Antigenotoxic Effects of *Zingiber officinale* Extract on *Saccharomyces cerevisiae* D7

Mohammed H. Z. Mutawakil

Biological Science Department, Faculty of Science, P. O. Box 80203, King Abdulaziz University, Jeddah, 21589, Saudi Arabia. mmutwakil@kau.edu.sa

Abstract: This study was designed to investigate the antigenotoxic potential of extract from *Zingiber officinale* (ginger) against anti-inflammatory drug (tenoxicam®) in *Saccharomyces cerevisia* strain D7. Three concentrations were prepared from tenoxicam® drug and ginger plant extract (1, 2, 4 µl/ml ml media) in the all treatments of *Saccharomyces cerevisia* strain D7. The result of anti-inflammatory drug showed decreasing in survival percentage more than the combined treatment or the ginger plant extract alone. Weak positive mutagenic activity was obtained using the two lowest concentrations (1,2 µl/ml media) only of tenoxicam® treatment which resulted in reversion and mitotic crossing over. Also, moderate mutagenic activity was obtained at the three loci under study at the highest concentrations in all treatments, expect the tenoxicam treatment, the mutagenic activity was high in all concentrations. These results suggest the mutagenic effect of tenoxicam® in the induction of reverted, converted and mitotic crossing over in *S. cerevisie* strain D7 and ginger plant extract have some antimutagenic potential.
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1. Introduction

Ginger (*Zingiber officinale*) is one of the more commonly used herbal supplements. Although it is often consumed for culinary purposes, many patients used it to treat a variety of conditions [**White, 2007**].

Hanafy (2010) found that ginger extract reduced the incidence of micro nucleated cells induced by Ehrlich Ascits Cells.

Tenoxicam has anti-inflammatory, analgesic, antipyretic properties and transfer the blood to the brain [**Jolliet *et al*., 1997**] and also inhibits platelet aggregation [**Blain *et al*., 2000**]. Oxicam is a potent inhibitor of prostaglandin biosynthesis. It may act as a scavenger for active oxygen at the site of inflammation. Therefore, it is widely used in the treatment of arthritis and pain [**Dammann, 1999; Vilegas *et al*., 2002**].

However, the treatment with non-steroidal anti-flammatory drugs such as tenoxicam® can cause genotoxic risk (DNA damages and sister chromatid exchanges). This genotoxicity may due to long time treatment or additional genotoxic factors (cytostatics, cigarette smoking, x-ray exposure) [**Kullich *et al*., 1990**]. Moreover, natural or herbal medicines, such as Ginger (*Zingiber officinale*) are traditionally preferable to be used in many cultures before taking drugs to treat nausea and vomiting of pregnancy or gastrointestinal symptoms [**Ernst and Pittler, 2000; Chandra *et al*., 2002; Jewell and Young, 2003; Borrelli, *et al*., 2005; Boone, 2005**].

The objective of this study the possible antigenotoxicity of ginger extract was assayed in *S.cerevisiae* D7 against anti-inflammatory drug (tenoxicam).

2- Material and Methods:

Chemicals:

Tenoxicam®, nonsteroidal anti-inflammatory drug from F. Toffmann-La Roche Ltd. Basel, Switzerland.

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

Yeast assay:

A diploid strain of *Saccharomyces cerevisiae* D7 (ade2-40/ade2-119, trp5-12/trp5-27, ilvl-92/ilvl-92, MATa/MATα) constructed by **Zimmermann *et al*. (1975)** was used. This strain makes it possible to investigate simultaneously the occurrence of reverse point mutations at the *ilv* locus, mitotic crossing-over at the *ade* locus and mitotic gene conversion at the *trp* locus.

Media:

The medium components have been described in detail by **Zimmermann *et al*. (1975).**

Testing assay:

As a testing procedure the assay according to **Zimmermann *et al*., (1975)** was used. D7 was tested for the frequency of spontaneous convertants and reverlants prior to every experiment. For each experiment a culture having a low content of convertionts (1-10 X 10^{-5}/plate) and revertants (1-10 x 10^{-6}/plate) was selected. The test culture was in the stationary phase and after washing it was suspended in phosphate buffer of pH 6.98. Cultures with a

density of 2 x 10^7 cells/ml were treated for 30 min with technical SCM at 28°C while shaking. The treatment was finished by centrifugation and washing 3 times in distilled water. Washed cell suspensions were plated on media, i.e., 1-2 x 10^6 cells/plate to investigate revertants on a selective medium without isoleucine, 1-2 x 10^5 cells/plate to investigate revertants on a selective medium without isoleucine, 1-2 x 10^5 cells/plate to investigate convertants on a selective medium without tryptophan and 2 x 10^2 to 2 x 10^3 (based on survival) on a sub maximal medium on which also mitotic crossing-over and other changes in adenine locus were evaluated. The plates were incubated at 28°C. Survival and mitotic gene conversion were evaluated after 5-7 days, reversions and mitotic crossing-over after 7-12 days.

Analysis and evaluation of data:

The frequencies of gene conversion, reverse mutation and mitotic crossin-gover were computed by dividing the number of convertant, revertant or mitotic crossing-over colonies. The consensus was that the increase in an end point under investigation up to two folds or more of the mean of control frequency is biologically considered as a significant response (**Brusick, 1980**).

3. Results:

Experimental data are reported in Tales 1-3. The results showed the following:

1- Tenoxicam at the highest concentration assay:

Tenoxicam at the highest concentration assayed (4 µl/ml) is active in inducing mitotic crossing-over, gene conversion and reverstants in *S. cerevisiae* D7 (Table 1).

Mitotic crossing over can be manifested by the occurrence of pink-red sector colonies of homaallelic type ade 2-40/ade 2-40 and ade 2-119/ade 2-119 from originally white colonies of heteroallelic strain ade 2-40/ade 2-119. Also, treatment with tenoxican alone shows a significant reduction in the number of survived cells.

2 - Ginger plant extract is slightly cytotoxic assay:

Ginger plant extract is slightly cytotoxic only at the high dosage 4 µl/ml and is not genotoxic in the 1-2 µl/ml range of assayed doses (Table 2). Ginger plant extract does not induce either mitotic crossing-over and conversion at the assayed doses (1, 2 µl/ml) but only at the high dosage (4 µl/ml) it is more effective in inducing revertants.

3 -Tenoxicam ginger plant extract mixture assay:

Tenoxicam® ginger plant extract mixture is slightly cytotoxic at the highest concentration assayed but does not show any genotoxic effect in the 1-2 µl/ml range of assayed doses (Table 3). The cytotoxic of tenoxicam® is lowered by the presence of ginger plant extract.

It the case of mitotic crossing-over, conversion and reversion, an inhibiting action by mixture of tenoxicam® and extract, even when considering genotoxicity in relation to cytotoxicity at tenoxicam® concentrations. These results indicate that the decrease in genotoxicity in the combined treatment is due to the reduced cytotoxicity.

Table (1): Effect of Tenoxicam on *S. cerevisiae* D7

Treatments µ/ml	Number of cell	Survival Percentage	Convertant			Revertant			Mitotic crossing over		
			Mut Freq	T/C	D. of Act	Mut Freq	T/C	D. of Act	Mut Freq	T/C	D. of Act
Control	7764	100%	(95)0.012	1	-	(134)0.017	1	-	(82)0.01	1	-
Tenoxicam (1µ/ml)	4616	59.5%	(62)0.013	1.1	-	(286)0.062	3.6	+	(57)0.012	1.2	-
Tenoxicam (2µ/ml)	3304	42.5%	(66)0.020	1.6	-	(400)0.121	7.1	+	(68)0.02	2	+
Tenoxicam (4µ/ml)	3676	47.3%	(108)0.029	2.4	+	(526)0.143	8.4	+	(96)0.026	2.6	+

C = Control level T = Treatment value
+ = 2-10 control level ++ = > 10 control level - = non significant
D. of Act. = Degree of activity, numbers between parentheses represents actual colony counts

Table (2): Effect of ginger plant extract on *S. cerevisiae* D7

Treatments µ/ml	Number of cell	Survival Percentage	Convertant			Revertant			Mitotic crossing over		
			Mut Freq	T/C	D. of Act	Mut Freq	T/C	D. of Act	Mut Freq	T/C	D. of Act
Control	7764	100%	(95)0012	1	-	(134)0.017	-	1	(82)0.01	1	-
Ginger extract (1µ/ml)	5624	72.4%	(83)0.015	1.3	-	(180)0.032	-	1.9	(97)0.017	1.72	-
Ginger extract (2µ/ml)	5260	67.7%	(91)0.017	1.4	-	(201)0.038	+	2.2	(101)0.019	1.9	-
Ginger extract (4µ/ml)	2900	37.35%	(81)0.028	2.3	+	(240)0.083	+	4.9	(109)0.037	3.7	+

C = Control level T = Treatment value
+ = 2-10 control level ++ = > 10 control level - = non significant
D. of Act. = Degree of activity, numbers between parentheses represents actual colony counts

Table (3): Effect of ginger plant extract (combined treatments) *on S. cerevisiae* D7

Treatments µ/ml	Number of cell	Survival Percentage	Convertant			Revertant			Mitotic crossing over		
			Mut Freq	T/C	D. of Act	Mut Freq	T/C	D. of Act	Mut Freq	T/C	D. of Act
Control	7764	100%	(95)0.012	1	-	(134)0.017	1	-	(82)0.01	1	-
Tenoxicam + ginger (1µ/ml)	4268	55%	(71)0.017	1.4	-	(112)0.026	1.5	-	(69)0.016	1.6	-
Tenoxicam + ginger (2µ/ml)	4044	52%	(73)0.018	1.5	-	(122)0.039	1.8	-	(74)0.018	1.8	-
Tenoxicam + ginger (4µ/ml)	3480	44.8%	(89)0.026	2.2	+	(150)0.043	2.5	+	(98)0.028	2.8	+

C = Control level T = Treatment value
+ = 2-10 control level ++ = > 10 control level - = non significant
D. of Act. = Degree of activity, numbers between parentheses represents actual colony counts

4. Discussion:

In the present work we have characterized two properties of ginger extract, genotoxicity and antimutagenicity using *Saccharomyces cerevisiae* D7. Ginger (*Zingiber officinale roscoc, Zingiber aceae*) is among the most frequently and heavily consumed dietary condiments throughout the world.

Besides it 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-sichness **(Mascolo *et al*., 1989; Mustafa *et al*., 1993; Surh, 1999 and Ahmed and Al-Twaty, 2009).**

Also, the present study was carried out to assess the genotoxicity effects of tenoxicam on *S.cerevisiae* D7 singly or combined with ginger extract.

Tenoxicam is one of the oxicams a special group of non-steroidal anti-inflammatory drugs. It is a potent inhibitor of prostaglandin biosynthesis (**Dammann, 1999**). And it is widely used in the treatment of arthritis and pain (**Vilegas *et al*., 2002**). Treatment with tenoxicam shows a significant reduction in the number of survived cells more than ginger extract. This set of results is in agreement with the results obtained by **kunglos and Aoyama (1991), and Al-Twaty (2008)**

Using in *S. cerevisiae*. Moreover, tenoxicam induced reversion at isoleucine (ilv) locus in all single treatments. On the other hand, negative results in the combined treatment with ginger extract. Only in the case of gene conversion and mitotic crossing-over induced by tenoxicam at the highest concentration. These results obtained with tenoxicam singly or combined with ginger extract are in keeping with data from the literature which indicate that this drug induced & mitotic crossing over, gene conversion and reverse mutation in *S. cerevisiae* D7 **(Badawy and Ali, 2000 and Keszenman *et al*., 2005).**

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 drugs **(Kaur and Kapoor, 2002).**

The aim of this investigation was to determine it extract of ginger reduce the genotoxic damage induced by tenoxicam drug in *S. cerevisiae*. From the resutls ginger plant extract induce some anti-mutagenic effect of tenoxicam drug in *S. cerevisiae*. Ginger extract is slightly mutagenic and induces reversion and mitotic crossing-over in *S. cerevisiae* D7 only in the high concentration but it does not induce either mitotic crossing over or mutation in lower concentrations. Moreover, combined treatments with tenoxicam did not show any genotoxic effect. Similarly, tests for mutagenicity in other systems were negative using ginger plant extract. **(Katiyar *et al*., 1996, Surh, 2002, Lu *et al*., 2003, Kim ,2004, Young *et al*., 2005 and Hanafy, 2010).** Also, **Miadokova *et al*., (2008)** investigated the potential antigenotoxic effect of the extract of artichoke *Cynara cardunculus* (CCE) using *S. cerevisiae* D7 and concluded that CCE is of particular interest as a suitable candidate for an effective chemopreventive agent. On the other hand, the potential genotoxicity and antigenotoxicity of non-photoactivated hypericin was investigatd in *S. cerevisiae* D7 it did not mutagenic and it did not exert any antimutagenic effects (**Miadokova *et al*., 2010**).

In conclusion these results suggest that tenoxicam has a mutagenic effect on is used in the treatment of *S. cerevisia*e D7 as indicated by mitotic crossing-over, gene conversion and reverse mutation. The results also demonstrated a protective effect (antimutagenic activity) of ginger extract against tenoxicam.

Corresponding author
Mohammed H. Z. Mutawakil
Biological Science Department, Faculty of Science, P. O. Box 80203, King Abdulaziz University, Jeddah, 21589, Saudi Arabia
mutwakil@Kau.edu.sa

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