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REVIEW

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Genotoxicity induced by medicinal plants

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Abstract

Background: The use of medicinal plants in curing diseases is an ancient culture still in use in many parts of the world. Many plants have been proven to have precise ethno-pharmacological relevance. On the contrary, many folkloric plants have also been found to possess DNA damaging effects. Hence, assessing the safety profile of medicinal herbs before being approved for use must be undertaken.

Main text: This review focuses on medicinal plants exerting genotoxicity effect within through in vivo studies on the bone marrow, erythrocyte or other organs on animal models and in vitro studies on bacterial cells or mammalian cell lines such as mammalian lymphocytes, human hepatoma cell line or HepG2, mouse lung fibroblast cell lines or human adenocarcinoma cell lines. This review has found that several medicinal plants possess genotoxic potentials and are not safe to use. The common methodologies several authors have used include the comet assay, micronucleus assay, bacterial reverse mutation assay, Ames test or Salmonella/microsome assay.

Conclusion: Plants that have been proven to be genotoxic are not reduced to a particular family, while groups including Fabaceae, Asteraceae, Euphorbiaceae, Rosaceae, Lamiaceae and Apocynaceae appear to be frequent. To avoid any mutation in its users, genotoxicity assessment of therapeutic plants appears to be required.

Keywords: Ames test, Comet assay, Genotoxicity, Medicinal plants, Micronucleus assay

Background

The ethno-social lives of different tribes of people throughout the world have seen to be inevitably reliant on plants and their products for therapeutic purposes. In many indigenous cultures, knowledge and practice of using herbal medicine has been passed from generation to generation. According to the World Health Organization (WHO), 70– 80% of the world population depends exclusively on herbs for their primary health care (Chan 2003; Muhammad et al. 2011; Sponchiado et al. 2016). In India, about 7000–7500 species of plants have medicinal usage in folk and documented systems of medicine (Pandey et al. 2013). It is believed that as many as 25,000 different formulations are prepared from these plants as a cure against various diseases and disorders (Pandey et al. 2013). Even in the process of developing new drugs, plant constituents constitute an important component as in vitro molecular syntheses are difficult, and most can be used as a prototype for the synthesis of new drugs.

It is a common belief that the herbal products are safe and more efficient than their allopathic counterparts which make its uses more prominent. However, since this assumption is false and harmful, toxicological research on herbal medications should be conducted (Kahaliw et al. 2018). Studies have revealed that some plants frequently used in folk medicine were potentially genotoxic (Marques et al. 2003; Ananthi et al. 2010; Melo-Reis et al. 2011; Regner et al. 2011; Shin et al. 2011; Sponchiado et al. 2016). The interaction of any toxic material may lead to numerous chromosomal aberrations including chromatid breaks, isochromatid breaks, gaps, chromosomal fragments, exchanges, and sister chromatid unions; even in case of the change in DNA structure. The consequences of such DNA impairment could be the establishment of and/or predisposition to diseases, increased morbidity/mortality, changes in heritable characteristics, and impaired reproductive capacity (Lázaro et al. 2010; Sponchiado et al. 2016).

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The leap in the biophysical techniques has made studies on toxicity diverse and advance. There were many assays following different methodologies and strategies that have been developed to make the toxicity study efficient. The establishment of these protocols has been based on studies demonstrating the correlation between carcinogenicity and mutagenicity, and the correlation of both parameters with genotoxicity (Dearfield et al. 1991; Waters et al. 1999). Since then, established processes for assessing the genotoxic risk associated with the use of medications, food additives, pesticides, industrial and environmental chemicals, as well as natural items such as medicinal plants and their oils, have been employed (Waters et al. 1999; Chen-Chen and Sena 2002; Costa et al. 2018).

There are several different species of plants that are being used to cure various diseases such as headache, respiratory infection, inflammation, gastrointestinal disorders, memory loss, stress, insomnia, anemia, diminishing eyesight and sexual impotency, dermatitis, diabetes, wounds and pains, and cancers. The genotoxic potentials have been recorded here. Any plant may have potential genotoxic capability in a limited region or throughout, and the chemical employed to make the extract, as well as the model organism utilised, have a significant impact on this conclusion in vivo or in vitro. Through this study, an effort has been made to find out the assays used and where and how they have been experimented on along with families where these plants belong.

Main text

Medicinal plants and genotoxic assays

The inherent knowledge of folkloric herbal medicine is a pivotal aspect of ethnicity which should be scientifically tested upon. Many familiar plants have been found to contain compounds bearing DNA damaging impact (Fig. 1). There are specific procedures and protocols known as "genotoxic assay" to ensure the safety profile of any suspected candidate (Fig. 2). Genotoxic assays have different endpoints, such as single- and double-strand breaks, point mutations, deletions, chromosomal aberration, micronuclei formation, DNA repair, and cell-cycle interactions (Ng et al. 2010). Different assays which have been found to be used while studying these plants are in vivo or in vitro comet assay (CA), micronucleus test (MN), Ames test or Salmonella/microsome assay or Bacterial reverse mutation assay (BRM), *Allium cepa* test (ACT), and chromosomal aberration assay (ChA). Vitotox assay (VA), yH2AX In-Cell Western Assay (WA), mouse lymphoma tk assay (MLtk), and lysogenic induction assay (LI) have also been used to find out genotoxic medicinal plants.

The choice of assay to be performed is a crucial decision in geno-toxicological studies. It's worth noting that genotoxicity should be assessed using a series of assays because no one test can identify total genotoxicity (Saravanan et al. 2020). Specificity in detection even comes along with models and extracts. An aqueous extract of nine medicinal plants known in natural Korean medicine under the name "Gumi ganghwaltang" demonstrated

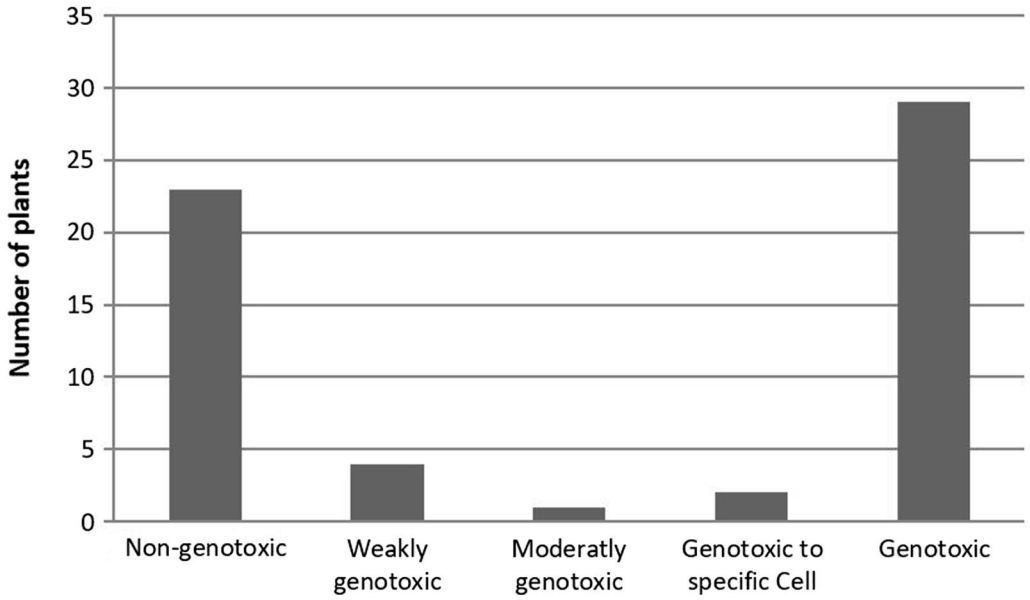

Fig. 1 Genotoxic behavior of medicinal plants

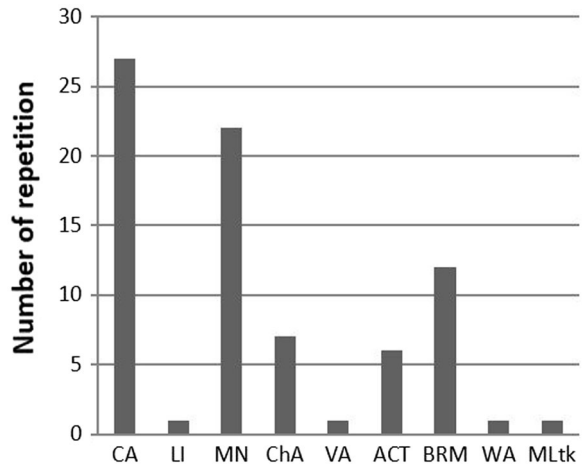

Fig. 2 Genotoxic assays used. CA, comet assay; LI, lysogenic induction assay; MN, micronucleus test; ChA, chromosomal aberration assay; VA, Vitotox assay; ACT, *Allium cepa* test; BRM, Bacterial reverse mutation assay; WA, Western Assay; MLtk, mouse lymphoma tk assay

genotoxicity in the Ames test and Chinese hamster lung cell culture (CHL cells) but was inactive for the micronuclei count in polychromatophylic mice erythrocytes (Shin et al. 2011; Durnev and Lapitskaia 2013). An aqueous extract of six plants "Pyungwisan," which belong to the same group of natural drugs, induced chromosome aberrations in a test with CHL cells in vitro (Shin et al. 2011; Durnev and Lapitskaia 2013).

Preparation of the extract from the desired parts of the plant is a major determining step in any scientific study. Different solvents were used to prepare them based on the physical and chemical properties of the constituents (Fig. 3). Solvents commonly used in extraction of medicinal plants are polar solvent (e.g., water, alcohols), intermediate polar (e.g., acetone, dichloromethane), and nonpolar (e.g., n-hexane, ether, chloroform) (Abubakar and Haque 2020). In general, extraction procedures include maceration, digestion, decoction, infusion, percolation, Soxhlet extraction, superficial extraction, ultrasound-assisted, and microwave-assisted extractions (Abubakar and Haque 2020). When the manner of extracts utilized in research is quantified, alcoholic extracts are shown to be the most popular.

In vitro studies

In vitro genotoxic assays represent simple, robust and time and cost-effective testing of targeted toxicity and underlying mechanisms (Dusinka et al. 2012). In vitro studies have been done using Ames test, in vitro comet and micronucleus test, in vitro chromosomal aberration assay, vitotox assay, mouse lymphoma tk assay on bacterial strains or mammalian cell lines such as HepG2 cell line, lymphocytes or peripheral mononuclear blood cell line following Organisation for Economic Co-operation and Development (OECD) guideline 473 (2016). Several workers have worked using both in vitro and in vivo assays.

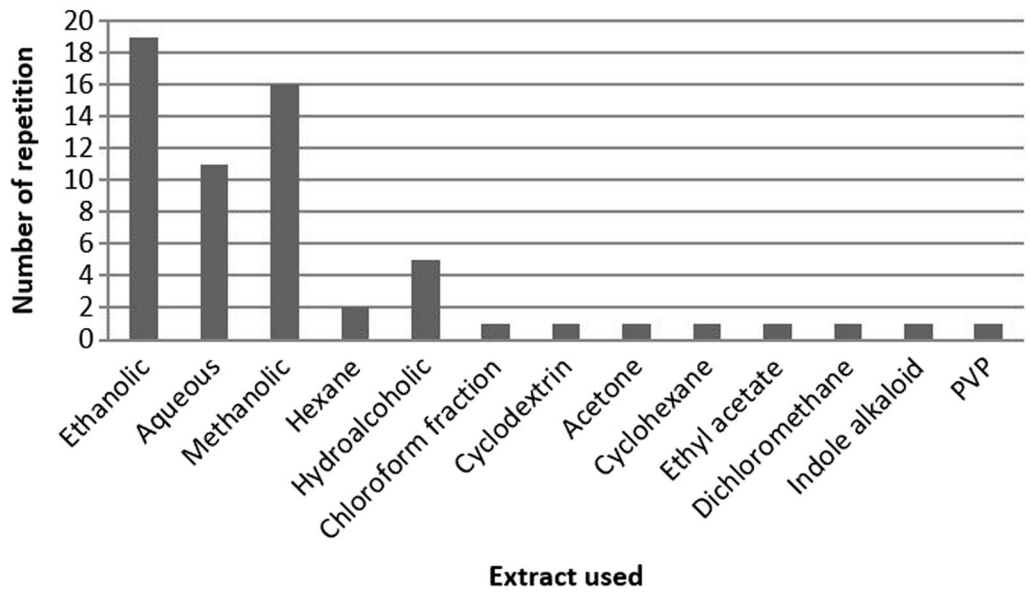

Fig. 3 Extracts used

Tsuboy et al. (2010), Maronpot (2015), Shin et al. (2015), Akhtar et al. (2016), Sharif et al. (2017), Quadros et al. (2017), Madikizela and McGaw (2017), Jeong et al. (2018a, b), Kon-Young et al. (2020), Saravanan et al. (2020), and Zhao et al. (2020) studied different plants using in vitro Bacterial reverse mutation assay or Ames test or *Salmonella*/microsome assay. It was based on induction of reverse mutation in the histidine gene, which enables the bacteria to synthesize histidine and form visible colonies in minimal histidine medium (Dusinka et al. 2012). The protocols that are followed are given by Maron and Ames (1983) and Mortelmans and Zeiger (2000).

The first ever toxicity (genotoxicity and cytotoxicity) study using Vitotox assay which was also a bacterial genotoxicity test was performed by Chichico-Hernandez et al. (2011) with 100% methanolic extracts of *Cassia fistula, Derris elliptica, Ficus elastica, Gliciridia sepium, Michelia alba, Morus alba, Pogostemon cablin* and *Ricinus communis* on two different strains of *Salmonella typhimurium* (TA 104) based on their SOS response and found genotoxic effect only on *P. cablin* and *R. communis*. One has a luciferase gene under the control of the recN promoter, which leads to light production when DNA is damaged (TA 104-recN2-4 strain or Genox strain) while the second one contains lux-genes under the control of a constitutive promoter so that the light production is not influenced by genotoxic compounds (pr1 or Cytox strain). It serves as an internal control wherein, if the light production goes up, the test compounds affect the lux gene in a different way than damaging the DNA. On the other hand, a decrease in light production would indicate a toxic response (Chichico-Hernandez et al. 2011).

The in vitro chromosomal aberration test identifies agents that cause structural chromosomal aberrations in cultured media. Kulkarni et al. (2010) used this technique with the methanolic extracts made from fruit and leaf of *Persea Americana* on human peripheral blood cells and found the occurrence of acrocentric associations and premature centromeric separation. Cell cultures are exposed to the test substance both with and without metabolic activation and are treated with a metaphase-arresting substance (colcemid or colchicine) before harvest (Dusinka et al. 2012) which then can detect the presence of aberrant chromosome microscopically.

Comet assay is found to be the most repeated assay. This method represents a rapid, sensitive, reliable, robust and relatively inexpensive way to study DNA damage (including DNA oxidation), and repair in different cell types both in vitro as well as in vivo (Dusinka et al. 2012). Sassi et al. (2016) performed in vitro comet assay with *Ceratonia siliqua* extract in murine leukemia cell; Maistro et al. (2019) with *Salix alba* bark extract on PBMC and HepG2 cells; Beeran et al. (2020) on human adenocarcinoma cell line with *Vernonia cinerea*, and Ahmadi et al. (2021) with the extract of *Ziziphora clinopodioides* on PBMC. Any breakage in DNA strand can be observed as that of comets when put under electrophoresis. The more is the damage in DNA; more is the number of comets formed and hence is a proof of more genotoxicity impact.

In vivo study

Compared with in vitro genotoxicity assay, in vivo genotoxicity assay has been used to verify in vitro assay result and definitely provide biological significance for certain organs or cell types (Kang et al. 2013). In vivo genotoxicity tests using tissues can be used when obtaining in vitro positive results that can reflect absorption, excretion, distribution and metabolism of chemicals but the in vitro test does not (Sasaki et al. 2002; Benigni et al. 2012; Kang et al. 2013). Most of the studies are anyway based on in vivo protocols of comet and micronucleus assay.

In vivo comet assay and micronucleus test can be performed on any type of animal tissue and even blood sample after the proper treatment on the model organism. Tsuboy et al. (2010) performed in vivo comet assay by withdrawing peripheral blood puncturing caudal vein following the guideline given by Tice et al. (2000) in *Coccoloba mollis*. Whether several other workers followed the protocols given by Singh et al. (1988) which was modified by Hartmann and Speit (1997), except for Boiera et al. (2010) who followed the modified protocol of Da Silva et al. (2000). One of the most popular protocol of micronucleus (MN) test is to observe the presence of micronucleus on bone marrow after flushing out the femurs as done by Regner et al. (2011) on the hexane extract of aerial parts of *Pterocaulon polystachyum*, Asare et al. (2012) with ethanolicc extract of *Phyllanthus niruni* and several others.

Another popular in vivo study is *Allium cepa* test where the *Allium cepa* root tip is studied after treatment for the presence of chromosomal aberration in mitotic stage. Ping et al. (2012), Almeida et al. (2016), Paw et al. (2020), Gogoi et al. (2020), Dey et al. (2021), de Souza et al. (2020) and Asita et al. (2021) studies genotoxicity using this assay on *Euphoria hirta, Jatropha gossypiifolia, Curcuma caesia, Cymbopogon khasianus, Aristolochia indica, Chaptalia nutants*, respectively.

Geno-toxicological findings

Genotoxicity study to explore the toxicity measures of different medicinal plants used in folkloric medicine culture have led quite a prominent way till now (Table 1). The detailed study of the literature mentioned is a proof of that. There were many in vitro and in vivo studies that

Table 1 Medicinal plants studied for their genotoxic potential

Sl no	References	Plants used	Family	Genotoxic assay	Result	Extract	Model organism/ cell line
1	Tsuboy et al. (2010)	*Coccoloba mollis*	Polygonaceae	Ames test, Comet assay, Micronucleus test	No genotoxic effect	Ethanol (95%)	HTC cell
2	Vidal et al. (2010)	*Echinodorus macrophyllus*	Alismataceae	Lysogenic induction assay	Genotoxic	Ethanol (70%)	*E. coli*
3	Boiera et al. (2010)	*Passiflora alata*	Passifloraceae	Comet and Micronucleus test	Genotoxic	Aqueous	Mice and Wistar Rat
4	Kulkarni et al. (2010)	*Persea americana*	Lauraceae	Chromosome aberration assay	Genotoxic	Methanol (50%)	Human peripheral blood cell
5	Chichioco-Hernandez et al. (2011)	*Cassia fistula*, *Derris eliptica*, *Ficus elastica*, *Gliciridia sepium*, *Michelia alba*, *Pogostemon cablin*, *Ricinus communis*	Fabaceae Fabaceae Moraceae Fabaceae Magnoliaceae Lamiaceae Euphorbiaceae	Vitotox assay	No genotoxic effect (genotoxic for *P. cablin* and *R. communis*)	Methanol (100%)	*Salmonella typhimurium*
6	Regner et al. (2011)	*Pterocaulon polystachyum*	Asteraceae	Comet assay	Genotoxic (Only in kidney tissue)	Hexane	Mice
7	Asare et al. (2012)	*Phyllanthus niruni*	Phyllanthaceae	Chromosome aberration assay	Non-genotoxic	Ethanol	Rat
8	Raymundo et al. (2012)	*Maytenus robusta*	Celastraceae	Comet assay	Weak genotoxic effect	Hydroalcohol	Mice
9	Ping et al. (2012)	*Euphorbia hirta*	Euphorbiaceae	*Allium cepa* assay	Dose dependent genotoxicity	Methanol	*Allium cepa* root
10	dos Santos et al. (2012)	*Bauhinia platypetala*	Leguminoceae	Alkaline comet assay	Genotoxic	Ethanol	*Saccharomyces cerevisiae*
11	dos Santos et al. (2013)	*Arrabidaea chica*	Bignoniaceae	Micronucleus and alkaline comet assay	No genotoxic effect	Chloroform fraction	Mice
12	Soumaya et al. (2013)	*Cyperus rotundus*	Cyperaceae	Chromosome aberration assay	Genotoxic	Methanol	BALB/c mice
13	Demma et al. (2013)	*Glinus lotoides*	Ranunculaceae	Comet assay	Genotoxic	Methanol 60%	Rat
14	Boldbaatar et al. (2014)	*Leptopyrum fumarioides*	Amaryllidaceae	Comet assay	Genotoxic	Methanol	Mice
15	Nair and Van Staden (2014)	*Boophone disticha*	Euphorbiaecae	(review)	Non-genotoxic	-	-
16	Njoya et al. (2014)	*Codiaeum variegatum*	Euphorbiacea	Comet assay	Non-genotoxic	Aqueous	Mice lymphoma, primary hepatic cell, HepG2 cell
17	Discon et al. (2015)	*Grewia tiliaefolia*	Tiliaceae	Comet assay	Non-genotoxic	Methanol	Wistar rat and human peripheral blood mononuclear cell
18	Maronpot (2015)	*Ashitaba Chalcone*	Apiaceae	Bacterial reverse mutation assay, chromosomal aberration, mouse micronuclear assay	Non-genotoxic	Cyclodextrin	*Salmonella typhimurium*, Mice

Table 1 (continued)

Sl no	References	Plants used	Family	Genotoxic assay	Result	Extract	Model organism/ cell line
19	Shin et al. (2015)	*Polygala tenuifolia*	Polygalaceae	Bacterial reverse mutation assay, chromosomal aberration, mouse micronuclear assay	Non-genotoxic	Ethanol 75%	*Salmonella typhimurium, E. coli*, mice, Chinese hamster
20	Eck-Varanka et al. (2015)	*Lythrum salicaria*	Lythraceae	Mussel Micronucleus test	Genotoxic	Aqueous	*Unio pictorum*
21	Akhtar et al. (2016)	*Terminalia citrina*	Combrataceae	Ames reverse mutagenicity assay	Genotoxic	Ethanol and aqueous	*Salmonella typhimurium*
22	Sassi et al. (2016)	*Ceratonia siliqua*	Fabaceae	Comet assay	No genotoxic effect	Aqueous	Murine Leukemia cell
23	de Oliveira et al. (2016)	*Morus alba*	Moraceae	Micronucleus test	Genotoxic	Ethanol	Swiss Mice
24	Mendonca et al. (2016)	*Cecropia pachystachyca*	Urticaceae	Comet and Micronucleus test	Genotoxic to brain tissue	Aqueous	Mice
25	Almeida et al. (2016)	*Jatropha gossypiifolia*	Euphorbiaceae	*Allium cepa* assay	Genotoxic (not in aqueous)	Ethanol	*Allium cepa* root
26	Nazari et al. (2017)	*Glycyrrhiza glabra*	Fabaceae	Review	Genotoxic at high doses	Aqueous Ethanol	Rat/mice
27	Sharif et al. (2017)	*Kalanchoe laciniata*	Crassulaceae	Ames and MTT assay	Weak genotoxic effect	Methanol and n-hexane	*Salmonella typhimurium*
28	Ishikawa et al. (2017)	*Doliocarpus dentatus*	Dilleniaceae	Comet and Micronucleus test	Not genotoxic	Ethanol	*Mycobacterium tuberculosis*
29	Quadros et al. (2017)	*Crataegus oxycantha*	Rosaceae	Ames and micronucleus test	Genotoxic at high doses	Methanol (70%)	Leukocyte and HepG2 cell
30	Madikizela and McGaw (2017)	*Pittosporum viridiflorum Hypoxis colchicifolia*	Pittosporaceae Hypoxidaceae	*Salmonella* microsome assay	No reported genotoxicity	Acetone, Ethanol (70%), aqueous	*Salmonella typhimurium*
31	Acesio et al. (2017)	*Annona muricata*	Annonaceae	Micronucleus test	Non-genotoxic	Ethanol	Mice
32	Yonekubo et al. (2018)	*Cratageus oxyacantha*	Rosaceae	Comet and Micronucleus test	Genotoxic at high dose	Methanol (70%)	Mice
33	de Oliveira et al. (2018)	*Vochysia divergens*	Vochysiaceae	Micronucleus test	Genotoxic at high concentration	Ethanol	Male Swiss mice
34	Kahaliw et al. (2018)	*Pterolobium stellatum, Otostegia integrifolia, Vernonia amygdalina*	Fabaceae Lamiaceae Asteraceae	Alkaline cometassay	Genotoxic	Methanol	HepG2 cells
35	Singh et al. (2018)	*Meyna spinosa, Oroxylum indicum*	Rubiaceae Bignoniaceae	Comet and Micronucleus test	MS (genotoxic); OI (non-genotoxic)	Hydroalcohol	BALB/c mice
36	Jeong et al. (2018a, b)	*Eriobotrya japonica*	Rosaceae	Bacterial reverse mutation assay, micronucleus assay	Non-genotoxic	Ethanol	*Salmonella typhimurium, E. coli*. Chinese Hamster, mice
37	Soleimani et al. (2019)	*Silybum marianum*	Asteraceae	Review	No genotoxic effect	-	*Salmonella typhimurium, E. coli*
38	de Medeiros et al. (2019)	*Cereus jamacaru*	Cactaceae	Micronucleus test	Genotoxic	Ethanol	Rat
39	Maistro et al. (2019)	*Salix alba*	Saliaceae	Comet assay	Low genotoxic effect; when not digested by Liver enzymes	Ethanol (70%)	Human peripheral leukocyte and HepG2

Table 1 (continued)

Sl no	References	Plants used	Family	Genotoxic assay	Result	Extract	Model organism/ cell line
40	Tsafantakis et al. (2019)	*Opuntia ficus indica*	Cacteceae	Comet and the yH2AX In-Cell Western Assay	Not genotoxic, but fruit flesh and seed extract -genotoxic	Aqueous	HepG2 cells
41	Rocha et al. (2019)	*Pityrocarpa monilifomis*	Fabaceae	Comet and Micronucleus test	Non-genotoxic	Cyclohexane, ethyl acetate, methanol	Mice
42	Boas et al. (2019)	*Mangifera indica*	Anacardiaceae	Comet and Micronucleus test	Non-genotoxic	Aqueous	Rat
43	Kon-Young et al. (2020)	GHX02 (A mixture) Gwaruin Haengin Hwangryeon Hwangkeum	Cucurbitaceae Rosaceae Ranunculaceae Lamiaceae	Comet assay, in vivo Micronucleus test, bacterial reverse mutation, in vitro chromosomal aberration assay	In vitro genotoxic In vivo non-genotoxic	Aqueous	Sprague–Dawley rats
44	Beeran et al. (2020)	*Vernonia cinerea*	Asteraceae	Comet assay	Genotoxic	Dichloromethane	Human adenocarcinoma
45	Paw et al. (2020)	*Curcuma caesia*	Zingiberaceae	*Allium cepa* assay	Negligible	Rhizome Oil	*Allium cepa* root
46	Gogoi et al. (2020)	*Cymbopogon khasianus*	Poaceae	*Allium cepa* assay	moderate effect	Methanol	*Allium cepa* root
47	Luo et al. (2020)	*Eucommia ulmoides*	Eucommiaceae	Micronucleus and chromosomal assay	non-genotoxic	Ethanol (70%)	Wistar rat and Mice
48	de Moura et al. (2020)	*Himatanthus drasticus*	Apocyanaceae	Comet assay	genotoxic	Aqueous	Mice
49	Szokalo et al. (2020)	*Smallanthus sonchifolius*	Asteraceae	Micronucelus test	Genotoxic at high doses	Aqueous	Chinese hamster, HepG2 cell
50	Saravanan et al. (2020)	*Kalanchoe pinnata*	Crassulaceae	Bacterial reverse mutation assay, Mouse Lymphoma Tk assay, Micronucleus test	Weak genotoxic effect	Ethanol	*Salmonella typhimurium*, Mice
51	de Quadros et al. (2020)	*Rubus rosifolius*	Rosaceae	Comet and Micronucleus test	Genotoxic	Methanol	HepG2 cells
52	Zhao et al. (2020)	*Alstonia scholaris*	Apocynaceae	Bacterial reverse mutation test, Mammalian chromosomal test, Micronucleus test	Not genotoxic	indole alkaloid extract	Salmonella typhimurium, Chinese hamster, Mice
53	Dey et al. (2021)	*Aristolochia indica*	Aristolochiacaeae	*Allium cepa* assay	Genotoxic	Methanol	*Allium cepa* root
54	de Souza et al. (2020)	*Chaptalia nutants*	Asteraceae	*Allium cepa* assay	Not genotoxic	Hydromethanol	*Allium cepa* root
55	Ahmadi et al. (2021)	*Ziziphora clinopodioides*	Lamiaceae	Comet assay	Dose dependent genotoxicity	Ethanol	Human blood leukocyte
56	Tavares et al. (2021)	*Sapindus saponaria*	Sapindaceae	Ames test	Genotoxic	10% (w/w) Insoluble polyvinylpyrrolidone (PVP)	HepG2 cells
57	Asita et al. (2021)	*Cannabis sativa*	Cannabaceae	*Allium cepa* assay	Genotoxic	Ethanol, methanol	*Allium cepa* root
58	Ribeiro et al. (2021)	*Vitex megapotamica*	Lamiaceae	Comet assay	non-genotoxic	Aqueous	Wistar rat
59	Ayubi et al. (2021)	*Zataria multiflora*	Lamiaceae	Comet assay	non-genotoxic	Hydroalcohol	Wistar rat

have been done so far with various plants. This does not only set forth the toxicity measures of the herbs but also explicit how different cultures and regions in earth have been inevitably dependent on herbs for physical well-being. This study has covered plants under families such as Fabaceae, Lamiaceae, Cactaceae, Euphorbiaceae, Apiaceae, Alismataceae, Passifloraceae, Lauraceae, Magnoliaceae, Amaryllidaceae, Cypreaceae, Leguminoceae, Cannabiceae, Sapindaceae, Asteraceae, Phyllanthaceae, Apocynaceae, Bignoniacaeae, Ranunculaceae, Tiliaceae, Polygalaceae, Combrataceae, Moraceae, Crassulaceae, Celastraceae, Magnoliaceae, Lythraceae, Combrataceae, Urticaceae, Dilliaceae, Pittosporaceae, Hypoxidaceae, Annonaceae, Vochysiaeae, Rubiaceae, Anacardiaceae, Saliaceae, Cucurbitaceae, Zingiberaceae, Poaceae, Eucommiaceae and Aristolochiaceae where plants belonging to the family "Fabaceae" and " Asteraceae" have appeared to be in the highest. Other families such as Euphorbiaceae, Rosaceae, Lamiaceae and Rosaceae are also found to be repeated. The reason of such high occurrence in genotoxic phenomenon might be their constituents. Pyrroliside alkaloids are widely distributed in plants from Asteraceae, Fabaceae and Borage (Boraginaceae) families (Durnev and Lapitskaia 2013) are found to have genotoxic compounds. Allyl thiocyanate is contained in the amount of 50–100 ppm from Brassicacae family (Durnev and Lapitskaia 2013) which is observed to bear mutagenic properties at the light of the genotoxicity study. Aristolchic acid, a major compound in Aristolochiaceae family has recently been classified in the International Agency for Research on Cancer (IARC) as a human carcinogen (Durnev and Lapitskaia 2013). Other compounds such as propenyl benzene, hydrazine, antheraquinones and their derivatives, allyl isothiocyanates and flavonoides as found in *Ashitaba* chalcone, coumarines and psoralens have already been tested and proven to possess genotoxicity inducing property. Genotoxicity study depends on the mode of extract being used. For example, *Jatropha gossypiifolia* has found to be genotoxic at other extracts except aqueous. *Cecropia pachystachyca* and *Pterocaulon polystachycum* showed genotoxicity only to mice brain and kidney tissues, respectively. Even each part of a plant might not bear similar genotoxic phenomenon, such as in *Opuntia ficus-indica*, where only the fruit and seed extracts were found to be genotoxic, whereas other parts were found to be negative of this aspect.

Most of the plants such as *Euphoria hirta*, *Glycyrrhiza glabra*, *Cratageus oxycantha*, *Vochysia divergens*, *Smallenthus sonchifolius, Ziziphora clinopodioides* have been found to be exerting dose dependent genotoxicity. Also, *Kalanchoe pinnata* and *K. lacinata* showed weak genotoxic effect whereas *Cymbopogon khasianus* shows moderate genotoxic effects.

It is a habitual aspect of people to believe in the naturally obtained products more than that of synthesized ones. But while setting to this aspect, its inherent toxicological properties tend to be ignored. Several workers have already done many experiments with the natural compounds obtained from plants yet many are still unknown. Many regular culinary ingredients have found to contain toxic compounds after experimentation. Regular studies have been adding new evidences on this aspect. Such as saponins, which were not mentioned among geno-toxicants before, are considered to be responsible in Chinese hamster ovary (CHO) cell cultures for the mutagenic activity of water and the organic extracts of the cortex of the medicinal plant Nauclea (Rubiaceae) (Liu et al. 2000). There are many controversies related to the extract used. An aqueous extract of nine medicinal plants known in natural Korean medicine under the name "Gumiganghwaltang" demonstrated genotoxicity in the Ames test and Chinese hamster lung cell culture (CHL cells) but was inactive for the micronuclei count in polychromatophylic mice erythrocytes (Shin et al. 2011). The study of 19 different extracts of 11 plants (*Arctium minus* (Hill) Bernh., *Ecballium elaterium* L., *Momordica charantia* L., *Plantago major* L., *Urtica dioica* L., *Viscum album* L., *Salvia triloba*, *Euphorbia rigida*, *Stachys lavandulifolia*, *Acteoside* and *Abies nordmannia*) that are considered as phyto-immunomodulators did not reveal a mutagenic effect using the strains TA98 and TA100 in the Ames test but indicated clear DNA damaging action toward lymphocytes of human peripheral blood in vitro (Durnev and Lapitskaia 2013). Even in case of in vitro and in vivo studies, some extracts that induce genotoxicity in vitro show negative result in vivo. According to Durnev and Lapitskaia (2013), in vivo studies suffer from a number of drawbacks due to the imperfection of their design from the point of view of the evaluation of real genotoxic risks for humans. First of all, the expressed discrepancy of the compound dosage used in an experiment and that that is actually consumed by a person is relevant. The second issue is the discordance of the regimes of introducing a compound into organism.

Conclusions

There might be many controversies and uncertainty lying down behind each experiment, but each toxicological experiment brings the safety of humans a step closer. Therefore, identifying the geno-toxicants and then taking the required measures for their sanitation is always necessary before switching onto any herbal products.

Abbreviations
CA: Comet assay; MN: Micronucleus test; BRM: Bacterial reverse mutation assay; ACT: *Allium cepa* Test; ChA: Chromosomal aberration assay; VA: Vitotox assay; WA: Western Assay; MLtk: Mouse lymphoma tk assay; LI: Lysogenic induction assay.

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Author contributions
ADS supervised the study, analyzed the data, edited and finalised the draft. AB conducted the search of literature, compiled the data and wrote the first draft. All authors read and approved the final manuscript.

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Availability of data and materials
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Declarations

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All the authors declare that they have no competing interests.

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