

THE PROTECTIVE POTENCY OF GREEN TEA AND GINGER EXTRACTS ON THE GENOTOXIC EFFECT OF MALATHION INSECTICIDE IN BONE MARROW CELLS OF MICE (*MUS MUSCULUS*)

[70]

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ABSTRACT

In present set of investigations the chemoprotective effect of green tea and ginger extracts has been evaluated using *in vivo* chromosomal aberrations assay in albino mice (*Mus musculus*). The organophosphate agropesticide malathion, 80% technical grade consider as a potent genotoxic agent, was given at a single dose 230 mg/kg b.w. (1/12 LD50) intraperitoneally. Pretreatment with 4 and 3% of freshly prepared green tea (GTI), ginger (GI) extracts, respectively and the mixture of both extracts (GTI+GI) were given through oral incubation for 6 days prior to malathion administration. Animals from all the groups were sacrificed at sampling times of 24 and 48 hours and their bone marrow cells were analyzed for chromosomal damages. The animals of the positive control group (Malathion alone) showed a significant increase in chromosomal aberrations both at 24 and 48 h sampling time. The green tea and ginger extracts, alone did not significantly induced aberrations at either sampling time, conforming their non-mutagenicity. However, significant suppressions in the chromosomal aberrations were recorded following pretreatment with green tea and ginger extracts administration. The antigenotoxic effects of both extracts separately and in mixture were also evident, as observed by significant increase in mitotic index, when compared to positive control group. Reduction in malathion induced clastogenicity by both extracts, was evident at 24 h and to a much greater extent at 48 h of cell cycle. Thus results of the present investigations revealed that green tea and ginger extracts have chemoprotective potential against malathion induced chromosomal mutations in albino mice.

Key words: Green tea, Ginger, Genotoxicity, Malathion, Anti-carcinogenic, Albino mice

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INTRODUCTION

Food may contain protective antimutagenic or anticarcinogenic substances, of which most are present in plants, e.g. glucosinolates in vegetables, vitamin C in fruit, and polyphenolic compounds in green tea and ginger (**DeMarini, 1998**).

Inhibition of mutagenesis or carcinogenesis is generally not based on one specific mechanism. Protection against cancer can occur at different stages of the complicated processes of carcinogenesis. Compounds and complex mixtures with antimutagenic activity have different modes of action and act in parallel at different levels. As inhibitors, they may prevent the formation of mutagens, such as the endogenous formation of nitrosamines. As blocking agents, they can prevent the biotransformation of premutagens into reactive metabolites by inhibiting metabolic activation, by stimulating detoxification enzymes, or by scavenging reactive molecules. As suppressing agents, they may modulate intracellular processes, which are involved in DNA repair mechanisms, tumor promotion and tumor progression (**Bailey and Williams, 1993 and Krul et al 2001**).

Tea (*Thea sinensis*), the most widely consumed beverage in the world next to water, has drawn attention as a source of antimutagenic compounds. Mechanisms

to explain this activity have been proposed and include direct binding to mutagens, modification of metabolic enzymes and antioxidant activity (**Weisburger, 1999**). Although in animal studies an inhibitory effect of tea on cancer incidence has been observed, the evidence for an effect on human cancer is not conclusive (**Kohlmeier et al 1997**).

This is partly due to lack of information on the bioavailability in the human digestive tract of the active components in tea, especially in combination with our common diet. Among many polyphenolic compounds isolated from green tea, (-)-epigallocatechin gallate (EGCG) is believed to be a key active constituent in terms of cancer chemoprotective potential (**Fujiki et al 1992, Komori et al 1993, Fujiki et al 1994, Fujiki et al 1996 and Conney et al 1997**). The strong antioxidative activity retained in this polyphenol has been confirmed in numerous *in vivo* and *in vitro* studies (**Ho et al 1992, Wei and Frenkel, 1993, Lin and Lin, 1997 and Yoshioka et al 1997**), which appears to contribute in part to the antimutagenic and anticarcinogenic effects of green tea. Thus, pretreatment of SENCAR mice with EGCG significantly ameliorated TPA-induced infiltration and diminished the formation of hydrogen peroxide and oxidized DNA bases including 8-hydroxy-2'-deoxyguanosine (8-OH-dG) and 5-hydroxymethyl-2'-

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deoxyuridine in the skin (**Wei and Frenkel, 1993**).

Ginger (*Zingiber officinale* Roscoe) 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. The oleoresin from rhizomes of ginger contains [6]-gingerol (1-[4'-hydroxy-3'-methoxyphenyl]-5-hydroxy-3-decanone) and its homologs as pungent ingredients that have been found to possess many interesting pharmacological and physiological activities, such as anti-inflammatory, analgesic, antipyretic, antihepatotoxic, and cardiotoxic effects (**Mustafa et al 1993 and Surh 1999**).

Malathion, an organophosphate insecticide, is the most commonly used insecticide. According to the **U.S. Environmental Protection Agency (US-EPA 2000)**, there is "suggestive evidence" that Malathion causes cancer. However, recent studies provide stronger evidence: a technical grade malathion insecticide caused breast cancer in laboratory animals, and malathion use by farmers is associated with an increased incidence of a type of cancer, non-Hodgkin's lymphoma (**Ernst, 2002 and Meulenber, 2002**).

Results from a variety of recent studies are consistent with the genotoxicity and the carcinogenicity of malathion insecticide. **Contreras and Bustos-Obregón, (1999) and Giri et al (2002)** showed that, malathion given orally caused genetic damage in laboratory mice. Another 2002 study,

from Egypt's National Research Center, showed that mice fed with stored wheat that had been treated with a commercial malathion insecticide developed two kinds of genetic damage. The damage occurred at all dose levels tested in this study (**Amer et al 2002**). **Blasiak and Stankowska (2001)** showed that malathion, its oxygen analogue malaoxon, and its isomer isomalathion cause genetic damage in human cells.

The aim of this study was to investigate the protective effects of aqueous extracts of green tea and ginger, which they used separately or in mixture, on the genotoxic effect of malathion insecticide in albino mice using cytogenetic end points.

MATERIAL AND METHODS

Green tea and ginger extracts

The green tea (GTI) and ginger (GI) infusions were freshly prepared everyday for the duration of *in vivo* study (4 g of green tea leaves/100 ml hot water and 3 g of ginger rhizomes/100 ml hot water). The concentrations of the natural extraction used in the present study were selected with reference to concentration range that has been used in previously published papers (**Surh, 1999 and Gupta et al 2002**). The green tea leaves and the ginger rhizomes were procured from local traditional oriental medicine market.

Chemicals

Malathion[S-(1,2-dicarboethoxyethyl) O,O-dimethyl phosphorodithioate]-technical grade (80% purity and the remainder 20% is other Malathion metabolic products as malaaxon [S-(1,2-

dicarboethoxyethyl)O,O-dimethyl phosphoro-thiolate] and its isomer isomalathion [S-(1,2-dicarboethoxyethyl) O,S-dimethyl phosphorodithioate]- has been supplied by Ministry of Agriculture, Land Reclamation Branch, while Colchicine was obtained from Sigma-Aldrich Chimie (Saint-Quentin Fallavier, France). All other chemicals used in the study were analytical grade.

Experimental animals

The experimental animals used in this work were adult males of the laboratory albino mice *Mus musculus* (18-28 g in weight). Animals were obtained from Giza Research Center of Optic Diseases. All animals were housed in plastic cages with wired covers and kept under normal laboratory conditions for different periods. The animals were fed on the standard commercial diet (ATMID company, Egypt), and provided with tap water.

Treatment schedule

The pre-treatment of animals with GTI, GI or GTI + GI mixture were performed along with malathion post-treatment to male mice. The animals were divided in to seven groups of 10 animals each. The animals of the group I were used as a control and no treatment was given. The animals of groups II and III were treated by GTI and GI respectively, through oral intubation for six days consecutively. The animals of the group IV were served as positive control and only malathion was given at the single dose of 230 mg/kg body weight (corresponding to 1/12 LD50) intraperitoneally. The dose used in the

present study was selected with reference to the LD50 and the dose range that has been used in previously published papers dealing with the mutagenicity of malathion in mice (**Salvadori et al 1988 and Blasiak et al 1999**). While the animals of the groups V, VI and VII, were given the pre-treatment of GTI, GI and GTI+GI respectively, through oral intubation for six days consecutively, and malathion treatment was given after 1 hour of the last dose of GTI, GI or GTI + GI mixture on the 6th day, as a single dose of 230 mg/kg b.w. intraperitoneally.

After completion of the treatment period, five animals from each group were sacrificed at sampling time of 24 h rest were sacrificed at 48 h, by cervical dislocation, Colchicine was given at the dose of 4 mg/kg b.w. intraperitoneally at 22 and 46 h respectively prior to sacrificing the animals. The bone marrow smears of animals in each group were prepared as per protocol of **Preston et al (1987)**. Slides were stained with Giemsa and well spread metaphases were analyzed for chromosomal aberrations. Mitotic index and Incidence of aberrant cells (in percentages) for each group were analyzed.

Also % suppressed aberrant cells were calculated as: $100 - (\% \text{ aberrant cells in each GTI, GI or GTI+GI mixture pre-treated and malathion post-treated groups} / \% \text{ aberrant cells in positive control (malathion treated) group}) \times 100$.

Statistical analysis

The data was analyzed for mean values and standard error for all groups, which were subjected to statistical comparison using student-t-test $P < 0.01$, was considered significant.

RESULTS

The results revealed that malathion when given at a single dose of 230 mg/kg b.w., once only (Gr.IV) caused a high incidence of all types of chromosomal aberrations in albino mice, including chromatid breakage, centromeric attenuation, centric fusion, end to end association, polyploidy and endomitosis. The mitotic index and the incidence of aberrant cells were decreased by 40.40% and increased by 27.00%, respectively, compared to control Gr.I ($P < 0.01$), indicating bone marrow cytotoxicity. However, each of green tea and ginger extracts (Gr.II and Gr.III, respectively) induced the lowest count of chromosomal aberrations significantly confirming its non-mutagenicity (Tables 1&2). Also, these extracts showed no cytotoxic effects, as there were no significant changes in the mitotic index and the incidence of the aberrant cells compared to the control group I (Fig. 1).

Moreover, when pretreatment of different extracts (GTI, GI and GTI+GI) was given prior to malathion treatment (in groups V, VI and VII, respectively), decreased rates of clastogenic changes were observed (Tables 1&2). All types of chromosomal aberrations induced by malathion, including breaks and other multiple damages were found to be reduced by GTI, GI and GTI+GI. The status of mitotic index was found to increase during first phase of cell cycle (24 hr. sampling time), 20.34% ($P < 0.01$) by GTI, 16.40% ($P < 0.01$) by GI and 16.48% ($P < 0.01$) by GTI+GI mixture, respectively (compared to positive control Gr.IV), indicative of their anticytotoxicity towards malathion (Table

2). The incidence of aberrant cells, which were found to be 38.80 ± 1.241 in malathion treated animals, was reduced to 30.80 ± 1.113 by GTI, 29.20 ± 0.583 by GI and 30.40 ± 0.927 ($P < 0.01$) by GTI+GI mixture (Table 2). A decrease in the number of aberrations per cell, both chromosome and chromatic type was observed in GTI, GI and GTI+GI pretreated and malathion post-treated groups. The calculated suppressive effect was 20.62% by GTI, 24.74% by GI and 21.65% by GTI+GI mixture, respectively (Fig. 1).

During second phase of cell cycle (48 hr sampling time) the incidence of all types of chromosomal aberrations and aberrant cells in positive control group, was found to be relatively low (37.60% and 35.20%, respectively, $P < 0.01$) but significantly higher than control group (Tables 1&2 and Fig. 1). The cytotoxic potential of malathion was still evident in Gr.IV, as there was a significant decrease in mitotic index (56.93%, $P < 0.01$). In Gr.II and Gr.III, no significant increase in aberrant cells and decrease in mitotic index was observed when compared to Gr.I, further indicating a non-mutagenic and non-cytotoxic response of green tea and ginger extracts (Tables 1&2). The incidence of aberrant cells was found to be 35.20 ± 0.663 in Gr.IV, but declined to 26.80 ± 1.020 $P < 0.01$ in Gr.V, 27.20 ± 0.917 $P < 0.01$ in Gr.VI and 25.80 ± 0.735 $P < 0.01$ in Gr.VII, respectively (Fig. 1). The chromosomal and chromatid type aberrations per cell were also inhibited by GTI, GI and GTI+GI pretreatment. Mitotic index at 48 hr sampling time, when compared with Gr.IV was found to be increased by 17.18%, 11.10% and 14.01% in Gr.V, Gr.VI and Gr.VII, respectively. The

inhibition of 23.86% in Gr.V, 22.73% in Gr.VI and 26.70% in Gr.VII of GTI, GI and GTI+GI pretreatment, respectively, against malathion induced cytogenetic damage was recorded (Fig. 1).

DISCUSSION

A considerable emphasis is being laid down on the use of dietary constituents as chemoprotective measure for control of genetic diseases (**Mitscher *et al* 1996**).

Bone marrow cytogenetics is a useful short-term technique, for elucidating the mechanism as well as to identify the substances for their clastogenic and anticlastogenic activity. Majority of the mutagenic/carcinogenic compounds e.g. polycyclic aromatic hydrocarbons, acts by generating electrophilic intermediates by microsomal enzymatic reactions causing mutation. These compounds generate alkylating metabolites. Following

Table 1. Protecting effects of green tea and ginger extracts pretreatment on malathion induced different types of chromosomal aberrations in mouse bone marrow cells

| Groups | Number of different types of chromosomal aberrations at 24 hr sampling time | | | | | | |
|--------|---|-------------|--------------|--------------|-------------|--------------|----------------|
| | Chr.B. | Cent. Att | Cent.Fu | E.E.Ass. | Polyp. | Endom. | Total |
| Gr.I | 18 (7.2%) | 3 (1.2%) | 4 (1.6%) | 2 (0.8%) | 2 (0.8%) | 4 (1.6%) | 33 (13.2%) |
| Gr.II | 24 (9.6%) | 6 (2.4%) | 2 (0.8%) | 1 (0.4%) | 2 (0.8%) | 5 (2.0%) | 40 (16.0%) |
| Gr.III | 20 (8.0%) | 5 (2.0%) | 1 (0.4%) | 2 (0.8%) | 3 (1.2%) | 6 (2.4%) | 37 (14.8%) |
| Gr.IV | 84 (33.6%) | 5 (2.0%) | 10 (4.0%) | 13 (5.2%) | 4 (1.6%) | 10 (4.0%) | 126 (50.4%) |
| Gr.V | 46 (18.4%) | 1 (0.4%) | 6 (2.4%) | 14 (5.6%) | 7 (2.8%) | 8 (3.2%) | 82 (32.8%) |
| Gr.VI | 60 (24.0%) | 1 (0.4%) | 5 (2.0%) | 13 (5.2%) | 4 (1.6%) | 9 (3.6%) | 92 (36.8%) |
| Gr.VII | 54 (21.6%) | 3 (1.2%) | 5 (2.0%) | 11 (4.4%) | 6 (2.4%) | 5 (2.0%) | 84 (33.6%) |
| Groups | Number of different types of chromosomal aberrations at 48 hr sampling time | | | | | | |
| | Chr.B. | Cent. Att | Cent.Fu | E.E.Ass | Polyp. | Endom. | Total |
| Gr.I | 18 (7.2%) | 3 (1.2%) | 4 (1.6%) | 3 (1.2%) | 2 (0.8%) | 4 (1.6%) | 34 (13.6%) |
| Gr.II | 20 (8.0%) | 3 (1.2%) | 5 (2.0%) | 2 (0.8%) | 2 (0.8%) | 5 (2.0%) | 37 (14.8%) |
| Gr.III | 16 (6.4%) | 1 (0.4%) | 2 (0.8%) | 3 (1.2%) | 3 (1.2%) | 5 (2.0%) | 30 (12.0%) |
| Gr.IV | 63 (25.2%) | 5 (2.0%) | 3 (1.2%) | 9 (3.6%) | 8 (3.2%) | 6 (2.4%) | 94 (37.6%) |
| Gr.V | 36 (14.4%) | 4 (1.6%) | 1 (0.4%) | 14 (5.6%) | 5 (2.0%) | 7 (2.8%) | 67 (26.8%) |
| Gr.VI | 52 (20.8%) | 8 (3.2%) | 1 (0.4%) | 10 (4.0%) | 6 (2.4%) | 7 (2.8%) | 84 (33.6%) |
| Gr.VII | 26 (10.4%) | 2 (0.8%) | 1 (0.4%) | 1 (0.4%) | 5 (2.0%) | 2 (0.8%) | 35 (13.8%) |

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Number of metaphase cells analyzed per animal group = 250 cells

Chr.B. = Chromatid Breakage Cent. Att. = Centromeric Attenuation Cent. Fu. = Centric Fusion

E.E.Ass. = End to End Association

Polp. = Polyploidy Endom. = Endomitosis

Gr.I = Negative control Gr.II = Tea control

Gr.III = Ginger control

Gr.IV = Malathion positive control Gr.V = Tea + malathion Gr.VI = Ginger + malathion

Gr.VII = Tea + ginger + malathion

Protective potency of green tea and ginger extracts

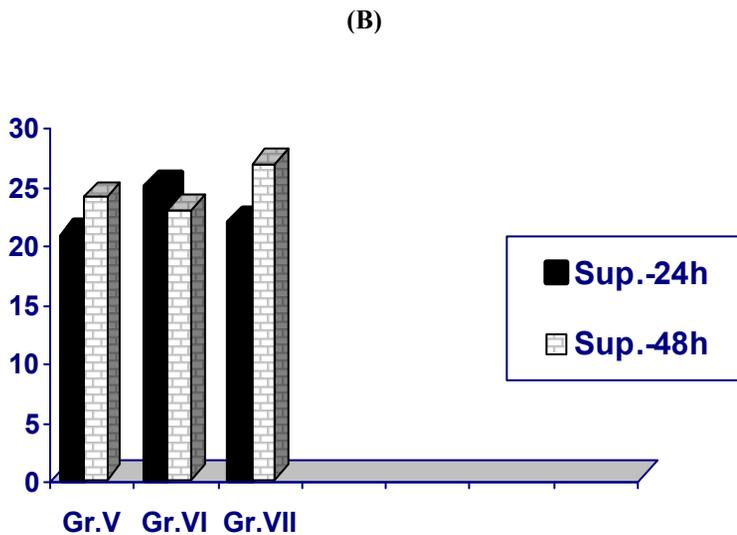
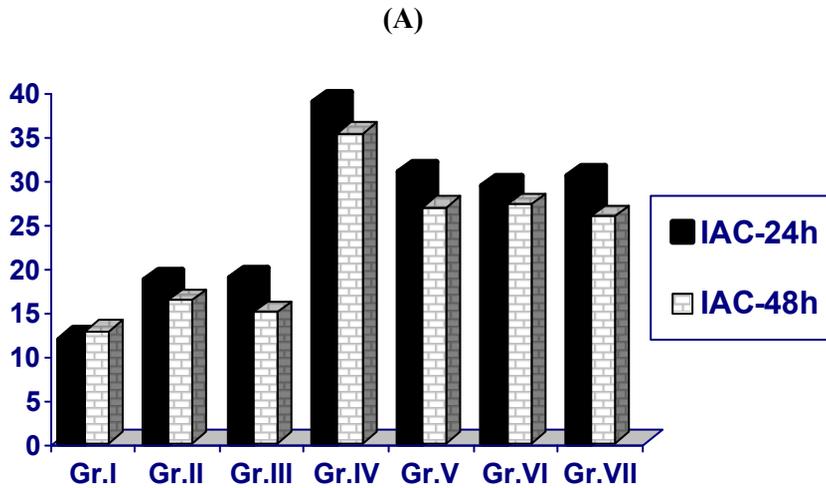


Fig. 1. Antimutagenic activity of green tea and ginger extracts in albino mice.
 (A) Incidence of aberrant cells (IAC) at sampling times 24 h and 48 h,
 (B) Suppressive effect (Sup.) of GTI, GI and GTI + GI on chromosomal aberrations at sampling times 24h and 48h

biological activation, resulting in formation of mutant cells (**Vainio et al 1992 and Schurz et al 2000**). Antigenotoxic agents especially those present in natural substances acts through different cellular pathways involving endogenous sequestration of mutagens by various enzymes (**Shukla and Taneja, 2002**).

The recorded results indicated, that animals treated once with the organophosphorous insecticide, malathion, at dose 230 mg/kg b.w. (Gr.IV), several-fold increase in the different types of chromosomal aberrations and frequency of aberrant cells with significant decrease in mitotic index were observed. This agrees with the previously reported ability of organophosphorous insecticides to produce chromosome aberrations (**Furlong et al 1993; Au et al 1999; Blasiak et al 1999; Heddle et al 1999 and Giri et al 2002**). The significant increase in the chromosomal abnormality and the significant decrease in the mitotic index may be due to gene damages. Since the organophosphorous insecticides are chemical alkylating agents (**Wild 1975 and Garaj-Vrhovac and Zeljezic, 2002**) alkylation of DNA bases either directly or indirectly via protein alkylation, is probably involved in the DNA disintegration (**Green et al 1974, Flora, 1998 and Shukla and Taneja, 2002**).

The present investigation revealed that the antimutagenic potential of green tea and ginger extracts against chromosomal damage, was induced by malathion. Earlier studies conducted with green tea, presence of many polyphenolic compounds, (-)-epigallocatechin gallate (EGCG), is believed to be a key active constituent in terms of cancer chemopreventive potential (**Conney et al 1997 and Surh, 1999**). The strong antioxidative activity retained in this polyphenol has been confirmed in numerous *in vivo* and *in vitro* studies (**Aucamp et al 1997 and Soliman and Mazzi, 1998**), which appears to contribute in part to the antimutagenic and anticarcinogenic effects of green tea. Also, **Surh, (1999) and Jodojin et al (2002)** indicated that, Tea consumption has been shown to moderately induce glutathione S-transferase, UDP-glucuronosyl transferase, NADPH-quinone oxidoreductase, and antioxidant enzymes, all of which could enhance the elimination of carcinogens.

While the oleoresin from rhizomes of ginger contains [6]-gingerol (1-[4'-hydroxy-3'-methoxyphenyl]-5-hydroxy-3-decanone) and its homologs as pungent ingredients, that have been found to possess many interesting pharmacological and physiological activities, such as anti-inflammatory, analgesic, antipyretic, antihepatotoxic, and cardiotoxic effects (**Mustafa et al 1993**). Gingerol has been

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found to possess substantial antioxidant activity, as determined by inhibition of phospholipid peroxidation induced by the FeCl₃-ascorbate system (Aeschbach *et al* 1994). The antioxidative properties of gingerol and other constituents of ginger have been confirmed in various *in vitro* and *in vivo* test systems (Kikuzaki *et al* 1994). Gingerol also exerts an inhibitory effect on xanthine oxidase (Chang *et al* 1994) responsible for generation of superoxide anion.

The results of the present study revealed that, GTI, GI and GTI+GI could exert different anticytotoxic and antimutagenic effects during both phases of cell cycle. However, GTI exerted the highest protective level at 24 h sampling time (Gr.V), but GTI+GI mixture (Gr.VII) at 48h sampling time. While high suppressive effects and low incidence of aberrant cells were observed at 48 h sampling time indicative of greater detoxification ability in later stages.

The present study concluded that, malathion used as commercial product can be considered as a genotoxic substance *in vivo*, because it produces, DNA disturbances, such as DNA breakage at sites of oncogenes or tumor suppressor genes, thus playing a role in the induction of malignancies in individuals exposed to this agent. Therefore, malathion can be regarded as a potential mutagen/carcinogen and requires further investigations. Also the study concluded that, each of green tea and ginger extracts exert antimutagenic effects of varied potency, dependent on sampling time. While the mixture of green tea and ginger extracts exerts the highest protective potency. Hence the oral administration of GTI, GI and GTI+GI is found to be capable of

preventing chromosomal aberrations caused by malathion. The protective effects of GTI, GI and GTI+GI towards malathion induced cytotoxic and cytogenetic damage implies as a good marker of their antimutagenic and antineoplastic activity.

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1019-1031، 13(3)، 2005، م. ش. ن. ي. ع. ع. م. ا. ج. ع. ي. ع. ا. ر. ز. ل. ن. و. ح. ب. ل. ن. ا. س. ا. ر. د. ق. ل. ب. ر. ع. ل. ك. ا. ع. م. ا. ج. ل. م. ا. ح. ت. ا. ق. ل. ج. م. ، 2005

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الذلل ادبع دمحم دمحم باهيا - أمش قهئ افماركأ

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