Cytotoxic Effects Induced by a nonsteroidal, anti-inflammatory compound on Root Tip Cells of *Vicia Faba* Plant

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Abstract: In this study ,mutagenic effects of four Piroxicam concentrations (0.5, 1, 1.5 and 2 mg/ 1ml water) on *Vicia faba* root tips and the recovery treatments with water for 24h were studied. Results revealed that all treatments reduced mitotic index; which reduction increase as Piroxicam concentrations increased. Also, the effect of Piroxicam in this trait persists after recovery treatments. Both different Piroxicam and recovery treatments caused unbalance mitotic phases percentages. And it increased as Piroxicam concentration increased. However, all recovery treatments lightly reduced the obvious trait, indicated that the mutagenic effect for Piroxicam compound persist under the recovery treatments. Stickiness was the major form of total abnormalities in both Piroxicam and recovery treatments indicating that Piroxicam can be intercalation with DNA leading to entanglement of chromatin threads. On the other hand, disturbed and C-metaphase were recorded the highest percentages after the sticky chromosomes in both Piroxicam and recovery treatments, meaning that Piroxicam has an effect on spindle formation in *Vicia faba* mitosis. However, Lagging chromosomes, bridges, fragments and breaks were slightly induced by some treatments. These results showed that the Piroxicam has the mutagenic effects on *Vicia faba* plant .
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1.Introduction

Nonsteroidal, anti-inflammatory drugs (NSAID) are group of agents with similar action but diverse chemical structures. These agents proved to be biologically active that affect diverse biological processes. It has been widely accepted that the mechanism of action of ($NSAID_s$) is the inhibition of prostaglandin synthesis, Diaz-Gonzalez and Sanchez – Madrid (1988). Recently, most ($NSAID_s$) can produce hepatoxicity, Planas et al.,(1990); Poniachik et al.,(1998). Piroxicam was also shown to prevent azoxy methane induced aberrant crypt foci and colon cancer in rats (Kulkarni et al., 1992; Singh et al., 1994; Pereira et al., 1996).Recent studies have suggested that apoptosis is a key phenomenon in the chemo preventive action of $NSAID_S$, Hara et al., (1997). Piroxicam is one of these compounds Biologically active compounds are of specific importance in the field of environmental mutagenesis. This investigation was aimed to study the possible effect of piroxicam on the genetic material on *Vicia faba* plant by detection of the capability of piroxicam to induce chromosomal aberrations.

2.Materials And Methods

2.1.Materials

2.1.1.1-Biological material:

Vicia faba (variety Giza 2) kindly produced from Crop Research Institute, Agricultural Research Center, Giza, Egypt.

2.1.2-Tested compound:

Piroxicam in a pharmaceutical grade form was used in the experiment of this study.

2.2. Methods

The root tips of *Vicia faba* seedlings were dipped through the holes of the porous cloth covering the jars containing four Piroxicam concentrations i.e. (0.50,1 ,1.50 and 2mg/1ml water) for 4 hours, then the roots were washed in running tap water. Water control was maintained simultaneously for assessment of spontaneous aberrations. Another group of treated *Vicia faba* roots were taken off after each treatment and washed with running water. Then put in jars containing tap water for 24h for recovery.

Treated and untreated control of *Vicia faba* root meristems of 2-3 ml length were excised, fixed in carnoy solution (3:1 absolute ethyl alcohol and glacial acetic acid) for 24 h., and then kept in70% ethanol in refrigerator at 4^oC until staining and examination. Aceto-carmine squash preparations were made and examined cytologically accorroding to Rank and Nielsen(1993). Five preparations from each treatment were examined to determine: mitotic index; different mitotic phase's percentages; total abnormalities percentages; abnormal cells frequencies in different mitotic phases and different types of abnormalities frequencies.

3.Results And Discussion

3.1-Mitotic Index and its phase's percentages

The results in Table (1) showed clearly that Piroxicam reduced mitotic index, which it reduced as Piroxicam concentration increased. Also, the effect of Piroxicam in this trait persists after recovery treatments. The mode of action of Piroxicam may be ascribed to its effect on spindle formation as this was clear from the high percentages of metaphase on the expense of (ana-telo) phases. Where, (pro, meta and ana-telo) phases % percentages recorded 51.30%, 22.08% and 26.62% in the control respectively. While these traits were recorded ranged: (39.65% - 54.52%); (20.40% - 35.79%) and (23.33% - 26.12%) after Piroxicam treatments. While they recorded: (40.08% - 53.66%), (21.46% - 33.46%) and (23.80% - 29.41%) after recovery treatments and this indicated that both Piroxicam and recovery treatments caused unbalanced mitotic phases percentages, and this alternated of the value of mitotic index. The reduction of mitotic index could be due to the effect of Piroxicam on the process of DNA synthesis is the essential requirement for the progress of the process of mitosis. These results are in agreement with many researches (El-Ashmawy et al., 1992; Badawy et al., 2000; EI-Soudy et al.,2001; Poli et al.,2002; Arkhipchuk et al.,2004;Singh et al.,2005; Cleik and Aras 2006; William and Elizabeth 2006; Kocamanand Topaktas 2009).

Table (1): Mitotic activity, total abnormalities % in treated *Vicia faba* root tips with different Piroxicam **concentrations after both direct (D) and recovery (R) treatments.**

Dose (mg/ml)		Examined cells No.	Dividing cells No.	Abnormal cells No.	Mitotic activity	Abnormalities %
Control		5979	462	10	7.73	2.16
0.50	**D**	5837	402	70	6.89	17.41
	R	5780	410	41	7.10	10.00
1	**D**	5696	332	81	5.83	24.39
	R	5538	289	68	5.22	23.53
1.50	**D**	6111	300	90	4.91	30.00
	R	5980	280	77	4.68	27.50
2	**D**	5971	285	123	4.77	43.16
	R	5690	257	106	4.52	41.25

3.2- Mitotic Abnormalities

All Piroxicam treatments caused mitotic abnormalities % in *Vicia faba* root tips, which it increased as Piroxicam concentration increased. Whereas, it ranged (17.41% - 43.16%) comparing with control (2.16%). On the other hand, the recovery treatments reduced the obvious trait, which it ranged (10% - 41.25%). Table (1), following the effect of recovery as an indication for effect persistence was investigated by many workers,(Kabarity et al.,1974; Mazrooli and Kabarity (1984) El-Nahas 1987).

Results from Table (2) showed that the percentages of abnormal (pro, meta and ana-telo) phases at control were: 0% , 8.82% and 0.81 %. While, they recorded as ranged with (0.55% - 7.96 %); (20.40% -35.79%) and (28.50% - 47.11%) after Piroxicam treatments, But they ranged after recovery treatments with the following percentages: (3.88% - 5.79%), (29.55% - 74.24%) and (14.71% - 55.80%) respectively

Table(2): Mitotic phases % and abnormality mitotic phases % in treated *Vicia faba* root tips with different Piroxicam **concentrations after both direct (D) and recovery (R) treatments .**

Dose (mg /ml)		Examined cell No.	Prophase			Metaphase			(Ana-Telo) Phase		
			No.	%	Ab%	No.	%	Ab%	No.	%	Ab%
control		462	237	51.30	-	102	22.08	8.82	123	26.62	0.81
0.50	**D**	402	215	53.48	-	82	20.40	48.78	105	26.12	28.51
	R	410	220	53.66	-	88	21.46	29.55	102	24.88	14.71
1	**D**	332	181	54.52	0.55	72	21.69	65.28	79	23.80	41.71
	R	289	138	47.75	5.79	66	22.84	56.06	85	29.41	27.00
1.50	**D**	300	160	53.33	4.38	70	23.33	71.43	70	23.33	47.11
	R	280	146	52.14	5.48	67	23.93	67.16	67	23.93	35.8
2	**D**	285	113	39.65	7.96	102	35.79	87.25	70	24.56	35.71
	R	257	103	40.08	3.88	86	33.46	74.42	68	26.46	55.8

From the obvious, results indicated that the most abnormalities were present in the (meta, ana and telo) phases. Also, metaphase stage was the more affected by all treatments than other stages, but prophase stage was less affected. These results were in agreement by many researches (Ma and Ma,1999; Chen et al.,2000 Hamed, 2001)-Different types of abnormalities percentages in treated *Vicia faba* root tips with different Piroxicam concentrations and recovery treatments were shown in Table(3). Stickiness was the major form of total abnormalities, which it ranged: (8.78% - 20.70%) and (4.39% - 18.68%) after Piroxicam and recovery treatments respectively, meaning that Piroxicam can be intercalation with DNA leading to entanglement chromatin threads(Maju et al.,1984; Makki, 2007). Stickiness was observed in metaphase (Fig.1:a) and anaphase (Fig.1:b) found to cover the whole chromosome complement leading to the appearance of chromatin masses where lost(Evandri et al.,2003; Usciati et al.,2004)attributed such stickiness to the process of de-polymerization of DNA, thus the chromosome surface becomes sticky.

On the other hand, disturbed and C-metaphase were recorded the highest percentages after the sticky chromosomes in both Piroxicam and recovery treatments with range of (1.74% - 7.37%), (1.22% - 7 %); (3.98% - 5%) and (2.08% - 3.89%) respectively.

Disturbed chromosomes were seen in (meta, and ana) phases (fig.1: c, d) as a result of the partial action of Piroxicam on the spindle formation. Consequently some chromosomes might lose their ability to attach with the spindle fiber. Whereas, the complete inhibition of Piroxicam on the spindle formation resulted in C-metaphase (Fig.1:e) in which the chromosomes lose their ability to continue to anaphase and are arrested at metaphase. These results are in agreement with many workers (Zaka et al.,2004; Sudhereer et al.,2007; Ann et al.,2012; Rina et al.,2012).

However, other abnormalities like: laggard, bridges, fragments and breaks were observed in some treatments with low percentages (Table3) (Fig.1: f,g,h,I,j, k).

We concluded that Piroxicam have mutagenic effect in *Vicia faba* root tip cells, which caused: reduced of mitotic index, unbalanced mitotic phases % and induced chromosomal abnormalities. Also, the mutagenic of Piroxicam compound persists under the recovery treatments.

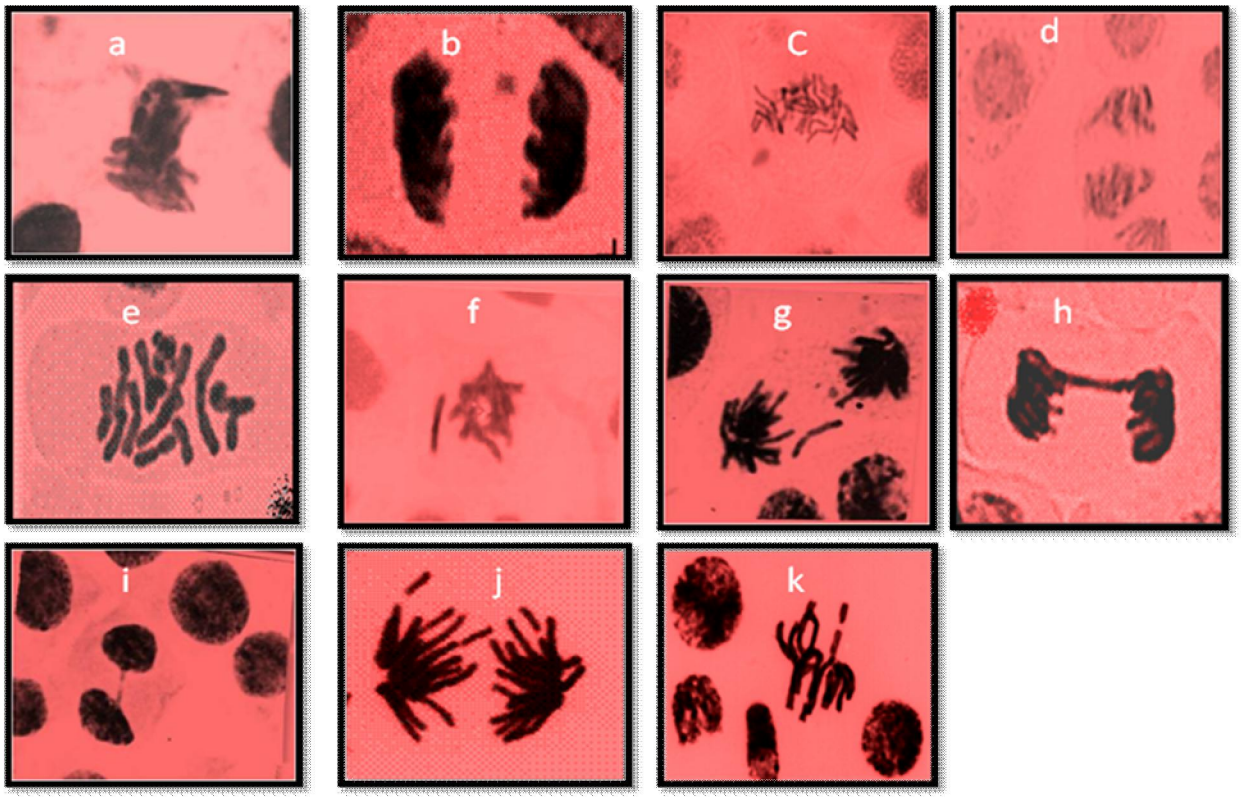

Fig.1: Different types of abnormalities in treated *Vicia faba* plant after Piroxicam **treatments. a and b: Sticky (meta and ana) phase; C and d: distusbed (meta and ana) phase; e: c- metaphase; f and g: Lagging (meta and ana); phase; h and i: bridges (ana and telo) phase; j and k : breaks (meta and ana) phase.**

Table (3): Different types of abnormalities percentages in treated *Vicia faba* root tips with different feldene concentrations after both direct (D) and recovery (R) treatments

Dose (mg /ml)		Abnormalities %	stickiness	Disturbed ch.	Cmeta phase	Laggring ch.	bridge	Fragments and breaks
control		2.16	0.22	1.08	-	0.87	-	-
0.50	D	17.41	8.71	1.74	3.98	1.49	1.49	-
	R	10.00	4.39	1.22	2.44	0.98	0.98	-
1	D	24.39	12.05	2.11	3.92	0.60	3.01	2.71
	R	23.53	11.07	4.15	2.08	3.81	1.38	1.03
1.50	D	30.00	16.66	3.67	5.00	3.33	0.67	0.67
	R	27.50	14.29	3.57	3.57	3.21	1.43	1.43
2	D	43.16	20.70	7.37	4.91	3.86	3.51	2.81
	R	41.25	18.68	7.00	3.89	3.89	4.67	3.11

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