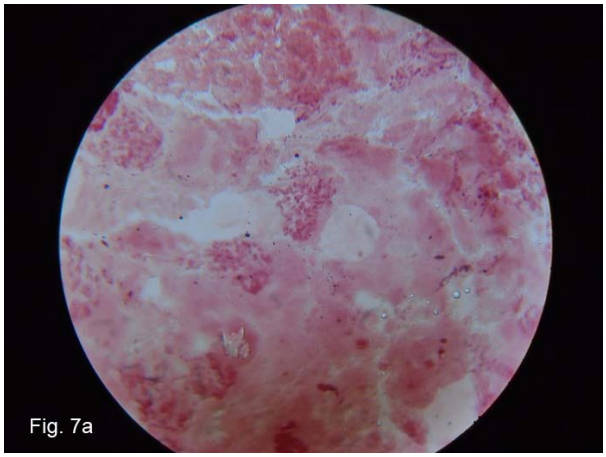

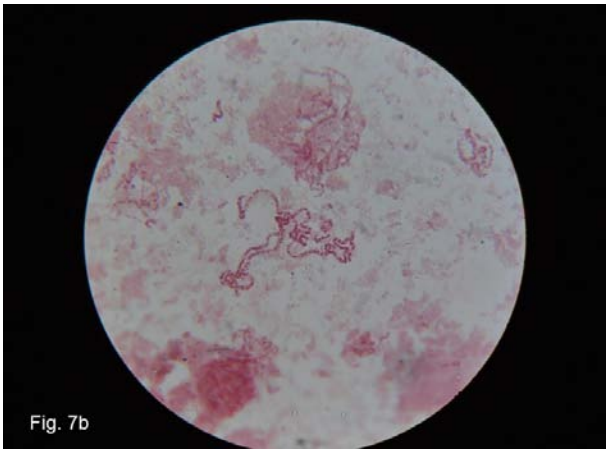

Figure 7. a, Salivary gland chromosome prepared from larvae growing on a strictly cannibalistic diet; b, Salivary gland chromosome prepared from larvae reared in a normal culture medium with only a basal level of cannibalism at play.

Acknowledgment: I am thankful to Dr. Debasis Mutsuddi, for his guidance, constant discussions and encouragement throughout the work. I am also grateful to Professor Subir Chandra Dasgupta, Head of the Department of Zoology, and Professor Mamata Roy, Principal in office at Maulana Azad College, Kolkata, for their great help and laboratory facilities provided

References: Ashburner, M., and H.D. Berendes 1978, Puffing of polytene chromosomes. *In*: *The Genetics and Biology of* Drosophila, vol. 3a, (Ashburner, M., and T. Wright, eds). Academic Press. New York, 325-421; Bhattacharyya, D., 2014, Dros. Inf. Serv. 97: 67-75; Gregg, T.G., A. McCrate, G. Reveal, S. Hall, and A.L. and Ryptra 1990, Biochemical Genetics 28(Nos. 3,4): 197-207; Lindsley, D.L., and E.M. Grell 1968, *Genetic Variations of* D. melanogaster. Carnegic Institute of Washington Publication. No 627; Rypstra, A.L., and T.G. Gregg 1982, Dros. Inf. Serv. 63: 116; Vijendravarma, R.K., S. Narasimha, and T.J. Kawecki 2013, Nature Communications 4: 1789 doi: 10.1038/ncoms 2744: 1-8.

Comparison of the genotoxic and antigenotoxic activity of three *Ipomoea* species with medicinal properties in *Drosophila melanogaster.*

Muñoz-Moya, Armando[1], Patricia Ramos-Morales[2], and Cristina Pérez-Amador[3]. [1]CCH-Sur, UNAM, [2]Laboratorio Genética y Toxicología Ambiental, [3]Laboratorio Química, Facultad Ciencias, UNAM, 04510, D.F., Mexico.

Abstract

Some plants are recognized for their therapeutical properties, which could be associated to the presence of one or more metabolites. The resins from the root from Ipomoea species are commonly used for their purgative activity, being *I. purga* the most effective. Nevertheless their low activity as purgative, *I. orizabensis* and *I. jalapa* are used to adulterate the officinal preparation. In spite of the medicinal activity, and the wide use of traditional resources, little is known about collateral effects of plants derivatives. The genotoxicity of three species of the *Ipomoea* (Convolvulaceae) was compared using the Somatic Mutation and Mitotic Recombination Test (SMART) of *Drosophila*. Larvae were chronically exposed to different concentrations of resins from *I. purga*, *I. jalapa,* or *I. orizabensis* before (Pretreatment) or after (Post treatment) acute exposure to sucrose 5% or N-

Nitrosodimethylamine (NDMA). None of the resins showed mutagenic activity. *I. purga* resin [100 ppm] reduces ($p < 0.05$) the frequency of somatic alterations only when it was administered before the NDMA treatment. *I. jalapa*'s resin increased ($p < 0.05$) the frequency of somatic alterations induced by NMDA treatment when it was administrated before, but was ineffective after it. Finally, *I. orizabensis* resin reduces ($p < 0.05$) the frequency of alterations when it was administered before (until 93%) or after (83%) the NMDA treatment. The *I. orizabensis* resin could contain a metabolite or metabolites with antimutagenic activity against the NDMA's induced damage.
Keywords: Antimutagenesis, Medicinal Plants, *Ipomoea*, *Drosophila*, SMART.

Introduction

The Jalapa Root constitutes one of the best known traditional resources of Mexico, due its medicinal properties. The Jalapa root was known mainly by its purgative and emetic activities; however, it seems also it has some role as anthelmintic and emmenagogue (Martinez, 1990). For its pharmacological properties it is classified as a drastic cathartic and hydragogue (Bauser, 1937). The use of the root of Jalapa goes back to the Hispanic towns, which took advantage of its properties as laxative resins and purgative (Pereda-Miranda, 1995). In particular, the *Ipomoea* genus, which is member of the Convolvulaceae Family, includes numerous medicinal and economically important species. The sweet potatoes varieties (sweet potato) from *I. batatas* (L) Lam. are appreciated by their nutritious roots; other species are known as ornamental in horticulture; and in agriculture like controllers of growth of grasses in the cane of sugar cultivations (Peterson and Harrison, 1991). It has been reported that the allelopathic principle of *I. tricolor* Cav is the glicoresin from the seeds (Anaya, 1990). The use of hallucinogenic seeds of *I. tricolor* in religious ceremonies of divination and in cure rituals was much appreciated in the prehispanic civilizations in Mexico and Central America (Hernandez-Carlos *et al.*, 1999). In pharmacological studies of extracts from these plants, it has been found activity as antimicrobial, analgesic, spasmogenic, spasmolytic, hypotensive, psychomimetic, and insecticide, among others (Bieber *et al.*, 1986).

The true root of Jalapa is *I. purga*; however, there exist resins obtained from roots of other species that are known as “false Jalapas” and they are also sold as “root of Jalapa" (Pedraza, 1982).

The "Root of Jalapa" complex includes *I. orizabensis* (Pelletan) Ledebour ex Steudel, *I. purga* (Wender) Hayne, and *I. jalapa* (L) Pursh, all herbaceous, tropical plants that prevail in the State of Veracruz (Chiconquiaco and Jalapa), Mex. They are known commonly as Root Escamonea of Mexico", Jalapa of Orizaba or Root of Jalapa". The active principle is the resin that acts as an energetic purgative (Cabrera, 1975), but details about the main derivative still remains unidentified. The resins are a complex mixture of fatty acids of 14C and 16C monohydroxi and dihydroxi moiety join to an oligosaccharide center by glycosidic links (glucose, ramnose, quinovose, and fucose), some of them sterificated with volatile organic acids (Bah and Pereda-Miranda, 1997). Commonly, the glicolipids also contains a macro cyclic lactone as part of their molecule (Noda *et al.*, 1987, 1990; Bah and Pereda-Miranda, 1997).

In spite of the medicinal activity, and the wide use of traditional resources, little is known about collateral properties of plant derivatives, as could be the genotoxic and antigenotoxic activities, that means, that one or more substances forming part of the plant, or some metabolite resulting from biotransformation through metabolic activity in the organism could disturb the quality or quantity of genetic material, or inclusive interfere with their genetic regulation (genotoxic activity). Alternatively, some substances can diminish the genetic damage associated to the exposition to chemical, physical or biological agents (antigenotoxic activity).

The genotoxic and antigenotoxic activities from three species of *Ipomoea* (Convolvulaceae) were compared using the Somatic Mutation and Mitotic Recombination Test (SMART) of *Drosophila*.

Methods

Chemicals:

N-Nitrosodimethylamine (NDMA) [CAS 62-75-9], Sigma; Tween 80 (polyethylene glycol-sorbitan monooleate) [CAS 9005-65-6], Sigma; Dimethylsulfoxide (DMSO) [CAS 67-68-5], Sucrose [CAS 57-50-1], Baker; Microchristalline Cellulose, Merck.

Officinal extraction of resins:

For the officinal preparation of resins from *I. purga*, *I. jalapa*, and *I. orizabensis*, root was pulverized and placed with ethanol into a Soxhlet for 4 h periods, three times. The ethanolic extracts were vaporized to obtain an extract which was mixed with 4 parts of water. The resins were separated, rinsed with water, and air dry.

Concentrations:

The solutions were prepared as described. Resins were dissolved separately with DMSO, stirring continuously until dissolving. The solution was stabilized with Tween 80, to avoid solution precipitation. Water was added slowly by dropping. The final concentrations of DMSO and Tween 80 were 1.5 % and 1 %, respectively. For each resin, the highest concentration dissolved before precipitation of the solution was chosen to be assayed. We adopted this criterion based in the fact that commonly this kind of treatments is used without physician assistant. Another hand, from previous experiments, we fixed in 10% the upper limit of mortality from experimental flies as the maximum mortality accepted for antigenotoxicity determinations. That point is important because otherwise the antimutagenic potential could be confused with cellular or organism death.

For *I. purga*, the highest concentration before precipitation was 500 ppm, three additional dilutions were assayed: 250, 100 and 50 ppm. For *I. jalapa*, it was 5000 ppm, and 2500, 500 and 250 ppm dilutions; the two lowest concentrations were chosen to compare with those from *I. purga*. Finally, for *I. orizabensis* it was 6250 ppm, and 5000, 4375, 3750 and 2500 ppm dilutions; as before, two concentrations were chosen to compare with *I. jalapa* concentrations. None of the resins reduced the viability larvae–imago (data no shown). As positive control and inductor of somatic mutation and mitotic recombination, a 12.5 mM solution of alkylating, promutagen N-Nitrosodimethylamine (NDMA) dissolved in 5 % sucrose was chosen. At this concentration, the viability larvae–imago is affected in less than 10 % and the frequency of somatic mutation is undoubtedly increased, as was preliminarily determined.

Drosophila *strains and matings:*

Two strains with markers on third chromosome were used:

i) *flr*^{3}*/In(3LR)TM3, ri p*^{p} *sep bx*^{34e} *e*^{s} *Bd*^{S} (brief, *flr*^{3}*/TM3, Bd*^{S}) flies. The recessive marker *flr*^{3} (*flare*, 3-38.8) is lethal in homozygous, and the balancer chromosome *TM3* (Third Multiple) is used for maintaining it. When expressed, *flr*^{3} produces amorphous, chitinous spots in comparison with a single regular tricoma in wild type flies. *TM3*, carrier of the autosomal, dominant, homozygous lethal marker *Bd*^{S} (*Beaded-Serrate*). *Bd*^{S}*/Bd*^{S}, is lethal and only *flr*^{3}*/TM3, Bd*^{S} flies are recovered.

ii) *mwh/mwh* flies. *mwh* (multiple wing hair) (3-0.0) is an autosomal recessive marker which produces multiple trichomes on the wing, instead of only one, as in wild type flies. For a detail description of markers see Garcia Bellido and Dapena (1974); Lindsley and Zimm (1990). 72 h old, *flr*^{3}*/TM3, Bd*^{S}, virgin females were mated with 48 h old, *mwh/mwh* males. Three days after mating, parents were transferred to fresh bottles during 8 h for egg laying. All the cultures were maintained at 25 ± 1°C and 60% humidity. The standard food for *Drosophila* was prepared with 1% agar, 10.5% corn meal, 7% sugar, 6% yeast, 0.4% Nipagin (10% dissolved in ethyl alcohol) and 0.4% Propionic Acid.

Experimental procedure:

Two types of exposures were chosen to determine possible differences due to the order in which the resins and the NDMA were given (Figure 1).

A. 1 ml of the resin to be assayed was poured into bottles containing larvae of 24 ± 4 h age and distributed homogeneously on the surface. At this age, larvae emerging from the eggs and are coated with the resin solution, or solvents (DMSO + Tween 80). After 48 h, these larvae (now from 72 ± 4 h age) were extracted using a 20% sucrose solution (Nöthiger, 1970) and were put into vials containing a nylon gauze at one side, and a rubber in the other to avoid that larvae escape. The vials were put into 10 ml beaker containing 60 mg of powder cellulose and 0.5 ml of 5% sucrose or 12.5 ml NDMA. After 6 h, larvae were rinsed with tap water and were put into fresh bottles with standard food for *Drosophila*, in which remained until emergence of adult flies. The final exposure was: 24 (larval age) × 48 (subchronic treatment with resins) × 6 h (acute treatment with sucrose or NDMA) (E = 24 × 48 × 6 h).

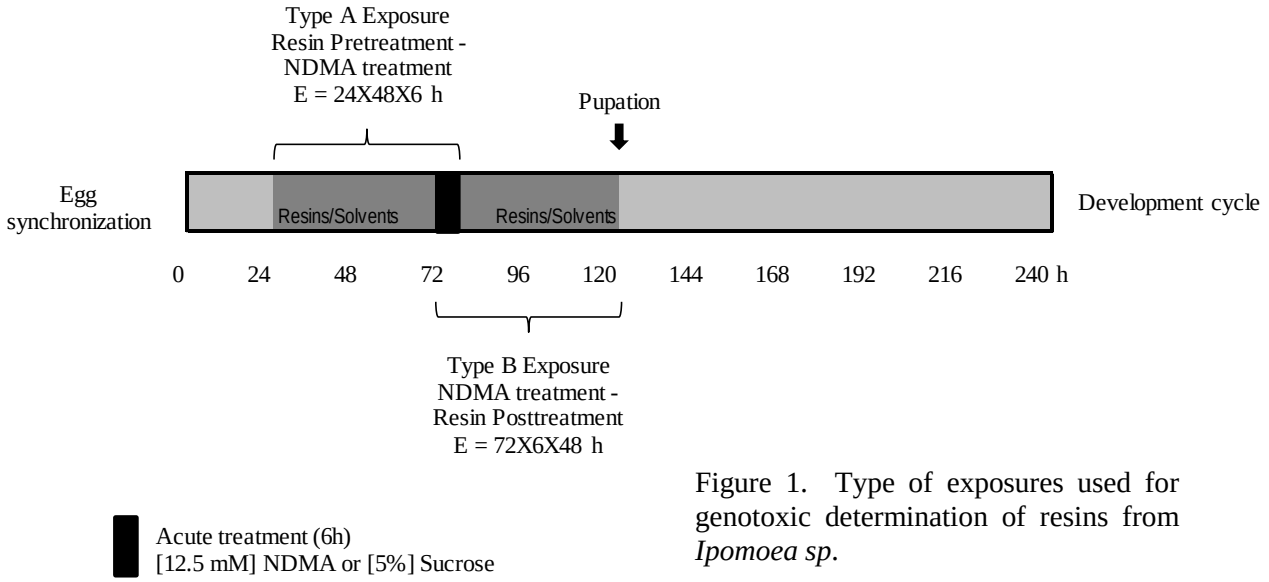

Figure 1. Type of exposures used for genotoxic determination of resins from *Ipomoea sp*.

B. In the other type of exposure, third instar larvae (72 ± 4 h age) were extracted with a 20% sucrose solution and treated with sucrose or NDMA as described previously. After the acute treatment, larvae were rinsed and put into bottles containing standard medium for *Drosophila* enriched with 1 ml of resin or solvent solution homogeneously distributed on the surface; the larvae remained in these bottles until the adults emerged. The final exposure was: 72 (larval age) × 6 (acute treatment) × 48 h (subchronic treatment) (E = 72 × 6 × 48 h). The experiments were done two times.

Adult flies recovered were counted, sexed, over anesthetized, and fixed in 70% ethanol. Wing slides were made putting a couple of wings from 10 females and 10 males using Faurè solution as described Graf *et al.* (1984). For each concentration, 120 wings were reviewed using a microscope at 40× magnification. The number, type and size of spots were scored. In addition, the total number of spots per fly was obtained.

Criteria for wing scoring:

In the SMART, transheterozygous larvae for two morphological markers affecting the trichome expression in the adult fly are used. The cells from the imaginal discs of the wings are mitotic proliferating but maintain undifferentiated until metamorphosis. In the wild type adult fly, each cell produces a single tricoma on the wing blade. Through larval development, the exposure to endotoxins can produce the loss of heterozygosity, leading to the expression of the recessive markers: *mwh* and flr^3, as described by Graf *et al.* (1984).

The genetic endpoints forming single spots are mainly punctual mutation, deletion, and recombination between markers. The recombination between the proximal marker flr^3 and the centromere (which has a role as an additional marker) lead to twins spots, with both markers forming part of a spot. Hence, twins spots indicate recombinogenic activity. Two spots are independent when separated by three or more wild type rows of trichome. Both sides of blade wing (dorsal and ventral) were scored for spots.

The spots were classified by size as small (1-2 trichome) and large (> 3 trichome). The number of trichome (cells) per spot allows us to determine the number of cell cycles occurring after the alteration in the original cell. So, the size of the spots can estimate the time of induction of the cellular clone, in absence of delay or cell death. The qualitative comparison of the distribution of the number of cells per clone also contributes to identify and discard false positives and false negatives diagnostics (Frei and Würgler, 1995).

Another side, the number of spots per fly gives an indication of individual susceptibility. In untreated flies, there are no spots in the flies, but occasionally one spot appears in one of the wings. Flies with two or more spots on the wings are less frequent. This analysis helps us to know whether the increase in the frequency of spots from experimental series has a biological meaning applicable to the population from which derived the treated flies, or it is associated to some particular genetic condition in some rare, exceptional organisms.

Statistical analysis:

1. For data processing, the SMART software was used (Frei and Würgler, unpublished). The frequency of small, large, twins and total spots from experimental and control series was compared through the Multiple Decision Procedure (Frei and Würgler, 1988) to determine a positive, negative, inconclusive, and weak positive diagnosis, with $\alpha/2 = 0.05$ (two tails) as critical region. To determine whether the *Ipomoea*'s resins are genotoxic to *Drosophila*, the frequency of spots from flies treated with resins from each one *Ipomoea* was compared to that from flies only exposed to solvents. To know if the resins modified the frequency of spots induced by NDMA, the frequency of spots from flies exposed to *Ipomoea*'s resin and NDMA, or NDMA and *Ipomoea*'s resins were compared separately to the frequency of spots from flies exposed only to NDMA.

2. The over dispersion in the distribution of the number of spots per fly from experimental and control series was compared with the non-parametric Kruskal-Wallis Test, and the differences between series were confirmed through the Dunn's Multiple Comparison Test (Sheskin, 2004).

Results

Genotoxicity Assays:

Type and frequency of spots. Tables 1 and 2 show the frequency and number of spots in transheterozygous *flr*^{3}/*mwh* flies pretreated or post treated with resins from *I. purga*, *I. jalapa*, and *I. orizabenzis*, respectively. None of the resins modifies in a significant manner the frequency of small, large and twins spots. Only slight deviations around the corrected control frequency of total spots, to their respective corrected controls were found, as is shown in Figure 2.

Spots per fly distribution. In experimental flies treated separately with each one of the resins, the spots distributed like in those unexposed, being the most of them spots free flies, a minor proportion showed one to three spots on their wings, and flies with 4 or 5 spots were rather rare. However, in treatments with the resin from *I. purga*, we observed a differential toxicity between first and third larval instars. For first instar larvae the treatment did not reduce larval viability, meanwhile for third instar larvae the treatment was toxic and interfered with the viability larvae-adult (data not shown).

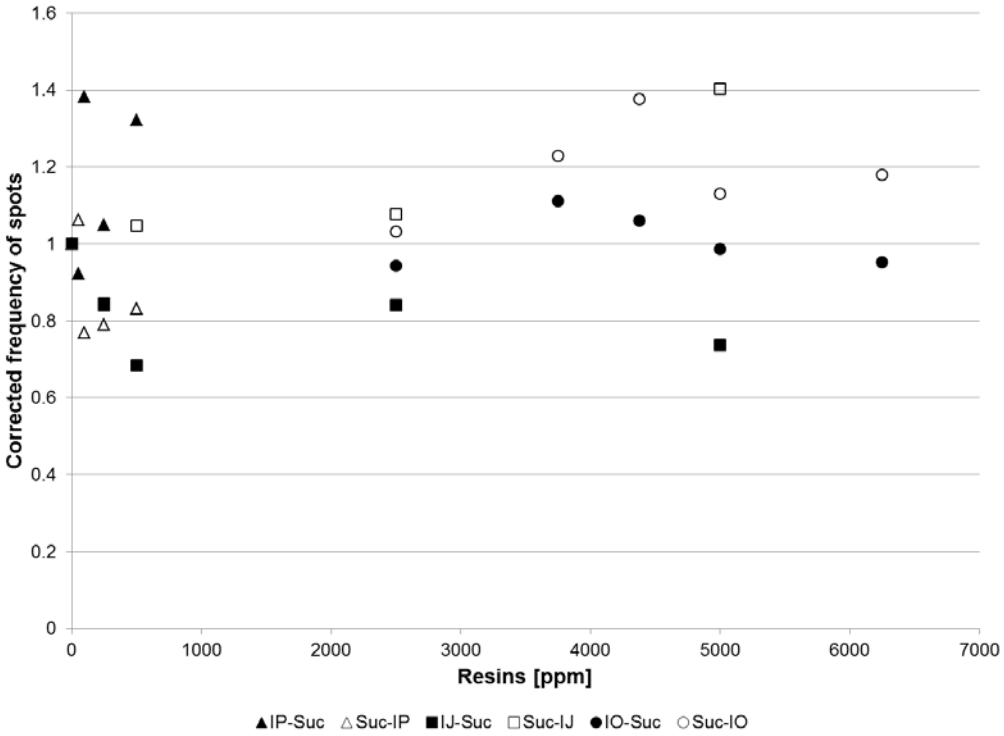

Figure 2. Corrected frequency of spots on the wings from flies exposed to resins from *I. purga*, *I. jalapa*, or *I. oriza-bensis* during larval development.

Table 1. Frequency and number of spots on the wings from flies pretreated with resins from *I. purga*, *I. jalapa* and *I. orizabensis* and treated with NDMA.

Ipomoea Extract	Number of Wings	Spots per Wing (Number of Spots) Statistical Diagnosis*															
		Small Single (m=2)			Large Single (m=5)			Twin (m=5)			Total (m=2)		
		Fr.	(Number)		Fr.	(Number)		Fr.	(Number)		Fr.	(Number)	
[ppm] *I. purga* + [5%] Sucrose [E: 24X48X6]													
0	118	0.24	(28)		0.03	(4)		0.00	(0)		0.27	(32)	
50	112	0.16	(18)	-	0.05	(6)	-	0.04	(4)		0.25	(28)	-
100	120	0.31	(37)	i	0.04	(5)	-	0.03	(3)		0.38	(45)	i
250	116	0.25	(29)	-	0.01	(1)	-	0.03	(3)		0.28	(33)	-
500	120	0.28	(34)	-	0.05	(6)	-	0.03	(3)		0.36	(43)	-
[ppm] *I. purga* + [12.5mM] NDMA [E: 24X48X6]													
0	120	0.83	(100)		1.09	(131)		0.15	(18)		2.08	(249)	
50	118	1.08	(127)	-	1.86	(219)	-	0.36	(43)		3.30	(389)	-
100	114	0.46	(53)	+	0.91	(104)	-	0.08	(9)	-	1.46	(166)	w
250	120	0.56	(67)	w	1.14	(137)	-	0.09	(11)		1.79	(215)	-
500	120	0.67	(80)	-	1.59	(191)	-	0.20	(24)		2.46	(295)	-
[ppm] *I. jalapa* + [5%] Sucrose [E: 24X48X6]													
0	120	0.23	(27)		0.04	(5)		0.05	(6)		0.32	(38)	
250	120	0.23	(27)	-	0.03	(4)	-	0.01	(1)	-	0.27	(32)	-
500	120	0.17	(20)	-	0.04	(5)	-	0.01	(1)	-	0.22	(26)	-
2500	120	0.18	(21)	-	0.07	(8)	-	0.03	(3)	-	0.27	(32)	-
5000	120	0.13	(16)	-	0.09	(11)	-	0.01	(1)	-	0.23	(28)	-
[ppm] *I. jalapa* + [12.5mM] NDMA [E: 24X48X6]													
0	108	1.66	(179)		3.88	(419)		0.78	(84)		6.31	(682)	
250	100	5.68	(568)	+	6.97	(697)	w	2.48	(248)	w	15.13	(1513)	+
500	120	4.00	(480)	+	7.64	(917)	w	2.46	(295)	w	14.10	(1692)	+
2500	120	3.43	(411)	+	6.68	(801)	w	2.08	(250)	w	12.18	(1462)	+
5000	120	1.61	(193)	-	4.52	(542)	-	1.08	(129)	-	7.20	(864)	-
[ppm] *I. orizabensis* + [5% Sucrose] [E: 24X48X6]													
0	120	0.10	(12)		0.04	(5)		0.01	(1)		0.15	(18)	
2500	106	0.06	(6)	-	0.08	(8)	-	0.01	(1)	-	0.14	(15)	-
3750	120	0.13	(15)	-	0.03	(3)	-	0.02	(2)	-	0.17	(20)	-
4375	88	0.11	(10)	-	0.05	(4)	-	0.00	(0)	-	0.16	(14)	-
5000	108	0.14	(15)	-	0.01	(1)	-	0.00	(0)	-	0.15	(16)	-
6250	56	0.11	(6)	-	0.02	(1)	-	0.02	(1)	-	0.14	(8)	-

[ppm] *I. orizabensis* + [12.5mM] NDMA [E: 24X48X6]														
0	116	1.11	(	129	)	3.08	(	357	)	0.66	(	76	)	4.84	(	562	)
2500	120	0.48	(	57	)+	2.19	(	263	)w	0.29	(	35	)w	2.96	(	355	)+
3750	120	0.43	(	51	)+	1.21	(	145	)w	0.23	(	27	)w	1.86	(	223	)+
4375	120	0.35	(	42	)+	0.63	(	75	)+	0.13	(	15	)+	1.10	(	132	)+
5000	120	0.31	(	37	)+	0.51	(	61	)+	0.08	(	9	)+	0.89	(	107	)+
6250	104	0.15	(	16	)+	0.15	(	16	)+	0.04	(	4	)+	0.35	(	36	)+

* Statistical Diagnosis according to Frei and Würgler (1992), α=β=0.05; two side test;-, negative; +, positive; w, weak positive; i, inconclusive; Fr., Frequency.

Table 2. Frequency and number of spots on the wings from flies treated with NDMA and postreated with resins from *I. purga*, *I. jalapa* and *I. orizabensis.*

		Spots per Wing (Number of Spots) Statistical Diagnosis*															
		Small Single (m=2)				Large Single (m=5)				Twin (m=5)				Total (m=2)			
Ipomoea Extract	Number of Wings	Fr.		(Number)		Fr.		(Number)		Fr.		(Number)		Fr.		(Number)	
[5%] Sucrose + [ppm] *I. purga* [E: 72X6X48]																	
0	120	0.30	(	36	)	0.02	(	2	)	0.00	(	0	)	0.32	(	38	)
50	116	0.26	(	30	)-	0.06	(	7	)-	0.02	(	2	)	0.34	(	39	)-
100	78	0.21	(	16	)i	0.04	(	3	)-	0.00	(	0	)	0.24	(	19	)i
250	76	0.17	(	13	)-	0.07	(	5	)-	0.01	(	1	)	0.25	(	19	)-
500	76	0.22	(	17	)-	0.04	(	3	)-	0.00	(	0	)	0.26	(	20	)-
[12.5 mM] NDMA + [ppm] *I. purga* [E: 72X6X48]																	
0	118	1.24	(	146	)	2.68	(	316	)	0.37	(	44	)	4.29	(	506	)
50	112	1.27	(	142	)-	3.33	(	373	)-	0.46	(	51	)-	5.05	(	566	)-
100	96	1.17	(	112	)-	2.88	(	276	)-	0.36	(	35	)-	4.41	(	423	)-
250	108	1.02	(	110	)-	3.86	(	417	)-	0.75	(	81	)-	5.63	(	608	)-
500	118	1.23	(	145	)-	3.01	(	355	)-	0.46	(	54	)-	4.69	(	554	)-
[5%] Sucrose + [ppm] *I. jalapa* [E: 72X6X48]																	
0	118	0.20	(	24	)	0.05	(	6	)	0.01	(	1	)	0.26	(	31	)
250	117	0.17	(	20	)-	0.03	(	4	)-	0.02	(	2	)-	0.22	(	26	)-
500	120	0.24	(	29	)-	0.03	(	4	)-	0.00	(	0	)-	0.28	(	33	)-
2500	120	0.23	(	27	)-	0.04	(	5	)-	0.02	(	2	)-	0.28	(	34	)-
5000	120	0.17	(	20	)-	0.04	(	5	)-	0.03	(	3	)-	0.23	(	28	)-
[12.5 mM] NDMA + [ppm] *I. jalapa* [E: 72X6X48]																	
0	120	1.93	(	231	)	2.56	(	307	)	0.61	(	73	)	5.09	(	611	)
250	120	1.66	(	199	)-	2.53	(	303	)-	0.56	(	67	)-	4.74	(	569	)-

500	120	1.44	(	173	)-	2.75	(	330	)-	0.53	(	64	)-	4.73	(	567	)-
2500	120	1.51	(	181	)-	2.50	(	300	)-	0.49	(	59	)-	4.50	(	540	)-
5000	118	1.49	(	176	)-	2.78	(	328	)-	0.56	(	66	)-	4.83	(	570	)-
[5%] Sucrose + [ppm] *I. orizabensis* [E: 72X6X48]																
0	118	0.14	(	16	)	0.03	(	4	)	0.00	(	0	)	0.17	(	20	)
2500	120	0.16	(	19	)-	0.01	(	1	)-	0.01	(	1	)-	0.18	(	21	)-
3750	120	0.17	(	20	)-	0.05	(	6	)-	0.00	(	0	)-	0.22	(	26	)-
4375	120	0.18	(	22	)-	0.03	(	4	)-	0.02	(	2	)-	0.23	(	28	)-
5000	120	0.13	(	15	)-	0.05	(	6	)-	0.02	(	2	)-	0.19	(	23	)-
6250	120	0.16	(	19	)-	0.03	(	4	)-	0.01	(	1	)-	0.20	(	24	)-
[12.5 mM] NDMA + [ppm] *I. orizabensis* [E: 72X6X48]																
0	120	2.58	(	310	)	2.88	(	345	)	0.43	(	52	)	5.89	(	707	)
2500	112	0.79	(	88	)+	1.47	(	165	)w	0.38	(	42	)-	2.63	(	295	)+
3750	116	0.93	(	108	)+	0.82	(	95	)w	0.06	(	7	)+	1.81	(	210	)+
4375	120	0.64	(	77	)+	0.72	(	86	)+	0.17	(	20	)w	1.53	(	183	)+
5000	120	0.41	(	49	)+	0.68	(	82	)+	0.10	(	12	)+	1.19	(	143	)+
6250	120	0.16	(	19	)+	0.72	(	86	)+	0.13	(	15	)+	1.00	(	120	)+

* Statistical Diagnosis according to Frei and Würgler (1992), α=β=0.05; two side test;-, negative; +, positive; w, weak positive; i, inconclusive; Fr., Frequency.

Antigenotoxicity assays:

For those assays, the promutagen NDMA was used and the frequency of spots was compared to that obtained from larvae exposed to NDMA and resins, either before or after the promutagen (Tables 1 and 2). The statistical diagnosis was made to value increasing or reduction in the frequency of spots in relation to that obtained from the positive control.

I. purga

Flies initially exposed to 50 ppm of *I. purga* resin and then to the promutagen showed on their wings more spots than flies exposed to the positive control, but not in sufficient number to increase the spots' frequency in a significant form. Treatments with 100 and 250 ppm reduce the frequency of spots, being this significant only at 100 ppm for small and total spots ($p < 0.05$). The higher concentration does not modify the number and type of spots. The post treatment with *I. purga's* resin does not affect the frequency of spots induced by the NDMA acute treatment.

I. jalapa

The resin from *I. jalapa* roots induced a different effect, which was associated to NDMA acute exposure and the age of larvae at treatment. For first instar larvae, the pretreatment with this resin increases in a significant manner the frequency of all type of spots ($p < 0.05$), except for the higher concentration assayed, as compared with that from NDMA treatment. For all the concentrations the effect was clearly positive for small and total spots and weak positive for large (except at 5000 ppm), and twins spots. For third instar larvae the response obtained was quite different, because the post treatment with the resin lack of any detectable activity. Here, the type and frequency of spots was similar to that from control positive flies.

I. orizabensis

The effect of treatments with this resin, administered before or after NDMA treatment, is to reduce, in a concentration dependent manner, the frequency of all type of spots ($p < 0.05$). For flies pretreated, the frequency of small and total spots was significant lower than that from positive control treatment; for large and twins spots, the reduction was weak positive for 2500 and 3750 ppm, but clearly positive since 4375 to 6250 ppm ($p < 0.05$). A similar effect was observed when post treatments with this resin were given. The frequencies of small and total spots were lower than those from positive control treatments ($p < 0.05$), but for large spots, the first two concentrations gave a weak positive reduction, and higher concentrations were clearly effective ($p < 0.05$). For twins spots, the administration of 2500 ppm of the resin does not reduce in a significant form the frequency of twins spots, but 3750, 5000, and 6250 ppm do reduce ($p < 0.05$); and 4375 ppm treatment only induced a weak positive reduction. Figure 3 show the corrected frequency of total spots from pre and post treatments with Ipomoea resins.

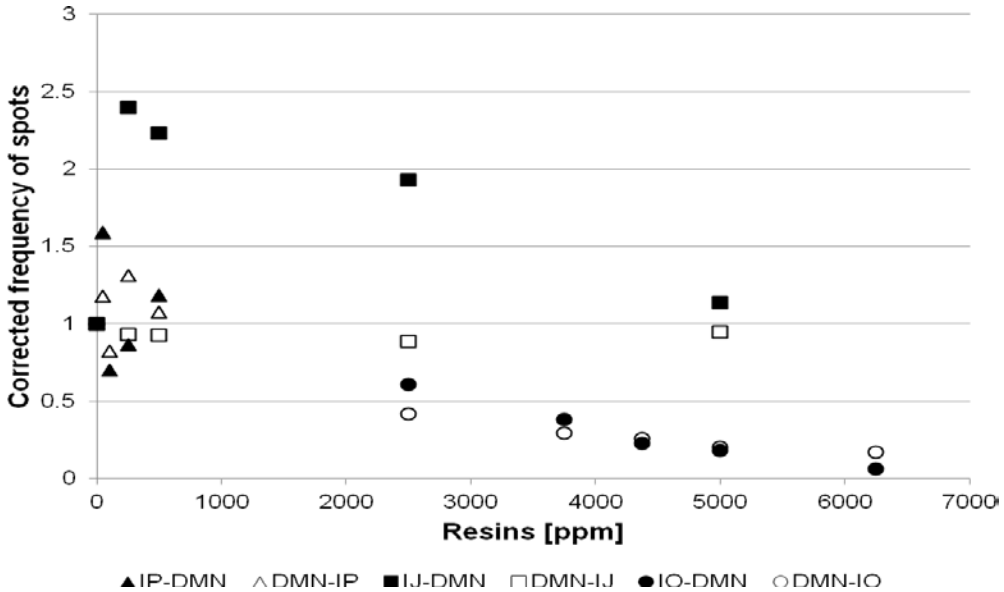

Figure 3. Corrected frequency of spots on the wings from flies exposed to NDMA and resins from *I. purga*, *I. jalapa*, and *I. orizabensis* during larval development.

Spots per fly distribution

I. orizabensis. This resin was the only one that showed antigenotoxic activity (Figure 4a and 4b). The Kruskal Wallis test found significant differences, which were confirmed through the Dunn's Multiple Comparison Test. The number of spots per fly, from flies exposed initially to *I. orizabensis* resin was different from that from positive control flies since 3750 ppm and higher ($p < 0.001$). Flies pretreated with 2500 ppm showed a different distribution compared with those from 4375 to 6250 ppm ($p < 0.001$). Also differences were detected between flies from 3750 *vs.* 5000 ($p < 0.01$) and *vs*. 6250 ppm pretreatments ($p < 0.001$). Finally, the distribution of the number of spots per fly from 4375 ppm pretreatment was different to that from 6250 ppm ($p < 0.05$) (Table 3). Another side, for post treatments, significant differences was detected between flies exposed to NDMA and NDMA + resin at either concentration ($p < 0.001$). Also dispersion was found among distributions from experimental series: 2500 *vs.* 4375 ($p < 0.01$), 5000 and 6250 ppm ($p < 0.001$), and finally those from 3750 *vs*. 6250 ppm ($p < 0.05$) (Table 4).

Discussion

The unrestricted exposure to plant derivatives for therapeutic use can imply some hazard to humans, because, in despite of the therapeutic effectiveness attributed to the plants, the knowledge about the type and proportion of substances that they contain and the chemical interactions among these substances and their metabolites, are rather scarce. Reports about medicinal properties of plants have been focused mainly on the

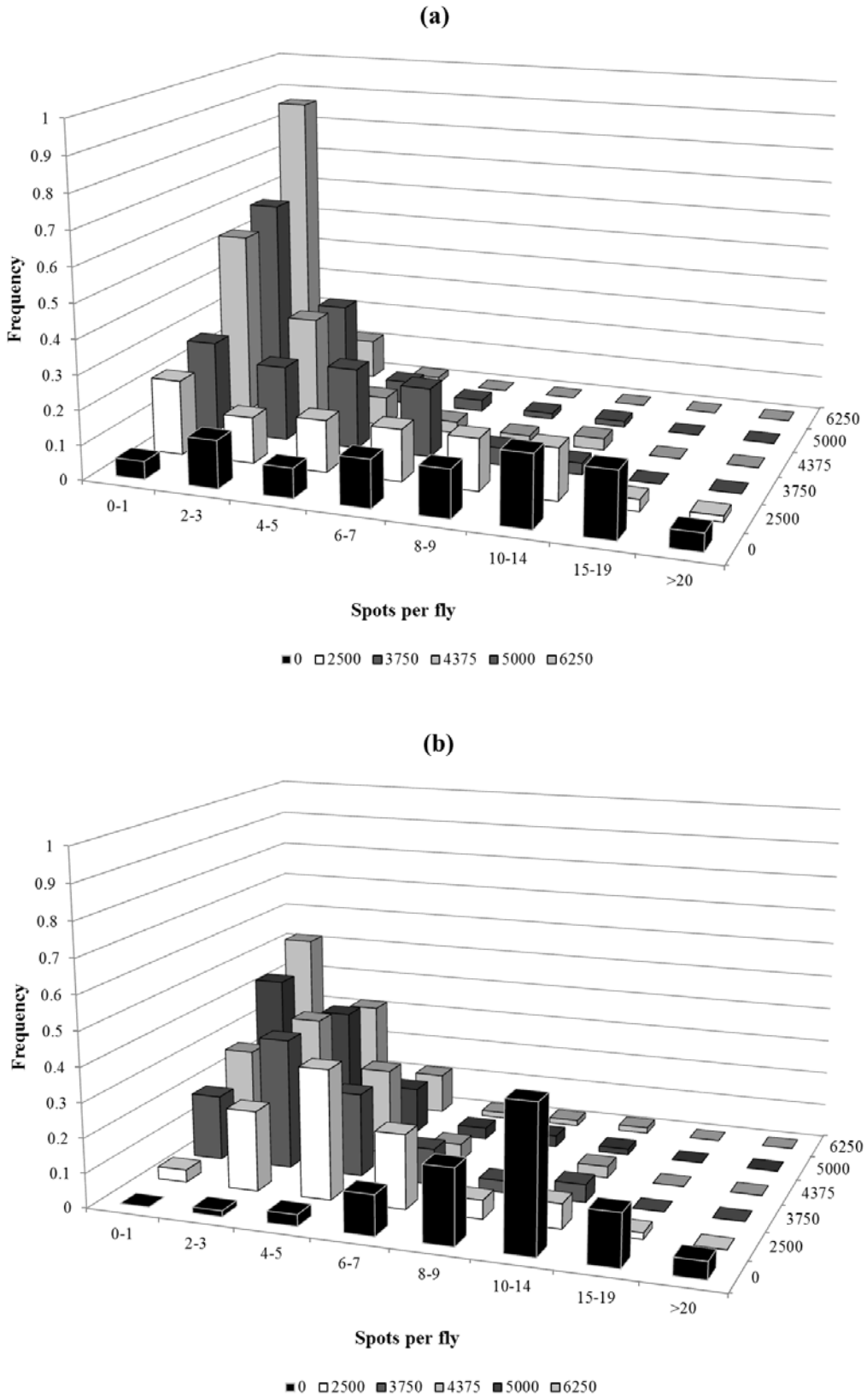

Figure 4. Frequency of spots per fly in flies exposed to *I. orizabensis* resin a) before or b) after an acute treatment with NDMA.

phytochemical composition of the plants, and the possibility of negative collateral effects associated to these kinds of practices have been not explored.

The genotoxic potential of three *Ipomoea* species was determined and compared through the SMART of *Drosophila melanogaster*.

Table 3. Comparison of the dispersion in the distribution of the number of spots per fly, from flies exposed to *I. orizabensis* - NDMA.

[ppm]	0	2500	3750	4375	5000
2500	ns				
3750	***	ns			
4375	***	***	ns		
5000	***	***	**	ns	
6250	***	***	***	*	ns

Statistical diagnosis according to the Kruskal Wallis Test. Differences confirmed through the Dunn Multiple Comparison; *, p<0.05; **, p<0.01; ***, p<0.001; ns, non significant.

Table 4. Comparison of the dispersion in the distribution of the number of spots per fly, from flies exposed to NDMA- *I. orizabensis*.

[ppm]	0	2500	3750	4375	5000
2500	***				
3750	***	ns			
4375	***	**	ns		
5000	***	***	ns	ns	
6250	***	***	*	ns	ns

Statistical diagnosis according to the Kruskal Wallis Test. Differences confirmed through the Dunn Multiple Comparison; *, p<0.05; **, p<0.01; ***, p<0.001; ns, non significant.

No clear evidence about genotoxic activity was observed in flies treated with each one of the resins, and only random variations around the control frequency of somatic mutation and mitotic recombination were recovered, but wide differences related with toxicity of them were evident, when the concentrations to be assayed were chosen. To select the concentration to be assayed, preliminary tests were run to determine the higher concentration after which 80% or more of the exposed flies survived. This criterion was adopted based in the fact that, in this kind of traditional practice, the supervision of concentration and dosage used is rather inexistent. In addition, we try to choose two concentrations overlapped in order to establish comparisons among the three species. However, the higher concentration was clearly different among the *Ipomea* species, which showed be toxic for *Drosophila* as follow: *I. purga* [500 ppm] > *I. jalapa* [5000 ppm] > *I. orizabensis* [6250 ppm]. For *I. purga*, concentrations upper 500 ppm were toxic for three instar larvae, but not for first instar larvae. That is supported by previous reports showing that metabolism of *Drosophila* larvae varies with age (Fuchs *et al.*, 1993). The *I. orizabensis* resin is not toxic at all.

Differences related to the purging potency among the three *Ipomoea* species were reported previously (McDonald *et al.*, 1997; Perez Amador *et al*., 1980, 1988; Meira *et al.*, 2012). 0.5 ml of a solution containing 0.2 g of the resin were used to instillate male rats, and the time elapsed since resin administration and excretion (diarrheic feces) was scored: *I. purga*, 30 min; *I. jalapa*, 45 min; *I. orizabensis*, 105 min. No feces from control males were recovered in the lapse scored (3 h). In addition, males exposed to *I. jalapa* showed toxicity symptoms like erected hairy, emetic reaction, unbalance, and posterior limbs distended.

Antigenotoxicity assays

There exist numerous reports about medicinal plant from Ipomoea genera, which in addition to their therapeutic effectiveness to reduce abdominal fever, dysentery, epilepsy, hydrocephalus, and meningitis (Martinez, 1990), some species contain glycosidic resins related to tumor inhibition. The ipolearoside, a glycoside extracted from the ethanolic fraction from whole plant *I. leari* showed a significant activity against the Walker carcinosarcoma 256 in rats (Bhakuni *et al.*, 1969; Sarin *et al.*, 1973); the intraperitoneal application of glycosides from *I. bahiensis* produced tumor growth inhibition against Sarcome 180 in mice (Bieber *et al.*, 1986); in mouse, the methanolic extract from *I. pes-caprae* exhibits antinociceptive activity against pain (De Souza *et al.*, 2000); the subcutaneous injection of the acid extract from *I. orizabensis* produces tumor damage against 37 sarcoma in mice (Bilkin and Fitzgerrald, 1952) and exhibit weak cytotoxicity against oral epidermoid carcinoma in humans (Hernandez-Carlos *et al.*, 1999).

In this study, the alkylating promutagen NDMA was used to induce somatic mutation and mitotic recombination in the wing version of the SMART, due: 1) this compound increases several times the spontaneous frequency of spots on the wings, making unambiguous the quantification of the antigenotoxic activity; 2) is a promutagen that implied several steps for their activation and posterior elimination and excretion, offering numerous opportunities to establish chemical interactions with compounds assayed for antigenotoxic potential; 3) its toxicity for the *in vivo* system of *Drosophila* is rather low (Ramos-Morales *et al.*, 2001). The effect of the administration of resins at two different ages allows us to distinguish genotoxic activity in two aspects: one related to the age of the larvae at treatment and the other independent of age.

The pretreatment with *I. purga* resin changes slightly the frequency of somatic spots obtained from flies exposed only to NDMA. The lower concentration of the resin seems to induce some spots, although the higher concentration produced a significant reduction in the frequency of spots on the wings, but this protection was less effective as the concentration increased. In contrast, no evident interaction was observed in post treatments.

For *I. japala*, we assume that some resin metabolites associated to the incomplete metabolism of first instar larvae enhanced the NDMA activity, because this enhancement does not persist when larvae were third instar at treatment. The metabolism of *Drosophila* is based in an enzymatic system dependent on Cytochrome P450, and the reactions implied in detoxification of genotoxins are quite similar to that from S9 microsomal fraction from mammal liver, and there exist reports about the genetically determined variation in the level and induction of Cytochrome P450 and the effect of NDMA in somatic cells or associated to developmental stages of the fly (Baars, *et al.*, 1980; Clark, 1982; Hällstrom, *et al.*, 1983, 1985).

Nevertheless the enhanced effect of NDMA after the exposure to the *I. jalapa* resin is evidence that this promutagen is efficiently detected and transformed by *Drosophila* larvae when possess a mature metabolism. It is possible that the resin from *I. jalapa* contain some compounds that, in combination with this promutagen, have a synergistic effect in younger larvae, maybe prolonged the half-life of metabolites produced, delayed their detoxification, or retarded the maturation of enzymatic larval system (Fuchs *et al.*, 1993). The absence of this synergistic effect when NDMA is administrated previous to the resin suggest that this response could be associated to initial steps implied in the biotransformation to this promutagen.

In our group, we have observed that the number of spots per fly is a reliable indicator of the metabolic activity in *Drosophila*. As more steps are involved in the biotransformation/detoxification of genotoxins, the number of flies carrying numerous spots increased, too. Third instar larvae of *Drosophila* exposed during 6 h to the alkylant mutagen N-nitrosodiethylamine [1-20 mM] became adults carrying up to 6 spots on their wings. In contrast, larvae treated as quoted to N-nitrosodimethylamine [1-50 mM], became adults showing up to 26 spots on their wings (Ramos-Morales *et al.*, 2001). The dispersion on the distribution of the number of spots per fly can be associated with metabolism genes from the population treated, and make evident the diversity in the individual susceptibility from organisms exposed to the same stimulus.

No more dispersion in the distribution of spots per fly, than that produced by treatment with NDMA, was observed in flies pretreated with the *I. purga* resin, and a weak dispersion was induced when the resin was given after NDMA. In contrast, a strong dispersion, as compared to the distribution of spots per fly induced by NDMA treatment, was recovered from the *I. jalapa* treatments, been higher from pretreatments, but also detected when the resin was administered after NDMA.

For *I. orizabensis*, an effect independent of larval age was observed. In both types of exposures, the resin from *I. orizabensis* reduced in a concentration dependent manner the NDMA genotoxicity, without some kind of toxicity. This similarity in the protection pattern observed suggests, that the components of this resin interfere with the biotransformation of NDMA, probably trapping the intermediary metabolites in a similar way as Vitamin-C does (Shankel *et al.*, 1987). We assume this on the fact that no more steps in metabolism implied in biotransformation/detoxification of NDMA activity were induced, as can be appreciated in Figure 4. The dispersion in the distribution of spots per fly was gradually lower, as the concentration of *I. orizabensis* resin increased, in both types of exposure; that suggests that no different steps in the metabolic pathway involved in the biotransformation of NDMA were implied. So, the frequency of spots recovered in pre- and posttreatments with *I. orizabensis* indicate that only a minor fraction of reactive metabolites escaped to be trapped by *I. orizabensis* resin. In this treatment, the viability larvae-adult was in the range of 80%, compared

to the number of flies recovered in concurrent negative controls, allowing us to discard treatment toxicity as the cause of the reduction in the number of spots.

In sum, the efficient reduction in the frequency of somatic mutation and recombination observed in *I. orizabensis*-NDMA treatments, altogether with the absence of toxicity, the characteristic distribution of the number of cells per spot, and the reduction in the number of spots per fly, without interference in the viability larvae-adult, allow us to propose that *I. orizabensis* resins contain substances with antimutagenic activity that effectively protect to *Drosophila* larvae from NDMA mutagenicity. It is important to know whether the antimutagenic activity detected in the *I. orizabensis* resin is efficient against other promutagens and mutagens from different chemical species. On the other side, more work is required to determine which compound or metabolite, or the interaction of some of them, is associated to the antimutagenic activity found in the *I. orizabensis* resin.

In *I. orizabensis*, the presence of scammonine I and II and orizabins V-VII, which are cytotoxic to some human epidermal carcinomas, and orizabins IX-XXI, which are cytotoxic to human colon cancer, has been reported (Meira *et al*., 2012)

Although progress has been made in the study of the activity of chemical derivatives of the genus *Ipomoea*, it is necessary to explore other species for which the information is rather scarce as in *I. jalapa*, *I. operculata*, and *I. parasitica*, among others.

Another side, the SMART and the *Drosophila* system, are confirmed as reliable tools to establish the potential mutagenic and antimutagenic of chemicals. Actually, *Drosophila* is one of the few *in vivo*, sensitive systems that provides of valious information about the different composition of complex mixtures, and the individual susceptibility of members from the same population exposed could help to find evidence about the early effect of genotoxins.

Acknowledgments: Workshop Students (93.1- 93.2), UNAM.

References: Anaya, A.L., 1990, J. of Chem. Ecology 16: 2145-2152; Baars, A.J., W.G.G. Blijleven, G.R. Mohn, A.T. Natarajan, and D.D. Breimer 1980, Mutation Research 72: 257-264; Bah, M., and R. Pereda-Miranda 1997, Tetrahedron 53: 9007-9022; Bauser, S.C., 1937, Torreya 37: 25-32; Bhakuni, D.S, M.L. Dhar, M.M. Dhar, B.N. Dhawan, and B.N. Mehrotra 1969, Indian J. Exp. Biol. 7: 250; Bieber, D.S., A.D.S. Alves, L.R.M.O. Correa, A.C. De Andrade, D.N.S. Carneiro, I.A. De Souza, J.F. De Mello, and H.J. Veith 1986, Phytochemistry 25: 1077-1081; Bilkin, M., and D.B Fitzgerrald 1952, J. Nat. Cancer. Inst. 13: 139-149; Cabrera, G.L., 1975, Yerbario mexicano. Gómez Gómez Hnos. Eds. México 239 pp; Clark, M., 1982, Mutation Res. 2: 89-97; De Souza, M.M., A. Madeira, C. Berti, R. Krogh, R.A. Yunes, and V. Cechinel-Filho 2000, R. Br., J. of Ethnopharmacology 69: 85-90; Frei, H., and F.E. Würgler 1995, Mutation Res. 334 (1995) 247-258; Frei, H., and F.E. Würgler 1988, Mutat. Res. 203: 297-308; Fuchs, S.Y., V.S. Spiegelmen, and G.A. Belitsky 1993, J. Biochem. Toxicology 8(2): 83-88; García-Bellido, A., and J. Dapena 1974, Molec. Genet. 128: 117-130; Graf, U., E. Würgler, A.J. Katz, H. Frei, H. Juon, C.B. Hall, and P.G. Kale 1984, Environ. Mutagen. 6: 153-188; Hällstrom, I., A. Blanck, and S. Atuma 1983, Chem. Biol. Interact. 46: 39-54; Hällstron, I., J. Magnusson, and C. Ramel 1985, Mutat. Res. 92: 61-168; Hernández-Carlos, B., R. Bye, and R. Pereda-Miranda 1999, J. Nat. Prod 62: 1096-1100; Lindsley, D.L., and G. Zimm 1990, Dros. Inf. Serv. 68: 382 pp; Martínez, M., 1990, Las plantas medicinales de México. Ed Botas. México pp 267-279; McDonald, A., J.S. Flores, J. Morales-Rosas, A.N. Garcia-Argaez 1997, Convolvulaceae: taxonomía y florística. Etnobotanica, Universidad Autónoma de Yucatán Merida, Yuc., 157 pp; Meira, M., E. Pereira da Silva, J.M. David, and J.P. David 2012, Brazilian Journal of Pharmacognosy 22(3): 682-713; Noda, N., T. Kogetsu, T. Kawasaki, and K. Miyahera 1990, Phytochemistry 11: 3565-3569; Noda, N., M. Ono, K. Miyahara, T. Kawasaki, and M. Okabe 1987, Tetrahedron 43: 3889-3902; Nöthiger, R., 1970, Dros. Inf. Serv. 45: 177; Pedraza, R.A., 1982, La raíz de jalapa INIREB informa. Comunicado No 54 sobre recursos bióticos del país. México; Pereda-Miranda, R., 1995, *In*: *Phytochemistry of Medicinal Plants*, (Arnason, J.T., R. Mata, and J.T. Romeo, eds.). Plenum Press: New York, pp 83-112; Perez-Amador, M.C., A.N. Garcia-Argaez, G. Varala, and F. Garcia-Jimenez 1988, Phyton 48: 97-99; Perez-Amador, M.C., E.A. Gonzalez, J. Marquez, J. Bailin, F. Garcia-Jimenez, and O. Collera 1980, Phyton 39: 85-94; Peterson, J.K., and H.F. Harrison 1991, J. of Chem. Ecology 17: 943-955; Ramos-Morales, P., M.G. Ordaz, A. Dorantes, H. Rivas, P. Campos, M. Martínez, and B. Hernández 2001, Drosophila is a reliable biomonitor of water pollution, *In*: *Biomonitors and Biomarkers as Indicators of Environmental Change: A Handbook*, Part II, (Butterworth, F.M., A. Gunatilaka,

and M.E. Gonsebatt, eds.), Kluwer Academic/Plenum Publishers, New York, pp. 257-299; Sarin, J.P.S, H.S. Garg, N.M. Khanna, and M.M. Dhar 1973, Phytochemistry. 12: 2461-2468; Shankel, D.M., P.E. Hartman, T. Kada, and A. Hollander 1987, Environmental Mutagenesis 9: 87-103; Sheskin, D.J., 2004, *Handbook of Parametric and NonParametric Statistical Procedures*, 3rd. ed., Chapman and Hall/CRC, New York, 1184 pp.