INTRODUCTION

1.1 General Introduction

The integrity of genome is the most important factor deciding the fate of a cell. DNA, the heritable macromolecule which constitutes the genome, is highly susceptible to damage by different chemical and physical agents, and this may affect its integrity and functionality. Prevention of damage as well as repair of the damages to the genome is highly essential to ensure the viability of healthy cells and continue their lineage. Cells are inherently provided with stringently controlled biochemical pathways to detect the modifications in DNA and to repair them, so as to ensure the fidelity of DNA replication and segregation of chromosomes correctly to the next generation of cells. Unrepaired damages lead to mutations and other abnormalities [1] which may further lead to carcinogenesis.

These carcinogens are majorly grouped into genotoxic and epigenetic (non-genotoxic). Genotoxic agents are carcinogens that usually are capable of causing direct damage to genetic material, which in turn can lead to mutagenic and/or clastogenic alterations [2]. Genotoxicity is a broad phenomenon that covers the ability of a substance, not only to alter the DNA, but also other cellular components that regulate the conformity of the genome and functionality and behaviour of chromosomes within the cell, for instance, the mitotic apparatus and topoisomerase enzymes. Genetic toxicology is the study of such agents, their mechanism of action and the consequences [3].

Humans persistently are exposed to quite a lot of environmental pollutants, industrial chemicals and effluents, heavy metals, cytotoxic drugs, dyes and colours used for different purposes that can lead to the development of different kinds of cancer [4–7]. Under conditions of extreme stress, the cellular biological machinery fails to overcome the genotoxic injuries caused by different agents, leading to malignancy. Toxicity of substances is manifested in diverse forms like DNA damage, Chromosomal aberrations, formation of micronuclei, cell death or abnormal cell growth leading to
tumour formation. Contact with such agents is often inescapable and creates a huge menace to human health.

The human body is innately equipped with molecular detoxification systems to defend against these toxins [8, 9]. But increased or repeated exposure to hazardous chemicals can lead to mutagenic events, as in the case of cancer chemotherapy. Cancer is caused by the genomic alterations leading to the accumulation of the abnormal cells which undergo uncontrolled cell division and can disperse to other tissues of the organism. Though chemotherapy remains a principle agent for the treatment of cancer and is expected to have the ability to differentiate between a normal and cancerous cell, toxicity issues to normal cells remain the major obstacles in its clinical use [1, 10, 11]. Fortifying the inherent defences with exogenous antigenotoxic agents becomes essential to prevent neoplasia. A viable approach in such situations is to help the individual to defend against mutagens and carcinogens by dietary supplementation with chemopreventive and chemoprotective agents [12, 13].

Several mechanisms of direct and indirect genotoxicity have been identified [14]. Oxidative stress is known to generate a multiplicity of DNA damage [15]. A shift in the prooxidant and antioxidant balance of the biological environment in favour of prooxidant state leads to oxidative stress. This occurs when cells are incessantly exposed to reactive oxygen species (ROS). There are contributed by both endogenous sources of normal metabolism and exogenous sources such as environmental pollutants, chemotherapeutic drugs and industrial chemicals. Oxidative DNA damage, combined with a malfunction of cellular DNA repair mechanisms, is extensively associated with carcinogenesis and other pathobiological conditions [16]. ROS may induce single- and double-stranded breaks, DNA crosslinks and base modifications, all of which are concerned in initiation and promotion of tumours. Under conditions of extreme oxidative stress induced by diverse sources, for instance chemotherapy, to tackle the genotoxic damage, use of antimutagens and antigenotoxins, from natural sources is a well-founded approach [17, 18].
It is crucial to investigate the genotoxicity of a compound, in the milieu of understanding its mechanism of carcinogenicity. Genotoxic carcinogens propound a larger peril to humans than non-genotoxic carcinogens [19]. Moreover, it is inevitable to understand the antigenotoxic properties of compounds, to be able to deploy them in strategies of fighting cancer. In this context, it is also of significance that the cytotoxic and anticytotoxic nature of substances is evaluated, to make certain their safety for therapeutic purposes. Such investigation of antimutagenic capabilities of natural products can lead to development of potential chemotherapeutic, chemopreventive agents.

Plants produce secondary metabolites in response to environmental stimuli. A vast number of these phytochemicals have been isolated and their benefits to human health have been documented. Crude extracts and biologically active compounds isolated from natural substances, especially those which are in use in ethnic medicinal systems, can be prolific sources of new drugs, with priceless application in treatment of various pathological conditions [20]. Topical health concerns have paved enormous deal of attention towards the natural antioxidants in plants for their medicinal and biological activities taking into consideration, the numerous shortcomings of synthetic compounds for human population [21]. Natural substances play a significant role in multiple biological mechanisms, contributing to their antigenotoxic and anticarcinogenic effects. The majority of these are known to wield their effects either by quenching ROS or by stimulating cellular defences. Plants are precious sources of such biologically active molecules. Research, across the world has been focussing on exploring the antigenotoxic, anticytotoxic and anticancer potentials of plant compounds. At the same time, it is highly important to evaluate their efficacy and safety as putative inhibitors, for clinical relevance. It also is important to understand the mechanisms involved therein [22].

The plant secondary metabolites, grouped as phenols and phenolic compounds have been shown to hold abundant therapeutic properties [23–25]. Phenolic compounds act as inhibitors of free radical production and also as free radical scavengers. They can also indirectly act by modulating the activity of enzymes with antioxidant, detoxifying
and repairing functions [13]. Several phenolic compounds are being studied for their possible use as antigenotoxic agents [13, 26–29]. Apocynin is one such phenol, renowned for inhibition of the complex NADPH-oxidase and is endowed with myriad applications in the treatment of inflammatory and oxidative disorders like arthritis, asthma and hypertension [9], which imply its potential use in treatment of several pathological conditions. Reports on the genotoxic and antigenotoxic abilities of apocynin are scarce. In view of the vast potential of this compound as a therapeutic agent, we chose to study the genotoxic and antigenotoxic properties, as well as the cytotoxic and anticytotoxic nature, of this compound.

Another major group of plant secondary metabolites with extensive biological and therapeutic performance are saponins. Saponins are the principle constituents in many herbal medicines and recognised for the health benefits offered by foods like soya [30]. Diosgenin is a steroidal saponin present in abundance in legumes and yams. It is extensively utilised in the pharmaceutical industry as starting point of various synthetic steroidal drugs. The antioxidant nature of this compound is well established [31]. The role of diosgenin and plant extracts containing diosgenin as an active principle, as chemopreventive or chemotherapeutic agents is being elaborately studied [32,33]. Efforts are being taken towards understanding the favourable role of diosgenin in case of metabolic diseases like hypercholesterolemia, obesity, dyslipidemia, inflammation, and diabetes [34]. An essential requirement in this process would be the understanding of genotoxic and antigenotoxic influences of diosgenin, on which very limited information is available. In this context, diosgenin was chosen as a part of this research work. In the present study, we investigated these two selected active plant constituents, apocynin and diosgenin, for their genotoxic and antigenotoxic properties. This could provide valuable leads in search of compounds that can reduce the occurrence of clastogenic and mutagenic alterations and consequent degenerative diseases.

Assessment of genotoxic and antigenotoxic nature of substances can be done by a series of in vitro and in vivo assay systems that became established over a period a time, as suitable indicators, under various international guide lines. A number of experimental systems are also available for ascertaining the cytotoxic and anticytotoxic
nature of substance. The experimental methodology for the current study was based on the recommendations in the various internationally accepted guidelines, chosen after reviewing a number of research reports in this area of science by researchers from different countries. Assays based on both animal and plant systems can be employed for a deeper understanding of the genotoxic nature of the selected plant compounds [35].

With this background, the current research work was proposed and carried out to explore the genotoxic/ antigenotoxic properties of apocynin and diosgenin against cyclophosphamide using the mouse bone marrow micronucleation test, measuring the haematological and oxidative stress parameters. The compatibility of apocynin and diosgenin with biological systems was further evaluated by testing their cytotoxic and anticytotoxic properties in cell culture systems. We also employed the Allium root tip meristem analysis, haemolytic and DNA fragmentation assay for a broad interpretation of the genotoxic and antigenotoxic properties of apocynin.

1.2 Objectives of the Study

Major Aim

The aim of the present investigation was to evaluate potential genotoxic and antigenotoxic effects of apocynin and diosgenin against chromosomal and genome damage induced by the chemotherapeutic agents in living cells and tissues, using in vitro and in vivo assays.

Objectives

1. To investigate the genotoxic and antigenotoxic potency of apocynin under cyclophosphamide induced oxidative stress and genotoxicity in experimental albino mice.

2. To investigate the genotoxic and antigenotoxic potency of diosgenin under cyclophosphamide induced oxidative stress and genotoxicity in experimental albino mice.

3. To investigate the possible cytotoxic and anticytotoxic potency of apocynin in cisplatin induced cytotoxicity by using MTT assay.
4. To investigate if apocynin has a selective cytoprotective efficiency towards normal cells compared to cancer cells

5. To investigate the possible cytotoxic and anticytotoxic potency of diosgenin in cisplatin induced cytotoxicity by using MTT assay.

6. To investigate if diosgenin has a selective cytoprotective efficiency towards normal cells compared to cancer cells

7. To investigate the protective effect of apocynin on cyclophosphamide induced chromosome aberrations in *Allium cepa* root tip meristem cells.

8. To investigate the possible effects of apocynin on haemolysis and DNA fragmentation in cell lines under cisplatin induced oxidative stress.