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A narrative review on genotoxic potential of medicinal plants used in Ayurveda

Research Article

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Abstract

Genotoxic substances are those which are capable to induce a destructive effect on a cell's genetic material. It can be either carcinogen, mutagen, teratogen or cytotoxic depending upon the dose, duration and mode of usage. Mutations may manifest in many forms as duplication, deletion or insertion. However, all mutagens are genotoxic, not all genotoxins induce mutations. Currently traditional medicines and plant drugs are used across the globe without strict medical supervision. In a country like India, folklore medicines play a pivot role in health sector. Irrational long-term usage of any plant-based drug are capable to induce adverse reactions. Till date there is no single hand information about the genotoxic effects of medicinal plants used in Ayurveda. Many of the drugs reported for toxicity are potent drug candidates in Ayurveda. From the available literature 184 articles reported the genotoxicity of medicinal plants. After shortlisting with the inclusion and exclusion criteria's 57 articles are found to be on genotoxic potential of medicinal plants. Present review reports genotoxic effect of 32 drugs used either as single or combination. It contains various array of drugs, for example poisonous drugs like *Arka, Dhattura*, *Mandookaparni* – which is a *medhya rasayana, Guduchi* – which is a potent immunomodulator and so on. Among them a few drugs like *Palandu, Tanduleeyaka, Misreya, Chandrasoora, Sariba, Manjishta, Dadima, Guduchi* etc. need special attention. This article tries to provide an insight on the reported genotoxic effect of plants used in Ayurveda.

Key Words: Genotoxicity, Cytotoxicity, Medicinal plants, Judicial usage, Ayurveda.

Introduction

Genotoxicity is a word related to genetics, which is defined as a substance which possess destructive effect on a cell's genetic material, either DNA or RNA affecting its integrity. The substances or drugs capable to induce genotoxicity on a living cell is called as genotoxins.(1). There are three primary effects that genotoxins induce in a living cell. It can be either carcinogen-cancer causing agents, mutagen-mutation causing agents or teratogen-birth defect causing agent. Generally, these genotoxins induces mutations which leads to a host of other problems from cancer to a wide variety of different diseases. Mutations can manifest in many forms as duplication, deletion or insertion of genetic information. However, all mutagens are genotoxic, not all genotoxic agents are mutagenic.(2) Ayurvedic medicines are very specific and generally prescribed after detailed examination by the physician. But currently there's a culture of self-practice and over the counter use of medicines, which is highly irrational. This ends up in the long-term use of some specific drugs irrespective of the need. This irrational long-term usage of medicines can produce many sorts of adverse effects due to the genotoxic potential of compounds present in them. So, this article highlights the importance of cautious usage of drugs.

Methods

In the current review, findings were extracted from PubMed, Web of Science, Scopus and Google Scholar databases from October 2021 – July 2022 by searching the key words including "genotoxicity" or "in vitro" or "in vivo" and "plants" or "medicinal plants". Publications in language other than English language and studies exclusively done on the active compounds were excluded.

Fig:1 Flow chart explaining the selection criteria for articles included in the review

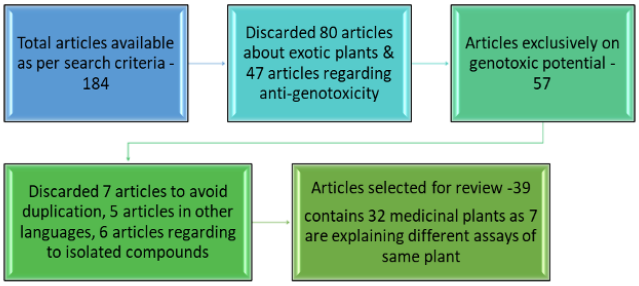

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Table:1 List of plants with reported genotoxicity – Botanical identity, Sanskrit name, Plant part, extract used, concentration, test performed, observation and results. [3-39]

Sl. no:	Botanical Identity	Plant part	Ex.	Conc.	Test	Result	
						Observation	Toxicity
1	*Alhagi pseudalhagi* (Bieb.) Desv. *[Yavasha]*(3)	WP	Al aq.	1,2.5, 5 µg/mL	Comet assay	DNA damage in tail length, percentage of DNA in the tail and tail moment	Genotoxic at 5 µg/mL
2	*Allium cepa L. [Palandu]*(4)	B	Aq	5,10, 15, 20mg/l	Allium cepa assay	Mitotic index of drug treated are lower than that of negative control.	Genotoxic at 15mg/l
3	*Amaranthus spinosus* Linn. *[Tanduleeyaka]* (5)	L	Aq.	0.05g/L, 0.1g/L, 0.5g/L, 1g/L	Allium cepa assay	Excessive reduction in mitotic index & extremely significant levels of clastogenicity on concentration dependant manner	Mild cytotoxic
4	*Aristolochia indica* Linn. *[Eeshwari]*(6)	R	Aq.	2.5, 5, 10mg/ml	Allium cepa assay	Significantly inhibited mitosis in dose dependant manner	Potent cytotoxic and genotoxic
5	*Azadirachta indica A Juss. [Nimba]*(7)	L	Aq.	10,15,20% conc.	Allium cepa assay	Exhibits mito-classic and chromate-classic effects	Potent mutagenic and carcinogenic
6..	*Calotropis procera* (Aiton) Dryand. *[Arka]* (8,9)	R	M, Ch, Aq.	2.5, 5, 10, 15, 25mg/ml	Chromosomal aberration assay	Significant alternation in the morphological appearance of chromosome.	Genotoxic
		L	PE	5,10,15,20mg/ml	Allium cepa root assay	Decreased percentage of mitotic index	Genotoxic
					Chromosomal aberration assay	Increased chromosomal aberrations like chromatid bridge, clumped metaphase, arrested telophase.	Mutagenic
7	*Capparis spinosa L. [Himsra]*(10)	Fl. Bud	Aq.	10,20,30g/L	Allium cepa assay	Dose dependant decrease in mitotic index.	Cytotoxic
8	*Citrullus colocynthis* (L.) *[Indravaruni]* (11)	L	Aq.	23,46,92g/L	Allium cepa test	Dose dependant increase in chromosome aberrations, micronucleus formation, inhibited mitotic index.	Cytotoxic Genotoxic
9	*Cyperus kyllingia* Endl. *[Mustha]* (12,13)	Rz	E	1, 10, 100, 1000ppm	Allium cepa assay	Significantly reduced the mitotic index in a dose dependant manner.	Genotoxic
		Aerial	Aq., EA, M, TOF	50, 200, 500 µg/ assay	Chromosomal aberration assay	It won't induce significant number of chromosome aberrations.	Non-genotoxic
10	*Datura metel* Linn. *[Dhathura]* (14)	L, R	M, Aq.	2,4,6,8 mg/ml	Allium cepa test	All extracts and all concentrations showed reduction in mitotic index and low proliferation index and aberrations like adherent nucleus, c-mitosis, anaphase bridge, binucleate cells and sticky cells.	Potent genotoxic
		Sd.			Chromosomal aberration assay	No statistically significant or dose related increase in the frequency of aberration.	
11	*Elephantopus scaber* Linn. *[Aanachuvadi]* (15)	L, R	Aq., M	1,50, 500, 1000µG/ML	Allium cepa assay	Dose dependant decrease of mitotic index. Significant levels of chromosomal aberrations like stickiness, bridges, c-mitosis and vagrant chromosomes.	Genotoxic

12	*Euphorbia hirta* Linn. *[Ksheerini]* (16,17)	WP	M	125,250,500,1000 µg/ml	Allium cepa assay	Dose dependant decrease in mitotic index and increase in chromosomal aberration.	Genotoxic and mito-depressive
			M	0.5, 1, 1.5, 2, 2.5, 3, 3.5µg/ml	Comet assay	DNA damage occurred and increase in amount of tail DNA	Genotoxic
			M	0.5, 1, 1.5, 2, 2.5, 3, 3.5µg/ml	Brine shrimp lethality assay	Mortality of shrimp were more in tested groups.	Cytotoxic
13	*Foeniculum vulgare var. vulgare* Mill. *[Misreya]* (18)	Sd.	Aq.	2,4,8%	Allium cepa assay	Inhibits mitotic division and induces chromosomal damage.	Genotoxic
14	*Hemidesmus indicus R Br. [Sariba]* (19)	R	E	2,4,8,16,32µg/ml	Sister Chromatid analysis Chromosome aberration assay Cytokinesis – block micronucleus assay	Significant reduction in the mitotic index and cytokinesis block proliferative index	Cytotoxic Genotoxic in higher doses
15	*Hydrocotyle asiatica L. [Mandookaparni]* (20)	WP	Aq.	1000µL	Ames test using TA97a, TA98, TA100, TA104	Showed mutagenic activity in TA98 strain with metabolic activation	Genotoxic
16	*Leucas indica* (Willd.) Linn. [*Dronapushpi*] (21)	L	Aq.	0.125, 0.25, 0.5,1, 2%	Allium cepa test	Inhibits mitosis of root meristem and shows clastogenic and anti-clastogenic abnormalities	Genotoxic
17	*Lepidium sativum* Linn. *[Chandrasoora]* (22)	Sd.	Aq.	200, 400, 800 mg/ kg	Sperm abnormalities, SSCP-PCR amplification Micronucleus assay	Significantly increased the sperm abnormalities like hookless-amorphous head, looped neck& midpiece and stickiness. Lead to point mutation in exon 5 of P53 gene in liver and colon tissues. Induces micro nucleated polychromatic erythrocytes.	Genotoxic – dose dependant
18	*Momordica charantia* Linn. [*Karavellaka*](23)	L	Aq.	50, 100, 150 mg/ kg	Micro nucleus assay	Significantly increased the frequency of MNPCE:PCE ratio, i.e the number of micro nucleated polychromatic erythrocytes were more when compared to polychromatic erythrocytes.	Genotoxic
19	*Myristica fragrans Houtt. [Jatiphala](24)*	Fr.	Aq.	1, 2, 4, 8%	Allium cepa assay	In a dose dependant manner, it inhibited the mitosis of root meristem	Genotoxic
20	*Nigella sativa* Linn. [*Kalajaji*] (25,26)	Sd.	Aq.	1, 4, 8mg/plate 2, 4, 9mg/ml 0.5265, 1.125, 2.25, 4.5, 9, 18 mg/ml	Vitotox test – TA104 Ame's assay – TA98, TA100 Comet assay Micronucleus assay In human C3A cells	Dose dependant cytotoxicity & genotoxicity	Genotoxic
			E	75&125µg	DNA fragmentation by Agarose gel electrophoresis on Oral cancer cell line	Shows genotoxicity on oral cancer cell-lines. Effective drug for oral cancer.	Genotoxic on oral cancer cell lines.

21	*Ocimum basilicum L. [Tulsi]* (27)	L	E-Aq	35.44, 3.544, 0.3544µg/mL	Cell proliferation and viability Alkaline comet DNA assay Chromosomal aberrations Micronuclei frequency	In all concentrations the plant extract induced chromosomal abnormalities. Dose dependant increase in formation of micronucleus.	Genotoxic
22	*Ocimum gratissimum L. [Ramatulsi]* (28)	L	Aq.	1, 2.5, 5, 10, 20%	Chromosomal aberrations Micronuclei frequency	Induces chromosomal aberrations and micro nuclei are formed in a dose dependant manner	Genotoxic
23	*Phyllanthus amarus* Schum & Thorm. [*Bhoomyamalaki*] (29)	St, L	Aq.	100, 200, 400, 800, 1600 mg/kg	Micronucleus assay Sperm morphology assay	The extract induced increasing frequency of micro-nucleated polychromatic erythrocytes and sperm abnormalities in a dose dependant manner.	Genotoxic
24	*Plumbago zeylanica* L. [*Sweta chitraka*] (30,31)	R	M	O..025, 0.050, 0.100mg/ml	Comet assay	In all concentrations significant DNA damage was observed	Genotoxic
			E	250, 500mg/kg	Micronucleus test	Results shows a significant increase in Micro nucleated polychromatic erythrocytes formation in treated groups.	Genotoxic
25	*Punica granatum* L. [*Dadima*](32)	Fr	E	0.45, 1, 2, 4, 6, 12, 18mg/ml	Ame's assay Saccharomyces cerevisiae assay Cytogenetic assay Sister chromatid exchange assay Chromosome aberration assay Micronucleus assay Sperm shape abnormality assay	The plant extract induced significant number of revertants. Failed to induce any gene-conversion No modification for mitotic or proliferation indices. A dose dependant increase of sister chromatid exchange per cell. A dose dependant increase in number of PCE-MN ratio has been observed. Abnormal sperms like amorphous, hookless, banana shaped sperms are observed.	Genotoxic
26	*Quassia indica* (Gaertn.) Nooteboom [*Gucchakaranja*] (33)	L	Aq.	2.5, 5 mg/kg	Allium cepa assay	Dose dependant decrease in mitotic index and presence of abnormal cells. Chromosomal abnormalities like chromosomal lagging, disruptive anaphase, irregularity in movement and arrangement of chromosomes, stickiness, vagrant chromosomes and polar deviation are found.	Genotoxic
27	*Rubia cordifolia* L. [*Manjishtha]* (34)	R	E	140, 280, 420, 560 mg/kg	Chromosomal aberration assay, Mitotic index, Proliferation of mice bone marrow cells	Dose and time dependant chromosomal aberrations are seen. Mitotic index is decreased in all treated concentration.	Genotoxic

28	*Ruta graveolens* Linn. *[Naagadamani]* (35)	R, St, Lf, Fr	M, E	100, 250, 500, 1000mg/kg	Allium cepa assay	All extracts are found to induce in vitro cytotoxic, mito-depressive clastogenic and non clastogenic activity and reduced mitotic index. Among the extracts ethanolic extract of leaf showed maximum genotoxic activity.	Genotoxic
29	*Salix alba* Linn. *[Jalavetasa]*(36)	St. Bk	E	2.5, 5, 10, 20, 50, 100, 200, 500 1000µg/ml	Comet assay	Mild genotoxicity has been observed.	Genotoxic
30	*Saussurea lappa* B C Clarke. *[Kushtha]*(37)	Rz	Aq.	0.5, 1, 1.5, 2 mg/dl	Cell line study	Increase in expression of proapoptotic genes: P53, 1kBα, BAX & TNF and decrease in the expression of apoptotic genes Bcl2, Survivin, and MMP-7	Genotoxic
31	*Tinospora cordifolia* (Willd.) Miers.*[Guduchi]* (38)	St	Aq.	10, 20, 30 mg/ml	Allium cepa assay	The extract increased the mitosis in root meristem at low doses, but reduced the mitosis in increased doses.	Non-genotoxic at lower doses

Results

There are about 32 plants used in Ayurveda are identified with various extend of genotoxic potentials. The details are summarised as table below.

Discussion

The results obtained are tabulated on the basis of assay used to analyse genotoxicity and extend of toxicity.
Based on assay used: (Table:2)

1. Allium cepa assay:
Allium cepa root meristem assay is used to detect genotoxicity, cytotoxicity and mutagenicity with specific endpoints as chromosome aberration, mitotic index and presence of micro nucleus respectively. It is a low-cost method and can be handled easily over other short-term assays. (39)
2. Ame's assay: It is a short-term bacterial reverse mutation assay specifically designed to detect a wide range of chemical substances that can produce genetic damage that leads to gene mutations. The test employs several histidine dependent Salmonella strains each carrying different mutations in various genes in the histidine operon. It functions as an initial screen to determine the mutagenic potential of new chemicals and drugs. The test is also used for submission of data to regulatory agencies for registration or acceptance of many chemicals, including drugs and biocides.(40)
3. Brine shrimp lethality assay: It is an important tool for the preliminary cytotoxicity assay of plant extract and others based on the ability to kill a laboratory cultured larva (nauplii). The nauplii were exposed to different concentrations of plant extract for 24 hours. The number of motile nauplii was calculated for the effectiveness of the extract. It is a simple, cost effective and requires small amount of test material. (41)
4. Chromosomal aberration assay: Chromosomal aberrations are the microscopically visible part of a wide spectrum DNA changes generated by different repair mechanisms of DNA double strand breaks (DSB).(42) The assay involves treatment of mammalian cells in culture with the test substance in the absence and in the presence of an exogenous metabolic system (S9 mix). The DSB in DNA can be induced directly or indirectly as a result of errors in replication or repair of DNS lesions. (43)
5. Comet assay: The single cell gel electrophoresis (the comet assay) is one of the most popular DNA damage assessment tools by virtue of its sensitivity, reliability, reproductability, adaptability and ease of use. (44) It detects the strand breaks and alkali-labile sites arising from the interaction of various damaging intermediates with DNA followed by the exposure of genotoxins. (45)

Table:2 Based on the assay used to assess genotoxicity

Sl. no.	Name of Assay	Name of drug
1	Allium cepa assay	*Allium cepa L., Amaranthus spinosus* Linn., *Aristolochia indica, Azadirachta indica A Juss., Calotropis procera, Capparis spinosa L., Citrullus colocynthis (L.), Cyperus kyllingia Endl., Datura metel Linn., Elephantopus scaber, Euphorbia hirta, Foeniculum vulgare var.vulgare Mill, Leucas indica, Myristica fragrans Houtt., Quassia indica, Ruta graveolens, Tinospora cordifolia, Tridax procumbens*
2	Ame's assay	*Hydrocotyle asiatica* L., *Nigella sativa*
3	Brine shrimp lethality assay	*Euphorbia hirta*
4	Chromosomal aberration assay	*Calotropis procera, Cyperus kyllingia* Endl., *Datura metel Linn., Hemidesmus indicus R Br., Ocimum basilicum L., Ocimum gratissimum L., Punica granatum, Rubia cordifolia L.*

5	Comet assay	*Alhagi pseudalhagi, Euphorbia hirta, Nigella sativa, Plumbago zeylanica, Salix alba*
6	Micronucleus assay	*Lepidium sativum, Momordica charantia, Nigella sativa, Ocimum basilicum* L., Ocimum gratissimum L., *Phyllanthus amarus, Plumbago zeylanica, Punica granatum*

Table:3 Classification of drugs based on toxicity

Sl. no.	Type of toxicity	Drugs
1	Dose dependant toxicity	*Allium cepa* L., *Amaranthus spinosus* Linn., *Capparis spinosa* L., *Citrullus colocynthis* (L.), *Cyperus kyllingia* Endl., *Elephantopus scaber, Euphorbia hirta, Foeniculum vulgare var. vulgare* Mill., *Hemidesmus indicus* R Br., *Hydrocotyle asiatica L., Leucas indica, Lepidium sativum, Momordica charantia, Myristica fragrans* Houtt., *Nigella sativa, Ocimum gratissimum* L., *Phyllanthus amarus* L., *Punica granatum, Quassia indica, Rubia cordifolia* L., *Tinospora cordifolia* (Willd.) Miers.
2	Potent toxicity	*Aristolochia indica, Azadirachta indica* A Juss., *Calotropis procera, Datura metel Linn., Ocimum basilicum* L., *Plumbago zeylanica, Ruta graveolens, Salix alba, Tridax procumbens*
3	Cytotoxic drugs	*Amaranthus spinosus* Linn., *Aristolochia indica, Capparis spinosa* L., *Citrullus colocynthis* (L.), *Hemidesmus indicus R Br.*

Among the 31 medicinal plants, 21 drugs exhibit dose dependant toxicity and nine exhibits potent toxicity and five shows significant cytotoxicity. Cell cytotoxicity refers to the ability of certain chemicals or mediator cells to destroy living cells. (46)

In the above listed drugs two drugs, *Calotropis procera* Linn. (47), *Datura metel* Linn.(48) are potent toxic plants as per *Ayurveda.* Its use has been strictly forbidden without proper purification. Another important species is *Aristolochia indica* Linn., which is a potent drug candidate against snake poison. A few drugs like *Hydrocotyle asiatica L., Punica granatum Linn., Rubia cordifolia L., Tinospora cordifolia* (Willd.) Miers. enlisted above are potent rejuvenators in Ayurveda.

After enumerating the predominant taste of the drugs enlisted above, it is evident that most of them are having bitter taste. In Samhita's it has been mentioned that excessive usage of bitter taste will results in *dhatu kshaya, anila vyadhi, bala kshaya, moha, bhrama, asya roukshya* by virtue of *roukshya, visada* and *khara guna's.* (49-50)

While describing about intelligent and quacker physicians, *Acharya Charaka* mentioned that the medicine will act as a poison, a weapon, as fire or thunderbolt if used in appropriately, whereas if used properly it functions as nectar. (51) If used properly as per *avastha* and *mātra*, *teeḳshna visha* will be an *uttama bheshaja*. (52) So special attention should be given during the long-term administration of plant-based drugs and also more importance must be given for ADR monitoring and reporting.

Conclusion

This article highlights the importance of cautious usage of plant-based drugs and also the relevance of pharmacovigilance in the present era. In the current scenario, there is a notion among the public that herbal drugs are safe and can be used without any restrictions. The current review gives an insight about the potent genotoxic effects of many relevant medicinal plants used in Ayurveda. Even though many of the plant samples induces genotoxicity, in mild doses they are potent drug candidates. Here we would like to emphasise the importance of judicial usage of plant-based drugs and educate the scientific community to remain conscious about the adverse reactions which can happen along with the administration of single drugs or combinations.

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List of Abbreviations

Abbreviations	**Definition**
B	Bulb
Fl. Bud	Flower bud
Fr.	Fruit
kg	Kilogram
L	Leaf
mg	Milligram
R	Root
Rz.	Rhizome
Sd	Seed
St	Stem
St. Bk.	Stem bark
WP	Whole plant

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