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Study of the mutagenic effect of tenoxicam and antimutagenic properties of ginger plant extract on root tips of *Vicia faba*

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ABSTRACT

In the present study the genotoxicity of tenoxicam the anti-inflammatory drug and ginger plant extract were investigated. Determination of the ability of antigenotoxicity of ginger plant extract through the combination with the tenoxicam on mitotic division in root tips of *Vicia faba* plant was carried out. In this study the meristematic cells of *Vicia faba* were treated by tenoxicam and ginger plant extract singly or combined with three different concentrations (10-20 and 40 mg/L DH_2O) for three different times (6-12-24 hours). In this study, the percentage of mitotic index decreased in single and combined treatments of tenoxicam at the highest concentration during different exposure periods. Also, tenoxicam in all concentrations and exposure periods singly or combined with ginger extract led to an increase of mutations frequency and chromosomal aberrations which included stickiness, fragments, breakage, bridges, lagging, disturbance, and micronuclei. These results suggested that tenoxicam drug singly or combined with ginger extract was found to induce chromosomal aberrations in root tip cells of *Vicia faba* and ginger plant extract failed to induce any mutagenis and antimutagenicity of tenoxicam drug in *Vicia faba.*

Key words: Tenoxicam, antimutagenic properties, *Vicia faba.*

INTRODUCTION

In the last few years, several vegetable products capable of modifying the activity of mutagens and carcinogens in various test systems have been identified; these include extracts of plants as well as plant derived products: pigments, vitamins, carotenes, phenolic, lactose, flavonoids and tannins (Dhir *et al*., 1993; Cozzi *et al*., 1997 and Lanar *et al*., 1999).

To date, a rather limited number of plants particularly of medicinal relevance have been screened for possible cytogenetic and genotoxic properties. Additional studies are needed to evaluate the possible antimutagenic, anticarcenogenic, and/or anticarcenogenic properties of plants and their derivatives.

Ginger (*Zingiber officinale Roscoe, Zingiberaceae*) is among the most frequently and heavily consumed dietary condiments throughout the world. Plants of ginger family have been widely used as spices and also traditional medicine. Ginger has been used extensively for more than 2500 years in China for conditions including headaches, nausea and colds (Grand and Lutz, 2000 and Dedov *et al*., 2002). Ginger extract failed to induce any mutagenicity (Ungsurungsie and Suthienkul, 1982), and even suppressed the genotoxicity of several carcinogens in bacterial and mammalian cells (Tarjan and Csukas, 1989).

The present study carried out in the framework of wider toxicological investigations, were designed to test the genotoxic and anti-mutagenic potential of tenoxicam and ginger plant extract.

Cytogenetic test was performed on root tips of *Vicia faba* in order to study tenoxicam and ginger effects on chromosome aberrations as well as its genotoxic and/or anti-mutagenic action.

MATERIAL AND METHODS

Chemicals

Tenoxicam (C13H11N3O4S2) non-steroidal anti-inflammatory drug from F. Ttoffmann-LaRoche Ltd. Basel, Switzerland.

Ginger plant extract from Kahira Pharmaceuticals and Chemical Industries Company, Egypt.

Cytogenetic assays

The cytogenetic *Vicia* test is recommended for detection of genotoxicity effects of environmental chemicals in plants by (Kanaya *et al*., 1994). It enables the simultaneous evaluation of numeric and structural chromosome changes. *Vicia faba* seeds were obtained from local nurseries. The roots were exposed for 6, 12, and 24 hours to 10, 20 and 40 mg/L of tenoxicam and ginger plant extract singly and combined.

Root meristems were examined at various intervals by thoroughly washing root tips and fixing them in carryon fixative (1:3- ethanol:glacial acetic acid) for 24 hours followed by washing in 70 % ethanol. Then, staining in acetocarmine and preserving them in 70 % ethanol. The root tips were squashed in 45 % acetic acid after macerating them in 1 N HCl for 5-10 minutes at maintained temperature of about 60°C (Dyer, 1979).

Cells were screened under a light microscope for mitotic phase and chromosomal aberrations. The mitotic index was expressed as the percentage of the number of divided cells to the total number of cells examined. The total number of chromosomal aberration was estimated in dividing cells. The abnormalities included cells with stickiness, fragments, breakage, brigdes and micronuclei.

The collected data of chromosomal aberrations were statistical analysis using (SPSS program).

RESULTS AND DISCUSSION

In the present work we have characterized two properties of ginger extract, genotoxicity and antimutagenicity. Ginger (*Zingiber officinale roscoe, Zingiber aceae*) is among the most frequently and heavily consumed dietary condiments throughout the world. Besides its extensive use as a spice, the rhizome of ginger has also been used in traditional oriental herbal medicine for the management of such symptoms as common cold, digestive disorders, rheumatism, neurologia, colic and motion – sickness (Mascolo *et al*., 1989; Mustafa *et al*., 1993 and Surh, 1999).

Table (1) shows the types and number of chromosomal aberrations obtained by exposing root tips cells of *Vicia faba*. We found that ginger extract did not influence mitotic activity of *Vicia faba* and did not significantly increase the frequency of chromosomal aberrations compared to control (in the three concentrations used for three different times). Similar results in other organisms have been reported by (Ungsurungsie and Suthienkul, 1982; Tarjan and Csukas, 1989; Surh *et al*., 1998; Surh, 2000; Someya *et al*., 2003; Bhandari *et al*., 2005 and White, 2007).

In other studies, however, the ginger extracts were found to induced chromosome breakage and other aberrations in root tip cells of onion (Abraham *et al*., 1976).

Nakamura and Yamamoto (1982) reported the mutagenicity of gingerol in Escherichia Coli B/r. The aliphatic chain moiety containing a hydroxyl group was proposed as an active part of gingerol responsible for its pungent constituent's gingerol and shogaol were tested in *Salmonella typhimurium* TA100 and TA1535. The gingerol and shogoal induced His^+ reversion in these bacteria in the presence of rat S-9 mix. (Nagabhushan *et al*., 1987). The ethanol extract of ginger was mutagenic in *S. typhimurium* TA98 and TA102 without metabolic activation (Mahmoud *et al*., 1992).

Tenoxicam is one of the oxicams a special group of non-steroidal anti-inflammatory drugs. Tenoxicam is a potent inhibitor of prostaglandin biosynthesis (Dammann, 1999). It is widely used in

Table 1: Percentage of chromosomal aberrations induced by ginger plant extract in root meristams of *Vicia faba*

Con. (mg/L)	Time (hours)	Total No. cells	Bridges	C-Metaphase	Ring chrom-osome	Star shape	Micronuclei	Lagging	Fragments	Distur-bance	Stickness	Total %
Control		1106	0.000	0.542	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.542
10	6	1173	0.131	0.436	0.801	0.000	0.568	0.946	0.986	0.942	0.261	5.071
	12	1280	0.130	0.234	0.410	0.000	0.140	0.640	0.562	1.953	2.984	7.053
	24	1115	2.616	0.934	0.062	0.000	0.654	0.616	0.679	0.629	0.012	6.202
20	6	1230	0.804	0.870	0.000	0.290	0.125	1.160	0.934	1.056	0.926	6.165
	12	1229	0.034	0.813	0.895	0.000	1.498	0.162	1.230	1.517	1.029	7.178
	24	1231	2.540	0.978	0.000	0.000	0.306	0.000	0.480	2.029	1.390	7.723
40	6	1284	0.828	0.538	0.236	0.355	0.775	0.893	0.710	0.786	3.372	8.493
	12	1291	0.250	0.250	1.174	0.000	0.512	0.489	1.424	0.720	2.526	8.345
	24	1147	2.639	0.580	0.000	0.000	0.527	0.739	0.580	2.988	1.290	9.343

the treatment of arthritis and pain (Vilegas *et al*., 2002). Table (2) shows the significant increase of chromosomal aberrations rates was scored after treatment with the same three different concentrations and three different times of tenoxicam.

Also, genotoxic effects (mitotic index reduction and cell growth inhibition) were observed after treatment with tenoxicam concentrations above 40 mg/L.

This is agreement with the genotoxicity found of piroxicam (the same chemical class as Tenoxicam) in *Saccharomyces cerevisia* (Badawy and Ali, 2000). Also, Giri and Mu Khopadhyay (1998) tested *in vivo* sister chromatid exchange (SCE) in bone marrow cells of mice for four pyrazolone derivatives (anti-inflammatory drugs) and they found that all four drugs showed a statistically significant increase in SCE in bone marrow cells when compared with control.

Moreover, an investigation was undertaken to determine anti-inflammatory drugs (4 compounds) had mutagenicity. Kuboyama and Fujii (1992) found that the (4 compounds) of anti-inflammatory drugs showed a DNA – damaging tendency.

On the other hand, many tests have been carried out on anti-inflammatory drugs about their safety. Kadotani *et al*., 1984 they were examined the tenoxicam drug by using *in vitro* bacterial systems (repair test and reversion test) and it was not mutagenic. And, Kullich *et al*., 1990 reported that tenoxicam and lornoxicam showed no influence on the SCE frequencies in therapeutic dosages in bone marrow cells of mice. However, Philipose *et al*., 1997 tested three anti-inflammatory drugs ibuprofen, ketoprofen and naproxen in the Ames mutagenicity assay and *in vivo* genotoxicity was tested by SCE in bone marrow cells of mice. Results showed no mutagenic effects in both testes.

Recently, there is interest in the development of chemoprevention agents against environmental mutagens. Natural products and naturally derived compounds from plants may have applications in controlling mutagenicity of some

Table 2: Percentage of chromosomal aberrations induced by tenoxicam drug in root meristams of *Vicia faba*

Con. (mg/L)	Time (hours)	Total No. cells	Bridges	C-Metaphase	Ring chrom-osome	Star shape	Micronuclei	Lagging	Fragments	Distur-bance	Stickness	Total %
Control		1442	0.000	0.542	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.542
10	6	1640	4.451	0.061	0.854	0.000	5.915	1.463	0.976	14.878	23.658	25.255
	12	1334	4,872	2.173	2.623	0.000	2.548	1.799	0.397	17.466	26.461	58.339
	24	1705	4.926	1.348	1.114	0.000	5.219	1.642	8.211	28.563	15.249	66.272
20	6	1067	3.467	0.187	0.187	0.000	14.995	1.312	7.497	19.306	9.185	56.136
	12	1366	2.708	0.000	0.585	0.000	3.733	0.000	0.219	13.543	20.351	41.139
	24	1403	3.278	0.214	0.071	0.000	12.900	0.926	2.423	16.464	16.037	52.313
40	6	575	3.304	0.696	0.696	0.347	3.652	0.695	1.217	7.478	16.869	34.953
	12	827	2.781	1.088	0.605	0.000	5.925	1.451	0.725	6.529	15.961	35.065
	24	1133	3.353	1.588	0.000	0.880	1.941	1.941	4.060	21.535	13.062	47.568

Table 3: Percentage of chromosomal aberrations induced by the combined treatments of ginger and tenoxicam in root meristams of *Vicia faba*

Con. (mg/L)	Time (hours)	Total No. cells	Bridges	C-Metaphase	Ring chrom-osome	Star shape	Micronuclei	Lagging	Fragments	Distur-bance	Stickness	Total %
Control		1442	0.000	0.542	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.542
10	6	1849	3.461	0.378	0.648	0.000	8.274	1.027	2.595	18.983	23.201	58.567
	12	1498	4.606	0.867	1.135	0.000	1.335	1.869	3.471	17.489	24.566	55.338
	24	1332	6.606	2.027	0.975	0.075	15.015	0.000	4.504	10.435	25.525	65.162
20	6	1119	1.966	1.966	1.072	0.000	2.234	0.536	5.093	15.013	12.421	40.301
	12	1554	2.123	0.450	0.321	0.000	0.772	0.128	2.960	16.924	12.419	36.097
	24	1263	2.216	0.554	2.375	0.000	1.187	0.475	3.879	27.278	14.726	52.490
40	6	1131	4.686	1.415	1.237	0.353	2.564	1.061	1.326	9.814	15.561	38.017
	12	941	2.763	1.062	0.000	0.000	5.951	2.019	0.956	7.332	14.771	34.854
	24	620	2.903	1.935	0.000	0.000	4.838	3.387	2.903	18.870	5.806	40.642

drugs. The protective action of fruits and vegetables has been attributed to the presence of anti-oxidants (Katt and Kushad, 2000; Prior and Cao, 2000 and Kaur and Kapoor, 2002).

Also, some flavonoids (a group of natural products) are able to cause DNA damage (Said *et al*., 1992 and Yamashita *et al*., 1999). The aim of this investigation was to determine if extract of ginger reduce the genotoxic damage induced by tenoxicam drug using the cytogenetic test (chromosomal aberrations and mitotic index) in root tips of *Vicia faba* plant.

From our results ginger plant extract failed to induce any anti-mutagenic effect of tenoxicam drug in *Vicia faba*.

Combined treatment with ginger plant extract and tenoxicam drug significantly increase, Table (3) showed that the combined treatment with ginger plant extract and tenoxicam drug significantly increases. The combination effect did not bring about antagonistic effect in comparison with the single treatment by tenoxicam alone but it showed synergistic effect. Ginger extract may have pro-oxidant actions. These results demonstrated that

Table 4: Mitotic index in root meristams of *Vicia faba* treated by tenoxicam, ginger plant extract and Combination of them

Treatments	Concentrations	Time (hours)	Total observed cells	Total dividing cells	Mitotic index %
	Control		7566	1106	14.62
Ginger	10 mg/L	6	8064	1173	14.55
		12	8450	1280	15.15
		24	8187	1115	13.62
	20 mg/L	6	8771	1230	14.02
		12	8299	1229	14.81
		24	8265	1231	14.90
	40 mg/L	6	8335	1284	15.40
		12	8345	1291	15.50
		24	8082	1147	14.20
Tenoxicam	10 mg/L	6	8289	1640	19.78
		12	7999	1334	16.71
		24	8321	1705	20.30
	20 mg/L	6	8177	1067	13.05
		12	8306	1366	16.47
		24	7423	1403	18.90
	40 mg/L	6	8267	575	6.95
		12	8033	827	10.29
		24	8402	1133	13.48
Ginger+Tenoxicam	10 mg/L	6	8674	1849	21.32
		12	7673	1498	19.52
		24	7957	1332	16.74
	20 mg/L	6	7182	1119	15.60
		12	8830	1554	17.60
		24	7565	1263	16.70
	40 mg/L	6	8169	1131	13.84
		12	8804	941	10.70
		24	7306	620	8.50

the pro-oxidant properties of ginger extract which are generally considered to be anti-oxidant may be involved with their mutagenic activities in combined treatment. These, results are in agreement with those reported by Yamashita *et al*. (1999) who fount that quartering induced extensive DNA damage via reacting with Cu (II).

Addition, Soudamini *et al*., 1995 found that spices like pepper, pippali, ginger and mustard increased the number of revertants in *Salmonella Typhimurium* strains TA100 and TA1535. However, treating root meristems of *Vicia faba* with aqueous extract of black pepper was fount to give clastogenic effects (Abraham and John, 1989). Furthermore, ginger extract were found to induce chromosome breakage and other aberrations in root tip cells of onion (Abraham *et al*., 1976).

From mitotic index data (Table 4) it is evident that ginger plant extract did not influence mitotic activity of *Vicia faba*. But tenoxicam singly or combined exhibited mitotic activity and the percentage of mitotic index decreased at highest concentration.

The cytogenetic *Vicia faba* test documented that tenoxicam single or combined with ginger plant extract exhibited the clastogenic effect on *Vicia faba* root tip meristams.

Finally, we conclude that ginger extract even though it showed genotoxic activity at combination with tenoxicam drugs is not a genotoxicant alone in root tips of *Vicia faba* and suggest that it is advisable to extend studies on this matter using other biological models.

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