**DISCUSSION**

Lee et al. (1986) studied the antioxidant activity of ginger. Cao et al. (1993) reported the scavenging effects of ginger on superoxide anion, and hydroxyl radical and hence the activity of 6-gingerol may be to scavenge $O_2^-$, which has already been reported in case of hypoxanthine oxidase system and the OH radical under UV exposure to $H_2O_2$ system. The scavenging effect of $O_2^-$ and OH$^-$ may, in fact, contribute toward explaining the pharmacological action of 6-gingerol (Lee et al., 1998) and determining the antitumor promotional activity of 6-gingerol, the major pungent principle of ginger, using a two-stage mouse skin carcinogenesis model. Application of 6-gingerol onto shaven backs of female ICR mice prior to each topical dose of 12-0-Tetradecanoyl Phorbor-13-Acetate (TPA), significantly inhibited 7, 12-dimethyl benz[a]anthracene-induced skin papillomagenesis. The compound also suppressed TPA-induced epidermal ornithine decarboxylase activity and inflammation. The antioxidant activity of 6-gingerol can further be studied with other assay systems.

Both neoplastic and mutagenic properties have been observed in MMS which is a monofunctional alkylating agent. It alkylates DNA at the N-7 position of guanine and the N-3 position of adenine. All these changes may give rise to abnormal base pairing at DNA replication (Mittal and Musarrat, 1990; Madrigal-Bujaidar et al., 1998). The production of methylguanosine lesions is attributed to the SCE end point, the lesions being related to tumor formation (Swenberg et al., 1985). SCE induction by MMS has been studied in earlier experiments with human lymphocytes showing positive results (Craig-Holmes and Shaw, 1977). CP is also an alkylating agent and after metabolic activation, it produces active mutagenic metabolic product phospharamide mustard (Ghaskadbi et al., 1992). It reacts with electron-rich area of susceptible molecules such as the DNA proteins (Krishna et al., 1986).
In this study, genistein is observed to lower the CAs and SCEs in MMS and CP induced genotoxicity, in the absence and also the presence of S9 mix in culture, respectively. Genistein may behave as free radical scavenger and hence, might react with metabolites which are carcinogenic before they are able to inflict DNA damaging effects. Inhibition of multiplication of neoplastic cells, including those in breasts, has been reported for genistein (Lamartiniere, 2000).

The results obtained in this study on genistein, a flavonoid, support the work done till now on natural products (especially genistein) in relation to their chemopreventive potential to reduce the genotoxicity induced by carcinogenic compounds. Therefore, genistein and other similar phytochemicals may be utilized commercially for pharmaceutical research as a model for chemopreventive drugs.

The use of stanozolol in sports is illegal and banned by the International Association of Athletics Federations. It enjoys large oral bioavailability due to a C17 alpha-alkylation which allows the hormone to survive first pass of liver metabolism. High doses of stanozolol could exert a proliferative effect on liver cells (Boada et al., 1999). Acute overdosage can produce nausea and gastrointestinal upset. Chronic use of stanozolol can cause menstrual irregularities and virilization in women, while impotence, premature cardiovascular disease and prostatic hypertrophy in men. Precocious prostatic cancer has been described after long term anabolic steroid abuse (Roberts and Essenhigh, 1986). Cases where hepatic cancers have been associated with anabolic steroid abuse have been reported (Overly et al., 1984). Also, androgen ingestion by a pregnant mother can cause virilization of a female fetus (Dewhurst and Gordon, 1984).

The trenbolone compounds have a binding affinity for the androgen receptor thrice as high as that of testosterone. Once metabolized, the drugs have the effect of increasing nitrogen uptake by muscles, leading to an increase in the secondary effects of stimulating appetite, reducing the amount of fat being deposited in the body, and decreasing the rate of catabolism. Some short term side effects include insomnia, high blood pressure and
increased aggression and libido. However, women suffer virilization effects even at small doses (http://en.wikipedia.org/wiki/trenbolone, 2007).

The only legitimate therapeutic indications for such anabolic steroids are in the replacement of male sex steroids in men who have androgen deficiency, e.g. due to loss of both the testes, in the treatment of certain forms of rare aplastic anaemia which are or may be responsive to anabolic androgens and, in case of certain countries to counteract catabolic states, e.g. after major trauma.

This experiment implies that the synthetic anabolic steroids, stanozolol and trenbolone, have the potential to cause genotoxic damage in human lymphocytes in vitro at higher dosage both in the presence and absence of S9 mix. Changes in chromosome structure due to a break or a swapping of chromosomal material are termed as CAs. Most of the CAs in cells are lethal, but many of them are also viable and can cause genetic effects, either somatic or inherited (Swierenga et al., 1991). These events can lead to the loss of chromosomal material at mitosis or to the inhibition of exact chromosome segregation at anaphase. The result of these changes is cell lethality (Tucker and Preston, 1996). In our experiment, we came across significant differences compared with control in the CA frequency at 40 and 60 \( \mu \text{M} \), with or without S9 mix. SCE is usually a more sensitive indicator of genotoxic effects than CA (Tucker and Preston, 1996). There is a correlation between the carcinogenicity and SCE inducing ability of many chemicals. Moreover, the SCE induction mechanism is heterogeneous and very different from the mechanism of CA induction (Gebhart, 1981). Androgenic steroids display teratogenic effects in all species that have been studied so far, and do so in a very predictable and consistent way (Juchau, 1997). Various psychological and physiological effects have been reported in both males and females among frequent users of androgens (McEvoy, 1995). There is little, if any, information available on the exact reasons for the genotoxic behavior of stanozolol and trenbolone. However, the present study is concurrent with the studies performed on synthetic steroids such as cyproterone acetate, ethynodiol diacetate, chlormadinone acetate, medroxyprogesterone acetate,
norgestrel and megestrol acetate that induced CAs and SCEs with or without metabolic activation system (Ahmad et al., 2001; Hundal et al., 1997; Siddique and Afzal, 2004a,b,c,d; Siddique et al., 2005a,b).

The outcome of most investigative experiments shows that stanozolol and trenbolone have the potential to be genotoxic and cytotoxic, especially at 40 and 60 µM, with or without metabolic activation, in cultured human lymphocytes. The evaluation of these genotoxicity tests is a useful tool for determining the toxic effects of potentially genotoxic chemicals, leading to identification of such carcinogenic agents. It is advisable to use the steroids studied here at their lowest effective dosage so that the risk to public health could be minimized. The risk of damage to human genetic material is a likely outcome of higher doses of these drugs.

Genistein is particularly effective in quenching free radicals produced by toxic agents and protects cells against oxidative damage especially with respect to DNA (Foti et al., 2005; Lee et al., 2000). It is a common precursor in the biosynthesis of antimicrobial phytoalexins and phytoanticipins in legumes, and an important nutraceutical molecule. It is also capable of inhibiting lipoprotein oxidation in vitro and suppressing formation of plasma lipid oxidation products in vivo. Extracts of ginger (including gingerol) have antioxidant activity through scavenging of superoxide and hydroxyl radicals and by inhibiting lipid peroxidation (Kikuzaki and Nakatani, 1993). It is also antibacterial, antifungal and used for common cold (Bode et al., 2001). The potency of ginger is due to gingerol which is an alcohol of oleoresin and the aroma is due to its oil (Hasenohrl et al., 1998). Gingerol is the major pharmacologically active component including a cause of apoptosis (Lee and Surh, 1998; Lee et al., 1998) in cancer cells. The results of the present study reveal that the selected dosages of genistein and gingerol are not genotoxic per se but reduce the genotoxic damage caused by oxandrolone and norethandrolone in human lymphocytes in vitro. The International Agency on Cancer (IAC), mainly on the basis of epidemiological studies classifies steroidal estrogen progestin combinations among agents carcinogenic to humans (Group 1), progestins as possibly carcinogenic (Group 2) and
androgenic anabolic steroids, as probably carcinogenic (Group 2A) (Martelli et al., 2003).

An increase in the frequency of chromosomal aberrations in peripheral blood lymphocytes is associated with an increased overall risk of cancer (Hagmar et al., 1994, 1998). The readily quantifiable nature of sister chromatid exchanges with high sensitivity for revealing toxicant-DNA interaction and the demonstrated ability of genotoxic chemicals to induce significant increase in sister chromatid exchanges in cultured cells has resulted in this endpoint being used as indicator of DNA damage in blood lymphocytes of individuals exposed to genotoxic carcinogens (Albertini et al., 2000). The above genotoxic endpoints are well known markers of genotoxicity and any reduction in the frequency of these genotoxic endpoints gives us indication of the antigenotoxicity of a particular compound (Albertini et al., 2000). Many products protect against xenobiotics either by inducing detoxifying enzymes or by inhibiting oxidative enzymes (Morse and Stoner, 1993).

The protective effect observed in the present study i.e. significant reduction in the frequency of cells with chromosomal damage and sister chromatid exchanges, may be due to the direct action of the compounds (i.e. genistein and gingerol).

The outcome of this experiment shows that norethandrolone and oxandrolone have the potential to be genotoxic and cytotoxic, especially at 30 and 40 μM, in cultured human lymphocytes and their genotoxicity is reduced significantly on applying genistein and gingerol separately, at appropriate dosage. The evaluation of these genotoxicity tests is a useful tool for determining the toxic effects of potentially genotoxic chemicals, leading to identification of such carcinogenic agents. It is advisable to use the steroids studied here at their lowest effective dosage so that the risk to public health could be minimized. The risk of damage to human genetic material is very likely at higher doses of these drugs. The effectiveness of genistein and gingerol as antimutagenic agents is an attribute that can be effectively used in making anticancer drugs.
The result of the present study reveals that TP is genotoxic only in the presence of metabolic activation (S9 mix + NADP). The first step may involve aromatic hydroxylation catalyzed by cytochrome p450 as in the case of other steroids (Fishman, 1983). Cytochrome p450 in liver S9 fractions plays an important role in activating promutagens to proximate and/or ultimate mutagens (Maron and Ames, 1983; Guengerich and Shimada, 1991).

Testosterone in human body can be converted to both estrogen (aromatization) as well as dihydrotestosterone. Estrogen is the main metabolic for many side-effects in the human body and it has also been reported for genotoxicity in various in vitro and in vivo experimental models (Dhillon et al., 1994; Hundal et al., 1997; Joosten et al., 2004; Siddique et al., 2005a).

In our present study, TP was found to increase CAs and SCEs at 20 and 40 µM. CAs are changes in chromosome structure resulting from a break or an exchange of chromosomal material. Most of the CAs observed in the cells are lethal but there are many other aberrations that are viable and can cause genetic effects either somatic or inherited (Swierenga et al., 1991). SCE is generally a more sensitive indicator of genotoxic effects than structural aberrations (Tucker and Preston, 1996). There is a correlation between the carcinogenicity and SCE inducing ability of large number of chemicals (Gebhart, 1981). The ready quantifiable nature of SCEs with high sensitivity for revealing toxicant-DNA interaction and the demonstrated ability of genotoxic chemicals to induce significant increase in SCEs in cultured cells has resulted into this endpoint being used as an indicator of DNA damage in blood lymphocytes of individuals exposed to genotoxic carcinogens. The above genotoxic endpoints are well known markers of genotoxicity and any reduction in the frequency of the genotoxic endpoints gives an indication of the antigenotoxicity of a particular compound (Albertini et al., 2000). Increase of chromosomal aberration has been associated with the increase in the incidence of formation of various carcinomas (Hagmar et al., 1994; Hagmar et al., 1998).
Natural plant products have been reported to reduce genotoxic effect of steroids in various in vitro and in vivo models. The genotoxic effects of steroids can be reduced by the use of antioxidants and natural plant products (Ahmad et al., 2004; Siddique and Afzal, 2005b; Siddique et al., 2005b; Siddique et al., 2006b; Siddique et al., 2007a,b,c; Beg et al., 2007; Siddique et al., 2008a,b). In this study, ECG reduces genotoxicity of TP in human lymphocytes. The reduction in genotoxic damage may be due to the possibility of the prevention of metabolic activation of TP by ECG. The selected dosage of ECG is potent enough to reduce genotoxicity. The concentrations studied here are higher than those of commonly used steroids. The higher concentration may be reached in some clinical conditions (Martelli et al., 2003) and this higher concentration may lead to genotoxic damage and may further increase the possibility of the development of various types of cancers (Albertini et al., 2000). ECG reduced genotoxic damage by the highest tested dosage thereby giving an indication of its protective role.