development, including tumour growth, angiogenesis, invasion, and immunosuppression in various in vivo and in vitro tumour cells [168, 170, 177, 213, 214]. In view of the immense therapeutic potential displayed by diosgenin, it is evidently necessary to understand the genotoxic and antigenotoxic nature of this compound. The present study aimed to reveal the nature of diosgenin towards induction of genotoxic damage using the Mouse erythrocyte micronuclei formation assay. CP was employed to induce genotoxic conditions in the experimental system. The other indicators of genotoxic damage that were studied include the level of hepatic lipid peroxidation, level of hepatic GSH and the haematological parameters.

In the current study against cyclophosphamide induced genotoxicity in mice bone marrow, diosgenin showed significant antigenotoxic activity, marked by reduction in the frequency of MnPCE caused by exposure to CP, in the experimental animals. Earlier Studies revealed that diosgenin has potent anticlastogenic effects on 7,12-dimethylbenz(a)anthracene treated hamsters[175]. Similar reports are provided by anticlastogenic effect of aqueous extract from water yam Dioscorea alata [215]. Diosgenin per se, did not aggravate formation of Mn.

Different modes of action are proposed for the biological effects displayed by diosgenin, involving a wide range of cellular modulating mechanisms [32]. It induces cell cycle arrest and apoptosis in osteosarcoma cells [33], diminishes matrix metalloproteinases and inhibits metastasis of prostate cancer cells [216] and suppresses 3-hydroxy-3-methylglutaryl CoA reductase expression in human colon cancer cells [217]. The antigenotoxicity of diosgenin, visible in our study, may probably be due to its antioxidant ability and also its effect on enhancing the GSH levels [170] which are also observed in the current study. As evidenced by other reports also, saponins with strong antioxidant capacities are capable of countering genotoxic damage caused by chemotherapeutic agents [170, 214, 215]. The modulatory effects of diosgenin against CP genotoxicity need further exploration to understand the interlaying molecular mechanisms.

Diosgenin was potent in normalizing the loss of WBC imposed by CP. The results of an earlier study suggest that diosgenin can modulate lymphopoiesis [173]. Other studies report that diosgenin and its analogues play a role in modulating
inflammatory mediators [218] through mechanisms that are yet to be deciphered. It conferred resistance to lymphocyte DNA damage under oxidative stress [31]. The present experimental results suggest diosgenin can moderate the leucopenia induced by treating the experimental animals with CP. The observation that diosgenin per se did not cause a lowering of total WBC count suggests it to be safe on the hematopoietic system. Diosgenin also did not affect the total RBC numbers and HB percentage.

In the experiments performed to further analyse the antigenotoxic nature of this sapogenin, diosgenin pretreatment was seen to significantly trim down the levels of LPO in the liver, as observed by reduced MDA quantities. Diosgenin was shown to lower peroxidation induced by CP in the urinary bladder of experimental animals in some previous reports also [169, 171, 175] which support our current observations.

An imbalance in the status of GSH in the liver homogenate is indicative of interruption of detoxification process by oxidative stress. The antiperoxidative potential of reduced glutathione on cyclophosphamide-induced lipid peroxidation was clearly demonstrated by Ray et al., (2010) [199]. In our experiments, diosgenin extended a protective effect on non-enzymatic antioxidant GSH besides the level of LPO. The GSH level was lowered significantly by CP treatment. The point that diosgenin is competent in offsetting CP-induced oxidative stress, is supported by the increased GSH and the decreased LPO levels seen in our study. These results are in agreement with other previous reports [171]. The molecular mechanism underlying this protective ability could be directly related to its notable potential to scavenge radical species [169]. Similar effects of diosgenin are reported by other workers where it reduced the plasma level of total cholesterol and conferred resistance to lymphocyte DNA damage under oxidative stress [31].

In the cell growth inhibition assays performed currently to evaluate cytotoxic and anticytotoxic nature of diosgenin, it has shown a significant cytotoxic action towards the cancer cell line HepG2. It also showed a synergistic effect with cisplatin by showing a dose dependent increase in the cisplatin induced cytotoxic activity on HepG2 cells, higher concentration of 100µM being the most effective, of the three tested concentrations. Plant extracts containing diosgenin and other steroid saponin constituents showed in vivo and in vitro anticancer activity [214]. Studies have shown
that diosgenin possesses potential prooxidant properties causing growth inhibition of Hep2 cells[103] and HepG2 cells[168], mediated by increased ROS generation and lipid peroxidation.

Diosgenin is shown to affect manifold steps of cell signalling associated with cell growth, differentiation and programmed cell death [32] in its chemopreventive activity. It was shown to suppress the expression of enzymes like metalloproteinases and kinases and also signalling molecules like nuclear factor kappa B (NF-κB) [216]. Diosgenin had enhanced the apoptotic effects of anticancer drugs paclitaxel and doxorubicin by lowering the production of STAT3- regulated gene products [172]. Different molecular mechanisms of action, responsible for the antiproliferative action of these steroidal saponins in vitro, on other cell lines are reported by other researchers also [32, 170, 176, 217].

On the other hand, reports of anticlastogenic nature of diosgenin and plant extracts containing steroidal saponins, mediated by antioxidant nature and enhancement of cellular antioxidants in different biological model systems are provided by other workers [175, 215, 219]. Studies by Das & Bharali (2014) reveal that diosgenin possesses significant free radical scavenging ability and acts as a bifunctional inducer of xenobiotic detoxifying enzymes [169]. In this context, a review of a number of scientific reports shows both anticlastogenic nature of diosgenin, especially in vivo and its cytotoxic nature on cancer cells in vitro. In the current study also similar results were obtained. Diosgenin is a natural compound with immense potential for therapeutic applications across a wide range of metabolic disorders and cancers [34]. The molecular mechanism of antigenotoxic activity as well as the synergistic cytotoxic ability of diosgenin with cisplatin can be further explored to understand its chemotherapeutic potential. This could prove valuable in clinical use of diosgenin against cancer and other metabolic disorders [173].

The other cell line employed in our present research was CHO-K1 whose growth was not affected by diosgenin, in any manner. It did not inhibit the growth of these cells in culture nor had synergistic effects with cisplatin; at the same time it did not affect the cytotoxic action of cisplatin on these cells, indicating a lack of cytoprotective effect. There are no previous reports of influence of diosgenin on non
cancerous cell cultures. We observed that though this sapogenin is able to cause death of HepG2 cells, it did not cause growth inhibition in the normal cells. This is an important observation in enabling the chemotherapeutic usage of diosgenin. In view of the previous reports, our findings demonstrate that diosgenin is bestowed with promising attributes that need further exploration and make it a potential candidate for application as antigenotoxic as well as chemotherapeutic agent in the chemotherapy strategies.