African Journal of Food Science Vol. 4(7), pp. 434 – 439, July 2010
Available online http://www.academicjournals.org/ajfs
ISSN 1996-0794 ©2010 Academic Journals

*Full Length Research Paper*

# Genotoxicity and antigenotoxicity studies of commercial *Argania spinosa* seed oil (argan oil) using the wing somatic mutation and recombination test in *Drosophila melanogaster*

**A. Dalouh*, S. Amkiss, S. N. Skali, J. Abrini and M. Idaomar**

Laboratoire de Biologie et Santé, Département de Biologie, Faculté des Sciences, Université Abdelmalek Essaâdi, BP 2121, 93002 Tétouan, Morocco.

Accepted 25 May, 2010

**Argan oil is receiving increasing attention due to its potential health benefits in the prevention of cardiovascular and cancer risk, but no information to date is available about its genotoxic or antigenotoxic effect. The genotoxicity and the antigenotoxicity of commercial argan oil and its unsaponifiable fraction (UF) against *Drosophila melanogaster* was evaluated using wing spots enumeration. Results showed that the argan oil (at 20% dosage) and the UF (at 0.112 g/l) were not genotoxic. Results on the effects of argan oil and UF on the mutagenic properties of methyl methanesulfonate (MMS) and ethyl carbamate (urethane) showed an inhibition rate of 54 and 43% against MMS and urethane, respectively, by argan oil and 56 and 75% against urethane and MMS, respectively, by UF. The result of the present study suggests that argan oil prevent mutations induced by urethane and MMS in *D. melanogaster*, as a consequence consumption of argan oil can prevent human DNA lesion induced by some environmental mutagens.**

**Key words:** Virgin argan oil, unsaponifiable fraction, *Argania spinosa*, *Drosophila melanogaster*, genotoxicity, antigenotoxicity.

## INTRODUCTION

Mutagenic agents can be chemical, viral or radiation. It is also possible that undetected environmental agents cause the so-called spontaneous mutations. Even the UV from sunlight can induce mutations which translate onto cancer (Schuch and Martins-Menck, 2010). Therefore, increasing number of scientists started investigating the mechanisms of antimutagenicity and anticarcinogenicity (Gebhart, 1974; Zieger, 2000; lee and Park, 2003, Waters et al., 1996). An antimutagene is any agent which reduces the frequency or the rate of the spontaneous or induced mutations independently of the mechanism of action (Waters et al., 1996; El Hamss and Idaomar, 2002).

The fruit fly *Drosophila melanogaster* is a well established model for the study of various compounds and mixtures antigenotoxicity due to its well-documented genetics and developmental biology (Mukhopadhyay et al., 2004; Gaivao et al., 1999). It is a eukaryotic organism with a short generation time (≈ 10 days at 25°C) and easily detectable genetically controlled morphological characters. A large number of mutants and genetically characterized stocks are available; its breeding is easy and inexpensive which allow the breeding of large populations easily. *D. melanogaster* is capable of activating promutagenes and procarcinogenes (Serikaya and Çakir, 2005).

*Argania spinosa* (Sapotacea) commonly called, Arganier in French and Argan tree in Arabic English is an endemic tree of South-Western Morocco and is the third

*Corresponding author. Email: dalouh_abdelkrim@yahoo.fr, idaomar@gmail.com.

**Abbreviations: MMS,** Methyl methanesulfonate; **URE,** urethane; ***D*, *Drosophila***; **UF,** usaponifiable fraction; ***Flr*,** flare; ***mwh*,** multiple wing hairs.

largest population of fruit trees in the sub-region. The 'oil of argan' obtained from seeds of *A. spinosa* form 9% of the annual Moroccan production of vegetable oils (Cherki et al., 2006). Virgin argan oil is well known for its cosmetic, pharmaceutical and nutritional virtues (Moukal, 2004). It is the source of 25% of the edible fats consumed by the population of South-Western Morocco (Benzaria et al., 2006). Argan oil is highly used in traditional medicine for the treatment of chicken pox, the teenage acne and rheumatism (Charrouf and Guillaume, 1999) and has beneficial effect on obesity risk prevention (Adlouni et al., 2008) and has antimalarial activity (ElBabili et al., 2008). Recent studies detected hypolipidemic, hypocholesterolemic and antihypertensive effects on rats subjected to argan oil enriched-diet (Berrougui et al., 2003; 2004). Furthermore, the hypolipidemic effect of argan oil was confirmed on another study on humans subjected to argan oil diet (Derouiche et al., 2005).

Virgin argan oil is characterized by its unique composition in fatty acids (Khallouki et al., 2003), 45% of monounsaturated fatty acids, 35% of polyunsaturated fatty acids and 20% of saturated fatty acids (Drissi et al., 2004). It is rich in phenolic compounds (3, 3 mg/kg), vegetable Sterols (295 mg/100 g) and tocopherols (637 mg/kg), (Berrougui et al., 2006). Phenolic compounds and sterols are known by their antioxidant and antimutagenic effects (Menat, 2006; De Mejıa et al., 1999). Thus, the tocopherol major present in the virgin argan oil is γ - tocopherol which is the tocopherol isomer of the most antioxidant and anticancerigene (Betti et al., 2006; Samane et al., 2006), among the sterols identified in the argan oil of the schottenol (5α-stigmasta-7-en-3β-ol) shows a anticarcinogenic activity (Yaghmur et al., 2001) the antiproliferative activity of polyphenole of argan oil was confirmed on prostate cancer (Bennania et al., 2009). To our knowledge no study on the mutagenicity and the antimutagenicity of the argan oil is made. The aim of this study is the evaluation of the genotoxicity and the antigenotoxicity of commercial argan oil using the somatic mutation and recombination test in *D. melanogaster*.

## MATERIALS AND METHODS

### Source of organisms, chemicals and preparation of argan oil extracts

The *D. melanogeste rmwh/mwh* and *flr*$^{3}$*/TM3,Bd*$^{s}$ strains, were kindly provided by Ricard Marcos (autonomous university of Barcelona). Virgin argan oil commercialized under the name tissaliwine® (produced by union of the Co-operatives of the Women for the production and the marketing of the of Argan oil and the agricultural produce (UCFA)), was purchased from Marjan supermarket, Rabat, Morroco. The unsaponifiable fraction (UF) was extracted according to procedures previously described by Samane et al. (2006) with slight modification (methanol instead of ethanol). To 50 g of argan oil, 500 ml of methanol and potassium hydroxide (KOH2N/MetOH analysis) was added, thoroughly mixed and then heated (at 44˚C) with backward flow for 1 h. Following addition of distilled water (500 ml) and cooling to room temperature, hexane (300 ml) was added. This mixture was shaken vigorously for 1 h and the partitioned hexane layer was collected. The remaining aqueous phase was extracted a second time more with hexane (300 ml). The hexane extracts were pooled, washed with water/methanol (50: 50), dehydrated over anhydrous $Na_2SO_4$ and filtered. The solvent was removed using a rotavapor. The residue was heated at 103˚C for 1 h. The pure 'argan oil' was diluted in tween 80 at 2% and ethanol 3%. The unsaponifiable fraction was diluted in methanol 3% and tween 80 at 2%. Ethyl carbamate (Urethane) ($C_3H_7NO_2$, PM 89.09 g/mol SERVA company Heidelberg, Germany), and methyl methanesulfonate (MMS) ($C_2H_6O_3S$; PM 110.13 g/mol, ACROS Organics company Belgium) were used as positive controls.

### Collection and treatment of insect larvae

From the cross between ≈ 400 ♀ *mwh/mwh* females and ≈ 200 ♂ *flr3/TM3;Bds* males eggs were collected for 8 h in culture bottles containing 20 g of baker's yeast, and incubated for 3 days at 25 ± 1°C and 60% relative humidity. The 72 ± 4 h old larvae were isolated from yeast using solution of NaCl 20% (100 ml) and placed in vials containing 1 g of the instant medium («Drosophila Instant Medium», Carolina Biological Supply Company, Burlington NC) and 3 ml of the test compound (argan oil, UF, argan oil and urethane or MMS, UF and urethane or MMS, MMS or urethane, solvent).

### Genotoxicity and antigenotoxicity studies

For genotoxicity study, *D. melanogaster* larvae (72 h old) were exposed to chronic treatment with argan oil or with the unsaponifiable fraction (UF), Larvae were feeding in 1 g instant medium containing 3 ml of argan oil (at 1, 3, 5, 10 or 20% dosage), or 3 ml of UF ( at 0.028, 0.056 or 0.112 g/l) during the rest of their larval life (48 h). For high concentrations of pure argan oil (10 and 20%), the larvae were removed from the medium of treatment after 48 h and were placed in instant mediums containing only the solvent to avoid the larval asphyxiation. For antigenotoxicity testing, 72 h old larvae were treated (by the method previously described in genotoxicity study) with 3 ml of solution containing the mutagen (urethane 10 mM or MMS 0,5 Mm ) and it's inhibitor (the argan oil at 10 or 20% oil or UF at 0.028; 0.056 or 0.112 g/l). A treatment including the mutagen or solvent only was used as positive or negative control, respectively. All the experiments were incubated at 25 ± 1°C and a relative humidity of approximately 60%. After eclosion, the adult flies were collected from exposure vials and stored in 70% ethanol.

### Determination of mutation clones?

The flies were collected from ethanol and examined under binocular magnifying glass. The flies having phenotype *Bd*$^{S}$ were eliminated (because they do not give information about recombination), only the wings of the wild flies (*mwh* $^{+}$, *flr* $^{3}$ */ mwh, flr*$^{+}$*)* were mounted in Faure solution (Gum arabic 30 g, Glycerol 20 ml, Hydrate chloride 50 g, distilled water 50 ml) and analyzed under a photonic microscope at 400× magnification for the occurrence of single spots (*mwh* or *flr* phenotype) or twin spots (*mwh* clone adjacent to *flr* clone). The spots *mwh* were not counted if they do not contained cells carrying more than two hairs (Graf et al 1989; Frei et al., 1985). The clones were classified on a table as small clones (*mwh* or *flr*, (1 - 2 mutated cells), large simple (more than 2 mutated cells) or twin clones. Single spots are due to mutational or recombinational events, while twin spots are produced by somatic recombination only.

**Table 1.** Results of the genotoxicity study of argan oil and its unsaponifiable fraction against *D. melanogaster*.

| Concentration (%) | Number of wings | spots per wing (number of spots) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Small singles | | Large singles | | Twins | | Total number of spots | |
| **Argan oil** | | | | | | | | | |
| Control | 120 | 78 | 0.65 | 8 | 0.06 | 4 | 0.03 | 90 | 0.75 |
| 1 | 40 | 22 | 0.55[a] | 0 | 0.00[a] | 1 | 0.02[a] | 23 | 0.57[a] |
| 3 | 40 | 25 | 0.62[a] | 2 | 0.05[a] | 1 | 0.02[a] | 28 | 0.70[a] |
| 5 | 40 | 19 | 0.47[a] | 3 | 0.07[a] | 0 | 0.00[a] | 22 | 0.55[a] |
| 10 | 30 | 28 | 0.93[a] | 0 | 0.00[a] | 0 | 0.00[a] | 28 | 0.93[a] |
| 20 | 38 | 41 | 1.08[b] | 0 | 0.00[a] | 0 | 0.00[a] | 41 | 1.08[a] |
| **Unsaponifiable fraction of argan oil g/l** | | | | | | | | | |
| Control | 40 | 25 | 0.63 | 1 | 0.02 | 0 | 0.00 | 26 | 0.65[a] |
| 0.028 | 40 | 10 | 0.25[b] | 3 | 0.07[a] | 0 | 0.00[a] | 13 | 0.32[a] |
| 0.056 | 40 | 18 | 0.45[a] | 0 | 0.00[a] | 1 | 0.02[a] | 19 | 0.47[a] |
| 0.112 | 37 | 18 | 0.48[a] | 4 | 0.11[a] | 1 | 0.03[a] | 23 | 0.62[a] |

a: no significant differences at $p>0.05$. b: significant differences at $p<0.05$.

**Statistical analysis**

The frequencies of mutations at various concentrations were compared pair wise with the corresponding negative control in the study of the genotoxicity and with those of urethane and methyl methanesulfonate (MMS) in the study of the antigenotoxicity. The statistical analysis was carried out using a programmed that employs the chi-square test (Frei et al., 1985; Frei and Würgler, 1995).

## RESULTS

Results of genotoxicity studies (Table 1) showed that argan oil at doses of 1, 3, 5, 10 and 20% and the unsaponifiable fraction (UF) at doses 0.028, 0.056 or 0,112 g/l does not increase the rate of wing spots of *D. melanogaster* (fruit fly) compared to the negative control. The distribution of spontaneous mutations represented by the predominance of small singles spots shows that the endogenous lesions of the genetic material of the treated larvae occur in the imaginal disc during the cells' last stage of differentiation, which corresponds to the last hours of the treatment. These same observations were made by other authors (Graf, 1995; Idaomar et al., 2002). Results also showed that the *mwh* spots were more frequent than *flr* spots. This could be due to recombination occurring between the *mwh* and *flr loci* (Graf et al., 1983; Graf, 1995; Zimmering et al., 1997).

Table 2 shows results of the combined treatment of *D. melanogaster* larvae with the argan oil or UF and urethane or methyl methanesulfonate. Results showed that, at 10%, argan oil significantly reduced the rate of the small singles spots (54%, $p < 0.001$) formation on the wings of the fruit flies, but there was no significant difference in the number of the large singles spots and the twin spots compared to the negative control. Results also showed that, combined treatment of *D. melanogaster* larvae with argan oil and urethane (20% dose) did not show any significant ($p > 0.05$) reduction in the number of small spots. Results of combined treatment of larvae with unsaponifiable fraction and urethane, showed a significant dose dependent reduction on the rate of the induced mutations, with a rate inhibition of 40% for the dose 0.056 g/l ($p < 0.05$) and 56% for the dose 0.084 g/l ($p < 0,01$).

The combined treatment of the larvae of *D. melanogaster* with argan oil and methyl methanesulfonate (MMS) showed that the argan oil at 10 and 20% significantly decreased the rate of the mutations induced by the MMS involving all the categories of the spots, with the inhibition very significant at 20% compared to the 10% dose, with a rates of inhibition of 20 and 43%, respectively. Results of combined treatment of the 72 h old larvae with the UF and the MMS shows that at 0.056 g/l UF inhibited ($p < 0.001$) the mutations (mainly the twins spots) induced by the MMS. At 0.112 g/l UF inhibited ($p < 0.001$) all the categories of the spots with a rate of inhibition of 75%.

## DISCUSSION

Chronic treatment of *D. melanogaster* larva has been used to investigate the genotoxicity of organ oil and its unsaponifiable fraction. This mode of treatment is most suitable for the study of the genotoxicity of the products for which genotoxicity is unknown (Graf et al., 1989). Results showed that argan oil and UF are not genotoxic, so the wing spots detected are due to spontaneous mutation. Results on the effects of argan oil and UF on the mutagenic properties of methyl methanesulfonate (MMS) and ethyl carbamate (urethane) showed an

**Table 2.** Results of the study of the antigenotoxic activity of the argan oil and its insaponifiable fraction against the mutations induced by urethane and the methyl methanesulfonate (MMS).

| Concentration | Number of wings | Number of spots (spots per wings) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Small singles | | Large singles | | Tween | | Total number of spots | | |
| **Control** | **160** | **(110)** | **0.69** | **(9)** | **0.05** | **(4)** | **0.02** | **(123)** | **0.77** | |
| **Argan oil** | | | | | | | | | | |
| URE 10 mM | 40 | (94) | 2.35 | (6) | 0.15 | (1) | 0.02 | (101) | 2.52 | |
| URE and 10% (v/v) | 40 | (41) | 1.02$^{d}$ | (3) | 0.07$^{a}$ | (2) | 0.05$^{a}$ | (46) | 1.15$^{d}$ | 54% |
| URE and 20% (v/v) | 40 | (74) | 1.85$^{a}$ | (7) | 0.17$^{a}$ | (3) | 0.07$^{a}$ | (84) | 2.10$^{a}$ | 17% |
| MMS 0.5 mM | 40 | (141) | 3.52 | (121) | 3.02 | (70) | 1.75 | (331) | 8.27 | |
| MMS and 10% (v/v) | 40 | (77) | 1.92$^{d}$ | (126) | 3.15$^{a}$ | 63) | 1.57$^{a}$ | (266) | 6.65$^{b}$ | 20% |
| MMS and 20% (v/v) | 31 | (48) | 1.55$^{d}$ | (67) | 2.16$^{b}$ | 29) | 0.93$^{c}$ | (147) | 4.74$^{c}$ | 43% |
| **Insaponifiable fraction of argan oil (g/L)** | | | | | | | | | | |
| URE 10 mM | 40 | (52) | 1.30 | (2) | 0.05 | (1) | 0.02 | (55) | 1.37 | |
| URE and 0.028 | 39 | (27) | 0.69$^{b}$ | (5) | 0.13$^{a}$ | (0) | 0.00$^{a}$ | (32) | 0.82$^{b}$ | 40% |
| URE and 0.084 | 40 | (20) | 0.50$^{d}$ | (2) | 0.05$^{a}$ | (2) | 0.05$^{a}$ | (24) | 0.60$^{c}$ | 56% |
| MMS 0.5 mM | 40 | (94) | 2.35 | (74) | 1.85 | (70) | 1.75 | (238) | 5.95 | |
| MMS and 0.056 | 40 | (69) | 1.72$^{a}$ | (59) | 1.47$^{a}$ | (23) | 0.57$^{d}$ | (151) | 3.77$^{d}$ | 36% |
| MMS and 0.112 | 40 | (32) | 0.80$^{d}$ | (21) | 0.52$^{d}$ | (7) | 0.17$^{d}$ | (60) | 1.50$^{d}$ | 75% |

a: no significant differences at p>0.05 ;b : significant differences at p<0.05 ; c : highly significant differences at p<0.01. d: very highly significant differences at p<0.001. Uret : urethane.

inhibition rate of 54 and 43% against MMS and urethane, respectively, by argan oil and 56 and 75% against urethane and MMS, respectively, by UF. Results on the effect of argan oil and UF on the mutagenic property of ethyl carbamate (urethane) showed an inhibition rate of 54% by argan oil and an inhibition rate of 56% by UF.

Urethane is mutagen and carcinogen, its metabolites, obtained after biotransformation, are responsible for such activity, from where the name promutagene (Zimmerli and Schlatter, 1991). The metabolic activation of urethane induces the formation of the carcinogenic compounds vinyl carbamate and the epoxy ethyl carbamate which are capable of inducing mutation (Peer, 2002). It's been earlier reported that the activity of P450 cytochrome-dependent enzymes in the metabolic activation of ethyl carbamate are responsible for mutation in *D. melanogaster* (Frölich and Würgler, 1999). The inhibitory effects of argan oil and its extract against the mutations induced by urethane could be a result of prevention of the formation of its metabolites, or by induction of the DNA repair system, or trapping and inactivation of urethane and/or its metabolites. Results on the effect of argan oil and UF on the mutagenic property of methyl methanesulfonate (MMS) showed an inhibition rate of 43% by argan oil and an inhibition rate of 75% by UF

Methyl methanesulfonate (MMS) belongs to the alkylating agents that are mutagens and carcinogenes (Drabløs et al., 2004). Alkylation in general is the nucleophilic substitution of a hydrogen atom by an alkyl group. The nucleophilic mechanism in MMS ($SN_2$ type) results in the formation of an intermediary between the nucleophilic acceptor and the electrophile (that is, between the DNA and the alkylating agent). The MMS is known for its capacity to react directly with the DNA *in vivo* and *in vitro* (Graf, 1995; Nivard et al., 1992; Madrigal-Bujaidar et al., 1998). The antigenotoxic effects of argan oil on MMS could be a result of the oil inducing the detoxification of enzymes or inducing the DNA repair system, or by the competition with MMS (electrophile) on the nucleophilic sites of the DNA. A similar mechanism (competition nucleophile-electrophile) that vitamin C inhibits genotoxicity of MMS in *Drosophila* (Kaya, 2003).

The first study of the antigenotoxicity by the wings SMART assay of *D. melanogaster* was published in 1989 by Negishi et al. (1989). Since then, the method was used in several studies of the antimutagenicity (Graf et al., 1998; Patenkovic et al., 2009). Thus in this work, the antimutagenic activity of argan oil in *Drosophila* was recorded. The antimutagenic activity of vitamin E is one of the significant components of argan oil and was earlier

reported by Nickolic et al. (2004); Betti et al. (2006) in there study on *Escherichia coli*.

During the last decades hundreds of antimutagenic and anticarcinogenic effects in several plant food substances have been reported (Knasmüller et al., 2002) an indication that food-based chemical substances can be used to protect against DNA damage and cancer. The presence of antioxidants and antimutagenic constituents in argan oil (Khallouki et al., 2003; El Babili et al., 2010; Nagy et al., 2009; Christet et al., 1997) shows that the oil could play a significant role in the protection of genetic materials against oxidative damages and other kinds of lesions which the human DNA is continuously exposed to (Valko et al., 2004; Bartsch and Nair, 2005).

## Conclusion

Our findings showed that argan oil prevents mutations induced by urethane and MMS in *D. melanogaster*, as a consequence consumption of argan oil that can prevent human DNA lesion induced by some environmental mutagens. Antimutagenic effect of argan oil needs to be more characterized by more genotoxicity assay using mammal and microbial organisnims,

### REFERENCES


Adlouni A, Christon R, Cherki M, Khalil A, ElMessal M(2008). The nutritional Benefits of Argan Oil in Obesity Risrk Prevention . Ather. Suppl. 9: 137-138.

Bartsch H, Nair J (2005). Accumulation of lipid peroxidation-derived DNA lesions: Potential lead markers for chemoprevention of inflammation-driven malignancies. Mut. Res. 591: 34-44.

Bennania H, Fietb J, Adlounia A (2009). Impacte de l'huile d'argan sur le cancer de la prostate: étude de l'effet antiprolifératif des polyphénoles. Revue Francophone des Laboratoire. pp. 23-26.

Benzaria A, Meskini N, Dubois M, Croset M, Némoz G, Lagarde M, Prigent AF (2006). Effect of dietary argan oil on fatty acid composition, proliferation, and phospholipase D activity of rat thymocytes. Nutrition 22: 628-637.

Berrougui H, Alvarez de Sotomayor M, Pérez-Guerrero C, Ettaib A, Hmamouchi M, Marhuenda E, Dolores Herrera M (2004). Argan (Argania spinosa) oil lowers blood pressure and improves endothelial dysfunction in spontaneously hypertensive rats. Br.itish J. Nutr. 92: 921-929

Berrougui H, Cloutier M, Isabelle M, Khalil A (2006). Phenolic-extract from argan oil (Argania spinosa L.) inhibits human law-density lipoprotein (LDL) oxidation and enhances cholesterol efflux from human THP-1 macrophages. Ather. 184: 389-396.

Berrougui H, Ettaib A, Herrera Gonzalez MD, Alvarez de Sotomayor M, Bennani-Kabchi N, Hmamouchia M (2003). Hypolipidemic and hypocholesterolemic effect of argan oil (*Argania spinosa* L.) in *Meriones shawi* rats. J. Ethnopharm. Macol. 89: 15-18.

Betti M, Minelli A, Canonic B, Castaldo P, Magi S, Aisa MC, Piroddi MD, Tomaso V, Galli F (2006). Antiproliferative effects of tocopherols (Vitamin E) on murinc glioma C6 cells: homologue-specific control of PKC/ERK and cyclin signalling. Free Radic. Biol. Med. 41: 464-472.

Charrouf Z, Guillaume D (1999). Ethnoeconomical, ethnomedical, and phytochemical study of Argania spinosa (L.) Skeels. J. Ethnophar. 67: 7-14.

Cherki M, Berrougi H, Drissi A, Adlouni A, Khalil A (2006). Argan oil: Which benefits on cardiovascular diseases? Pharm. Res. 54: 1-5.

Christen S, Woodall AA , Shigenaga MK, Southwell-Keely PT, Duncan M W, Ames BN (1997).γ-Tocopherol traps mutagenic electrophiles such as NOx and complements α--tocopherol: Physiological implications. *Proc*. Natl. Acad. Sci. USA, 94: 3217-3222.

De Mejıa EG, Castano-Tostado E, Loarca-Pina G (1999). Antimutagenic effects of natural phenolic compounds in beans. Mut. Res. 441: 1-9.

Derouiche A, Cherki M, Drissi A, Bamou Y, El Messal M, Idrissi-Oudghiri A, Lecerf JM, Adlouni A (2005). Nutritional Intervention Study with Argan Oil in Man: Effects on Lipids and Apolipoproteins. Ann. Nutr. Metab. 49:196-201

Drabløs F, Feyzi E, Aas PM,Vaagbø CB, Kavli B, Bratli MS, Peña-Diaz J, Otterlei M, Slupphaug G, Krokan HE (2004). Alkylation damage in DNA and RNA-repair mechanisms and medical significance. DNA Rep. 3: 1389-1407.

Drissi A, Girona J, Cherki M, Godàs G, Derouiche A, El Messal M, Saile R, Kettani A, Solà R, Masana L, Adlounia A (2004). Evidence of hypolipemiant and antioxidant properties of argan oil derived from the argan tree (Argania spinosa). Clin. Nutr. 23:1159-1166.

El Babili F, Bouajila J, Fouraste I, Valentin A, Mauret S, Moulis C (2009). Chemical study, antimalarial and antioxidant activities, and cytotoxicity to human breast cancer cells (MCF7) of Argania spinosa. Phytmed. 17:157-160.

El Hamss R, Idaomar M (2002). Antimutagènes et anticarcinogènes, identification et mécanismes d'action des xénobiotiques alimentaires. Thérapie :57::512-517.

Frei H, Wiirgler FE, Juon H, Hall CB, Graf U (1985). Aristolochic acid is mutagenic and recombinogenic in *Drosophila* genotoxicity tests. Arch Toxicol. 56:158-166.

Frei H, Würgler FE (1995). Optimal experimental design and sample size for the statistical evaluation of data from somatic mutation and recombination tests (SMART) in Drosophila**.** Mut. Res. 334: 247-258.

Frölich A, Würgler FE (1990). Genotoxicity of ethyl carbamate in Drosophila Wing spot test: dependence on genotype-controlled metabolic capacity. Mut. Res. 244: 201-208.

Gaivao I, Sierra LM, Comendator MA (1999) The w/w+ SMART assay of Drosophila melanogaster detects the genotoxic effects of reactive oxygen species inducing compounds. Mut. Res. 440: 139-145

Gebhart E (1974). Antimutagens Data and problems.Hum. gen.etik 24: 1-32.

Graf U (1995). Analysis of the relationship between age of larvae at mutagen treatment and frequency and size of spots in the wing somatic mutation and recombination test in Drosophila melanogaster. Experientia 51: 168-173.

Graf U, Abraham SK, Guzmán-Rincón J, Würgler FE, (1998). Antigenotoxicity studies in *Drosophila melanogaster*. Mut. Res. 402: 203-209.

Graf U, Frei H, Kägi A, Katz AJ, Würgler FE (1989). Thirty compounds tested in the Drosophila wing spot test. Mut. Res. 222: 359-373.

Graf U, Juon H, Katz AJ, Frei HG, Würgler FE (1983). A pilot study on a new Drosophila spot test. Mut. Res. 120: 233-239

Idaomar M, El Hamss R, Bakkali F, Mezzoug N, Zhiri A, Baudoux D, Muñoz-Serrano A, Liemans V, Alonso-Moraga A (2002). Genotoxicity and antigenotoxicity of some essential oils evaluated by wing spot test of Drosophila melanogaster. Mut. Res. 513: 61-68.

Kaya B (2003). Anti-Genotoxic Effect of Ascorbic Acid on Mutagenic Dose of Three Alkylating Agents. Turk. J. Biol. 27: 241-246.

Khallouki F, Younos C, Soulimani R, Oster T, Charrouf Z, Spiegelhalder B, Bartsch H, Owen RW (2003). Consumption of argan oil (Morocco) with its unique profile of fatty acids, tocopherols, squalene, sterols and phenolic compounds should confer valuable cancer chemopreventive effects. Eur. J. Can. Prev. 12: 67-75.

Knasmüller S, Steinkellner H, Majer BJ, Nobis EC, Scharf G, Kassie F, (2002). Search for dietary antimutagens and anticarcinogens :methodological aspects and extrapolation problems. Food Chem. Toxol. 40: 1051-1062.

Llee BM, Park KK (2003). Beneficial and adverse effects of chemopreventive agents. Mut. Res. pp.523-524.

Madrigal-Bujaidar E, Diaz Barriga S, Cassani M, Marquez P, Revuelta P (1998). *In vivo* and *in vitro* antigenotoxic effect of nordihydroguaiaretic acid against SCEs induced methyl methanesulfonate. Mut. Res., 419: 136-168.

Menat É (2006). Les polyphenols de the, du vin et du cacao. Phytothér.

1: 40-45.
Moukal A (2004). L'arganier, Argania spinosa L. (skeels), usage thérapeutique, cosmetique et alimentaire. Phytothér. 5: 135-141.
Mukhopadhyay I, Kar Chowdhuri D, Bajpayee M, Dhawan A (2004) Evaluation of in vivo genotoxicity of cypermethrin in Drosophila melanogaster using the alkaline Comet assay. Mutgenesis. 19: 85-90.
Nagy M, Križková L, Mučaji P, Kontšeková Z, Šeršeň F, Krajčovič J (2009). Antimutagenic Activity and Radical Scavenging Activity of Water Infusions and Phenolics from Ligustrum Plants Leaves. Molecules 14: 509-518.
Negishi T, Arimoto S, Nishizaki C, Hayatsu H (1989). Inhibitory effect of chlorophyll on the genotoxicity of 3-amino-1-methyl-5H –pyrido[4,3-b]indole (Trp-P-2). Carcinogenesis 10: 145-149.
Nikolic B, Stanojevic J, Mitic D, Vuković-Gačić B, Knežević-Vukčević J, Simić D (2004). Comparative study of the antimutagenic potential of Vitamin E in different *E. coli* strains Mut. Res. 564: 31-38.
Nivard MJM, Pastink A, Vogel EW (1992). Molecular Analysis of Mutations Induced in the vermilion Gene of Drosophila melanogaster by Methyl Methanesulfonate. Genetics 131: 673-682.
Patenkovic A, Stamenkovic-Radak M, Banjanac T, Andjelkovic M (2009). Antimutagenic effect of sage tea in the wing spot test of Drosophila melanogaster. Food and Chem. Tox. 47:180-183.
Peer S (2002). Toxicology and carcinogenesis studie of urethane, ethanol and urethane. NIH Publication, 02-4444
Samane S, Noe J, Charrouf Z, Amarouch H, Haddad PS (2006). Insulin-sensitizing and Anti-proliferative Effects of Argania spinosa Seed Extracts. Evid Based Complement Alternat. Med. 3: 317–327.
Schuch AP, Martins-Menck CF, (2010). The genotoxic effects of DNA lesions induced by artificial UV-radiation and sunlight. J. Photochem. Photobio. 99: 111-116.
Serikaya R, Çakir Ş (2005). Genotoxicity testing of four food preservatives and their combination in the Drosophila wing spot test. Environ. Tox. Pharm. 20: 424-430.
Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J (2004). Role of oxygen radicals in DNA damage and cancer incidence. Mol. Cel. Biochem 266: 37-56.
Waters MD, Stack HF, Jackson MA, Brockman HE, De Flora S (1996). Activity profiles of antimutagens: in vitro and *in vivo* data. Mut. Res. 350: 109-129.
Yaghmur A, Aserin A, Mizrahi Y, Nerd A, Garti N (2001). Evaluation of Argan Oil for Deep-Fat Frying, LWT 34: 124-130.
Zieger E (2000). Death and antimutagenicity. Mut. Res. 466: 125-127.
Zimmering S, Cruces MP, Pimentel E, Arceo C, Carrasco G, Olvera O (1997). On the recovery of single spots with the flr phenotype in the wing spot test in Drosophila. Mut. Res. 379: 77-82.
Zimmerli B, Schlatter J (1991). Ethyl carbamate: analytical methodology, formation, biological activity and risk assessment. Mut. Res. 259: 325-350.