GENETIC EFFECT OF OXICAM AND/OR GINGER EXTRACT

ON *DROSOPHILA MELANOGASTER*

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

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

Ginger (*Zingiber officinale*) is one of the more commonly used herbal supplements. Although it is often consumed for culinary purposes, many patients used it to treat a variety of conditions [White, 2007]. This needs further investigation, therefore, the present study was carried out to evaluate the genetic effect of Ginger (*Zingiber officinale*) and/or Oxicam, by using two test systems; the Sex Linked Recessive Lethal test (SLRL) on germ cells of *Drosophila melanogaster*, which is known to be efficient in detecting chemical mutagens, and by estimating their effects on the activity of cholinesterase enzyme which is a sensitive tool in mutagenicity testing.

MATERIALS AND METHODS

1. *Materials*

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

a.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, Egypt This strain was repeatedly tested to determine its spontaneous sex-linked recessive lethals(SLRL).

b. Mullar-5(M_5)

A marker strain of *Drosophila melanogaster* usually 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 .

Chemicals:

a. Oxicam (Tiloctil) is non steroidal anti inflammatory and pyretic properties drug.
b. Cholinesterase detection kit for estimation of the activity of the cholinesterase enzyme (ChE) from Quimica Clinica Aplicada.

2- Methods

Two methods were used in the present study to assess the mutagenic effect of Oxicam drug and Ginger plant extract.

a. Mullar (1972) and Brusick (1980) for *Drosophila* sex-linked recessive lethal (SLRL) assay.
b. The estimation of the activity of the cholinesterase enzyme according to Augustinson, 1961.

In this investigation, Oregon-R strain of *D.melanogaster* males were treated as follows:

- Single treatment with Oxicam drug (from F.Hoffmann – La Roche Ltd. Basel, Switzerland) with two concentrations of (2ml/100ml and 4ml/100ml) in the media was applied.
- Single treatment with Ginger (from Kahira Pharmaceuticals and Chemical Industries Company, Egypt) with the same two concentrations of (2ml/100ml media and 4ml/100ml) was also done.
- The combined treatments with Oxicam(2ml/100ml and 4ml/100ml) and Ginger extract(2ml/100ml media and 4ml/100ml) were carried out.
- SLRL have been estimated and three categories were analyzed for cholinesterase enzyme activity, F1 and F2 heterozygous females and F2 wild type males.

Cholinesterase was estimated by using spectrophotometric analysis.

Samples were prepared by homogenizing the whole body of 100 adults in 1.0 ml of refrigerated phosphate buffer (ph 7.2) with glass homogenizer, then centrifugated at 8.000 rpm for about 1 minute at 4C°. The particulated material was discarded, and then 40 µl of the supernant was transferred in a test tube. The kit of ChE was added and the mixture was shaken vigorously to avoid bubble formation during the meauserment of transmission. The transmission was then measured at 405 mµ using spectronic spectrophotometer model.

Statistical analysis

1- Kasten Baum and Bowman test was used to test significancy of sex-linked recessive lethal induction [Wurgler *et al*., 1975].
2- ANOVA test (SPSS programe) was applied for testing significancey of enzyme estimates.

RESULTS AND DISCUSSION

1. *Induction of sex-linked recessive lethal (SLRL):*

The results of SLRL that were obtained after the treatment with two concentrations of Oxicam or Ginger plant extract (2ml and/or 4ml /100ml of medium) are summarized in Table (1) and presented graphically in Figure (1). The control mean results for the four examined broods showed about 0.14% mutation. This class of mutation is termed spontaneous mutation, which was previously established by AL-Tawaty (1990) and Youssef (1993).

When comparing the results after the treatment with Oxicam alone (2%) with the control one, it was found that the percentage of the mutations occurred with this medicine was 0.13%, while it was 0.14% for the control (Table 1 and Figure1). However, non of the phases exhibited statistical significant increase in the number of mutations. And when comparing the results after treatment with Oxicam (4%) with the results of the control, it was found that the percentage of the mutations occurred with this medicine was 0.18% as shown in Table (1) and Figure (1). No significant differences were obtained for the different dosages used in this study. Therefore, oxicam is not a mutagen, and this result is concordant with Niikawa and Nagase (2007).

From the previous results, it indicated that Oxicam did not cause an increase in the SLRL. Due to the fact that this medicine is a harmful factor that splits DNA, so it may lead to

the death of the cells bearing the genetic harms rather than participating in the formation of gametes or (zygotes) or that the reformation processes may reduce the severity of this effect or that these compounds are unable to stimulate this type of mutations (Eiche *et al*., 1990; Youssef, 1993). The last reason for the obtained results may be the usage of low dosages which may not be able to cause the ceasing of the cell in G2 phase and obstructing it to enter into the process of Mitotic division, and thus preventing the formation of the spindle strings that lead to the cells accumulation in the Mitotic division. Thus, the presence of the divided cells increase, whereas the high concentrations affect the cells in S phase before entering into the division process (Choudhury *et al.*, 2000). However, this does not exclude its genetic toxicity that was pointed out through the results of the previous studies.

From the results of the treatment with Ginger extract that was applied for *D. melanogaster* males, it was pointed out that when using this extract with 2% concentration, there was no significant increase in the percentage of mutations (SLRL) in all the four broods when compared with the control. The mutation percentage in the treated individuals was 0.05% while it was 0.14% in the control (Table1 and Figure1). Whereas results of 4% Ginger extract treatment pointed out that the percentage of mutation for the four broods is 0.15%, compared to 0.14% for the control and there was no significant increase as shown in Table(1) and Figure(1). These results agreed with Flammang *et al.*, 2004, Raju *et al*., 2004, Tellez *et al*., 2007 and Furlanetto *et al*., 2007. It can be concluded that the Ginger extract has no mutagenic effect with the used dosages. However, the contradiction between the present study may be due to the dosage used in the current experiments or as a result of the little sensitivity of *D. melanogaster* to the used dosages, as there is no significant increase in the mutations of SLRL.

The results of the combined treatment with Ginger extract with Oxicam with 2% or 4% concentrations for each of them pointed out that there is no significant change of SLRL mutations in all stages of spermatogenesis, compared to the control as shown in Table(1) and Figure (1). These results are in accordance with several studies, like Salikhova *et al*., (1994), Hassan, (1998), Ng and Figg (2003), Panwar *et al*., (2005) and AL- Tawaty *et al.*, (2007). The previous results indicated that the treatments of *D. melanogaster* with the different combined treatments with Ginger extract and Oxicam with the used dosages have no mutagenic effect, in spite of the increase of the percentage of the SLRL mutations sometimes, yet it is insignificant.

2. *Estimation activity of ChE enzyme*

The second part of this investigation was carried out to estimate the activities of the enzyme ChE in some insects of two generation of SLRL (F1 females, F2 bar eye females and F2 wild type male) after different treatments with Oxicam drug and Ginger plant extract. As shown in table(2) and Figure(2), the two concentrations of Oxicam caused change in enzyme activities.

Oxicam 2% induced significant difference (increase or decrease) in enzyme activity only in the spermatocytes stage (third brood) in all the three categories of *Drosophila melanogaster*. It is of interest to note here that spermatocytes stage of this strain showed higher sensitivity to the mutagenic effect of Oxicam 2% than other spermatogenesis stages.

Oxicam 4% induced significant difference from the control for both generations in all spermatogenesis stages (broods) of the three categories of *D. melanogaster* which proves the mutagenic potentiality of the drug in this concentration. These results are in accordance with Tutor- Crespo *et al.* (2004).

The results of the Oxicam treatments (2% & 4%) with Ginger plant extract are summarized in Table (2) and presented graphically in Figure (2). These results showed an significant increase or decrease in the enzyme activity compared to the control in all broods of

the three categories of *D. melanogaster* except the F2 wild type male in the third brood at the 2% concentration, the increase of enzyme activity was nonsigniticant. This result is in accordance with several studies, Rahman *et al*., (2001); Das *et al*., (2002); Rahman *et al*., (2004); Oh *et al*., (2004); Viegas *et al*., (2005). Through the previous presentation of the results , it is pointed out that the Ginger extract is the cause of the significant differences in ChE enzyme activity when experimented on *D. melanogaster* and this needs to be more emphasized by using dosages less than those used in this experiment to know the essence and fact of the activity of the Ginger extract, and we recommend to be cautious when using Ginger or the herbal plants that we used to utilize daily.

The results of the combined treatments with Oxicam and Ginger plant extract on the enzyme activity caused significant effects in all broods in *D. melanogaster*.

In conclusion, Oxicam 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 proves its ability to cause mutations. The treatment with Ginger plant extract didn't cause any significant increase in the SLRL mutations but gave a high significant difference when estimating the enzymatic activity of ChE which brings to attention the necessity of codification of its use, because it might has dangerous effects on human when uses in high doses but we can't judge on its mutagenic effect before using a highly sensitive tests more than those used in this experiment.

SUMMARY

In the present study, *Drosophila melanogaster* males were treated with either Oxicam or Ginger plant alone and then with combination of both to study their mutagenic effects. Two tests were used for the detection of the mutagenic effect, Sex Linked Recessive Lethal test (SLRL) and estimation of cholinesterase enzyme activities (ChE). The results indicated that both of Oxicam and Ginger didn't increase SLRL in single and combined treatments. In contrast, estimation of ChE activities showed significant effects in all stages of spermatogenesis with single and combined treatments, except some broods of females in the first generation. We concluded that, estimation of ChE enzyme activity was found to be more sensitive for the detection of the mutagenic effect than SLRL.

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Table (1): Identification of sex linked recessive lethals occurring spontaneously and after different treatments with Oxicam and/or Ginger plant extract in *D.melanogaster*.

Treatments	Sperms B1			Spermatides B2			Spermatocytes B3			Spermatogonia B4			Total		
	N.	L.	%	N.	L.	%	N.	L.	%	N.	L.	%	N.	L.	%
Control	906	1	0.11	931	1	0.10	888	2	0.22	815	1	0.12	3540	5	0.14
Oxicam 2%	900	1	0.11	942	1	0.10	969	1	0.10	997	2	0.2	3808	5	0.13
Oxicam 4%	963	2	0.20	889	1	0.11	976	2	0.20	981	2	0.20	3809	7	0.18
Ginger 2%	965	1	0.10	943	1	0.10	891	-	-	850	1	0.10	3649	3	0.08
Ginger 4%	988	1	0.10	972	2	0.20	865	2	0.23	916	1	0.10	3751	6	0.15
Oxicam & Ginger 2%	805	4	0.49	910	1	0.10	897	2	0.22	854	2	0.23	3466	9	0.25
Oxicam & Ginger 4%	763	7	0.91	851	3	0.35	830	2	0.24	795	3	0.37	3239	15	0.46

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

Table 2: Effect of Oxicam and Ginger plant extract with different treatments on Cholinesterase (ChE) activity in the three categories of D.melanogaster

Category		ChE activity (units)*						
		Control	Oxicam 2%	Oxicam 4%	Ginger 2%	Ginger 4%	Oxicam & Ginger 2%	Oxicam & Ginger 4%
F1 ♀	B1	22827	24908	13482 **	8456 **	9382 **	5963 **	14952 **
	B2	24637	28257	17419 **	26738 **	23561 **	31514 **	20436 **
	B3	13767	32819 **	11654	35743 **	29584 **	39738 **	9465 **
	B4	30153	31818	29732	37382 **	38753 **	42886 **	22140 **
	Mean	22846	29450.5	18071.25	27079.75	25320	30025.25	16748,25
F2 ♀	B1	37616	39572	32820 **	41356 **	46792 **	40835	38945 **
	B2	26000	27532	20791 **	34913 **	37490 **	36942 **	39814 **
	B3	52753	34841 **	43084 **	15709 **	18927 **	38954 **	20586 **
	B4	31509	38721	17432	49008 **	50483 **	53696 **	52794 **
	Mean	36969.5	35166.5	26531.75	35246.5	38423	4260675	38034,75
F2 ♂	B1	53054	54732	30458 **	9147 **	11356 **	56342	24784 **
	B2	50227	52617	41837 **	28183 **	30476 **	41894 **	28376 **
	B3	34809	51430 **	20159 **	36912	38865 **	35016	14538 **
	B4	32363	35697	29974 **	17516 **	22853 **	64840 **	24534 **
	Mean	42613.2	48619	30607	18999.5	25887.5	49523	23058

*one unit of ChE activity is expressed as one μ g of acetylcholine(substrate) reacting with ChE in on ml of 100 flies homogenate for one hour incubation at37̊c.

*P 0.05 ** P 0.01

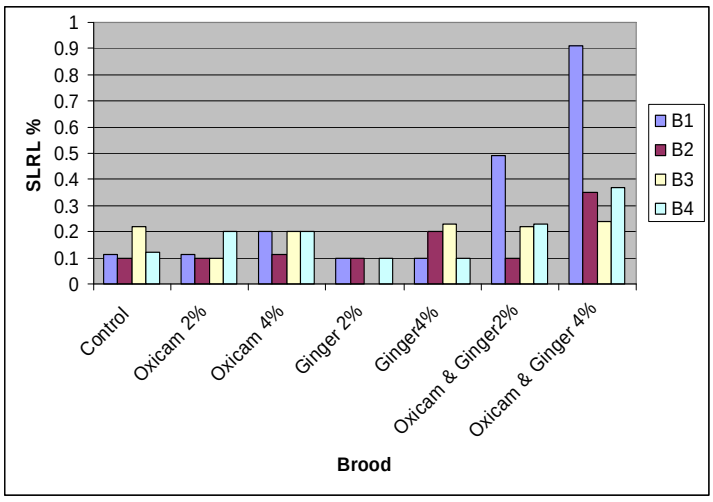

Fig (1): Sex linked recessive lethals in four broods of *D.melanogaster* occurring spontaneously and after treatments with Oxicam or/and Ginger plant extracts.

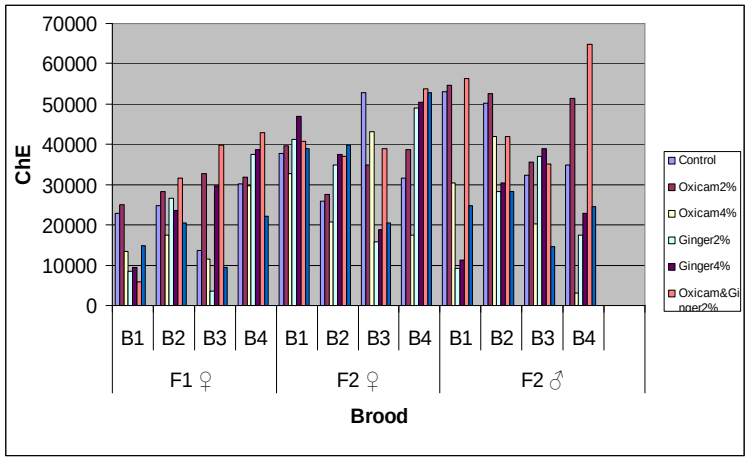

Fig.(2): Effect of Oxicam and Ginger plant extract with different treatments on cholinesterase (ChE) activity in three categories of *D.melanogaste*