Mutageneic and antimutagenic effects of some plant extracts *in Drosophilla melanogaster*

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Abstract: This study was designed to investigate the mutagenic potential of the anticancer drug vincristine and some plant extracts (fennel and parsley) on *Drosophilla melanogaster* using two test systems: the sex linked recessive lethal (SLRL) and the estimation of the activity of cholinesterase enzyme (ChE) in F1 and F2 bar eye females and F2 wild type males. A wild type strain Oregon-R (or-R) male flies of *D.melanogaster* were treated on a medium containing a concentration of only one of the three agents, followed by a combined treatment in an alternative way of fennel extract or parsley extract followed by vincristin, then vincristin followed by fennel extract or parsley extract and finally the three agents together. The results obtained, showed non significant increase in the percentage of the S.L.R.L in all stages of spermatogenesis in all treatments. Meanwhile, vincristine as a single treatment or combined with fennel or parsley extracts showed genotoxic effects in the three categories of the two generations of S.L.R.L: F1 females heterozygous F2 bar eye females and F2 wild type males on the genetic background of ChE in all treatments. [Nature and Science 2010;8(4):77-82]. (ISSN: 1545-0740].

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1. Introduction

In the past, most of the studies on the genetic effects of anticancer drugs have been concentrated on cytogenetic damage (Clements etal., 1990). However, it is obviously important to learn more about the different types of mutagenic lesions induced by anticancer drugs. Marselos and Vainio(1991) reported that most of the cancer chemotherapeutic agents are mutagenic and carcinogenic. Vincristine (VCR) is a widely used anticancer drug in Arab countries.It contains the active substance vincristine sulphate.

Vincristine sulphate is a dimeric alkaloid found in the leaves of the plant *Catharanthus roseus,* Downing (2000). The natural vinca alkaloids and their synthetic derivatives are used as antineoplastices. These agents act by reacting with tubulin, altering the microtubule organization and dynamics disturbing the mitotic spindle and subsequently causing cell aneuploidy,Downing, (2000) and Ramirez et al. (2004) .The genotoxicity of VCR has been tested several times in vitro and in vivo in lower organisms. Various aspects of its effect have been reviewed on several occasions, Degraeve, (1978) & Kirsch-Volders,and Parrt (1996).

The available information on its genotoxicity is found to be contradictory to each other. Moreover in most of the earlier studies, either the doses tested were unusually high or the data generated were after chronic exposures to the chemical.Furthermore, Gonzalcz-Cid *et al*. (1999) found that VCR and vinorelbine (VRB) induced a significant increase in micronuclei (MN) frequencies in binucleated (BN) cells, as well as produced slowing of the cell cycle, causing a decrease in the percentage of BN cells in cultured human lymphocytes. Also, Tiburi *et al*. (2002) reported that vincristine (VCR), inblastine (VBL) and vinorelbine (VNR) induced genetic toxicity causing increments in the incidence of mutational events, as well as in somatic recombination in *Drosophila melanogaster*.

Now, the world is directed to depend on nature to decrease the side effects of the drugs. Herbal medicine is the oldest form of health care known to man kind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization, the plant kingdom has provided an endless source of medicinal plants .Herb plants produce and contain a variety of chemical substances that act upon the body. Therefore, discovery and exploration of compounds possessing antimutagenic and anticarcinogenic properties are of great importance. Many substances with antimutagenic activity have been found by several investigators Soudamini et al., (1995) and Xie et al., (2006) . The present study was designed to detect the mutagenic effects of vincristine (VCR) anticancer drug and antimutagenic effects of some plant extracts in *Drosophila melanogaster* using two test systems, the sex liked recessive lethal mutations test (SLRL) and the estimation of the activity of Cholinesterase enzyme (ChE).

2. Materials and Methods

2.1-Materials:

2.1.1 Strains:

Two strains of *D.melanogaster* were used in the present study:

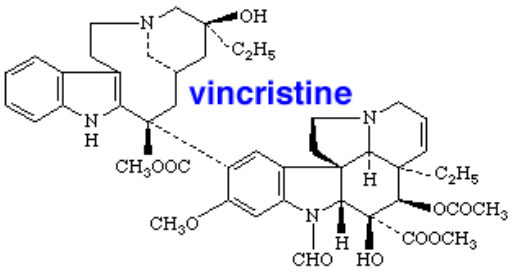
a- Muller-5 (M-5):
A marker strain of *D.melanogaster* used for the detection of Sex Linked Recessive Lethal mutations. Its X-chromosome carries a dominant marker bar eye (B) and a recessive mutant eye color, white apricot (W^a). It has also two inversions, the first is scute (Sc^{8r}) inversion and the second designated (in-s) is included in the first inversion.

b-Oregon- R (O-R):
This stock is a wild type strain that has always been used in *Drosophila* laboratories. It was obtained from the department of Genetics, Ain Shams University, Cairo, A.R.E. This strain was repeatedly tested to determine its spontaneous Sex-linked recessive lethal (S.L.R.L).

2.1.2.Chemicals:
a- Vincristine sulfate (Oncnvin):
Tablets product by Faulding Pharmaceuticals Pic/ Warwickshire CV31 3RW, United Kingdom.
(It is a dimeric alkaloid found in the leaves of the plant *Vinva rosea*.

b-Fennel (*Foeniculum vulgare Mill*)
The essential oil of the most important fennel variety (var. dulce) contains anethole (50 to 80%), limonene (5%), fenchone (5%), estragole (methyl-chavicol), safrole –pinene (0.5%), camphene, pinene, myrcene and p-cymene. **Parsley (*Petroselinum sativum)*** :
In this study, Parsley oil had been used for (CAP FARM) company no.22977/2002,Cairo, Egypt.

d- Kit for Cholinesterase estimation:
This kit was obtained from QUIMICA



CLINICA APLICADA for the estimation of the activities of the enzyme Cholinesterase (CHE).

2.2. Methods:

2.2.1.Two test systems were employed in this study:
a- Sex Linked recessive lethal (SLRL) assay for *Drosophila;* Mullar.(1972) and Brusick.(1980)
b-Estimation of enzyme Cholinesterase (ChE) activity in *Drosophila* using spectrophotometric analysis; Perparation of samples for Cholinesterase (ChE) activity estimation was carried as follow:
Sample prepared by homogenizing the whole body of 100 adults in 1.0 ml of refrigerated phosphate buffer (PH7.2)with glass homogenizer, centrifugation at 8.000 rpm for about 1 minute at 4Cºwas carried out . The particulated material was discarded, then 40 ul of the supernant was transferred in test tube. Use the kit of ChE as instruction .Measurment of transmission was done at 405 mu using spectronic spectrophotometer model.
2.2.2. Oregon-R of *D.melanogaster* males were treated as follows:
a. Single treatment of VCR with a concentration of 2ml/100 ml medium.
b. Single treatment of fennel with a concentration of 2ml/100 ml medium.
c. Single treatment of parsley with a concentration of 2ml/100 ml medium.
d. Combined treatments with VCR and parsley extract by arrangement of vinncristin followed by parsley extract then parsley extract followed by vinncristin and finally the two agents together.
e. Combined treatments with VCR and fennel extract by the arrangement of vinncristin then fennel extract, fennel extract followed by vinncristin and finally the two agents together.
3-SLRL have been estimated and three categories were analyzed for enzyme activity: F1, F2 females heterozygous and wild type males.

2.2.3. **Statistical Analysis:**
a-Significance of sex-linked recessive lethal results was detected by Kasten Baum and Bowman test Wurglar et al., (1975).
b- ANOVA test (SPSS program) was applied to determine significe of enzyme estimation.

3. Results

3.1 Induction of Sex-Linked Recessive Lethal:

a - The results obtained from the SLRL test after treatment with the one concentration of VCR (2ml/100ml of medium) are summarized in table (1). The frequencies for all broods were not significantly different from the control frequencies. Thus, it would be considered as conclusive results. Similarly, treated males with fennel and parsley extract as a single dose induced lethal mutation with a frequency of about 0.0 % in the first brood, and in the second brood about

0.12%, and about 0.40% in the third brood, with no lethality at the fourth brood. The frequencies for all broods were not significantly different from the control ones.

In addition, from table (1), it was noticed that data obtained showed that the single and combined treatments using sex linked recessive lethal mutations are inactive, producing a statistically insignificant increase in the frequency of total SLRL

3.2. Estimation activity of ChE enzyme:

This estimation was carried out in some insects of two generation of SLRL. VCR caused change in ChE activities in F1 females, F2 bar eye females and F2 wild type male due to its mutagenic potentiality. Statistical analysis indicated that the difference of F1 females, F2 females and F2 males with the control were significant (table2)

Table 1: Identification of sex linked recessive lethals occurring spontaneously and after different treatment with vincestine fennel and parsley plant extract in *D. melangaster*

Treatment	Sperms B1			Spermatides B2			Spermatocytes B3			Spermatogonia B4			Total		
	N.	L.	%	N.	L.	%	N.	L.	%	N.	L.	%	N.	L.	%
Control	906	1	0.11	913	1	0.10	88	2	0.22	815	1	0.12	3540	5	0.14
VCR 2%	1079	4	0.37	847	1	0.11	941	1	0.01	926	1	0.10	3793	7	0.18
Fennel plant Extract 2%	748	0	0.0	792	1	0.12	744	3	0.04	912	0	0.0	3232	4	0.12
Fennel 2% then VCR 2%	983	5	0.50	839	4	0.47	964	4	0.14	975	0	0.0	3761	13	0.34
VCR2% then Fennel 2%	852	0	0.0	847	1	0.11	986	3	0.03	915	2	0.21	3600	6	0.16
VCR2% and Feneel2% together	955	4	0.41	1018	3	0.29	820	1	0.12	1074	5	0.46	3867	13	0.33
Parsley 2%	632	4	0.6	402	2	0.4	344	3	0.8	268	-	0	1674	10	0.6
Parsley 2% then VCR2%	963	3	0.3	886	-	-	913	1	0.1	935	3	0.3	3697	5	0.3
VCR2% then Parsley 2%	968	1	0.1	762	6	0.7	853	-	-	941	1	0.1	3524	8	0.2
VCR2% and Parsley 2% together	910	3	0.3	955	3	0.3	1004	5	0.5	1030	2	0.2	3899	13	0.3

N.= Number of tested chromosomes, L.= Number of lethal mutations (SLRL), %= Frequency of SLRL

Table 2: Effect of vincerestine, fennel plant extract and parsley plant extract with different treatments on Cholinesterase (ChE) activity in the three categories of *D. melanogaster*

Category		ChE activity (units)*									
		Control	VCR	Fennel	Fennel then VCR	VCR then Feneel	VCR and Fennel	Parsley	Parsley then VCR	VCR then Parsley	VCR and Parsley
F1 ♀	B1	22827	14331**	28067	49419**	27148**	40551**	22945	39340**	75903**	51245**
	B2	24637	16518**	21338	90171**	65967**	46229**	25082	37722**	87720**	14672**
	B3	13767	13765**	12262	60206**	66868**	79104**	14226	41424*	64416*	36478*
	B4	30153	28650**	32390	46784**	68756**	62588**	31674	28486**	53925**	72876**
	Mean	22846	18314	23514	61645	57184.7	57118	23482	146972	281965	175273
F2 ♀	B1	37616	19866**	35153	38774**	47288**	47311**	41981	36743	70491	43818
	B2	26000	21002**	28889	33430**	77540**	76087**	25226	45900**	59707**	27049**
	B3	52753	19023**	55334	16048**	18342**	16874**	51016	14626**	22729**	25148
	B4	31509	26746**	38847	88948**	55004**	86773**	32656	16307**	14341**	23460**
	Mean	36969.5	21659.2	39556	44300	49543.5	56761.2	30447	39469**	14104	65711**
F2	B1	5305	31311**	15873	45176**	72481**	48606**	16235	16302	10883	11405

♂	B2	50227	43459**	46946	76124**	42499**	40377**	40588	29057	27720	35351
	B3	32363	21645**	37702	50637**	50499**	55493**	32957	66926**	25544**	74011**
	B4	34809	32910**	33537	49901**	65812**	42718**	33281	16055**	46627**	96634**
	Mean	33176	32312.2	33515	55459.5	57822.7	46798.5	30765	32090	27694	54350

* one unit of ChE activity is expressed one Ug of acetylcholine (substrat) reacting with ChE in on ml of 100 flies homogenate in one hour incubation at 37°C. * P 0.05 ** P 0.01e

4. Discussion

Results obtained from the SLRL test after treatment with the one concentration of VCR (2ml/100ml of medium) showed that the frequencies for all broods were not significantly different from the control frequencies. Thus, it would be considered as conclusive result.This result agreed with that obtained by Tood *et al*. (1983**)**, who found that the VCR produced many chromosomal effects but it is in the main, not mutagenic. Also, Clements *et al.* (1990) found that vincristine did not give positive results in the white-ivory somatic mutation test in *Drosophila.* However, positive results have been observed in somatic mutation and recombination test (SMART) of *Drosophilla melanogaster* (Tiburi,et al., 2002).

In addition,results showed that fennel and parsley extract had no mutagenic effect on *D.melanogaster* and that the frequencies for all broods were not significantly different from the control ones .These results agreed with that obtained by Zheng *et al*,(1992) who isolated five natural products compound from Umbelliferae, these compounds induced the detoxifying enzyme glutathione S-transferase (GST) in several mouse target tissues and the tumor was reduced from 68% to 11%. Also, the antioxidant activity of the fennel oils was evaluated as well as the antimicrobial activity, **(**Ruberto,2000**)**.Moreover, these results disagreed with the results obtained by Sanchez-Lamar, *et al*.(2002),who found that *Phyllantus orbicularis* plant extract induced micro nuclei and abnormal anaphase in Chinese hamster ovarian (CHO) cells. Moreover, the results of parsley extract agreed with that of Miller *et al*.(1983) who found that parsley and myristicin component didn't induce carcinogenic activities in the mouse and rat male livers.Also Nakashima,(1989), found that parsley can inhibit 88% of the mutagenicity in extracts from roasted beef Reults of applying the combined treatments (pre, co and post): fennel or parsley followed by vincristine; vincristine followed by fennel or parsley and finally the three components together showed non significant results .These results agreed with that of Shukla and Tanega (2005)who found that the pretreatment with garlic extract for 5 days prior to cyclophosphamide (CP) shows significant decrease in the chromosomal aberrations in Swiss albino mice. Moreover, it has been found that orange juice reduced the extent of DNA damage induced by Methyl methanesulfonate (MMS) in the pre and post-treatment by using comet assay in peripheral white blood cell, Franke et al., (2005).Also the pre- treatment with tomato and garlic significantly reduced the frequencies of N-methyl-N-nitro- nitrosoguanidine (MNNG) which induced bone marrow micronuclei in male swiss mice Kumaraguruparan et al., (2005).

Vincristine has been reported to be cytotoxic, namely as far as accumulation of mitotic figures, arrested of cells at metaphases with highly contracted chromosomes but failing of chromatid separation C-mitotic effects, inhibition of tubulin polymerization, disruption in the formation of microtubules and movement of chromosome, Degraeve (1978),Kirsch-Voldersand Parrt(1996) and Millerand Adler (1989).Thus simultaneous measurement of genotoxicity and cytotoxicity at different doses and exposure times may be an important consideration in the evaluation of genotoxicants. Also, the small numbers of biochemical and genetic investigations do not permit establishment of an exact mechanism of herbal therapies and antimutagenic action.

Further experiments are required to determine whether these substances are scavengers of genotoxic species or if their antimutagenic potential is demonstrated in more complicated ways, Xie, et al., (2006) and Andrew,(1997) Although the protective effects of coffee against somatic mutation and mitotic recombination induced by cyclophosphamide (CPH), mitomycin C (MMC) and urethane (URE) were evaluated in the wing spot test in *Drosophila melanogaster*, coffee showed significant dose-related inhibitory effects on the genotoxicity of MMC. The same protective effect was also observed with one concentration of coffee in combination with CPH (Abraham, and Graf, 1996).

Our results showed that VCR caused change in ChE activities in F1 females, F2 bar eye females and F2 wild type male due to its mutagenic potentiality. Statistical analysis indicated that the difference of F1

females, F2 females and F2 males with the control were significant.These results agreed with that of Kozik and Szczech,(1983) who observed that administration of therapeutic doses of vincristine to young rats brings about a drop of the neuronal AChE activity.

Meanwhile, niether the single treatment with fennel plant extract or parsley plant extract did not induce significant difference with the control of both generations in all broods.

The results showed nonsignificant increase in enzyme activity, which did not agree with the finding of Atta-ur-Rahman *et al* (2004) who mentioned that five steroidal alkaloids isolated from ethanolic extract of *Savcococca saligna* possessed cholinesterase inhibitory potential. Also Orhan *et al.*(2004)found that, the fumaria extracts displayed highly potent inhibition against both of the activity of AChE (Acetylcholinesterase) enzymes.

The combined treatments with fennel plant extract or parsley in different combination with vincrestine (pre, co and post treatments) showed a significant increase in enzyme activity for both generations in all broods.

5. Conclusion

vincrestine drug failed to increase the percentage of SLRL mutations and gave a non conclusive result. In contrast, it did record a significant difference when estimating the enzymatic activity of ChE which proved its ability to cause mutations. The treatment with both extracts of fennel and parsley plants didn't cause any significant increase in the SLRL mutations and no significant difference when estimating the enzymatic activity of ChE. Moreover, fennel and parsley extracts failed to induce any antimutagenic effect of vincrestine drug in *Drosophila melanogaster*.

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