Evaluation of the Effect of Zinc on Fipronil Induced Hepatic Toxicity in Adult Male Albino Rats

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Abstract

Background: Fipronil showed toxicity in mammals and in insects, it was documented to have adverse effects to organs such as liver. Zinc homeostasis is primarily regulated in the liver, so the chronic liver damage results in impairment of zinc homeostasis and eventually zinc deficiency.

Aim: to study protective effect of zinc on liver toxicity induced by fipronil in adult male albino rats through biochemical, histopathological and genotoxic studies.

Main methods: In the present study, forty adult healthy male albino rats weighing 180 - 200 gm, were used. They were divided into 4 groups as following:Group I(control group): It was subdivided into 2 groups each of 8 rats; Group IA (negative control group) 8 rats:
Rats received no medication, only regular diet and water to measure the basic parameters for 6 weeks, Group IB (positive control group) 8 rats: Each rat treated with 1 ml distilled water (the vehicle of fipronil and zinc) by oral gavage once daily for 6 weeks. Group II (zinc group) 8 rats:
Each rat was gavaged orally with 2mg/kg bw zinc as powder dissolved in 1 ml distilled water once daily for 6 weeks.Group III (fipronil group) 8 rats: Each rat was gavaged orally with 9.7 mg/kg bw fipronil dissolved in 1 ml distilled water once daily for 6 weeks which equals 1/10 of LD50.Group IV (zinc+fipronil) 8 rats: Each rat was gavaged orally with 2mg/kg bw zinc as powder dissolved in 1 ml distilled water. An hour later; Fipronil was gavaged orally with 9.7mg/kg bw fipronil dissolved in 1 ml distilled water once daily for 6 weeks. At the end of 6 weeks, all rats were anesthetized then subjected to blood samples collection for estimation of serum biomarkers of liver function tests (ALT, AST, ALP & LDH), oxidative stress (MDA) and Antioxidant biomarkers SOD &GPX).

Results: The results of the present study revealed that zinc administration with FPN resulted in a significant decrease in the mean values of serum ALT, AST & ALP & LDH of Zinc with fipronil group when compared with FPN treated group, zinc administration with FPN resulted

in a very highly significant decrease in the mean values of serum (MDA , GPX &SOD) of Zinc with fipronil group when compared with FPN treated group, The results revealed some improvement in histopathological changes in zinc+FPN treated group indicated by normal central vein, minimal dark pyknotic nuclei with mild vacuolation and minimal blood sinusoidal congestion, minimal cellular infiltration.

Conclusion: Our findings prove that Zinc plays an important role in protection against fipronil liver toxicity evidenced by improving the biochemical, histopathological and comet assay results in groups treated with zinc and fipronil.
Keywords: Fipronil, liver toxicity

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Introduction:

Fipronil (FPN) is a broad-spectrum insecticide belonging to phenopyrazole family, used for control of a wide range of agricultural pests protecting many crops including rice, cotton, corn, wheat, grass, straw, and others. It is used also to control a lot of public health and veterinary pests, including mosquito, locusts, ants, cockroaches, fleas and ticks **(1).**

Fipronil is neurotoxic to insects and the primary mode of action refers to interfere with the passage of chloride ions through the γ-amino butyric acid (GABA) chloride channels as well as glutamate-activated chloride channels of the central nervous system causing uncontrolled hyperexcitation of insects at low doses and convulsions leading to insect death at high doses **(2).**

Further, there is no known fipronil resistance in target insects—a phenomenon that has been shown to occur for most other pesticides. This, in turn, has led to a rapid increase in the usage of fipronil, which accounts for approximately 10% of the global pesticide market **(3)**

Fipronil is primary metabolized by mammalian liver which is considered the central organ for metabolism connecting the digestive tract with the general circulation **(4).**

Zinc is a nutritionally fundamental trace element, essential to the structure and function of numerous macromolecules, including enzymes regulating cellular processes and cellular signaling pathways. Zinc also modulates immune response and exhibits antioxidant and anti-inflammatory activity. Zinc retards oxidative processes on a long-term basis by inducing the expression of metal-binding cysteine-rich proteins called metallothioneins. Furthermore, zinc increases the activation of antioxidant proteins and enzymes, such as glutathione and catalase **(5).**

We aimed at this study to to study protective effect of zinc on liver toxicity induced by fipronil in adult male albino rats through biochemical, histopathological and genotoxic studies.

Materials and methods

- **Material: -**

I-Chemicals: -

A) -Fipronil:

Fipronil (Contrado powder 5%) a preparation obtained from **StarChem Industrial Chemicals Company** and manufactured by Zhejiang Yongnong Chem.Co., China. It was in the form of white powder, dissolved in water, stable only for 24 hours after dissolvation so, it was freshly prepared before every use.

B) -Zinc:

Zinc was obtained from Elgomhoria Pharmaceuticles Co (Elsawaf st., Zagazig, Egypt) in the form of White odorless powder of zinc sulphate heptahydrate ($ZnSO4 \cdot 7H2O$) soluble in distilled water.

C)- Distilled water:

Obtained from El- Nasr Co, Egypt and used as a solvent for both fipronil and zinc.

D)- Reagents and commercial kits:

-Kits for estimation of Alanine transaminase (ALT), Aspartate transaminase (AST) , Alkaline phosphatase (ALP), Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Malondialdehyde (MDA) , ELIZA kits for estimation of triiodothyronine (T3) and thyroxine (T4), were purchased from Bio diagnostic Co. ELIZA Kits for estimation of thyroid stimulating hormone (TSH) were purchased from Calbiotech chemical company in Cairo

-Kits for estimation of lactate dehydrogenase (LDH) were purchased from Egyptian Company for Biotechnology.

II-Animals: -

Forty male adult albino rats, aged about 6 weeks and weighing 180 - 200 gm, were obtained from the animal house of Faculty of Medicine, Zagazig University.

The adult male albino rat was the animal of choice for this experiment because of metabolic similarities with human **(6).** It is extremely valuable in duplicating the response of human to drugs. Its numerical availability provides ground for its use in order to obtain relevant statistical evaluation **(7).**

N.B: One month injection in rats is equivalent to 24 months in human being **(8).**

The Institutional Animal Care and Use Committee (IACUC), Zagazig University has approved the design of the experiment. According to mean difference, sample size was calculated to be 40 rats using open Epi program in Community Medicine department, Faculty of Medicine, Zagazig University.

Before starting the experiment, all animals subjected to 2 weeks of passive preliminaries for house acclimatization, to ascertain their physical wellbeing and to exclude any diseased animal.

All animals received human care in compliance with the animal guidelines and ethical regulations in accordance with" The Guide for The Care and Use of Laboratory Animals **(9).**

The animals were kept in plastic mesh cages with solid bottom. These cages contained wood shavings in the bottom as bedding which were changed frequently to keep the animals

clean. Overcrowdness and isolation were avoided. Animals were allowed free access to solid food and water in their home cages. There was proper ventilation in the animal house and in the cage. The room was maintained with 12h-light/dark cycle.

All experimental procedures were ethically approved by The Ethical committee for scientific research of Faculty of Medicine, Zagazig University and performed according to the institutional guidelines for the care and use of laboratory animals, which are in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

- **Methods: -**

Experimental design: -

The rats were divided into 4 groups as following:

***Group I(control group):** contains 16 rats which was subdivided into 2 groups each of 8 rats

- **Group IA (negative control group):**

Rats will receive no medication, only regular diet and tap water to measure the basic parameters for 6 weeks.

Group IB (positive control group):

Each rat was treated with 1 ml distilled water (the vehicle of fipronil and zinc) by oral gavage once daily for 6 weeks.

***Group II (zinc group) (8 rats):**

Each rat was gavaged orally with 2mg/kg zinc as powder dissolved in distilled water once daily for 6 weeks according to **Goel et al., (10).**

***Group III (fipronil group) (8 rats):**

Each rat was gavaged orally with 9.7 mg/kg bw fipronil dissolved in distilled water once daily for 6 weeks which equals 1/10 of LD50 according to **Tomlin (11)** who reported that oral LD_{50} of fipronil is 97 mg/kg in rats.

***Group IV (zinc+fipronil) (8 rats):**

Each rat was gavaged orally with 2mg/kg zinc as powder dissolved in distilled water. An hour later; Fipronil was gavaged orally with 9.7mg/kg bw dissolved in distilled water once daily for 6 weeks according to **Swelam et al., (12)**.

At the end of 6 weeks, all rats were subjected to the following:

Each rat was anesthetized by ether inhalation and blood samples was collected from the venous retro-orbital plexus for estimating: -

- **liver function tests:** alanine amino transferase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH).
- **thyroid function tests:** thyroid stimulating hormone (TSH), triiodothyronine (T3) & thyroxine (T4).
- **oxidative stress biomarkers assay:** Malondialdehyde (MDA)**,** superoxide dismutase (SOD) and Glutathion peroxidase (GPX)**.**

Then rats were sacrificed by cervical dislocation, thyroid and livers were immediately dissected out and grossly inspected to assess any gross abnormalities. The thyroids and livers

were divided into two parts, the first part was fixed in 10% formalin for histopathological examination under light microscope. The second part was put in normal saline then kept frozen at –20ºC for Comet assay to evaluate genotoxic effects of fipronil.

- **Biochemical Studies:**

- **Method used for blood samples collection:**

Venous blood samples were collected from animals by means of micro-capillary glass tubes from the retro-orbital plexuses in accordance with the procedure described by **Johnson, (6).**

The animal was held in the left hand and grasped from the back, while enclosing the neck and exerting slight pressure by the thumb and index fingers. This would cause engorgement to the veins of the retro-orbital plexuses with slight protrusion of the eyeball. A glass tube with capillary orifice of 0.6 mm was inserted into the orbit of the eye at an anterior angle. Then it was rotated to drill through the conjunctiva in the direction of the site of optic nerve. The plexus would be reached at depth of 4.5 mm, and because of venous congestion, the blood spontaneously shoots into the capillary tube.

Evaluation of DNA damage:

(1)For viewing the DNA damage, observations were made of EtBr-stained DNA using a 40x objective on a fluorescent microscope.

(2)A Komet 5 image analysis software developed by Kinetic Imaging Ltd. (Liverpool, UK) linked to a charge coupled device (CCD) camera was used to determine the quantitative and qualitative extent of DNA damage in the cells by measuring the length of DNA migration [tail length] and the percentage of migrated DNA in the tail [tail DNA %]. Finally, the program calculates tail moment [correlation between tail length and tail DNA %]. Generally, images of 100 (50 X 2) randomly selected cells are analyzed per sample. The mean value (for 100 cells) was calculated.

Statistical analysis

The obtained results were tabulated as mean ± SE. Statistical analysis was performed using one-way analysis of variance (ANOVA), One Way Analysis of variance (ANOVA) test for comparison of means of multiple independent groups of normally distributed data, Least Significance Difference (LSD) test is used for comparison between different groups, Paired t-test is used for for comparison of means of one group at different time interval, Chi- square test (X2) is used to find the association between row and column variables, For all above-mentioned statistical tests, the threshold of significance was fixed at 5% level (P-value).P value of > 0.05 indicates non-significant results. P value of < 0.05 indicates significant results. P value of < 0.01 indicates high significant results. P value of < 0.001 indicates very high significant results.

Results

The present study was carried out on 40 adult male albino rats. The rats were divided into 4 groups: control group which comprised both negative control group (received only

regular diet and water) and vehicle control group (received 1 ml of distilled water), zinc treated group (with a dose 2mg/kg once daily by oral gavage), fipronil group (treated with a dose 9.7 mg/kg once daily by oral gavage) and zinc with fipronil group treated with zinc and after one hour treated with fipronil with the same previously mentioned doses.

I- Biochemical results:

(A) Biochemical parameters of control groups:

As comparing the laboratory results of biochemical tests regarding serum biomarkers of liver functions, thyroid functions and oxidative stress biomarkers of the negative control (group Ia) and the vehicle control (group Ib) were within normal values. There was no statistically significant difference between them as well as within the same group all over the period of the study (p>0.05).So we used negative control group as a standard reference for comparison with other groups **(Tables 1, 2, 3).**

Table (1): Statistical comparison between negative and **positive control groups as regard mean values of serum alanine amino transferase (ALT), aspartate transaminase(AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) at the end of six weeks by using student t-test.**

Group N=8 / parameter	-ve Control	+ve Control	t	p-value
	Mean±SD			
ALT (U/L)	28.88±4.7	31.25±4.65	1.015	0.327 NS
AST (U/L)	26.88±3.91	26.00±3.74	0.457	0.654 NS
ALP (U/L)	53.75±9.36	53.25±7.21	0.120	0.907 NS
LDH (U/L)	261.88±25.06	263.75±22.79	0.157	0.878 NS

N.B All values are expressed as mean±SD. (SD: standard deviation)
N : Number of rats in each group was 8 rats.
NS: P >0.05 =non significant. t:t- test
U/L= unit per litre

Table (2): Statistical comparison between negative and **positive control groups as regard mean values of serum thyroid stimulating hormone (TSH), triiodothyronine (T3) and thyroxine (T4) using at the end of six weeks by student t- test.**

parameter	-ve Control	+ve Control	t	p-value
	Mean±SD			
TSH (ng/ml)	**0**.91±**0**.09	**0**.92±**0**.06	0.228	0.823 NS
T3 (ng/ml)	1.52±**0**.39	1.47±**0**.37	0.267	0.793 NS
T4 (µg/dl)	8.55±1.12	8.21±1.07	0.625	0.542 NS

N.B All values are expressed as mean±SD. (SD: standard deviation)
N:Number of rats in each group was 8 rats.
NS: P >0.05 =non significant.
t: t- test
µg/dl = microgram per deciliter.
ng/mg = nanogram per milligram.

Table (3): Statistical comparison between negative and **positive control groups as regard mean values of serum malondialdehyde (MDA), Glutathione peroxidase (GPX) and superoxide dismutase (SOD) at the end of six weeks by using student t- test.**

parameter	-ve Control	+ve Control	t	p-value
	Mean±SD			
MDA (nmol/ml)	**0**.66±**0**.18	**0**.61±**0**.15	0.617	0.547 NS
GPX (mu/ml)	170.38±11.33	170.63±11.89	0.051	0.960 NS
SOD (U/ml)	292.13±19.13	292.63±20.26	0.043	0.966 NS

N.B All values are expressed as mean±SD. (SD: standard deviation)
N:Number of rats in each group was 8 rats.
NS: P >0.05 =non significant.
t: t- test
nmol/ml = nanomole per millilitre.
U/ml= unit per millilitre.

B) Biochemical parameters of treated groups (zinc (II), Fipronil III) and Zinc with fipronil (IV) group**):**

Liver function tests:-

1) **serum alanine amino transferase (ALT):**

There was a very highly significant difference (p< 0.001) in the mean values of serum ALT among negative control, fipronil, zinc and Zinc with fipronil groups by ANOVA test as shown in **(Table 4 & Figure 1).**

There was a very highly significant increase (p< 0.001) in the mean values of serum ALT of fipronil treated group when compared with negative control group. Also there was a very highly significant decrease (p< 0.001) in the mean values of serum ALT of Zinc with fipronil group when compared with fipronil treated group by LSD test as shown in **(Table 4 & Figure 1).**

Moreover, there was a significant difference (p< 0.05) in the mean values of serum ALT of Zinc with fipronil group when compared with negative control group by LSD test as shown in **(Table 4 & Figure 1).**

2) **Serum aspartate transaminase (AST):**

There was a very highly significant difference (p< 0.001) in the mean values of serum AST among negative control, fipronil, zinc and Zinc with fipronil groups by ANOVA test as shown in **(Table 5& Figure 1).**

There was a very highly significant increase (p< 0.001) in the mean values of serum AST of fipronil treated group when compared with negative control group. Also there was a very highly significant decrease (p< 0.001) in the mean values of serum AST of Zinc with fipronil group when compared with fipronil treated group by LSD test as shown in **(Table 4 & Figure 1).**

Moreover, there was a non-significant difference (p> 0.05) in the mean values of serum AST of Zinc with fipronil group when compared with negative control group by LSD test as shown in **(Table 4 & Figure 1).**

3) **Serum alkaline phosphatase (ALP):**

There was a very highly significant difference (p< 0.001) in the mean values of serum ALP among negative control, fipronil, zinc and Zinc with fipronil groups by ANOVA test as shown in **(Table 4 & Figure 10).**

There was a very highly significant increase (p< 0.001) in the mean values of serum ALP of fipronil treated group when compared with negative control group. Also there was a very highly significant decrease (p< 0.001) in the mean values of serum ALP of Zinc with fipronil group when compared with fipronil treated group by LSD test as shown in **(Table 5 & Figure 1).**

Moreover, there was a non-significant difference (p> 0.05) in the mean values of serum ALP of Zinc with fipronil group when compared with negative control group by LSD test as shown in **(Table 5 & Figure 1).**

4) Serum lactate dehydrogenase (LDH) :

There was a very highly significant difference (p< 0.001) in the mean values of serum LDH among negative control, Fipronil, Zinc and Zinc with fipronil groups by ANOVA test as shown in **(Table 4& Figure 10).**

There was a very highly significant increase (p< 0.001) in the mean values of serum LDH of fipronil treated group when compared with negative control group. Also there was a very highly significant decrease (p< 0.001) in the mean values of serum LDH of Zinc with fipronil group when compared with fipronil treated group by LSD test as shown in **(Table 5 & Figure 10).**

Moreover, there was a non significant difference (p> 0.05) in the mean values of serum LDH of Zinc with fipronil group when compared with negative control group by LSD test as shown in **(Table 5 & Figure 10).**

Table (4): Statistical comparison among negative control, zinc, fipronil, and zinc with fipronil groups as regard mean values of serum alanine amino transferase (ALT) ,aspartate transaminase(AST), alkaline phosphatase (ALP) and lactate dehydrogenase(LDH) at the end of six weeks by using ANOVA test.

Group N=8 / Parameter	-ve Control	Zinc	Fipronil	Zinc+fipronil	F	P-value
	Mean ± SD					
ALT (U/L)	28.88±4.7	29.5±5.26	130.13±27.29	45.13±10.48	82.861	<0.001**
AST (U/L)	26.88±3.91	27.25±4.39	157.50±36.27	43.38±9.33	88.293	<0.001**
ALP (U/L)	53.75±9.36	50.75±4.89	518.00±97.67	105.25±21.41	160.86	<0.001**
LDH (U/L)	261.88±25.06	263.25±21.29	857.00±245.93	349.00±55.76	40.397	<0.001**

N.B All values are expressed as mean±SD. (SD: standard deviation)
N: Number of rats in each group was 8 rats.
**: statistically highly significant (P <0.001)
U/L= unit per litre

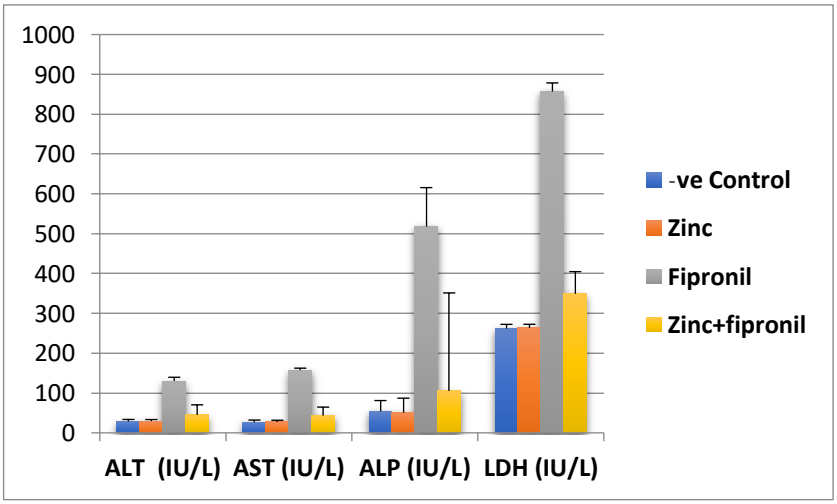

Figure (1):Bar chart showing comparison among negative control, zinc, fipronil, and zinc with fipronil groups as regard mean values of serum alanine amino transferase (ALT) ,aspartate transaminase(AST), alkaline phosphatase (ALP) and lactate dehydrogenase(LDH).

Table (5): Least significance difference (LSD) for comparison between mean values of serum alanine amino transferase (ALT), aspartate transaminase(AST), alkaline phosphatase (ALP) and lactate dehydrogenase(LDH) of negative control, zinc, fipronil, and zinc with fipronil groups at six weeks of the study.

Parameters	Group N=8	Zinc (29.5±5.26)	Fipronil (130.13±27.29)	Zinc+fipronil (45.13±10.48)
ALT (U/L)	-ve control (28.88±4.7)	**0**.934 NS	<0.001**	0.039*
	Zinc (29.5±5.26)		<0.001**	0.047*
	Fipronil (130.13±27.29)			<0.001**
AST (U/L)	Group	Zinc (27.25±4.39)	Fipronil (157.50±36.27)	Zinc+fipronil (43.38±9.33)
	-ve control (26.88±3.91)	**0**.969 NS	<0.001**	0.093 NS
	Zinc (27.25±4.39)		<0.001**	0.100 NS
	Fipronil (157.50±36.27)			<0.001**
ALP (U/L)	Group	Zinc (50.75±4.89)	Fipronil (518.00±97.67)	Zinc+fipronil (105.25±21.41)
	-ve control (53.75±9.36)	**0**.906 NS	<0.001**	0.050 NS
	Zinc (50.75±4.89)		<0.001**	0.039*
	Fipronil (518.00±97.67)			<0.001**

	Group	Zinc (263.25±21.29)	Fipronil (857.00±245.93)	Zinc+fipronil (349.00±55.76)
LDH (U/L)	-ve control (261.88±25.06)	0. 983 NS	<0.001**	0.181 NS
	Zinc (263.25±21.29)		<0.001**	0.188 NS
	Fipronil (857.00±245.93)			<0.001**

NS: statistically non significant (p>0.05). U/L= unit per litre
*: statistically significant (p<0.05).
**: statistically highly significant (P<0.001).
N:Number of rats in each group was 8 rats.

Oxidative stress biomarkers:-

Malondialdehyde (MDA):

There was a very highly significant difference (p< 0.001) in the mean values of serum MDA among negative control, Fipronil, Zinc and zinc with fipronil groups by ANOVA test as shown in **(Table 6& Figure 2).**

There was a very highly significant increase (p< 0.001) in the mean values of serum MDA of fipronil treated group when compared with negative control group. Also there was a very highly significant decrease (p< 0.001) in the mean values of serum MDA of zinc with fipronil group when compared with fipronil treated group by LSD test as shown in (**(Table 7& Figure 2).**

Moreover, there was a non-significant difference (p> 0.05) in the mean values of serum MDA of zinc with fipronil group when compared with negative control group by LSD test as shown in **(Table 7 & Figure 2).**

Serum Glutathione peroxidase (GPX):

There was a very highly significant difference (p< 0.001) in the mean values of serum GPX among negative control, Fipronil, Zinc and zinc+fipronil groups by ANOVA test as shown in **(Table 6).**

There was a very highly significant decrease (p< 0.001) in the mean values of GPX of fipronil treated group when compared with negative control group. Also there was a very highly significant increase (p< 0.001) in the mean values of GPX of zinc with fipronil group when compared with fipronil treated group by LSD test as shown in **(Table 7).**

Moreover, there was a non-significant difference (p> 0.05) in the mean values of serum GPX of zinc with fipronil group when compared with negative control group by LSD test as shown in **(Table 7).**

Superoxide dismutase (SOD):

There was a very highly significant difference (p< 0.001) in the mean values of serum SOD among negative control, Fipronil, Zinc and zinc with fipronil groups by ANOVA test as shown in **(Table 6).**

There was a very highly significant decrease (p< 0.001) in the mean values of serum SOD of fipronil treated group when compared with negative control group. Also there was a very highly significant increase (p< 0.001) in the mean values of SOD of zinc with fipronil group when compared with fipronil treated group by LSD test as shown in **(Table 7).**

Moreover, there was a non-significant difference (p> 0.05) in the mean values of serum SOD of zinc with fipronil group when compared with negative control group by LSD test as shown in **(Table 7).**

Table (6): Statistical comparison among negative control, zinc, fipronil and zinc with fipronil groups as regard mean values of serum malondialdehyde (MDA), Glutathione peroxidase (GPX) and superoxide dismutase (SOD) at the end of six weeks by using ANOVA test

Group N=8 / Parameter	-ve Control	Zinc	Fipronil	Zinc+fipronil	F	P-value
	Mean ± SD					
MDA (nmol/ml)	0.66±0.18	0.72±0.21	6.38±1.13	0.80±0.15	187.217	<0.001**
GPX (mu/ml)	170.38 ±11.33	172.00 ±11.55	100.50 ±15.98	159.50 ±9.55	60.293	<0.001**
SOD (U/ml)	292.13 ±19.13	291.25 ±21.67	106.13 ±10.95	275.63 ±24.85	166.360	<0.001**

N.B: All values are expressed as mean±SD. (SD: standard deviation)
N:Number of rats in each group was 8 rats.
**: statistically highly significant (P <0.001)
nmol/ml = nanomole per millilitre.
mu/ml = milliunit per millilitre.
U/ml= unit per millilitre.

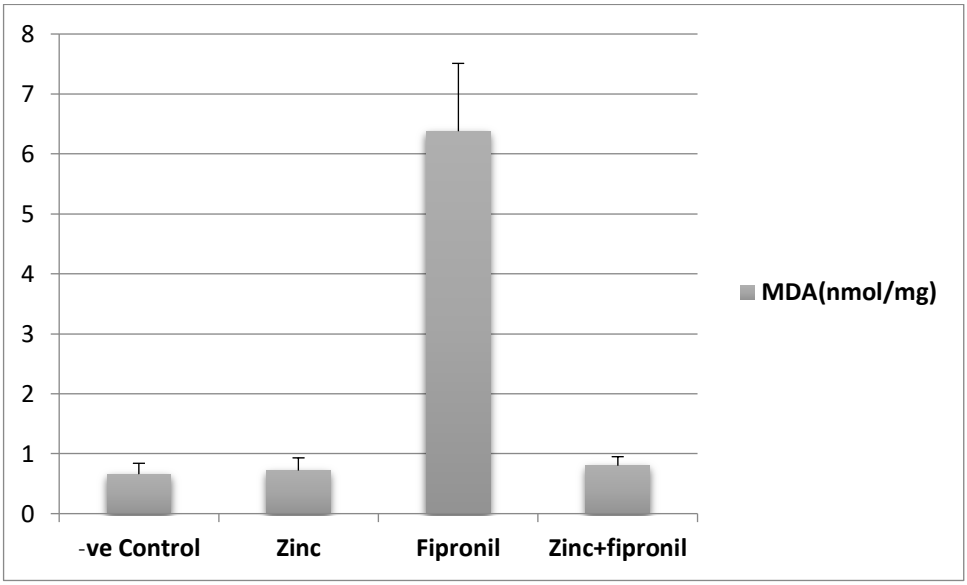

Figure (2):Bar Chart showing comparison among negative control, zinc, fipronil and zinc with fipronil groups as regard mean values of serum malondialdehyde (MDA).

Table (7): Least significance difference (LSD) for comparison between mean values of serum malondialdehyde (MDA), Glutathione peroxidase (GPX) and superoxide dismutase (SOD) of negative control, zinc, fipronil, and zinc with fipronil groups at six weeks of the study.

Parameters	**Group N=8**	**Zinc (0.72±0.21)**	**Fipronil (6.38±1.13)**	**Zinc+fipronil (0.80±0.15)**
MDA (nmol/ml)	**-ve control (0.66±0.18)**	0.836 NS	<0.001**	0.629 NS
	Zinc (0.72±0.21)		<0.001**	0.795 NS
	Fipronil (6.38±1.13)			<0.001**
GPX (mu/ml)	**Group**	**Zinc 172.00±11.55)**	**Fipronil 100.50±15.98)**	**Zinc+fipronil 159.50±9.55)**
	-ve control (170.38±11.33)	**0.946 NS**	<0.001**	0.089 NS
	Zinc (172.00±11.55)		<0.001**	0.052 NS
	Fipronil (100.50±15.98)			<0.001**
SOD	**Group**	**Zinc (291.25±21.67)**	**Fipronil (106.13±10.95)**	**Zinc+fipronil (275.63±24.85)**

(U/ml)	**-ve control** (292.13±19.13)	**0**.817 NS	<0.001**	0.107 NS
	Zinc (291.25±21.67)		<0.001**	0.126 NS
	Fipronil (106.13±10.95)			<0.001**

NS: statistically non significant (p>0.05). **mu/ml** = milliunit per millilitre.
: statistically highly significant (P<0.001). **nmol/ml = nanomole per millilitre.
N:Number of rats in each group was 8 rats **U/ml=** unit per millilitre

II- Histopathological results:

Liver

Control group (I) and zinc treated group (II):

Histopathological examination of the liver sections of adult male albino rats of negative control group (IA), vehicle control group (IB) and zinc treated group (II) showed the same histological features without any observable histopathological finding.

Macroscopic features:

Normal appearance of the liver was noticed with no cystic changes or abnormal masses, as well as cut sections were normal.

Microscopic features:

Examination of H&E stained liver sections of these group showed normal hepatic lobular architecture; hexagonal or pentagonal lobules with central veins and peripheral hepatic triads (portal areas) contained branches of the portal vein, hepatic artery, and bile duct within connective tissue stroma. Hepatocytes are arranged in cords running radially from the central vein. They had stippled appearance of the acidophilic cytoplasm and contained large pale vesicular nuclei. These cords were separated by sinusoidal spaces that were lined with the endothelial cells

Fipronil treated group (III):

Macroscopic features:

Macroscopic examinations of all liver specimens revealed no changes as compared to control group.

Microscopic features:

Examination of H&E stained liver sections of this group revealed morphological alterations in the form of hepatocytes' nuclei with characteristics of cell death processes with marginalized chromatin, hepatocytes with cytoplasmic vacuolization, periportal fibrosis with inflammation and hemorrhage in central vein

zinc with fipronil treated group(IV):

Macroscopic features:

Macroscopic examination of all liver specimens revealed no changes as compared to control group.

Microscopic features:

Microscopic examination revealed some improvement in histopathological changes indicated by normal central vein, minimal dark pyknotic nuclei with mild vacuolation, minimal blood sinusoidal congestion and minimal cellular infiltration.

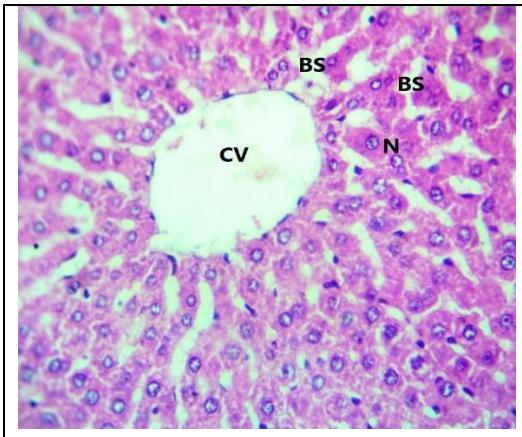

Fig. 3 A photomicrograph of a section in liver obtained from an adult male albino rat of the negative control group (group IA) showing normal hepatocytes containing pale nuclei (N) radiating from the central vein (CV) and separated by blood sinusoids (BS) (H&E x400).

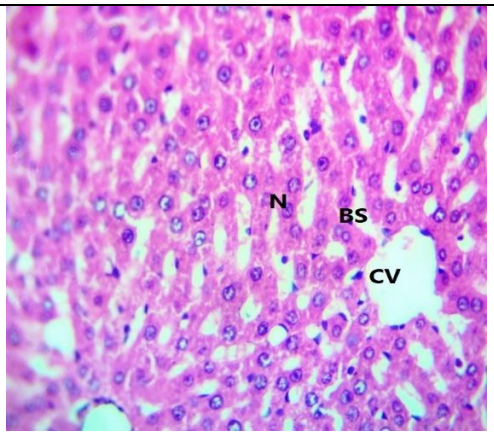

Fig. 4 A photomicrograph of a section in liver obtained from an adult male albino rat of zinc group (group II) showing normal hepatocytes (N) radiating from the central vein (CV) and separated by blood sinusoids (BS) (H&E x400).

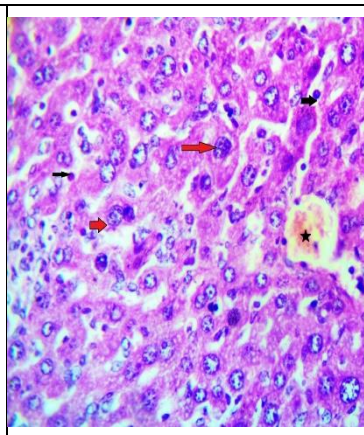

Fig. 5 A photomicrograph of a sec obtained from an adult male all fipronil group (group III) showing central vein (asterisk), hyperchromatism (red arrow) and (black arrow) (H&E x400).

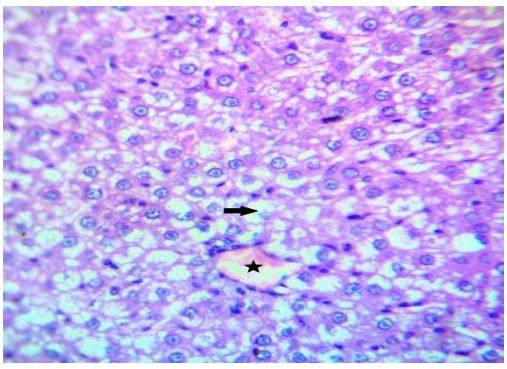

Fig. 6 A photomicrograph of a section in liver obtained from an adult male albino rat of fipronil group (group III) showing congested central vein (asterisk) and cytoplasmic vacuolar degeneration (black arrow) (H&E x400)

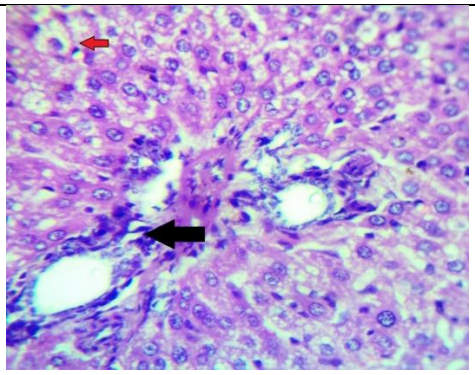

Fig. 7 A photomicrograph of a section in liver obtained from an adult male albino rat of fipronil group (group III) showing cytoplasmic vacuolar degeneration (red arrow), periportal fibrosis and inflammation (black arrow) (H&E x400)

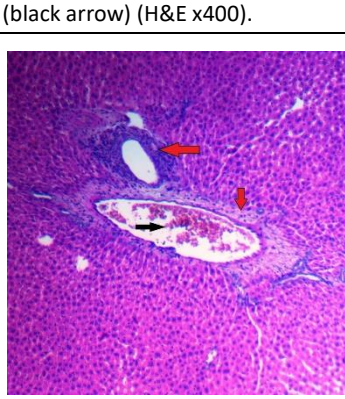

Fig. 8 A photomicrograph of a sec obtained from an adult male all fipronil group (group III) showing in with fibrosis (red arrow) and hemor central vein (black arrow) (H&E x10

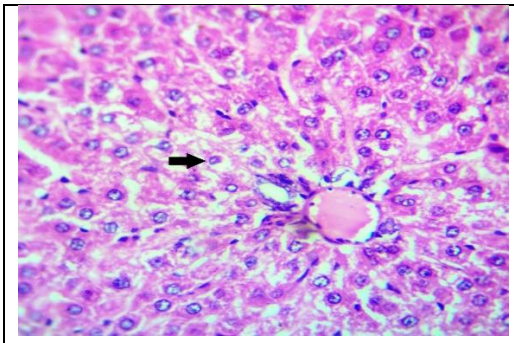

Fig. 9 A photomicrograph of a section in liver obtained from an adult male albino rat of zinc with fipronil group (group IV) showing remarkable improvement of hepatic tissue and central vein with mild cytoplasmic vacuolar degeneration (black arrow) (H&E x400)

III- Comet assay results:

Regarding the oxidative DNA damage caused by fipronil, the present study tested the *in vivo* genotoxic potential of fipronil in rats using the single cell gel electrophoresis (comet assay). The result revealed that oral administration of fipronil causes a time dependent increase in DNA damage in the thyroid and liver of adult male albino rats indicated by the damaged nuclei. The parameters used to measure DNA damage in the cells were the following: % of tailed nuclei, % of untailed nuclei, tail length (length of DNA migration), tail DNA % (percentage of migrated DNA in the tail) and unit tail moment (correlation between tail length and tail DNA %).

Comet assay of the liver:

The results of the present study showed no significant difference (P>0.05) among negative control group IA and the vehicle control group IB as well as within the same group as regard mean values of % of tailed nuclei, % of untailed nuclei, tail length, tail DNA % and unit tail moment in liver after 6 weeks of exposure. So, we used negative control group as a standard reference for comparison with other groups (Table 8).

Table (8): Statistical comparison between negative and **positive control groups as regard mean values of comet parameters in the liver at the end of six weeks by using student t-test.**

Group N=8 / parameter	-ve Control	+ve Control	t	p-value
	Mean±SD			
Tailed nuclei %	4.38±0.83	3.83±0.66	1.465	0.165 NS
Un tailed nuclei %	95.63±0.83	96.18±0.66	1.465	0.165 NS
Tail length (µm)	1.15±0.11	1.16±0.11	0.228	0.823 NS
Tail DNA %	1.26±0.13	1.23±0.15	0.527	0.606 NS

Unit tail moment	1.30**±0**.06	1.29**±0**.07	0.591	0.564 NS

N.B All values are expressed as mean±SD. (SD: standard deviation)
N:Number of rats in each group was 8 rats.
NS: P >0.05 =non significant.
%: percent
µm:Micrometer
t : t-test

There was a very highly significant difference (p< 0.001) in the mean values of % of tailed nuclei, % of untailed nuclei, tail length, tail DNA % and unit tail moment of the liver among negative control, Fipronil, Zinc and zinc with fipronil groups by ANOVA test **(Table 9 & Figs 10&11).**

Liver specimens of fipronil treated group revealed a very highly significant increase (p< 0.001) in % of tailed nuclei, tail length, tail DNA % and unit tail moment and a very highly significant decrease in % of untailed nuclei when compared with those of negative control group by LSD test as shown in **(Table 10 & Figs 10&11).**

Liver specimens of zinc+fipronil treated group revealed a very highly significant decrease (p< 0.001) in % of tailed nuclei, tail length, tail DNA % and unit tail moment and a very highly significant increase in % of untailed nuclei when compared with those of fipronil group by LSD test as shown in **(Table 10 & Figs 10&11).**

Moreover, the Liver specimens of zinc with fipronil treated group revealed significant differences in all comet parameters when compared with those of negative control group except tail moment parameters revealed non-significant differences by LSD test as shown in **(Table 10 & Figs 10&11).**

The comet assay results can be illustrated in the form of fluorescent photomicrographs as shown in **Figures (12-14).**

Table (9): Statistical comparison among negative control, zinc, fipronil and zinc with fipronil groups as regard mean values of comet parameters in the liver at the end of six weeks by using ANOVA test.

Group N=8 / Parameter	-ve Control	Zinc	Fipronil	Zinc+fipronil	F	P-value
	Mean ± SD					
Tailed nuclei %	4.38**±0**.83	4.06±1.21	13.75±1.38	5.81±1.41	109.629	<0.001**
Un tailed nuclei%	95.63**±0**.83	95.94±1.21	86.25±1.39	94.19±1.41	109.629	<0.001**
Tail length (µm)	1.15**±0**.11	1.21**±0**.10	2.72**±0**.09	1.296**±0**.197	255.109	<0.001**
Tail DNA %	1.26**±0**.13	1.25**±0**.09	3.09**±0**.36	1.54**±0**.23	121.681	<0.001**
Unit tail moment	1.45**±0**.19	1.50**±0**.13	8.41±1.14	2.02**±0**.44	237.573	<0.001**

N.B All values are expressed as mean ±SD. (SD: standard deviation)
N:Number of rats in each group was 8 rats.
**: statistically highly significant (P <0.001)

%: percent
µm:Micrometer

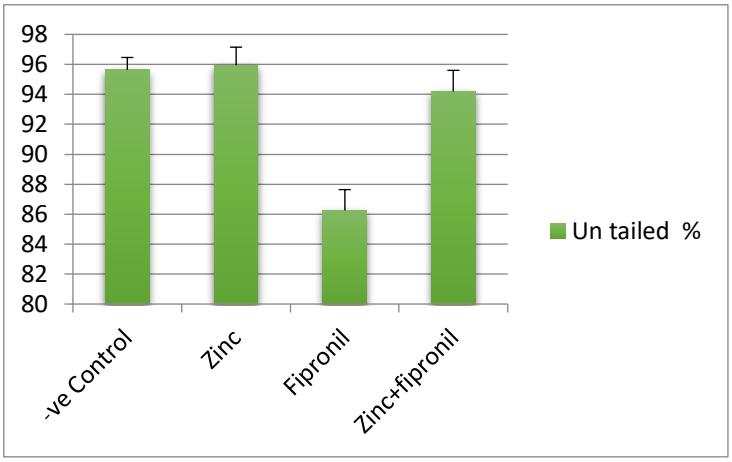

Figure (10):Bar chart showing comparison among negative control, zinc, fipronil and zinc+fipronil groups as regard mean values of untailed nuclei % in the liver.

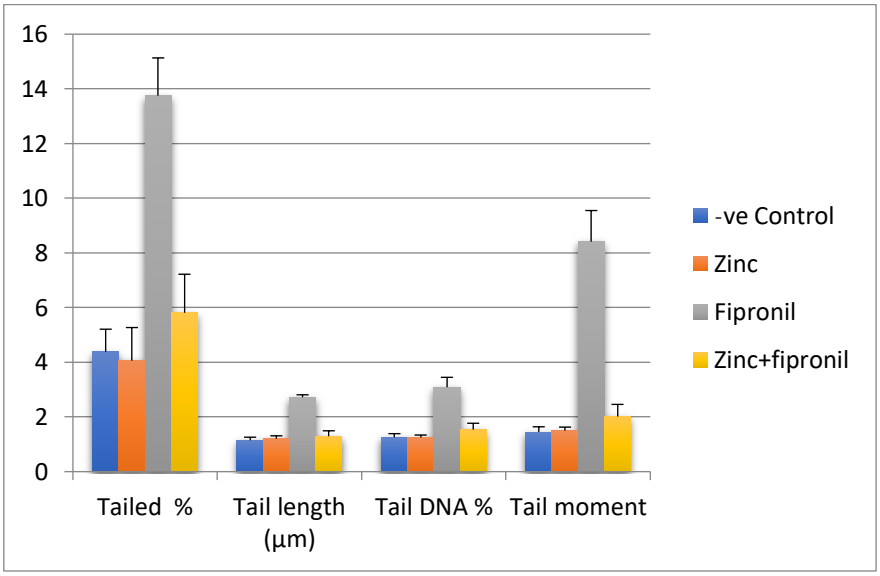

Figure (11):Bar Chart showing comparison among negative control, zinc, fipronil and zinc with fipronil groups as regard mean values of comet parameters in the liver.

Table (10): Least significance difference (LSD) for comparison between mean values of comet parameters in the liver of negative control, zinc, fipronil, and zinc with fipronil groups at six weeks of the study.

Parameters	Group N=8	Zinc (4.06±1.21)	Fipronil (13.75±1.38)	Zinc+fipronil (5.81±1.41)
Tailed nuclei %	-ve control (4.38±0.83)	0.616 NS	<0.001**	0.027*
	Zinc (4.06±1.21)		<0.001**	0.01**
	Fipronil (13.75±1.38)			<0.001**

Un tailed nuclei %	**Group**	**Zinc (95.94±1.21)**	**Fipronil (86.25±1.39)**	**Zinc+fipronil (94.19±1.41)**
	-ve control (95.63±0.83)	**0.616 NS**	**<0.001****	**0.027***
	Zinc (95.94±1.21)		**<0.001****	**0.01****
	Fipronil (86.25±1.39)			**<0.001****
Tail length (µm)	**Group**	**Zinc (1.21±0.10)**	**Fipronil (2.72±0.09)**	**Zinc+fipronil (1.296±0.197)**
	-ve control (1.15±0.11)	**0.345NS**	**<0.001****	**0.032***
	Zinc (1.21±0.10)		**<0.001****	**0.207 NS**
	Fipronil (2.72±0.09)			**<0.001****
Tail DNA %	**Group**	**Zinc (1.25±0.09)**	**Fipronil (3.09±0.36)**	**Zinc+fipronil (1.54±0.23)**
	-ve control (1.26±0.13)	**0.878 NS**	**<0.001****	**0.017***
	Zinc (1.25±0.09)		**<0.001****	**0.012***
	Fipronil (3.09±0.36)			**<0.001****
Unit tail moment	**Group**	**Zinc (1.50±0.13)**	**Fipronil (8.41±1.14)**	**Zinc+fipronil (2.02±0.44)**
	-ve control (1.45±0.19)	**0.853 NS**	**<0.001****	**0.077 NS**
	Zinc (1.50±0.13)		**<0.001****	**0.111 NS**
	Fipronil (8.41±1.14)			**<0.001****

NS: statistically non significant (p>0.05) **: statistically highly significant (P<0.001).
N:Number of rats in each group was 8 rats µm:Micrometer %: percent

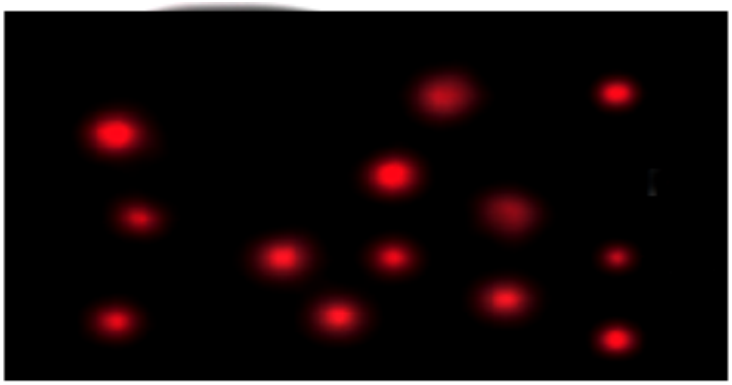

Figure (12): A photomicrograph by fluorescent microscope showing liver cells nuclei from adult male albino rats of negative control group (IA), DNA in most cells is tightly compressed and maintains the circular disposition of normal nucleus.

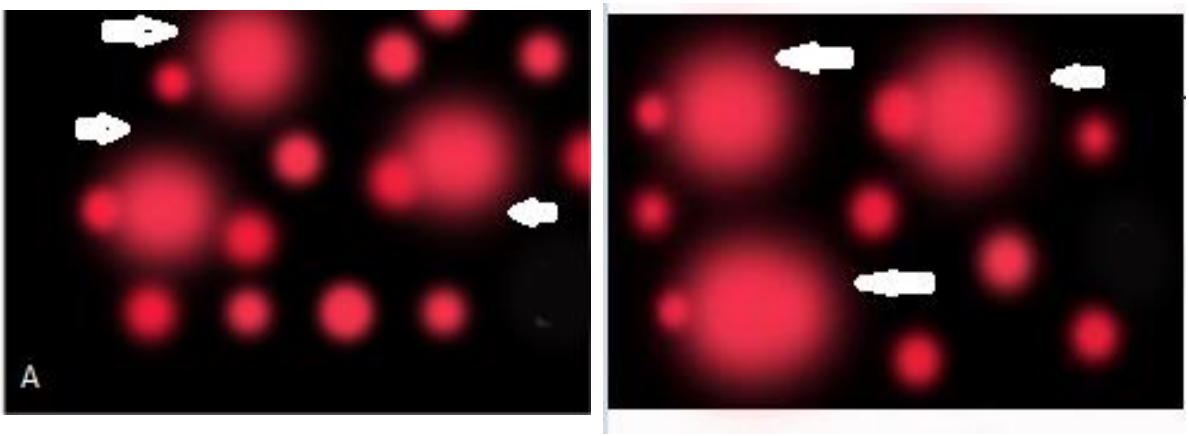

Figure (13): A photomicrograph by fluorescent microscope showing liver cells nuclei from adult male albino rats of fipronil group (group III),the comet has residual head and long dense tail pattern since most DNA migrated to tail (white arrow).

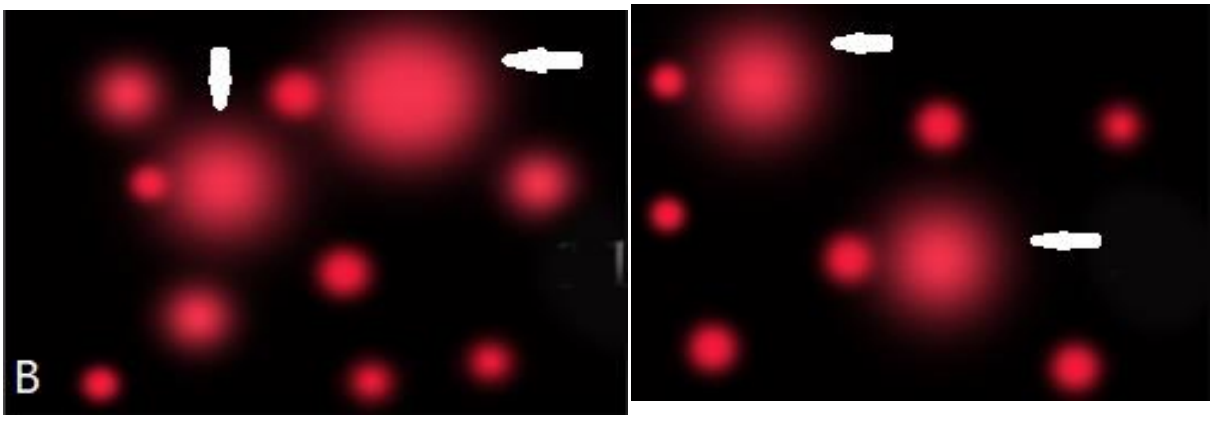

Figure (14): A photomicrograph by fluorescent microscope showing liver cells nuclei from adult male albino rats of zinc with fipronil group (group IV), the comet has residual head but shorter and less dense tail pattern as compared to fipronil treated group (white arrow).

Discussion:

Fipronil (FPN) is a phenylpyrazole insecticide widely used in agricultural, veterinary and public hygiene fields to control many insects. It is more effective than classical insecticides such as pyrethroids, organophosphates and carbamates **(13).**

Fipronil or its metabolites, exert neurotoxic effects through suppression of the inhibitory effect of gamma aminobutyric acid (GABA) by targeting $GABA_A$-regulated chloride channels. This inhibits chloride influx into nerve cells leading to hyperexcitation, paralysis, and death of insects **(14).**

Liver is one of the main target organs for FPN toxicity. Moreover, Exposure to fipronil caused thyroid tumors in rats via hypertrophy of thyroid follicles and altered the integrity of follicular cells, thyroid tissue and even the chemical composition of the colloid in mice **(15).**

Zinc is is necessary for proper liver function. It has antioxidant, anti-inflammatory, antiapoptotic properties and it yielded antagonistic effects on tumorigenesis by regulating many DNA repair genes **(16 & 17).**

Biochemical studies

1) Effect of fipronil on serum biomarkers of hepatic functions (ALT, AST & ALP &LDH):

The serum enzyme markers such as ALT, AST, and ALP are recommended for the assessment of hepatocellular injury in preclinical studies as it is considered a more specific and sensitive indicator of liver damage.

Low levels of ALT , AST and ALP are normally found in the blood, but when the liver is damaged or diseased, it releases these biomarkers with subsequent increase in their levels **(18).**

Serum alanine transaminase is a cytoplasmic enzyme presents in a higher concentration in the liver tissues than any other tissues in the body. It is relatively specific to the liver and early affected by hepatotoxicity , it is used as an excellent marker of cellular necrosis **(19, 20).**

The elevated activities of AST and ALT in serum are indicative of cellular leakage and loss of the functional integrity of cell membranes in the liver **(21).**

Alkaline phosphatase (ALP) enzyme is widely used as an indicator of hepatobiliary disease **(22).**

LDH enzyme is used to evaluate tissue damage of the affected organ and serum LDH is a biomarker of liver tissue lesions. Cell necrosis leads to increased LDH in tissue and serum. LDH release in the blood is an indicator of cell death and disintegration of cell membrane **(23).**

In the present study, there was a very highly significant increase ($P<0.001$) in the mean values of liver enzymes (ALP, AST, ALT and LDH) (p when compared with the negative control group at the end of the study.

The present results are in agreement with **Abou-Zeid et al. (13)** who indicated significant elevations in the activates of ALT, AST, ALP, and LDH after daily administration of fipronil at a dose level of 9.7 mg/kg for 28 days.

Also, these results are consistent with **Mossa et al. (24)** who found that FPN administered to albino male rats at concentrations 0.1, 1, and 10 mg/L in drinking water for 45 days induced highly significant increase of the activities of serum AST, ALT, ALP, and lactate dehydrogenase (LDH).

Similar findings were also reported by **Eid et al. (25)** who reported similar conditions of elevated AST, ALT, and ALP activities in the serum of adult male albino rats following oral administration of fipronil at a dose of 5 mg/kg b.w. by stomach tube 6 days a week for 28 days. They explained that elevation by oxidative stress induced by FPN, which led to hepatocytes damage, decreased blood protein production, and leakage of its enzymes into the circulation. Likewise, **Kartheek and David, (26)** reported that FPN caused a significant elevation in the levels of AST, ALT, and ALP in the serum of Wistar rats.

2) Effect of zinc and fipronil on serum biomarkers of hepatic functions (ALT, AST & ALP &LDH):

The results of the present work demonstrated that zinc administration with FPN resulted in a very highly significant decrease ($P<0.001$) in the mean values of serum ALT, AST & ALP &LDH of Zinc with fipronil group when compared with FPN treated group.

In agreement with our results, the study of **Mard et al. (27)** showed that pretreatment with ZnSO4 significantly decreased the increased serum concentrations of liver enzymes including AST,ALT, ALP, and LDH following hepatic ischemia-reperfusion (IR) injury. Ischemia was induced by surgery for 45 min followed by 60 min reperfusion with hepatic pedicle clamping.

In addition, these results are consistant with the studies of **Goel & Dhawan , (28)** and **Naqvi, (29)** who revealed that zinc pretreatment decreases the increased serum levels of liver enzymes following drug-induced hepatotoxicity such as chlorpyrifos and acetaminophen

Moreover, the results of **Yoshioka et al., (30)** indicated that multiple pretreatment with Zn reduced the increased ALT and guarded against acute pathological changes induced by CCl4 in the liver and postulated that the protection may be mediated by metallothionine induction.

<u>Effect of zinc and fipronil on serum oxidative stress biomarker (MDA) and antioxidative stress biomarkers (GPX &SOD):</u>

The results of the present work demonstrated that zinc administration with FPN resulted in a very highly significant decrease ($P<0.001$) in the mean values of serum MDA and a very highly significant increase ($P<0.001$) in the mean values of serum GPX & SOD of Zinc with fipronil group when compared with FPN treated group.

These results coincide with **Swelam et al., (12)** who reported that Co-administration of zinc at at concentration 227 mg/l to FPN-treated rats at a dose 2.0 mg/kg for 45 days through drinking water improved oxidative stress biomarkers such as GST (glutathione-s-transferase), SOD, CAT and GPx in the liver and kidney as compared to FPN treated rats.

Similar antioxidaive effects of zinc were observed by **Fedala, et al., (17)** who reported that co-treatment of Potassium dichromate **(**$K_2Cr_2O_7$) with Zinc improved oxidative stress biomarkers (SOD, MDA &GPX) in Wistar albino pregnant rats

Moreover, zinc antioxidant effect against other types of pesticide was observed by **Mansour et al., (31)** who demonstrated that zinc supplementation could alleviate oxidative stress exerted by low doses of individual pesticides (Methomyl , Abamectin) and their combination.

Further to this, Zn was found to exhibit a protective effect against nickel-induced glutathione and lipid peroxidation in brain cells of mice **(32).**

The present findings are in a line with the results of **Nazem et al., (33)** who reported that zinc supplementation increased both gene expression and enzyme activity of SOD as well as the levels of insulin in overweight type 2 diabetes patients and suggested that through the up regulation of SOD gene, zinc might be able to directly increase the activity of SOD enzyme, improve its function and ultimately enhance the antioxidant defense system.

As regard zinc effects on SOD, Zn is a structural component of SOD, which converts two superoxide ions into molecular oxygen **($O^{\cdot}_2$)** and hydrogen peroxide (H_2O_2) thus reducing its toxicity and convert a highly reactive species to a less harmful one **(34).**

According to **Álvarez-Barrios et al., (2021),** Zinc has a direct antioxidant effect by occupying iron and copper binding sites on lipids, proteins and DNA.

Moreover, the observed normalization of GPX activity after zinc treatment can be attribued to metallothionein inducion by zinc which is very rich in cysteine, an excellent scavenger as zinc is a potent inducer of metallothionein expression **(35)**, or due to indirect action of zinc in reducing the levels of reactive oxygen species **(12).**

In addition, zinc is an inhibitor of NADPH oxidases which catalyze the production of $O^{\cdot}_2$ from oxygen by using NADPH as the electron donor **(36).**

Moreover, zinc increases GSH synthesis by stimulating glutamyl-cysteine ligase expression **(37).**

Histopathological results:

Effect of fipronil on histopathological changes of liver:

The liver is the body's second largest organ. It plays a major role in metabolism and has a number of functions in the body, including glycogen storage, plasma protein synthesis, hormone production and detoxification of xenobiotics such as pesticides **(38).**

The hepatic histopathological findings in the current work confirmed the results obtained for the effect of FPN on alteration of liver function tests (AST, ALT, ALP& LDH).

As compared to control groups, FPN exposure induced several histopathological alterations in the liver in the form of hepatocytes with cytoplasmic vacuolization, inflammation and fibrosis around the portal area and central vein as well as hemorrhage in central vein, The nuclei of the hepatocytes showed hyperchromatism which could be associated with cell death processes due to the toxicity of the chemical products present in the body.

These results were in accordance with **Elgawish et al., (39)** who reported that administration of fipronil orally with a dose 9.7 mg/kg for 6 weeks caused inflammation and necrosis in the liver of rats mainly in the portal region, hydropic degeneration in hepatocytes and signs of cell death and apoptosis as chromatin marginalization and compaction.

Also, our results are supported with **Khalaf et al., (2)** who reported hepatic pathological changes as vacuolar degeneration of the hepatocytes surrounding the dilated central vein with congestion and dilatation in the bile duct upon administration of FPN (10.5 mg/kg) for 30 days in male rats.

These results are in agreement with **Refaie et al., (40)** who observed inflammation around the central vein and the portal area have inflammatory cells around their vessels in female rats after receiving fipronil orally at adose 9.7mg/kg b.wt. for 28 consecutive days.

The results also in agreement with **Elazab et al., (41)** who reported that oral fipronil with a dose (19.4 mg/kg BW; 1/5 of the oral LD_{50}) for 5 days exhibited disorganization in their hepatic cords with the formation of broad fibrous septa separating the hepatic lobules and leukocyte cell infiltration, besides congested blood vessels, the hepatocytes showed focal coagulative necrosis.

Furthermore, the observations of present work were confirmed by **Mossa et al ., (24)** who reported that after 45 days of exposure to fipronil in the drinking water at three concentrations 0.1, 1 and 10 mg/L, Severe histopathological alterations, including degeneration, infiltration, inflammatory cells, cell proliferation and focal hepatic hemorrhage were noted in the liver of male rats exposed to 10 mg/L of FPN. Rats exposed to 1 mg/L of FPN showed degeneration of hepatocytes and portal infiltration with inflammatory cells. Mild alterations, including, congestion, vacuolization and cystic dilation of bile duct were noted in the liver of male rats exposed to 0.1 mg/L of FPN.

In the present work, cytoplasmic vacuolations were showed in FPN treated rats. Similarly, **EL-Ballal et al., (42)** described hydropic degeneration of hepatocytes around central vein in fipronil treated rats with a dose (9.7 mg/kg) by oral gavage 3 times weekly for 8 weeks.

Correlating the histoarchitectural findings with antioxidant outcome, it is ascertained that decline in enzymatic threshold of ROS scavenging enzymes might have triggered the oxidation reactions thereby causing the catastrophic damage to the cells and tissues **(26).**

Effect of zinc and fipronil on histopathological changes of liver:

The results revealed some improvement in histopathological changes in zinc+FPN treated group indicated by normal central vein, minimal dark pyknotic nuclei, vacuolation, minimal blood sinusoidal congestion, few cell infiltration.

These results are in agreement with **Zia and Fatima (43)** who reported that zinc significantly decreased the histopathological changes induced by isoniazide and rifampicin such as steatosis, congestion, sinusoidal distension and kupfffer cell.

These results coincide with **Naqvi et al., (29),** who reported that the characteristic histological changes associated with acetaminophen toxicity such as centrizonal necrosis, steatosis, and sinusoidal enlargement were significantly prevented by zinc sulphate in a dose-dependent manner.

In agreement with our results, **Goel et al., (10)** have reported that zinc improved the histomorphological features of the liver of male rats in chlorpyrifos induced liver injury.

The results are also in accordance with **Shaikh et al., (44)** who observed that zinc reduces steatosis, necrosis and fibrosis in liver of rabbits induced by carbon tetrachloride by increasing the antioxidant levels of glutathione and catalase and also by decreasing the levels of superoxide dismutase.

III- Comet assay results:

The interaction of free radicals, aldehydes derived from lipid peroxidation and protein carbonyls with DNA may lead to the hydrolysis of chemical bonds, resulting in DNA fragmentation **(45).** Hence, metals tend to bind primarily with DNA and nuclear proteins, thus leading to the oxidative deterioration of biomolecules **(46).**

Effect of fipronil on comet assay results:

One of the key disciplines governing risk assessment of substances for human health is genotoxicology due to the fact that classic genotoxic substances lead to carcinogenesis **(47).** Genotoxicity testing, the evaluation of the carcinogenicity and mutagenicity of substances are the most important part of the safety testing of chemical compounds **(48).**

In recent years, single cell gel electrophoresis "the comet assay" has been shown to be a very sensitive technique and a useful tool that is being widely used to detect genetic damage at individual cell level and in human biomonitoring **(49).**

In the present study, comet assay performed on the liver specimens of FPN treated group revealed a very highly significant increase in % of tailed nuclei, tail length, tail DNA % and unit tail moment and a very highly significant decrease in % of untailed nuclei when compared with those of the negative control group.

The present study showed that FPN is a genotoxic substance manifested by hepatic DNA damage which was evaluated by comet assay. Densitometric and geometric parameters of the comets as determined using image analysis software revealed a very highly significant increase in % of tailed nuclei, tail length, tail DNA % and unit tail moment and decrease in % of untailed nuclei after FPN administration.

An excess of ROS could significantly increase DNA fragmentation **(50).** Similarly, **Khan et al., (51)** have found that higher doses of fipronil (5 and 10 mg/kg b.w.) orally for 4 weeks markedly reduced the DNA integrity of spermatozoa along with excessive ROS generation suggesting that FPN causes male reproductive toxicity through oxidative stress-induced DNA damage to spermatozoa.

In agreement with these results**, Al-Harbi, (52)** reported that FPN exposure to male rats at concentration 10 mg/L in drinking water for 30days resulted in damaged DNA strand breaks and damaged nuclei with appearance of more than one apoptotic cells with large tail and small head.

Similar toxic effects were observed by **Aldayel et al., (53)**, who detected a significant elevation in comet assay DNA damage %, DNA% in tail, and tail length in the lymphocytes of male rats received 9.7 mg/kg FPN for 30 days.

In addition, **Çelik et al., (54)** reported that by using the alkaline comet assay, all the doses of the FPN (0.7, 0.3, 0.1 mg/mL) induced DNA damage in human peripheral blood lymphocytes in vitro.

Moreover, the results of the present study are in a line with those of **Badgujar et al., (55)** who gavaged adult male and female mice with various doses of fipronil (2.5, 12.5, and 25 mg/kg body weight) to evaluate micronucleus test, comet assay and chromosome aberration (CA) and treated another group of animals with vitamin E orally (400 mg/kg bw) for 5 days prior to administration of fipronil (12.5 mg/kg). They reported that fipronil insecticide has clastogenic and mutagenic effects in male as well as female mice and attributed that to ROS-mediated oxidative stress.

In the same context, **Zhou et al., (56)** demonstrated FPN induced apoptosis and cell cycle arrest in porcine oocyte maturation because of increased ROS levels and DNA damage suggesting that the FPN may have potential detrimental effects on the female mammalian reproductive system.

In contrast, the study performed by **de Oliveira et al., (57)** revealed that doses of 15 mg/kg and 25 mg/kg of fipronil did not have genotoxic effects. Only the highest dose tested (50 mg/kg) induced DNA damage 24 h after exposure, indicating the mutagenic potential of fipronil.

2) <u>Effect of zinc and fipronil on comet assay results:</u>

In the present study, comet assay performed on liver specimens of zinc+fipronil treated group revealed a significant decrease in % of tailed nuclei, tail length, tail DNA % and unit tail moment and a significant increase in % of untailed nuclei when compared with those of the negative control group.

The results were in accordance with **Garcia et al., (58)** who stated that zinc therapy was able to prevent the increased DNA damage observed in spermatozoa from rats of the cigarette- smoking group as seen in the comet assay results and explained that by zinc antioxidant properties which can protect cells against ROS-provoked oxidative and electrophilic stress, damaging DNA or other important structures such as proteins or cell membranes.

These results are concomitant with the results of **Fedala et al., (17)** who found that zinc treatment has genoprotecive effect against $K_2Cr_2O_7$-induced hepatic DNA damage.

In addition, the study of **Song, et al., (59)** confirmed that both severe and marginal zinc deficiencies in vivo increased oxidative stress, impair DNA integrity and increase DNA damage in rat peripheral blood cells and that damage could be reversed upon zinc repletion.

Furthermore, zinc exhibited a marked impact in maintaining DNA integrity by preventing its oxidative damage and promoting its repair **(60).** Thereby, the prophylactic treatment with Zn promoted DNA repair in HeLa cells by revoking the inhibition of DNA-protein interactions exerted by cadmium **(61).**

Conclusion

In conclusion, this present study demonstrated that Fipronil is hepatotoxic substance as indicated by a significant increase in hepatic function tests along with marked histopathological change. Different mechanisms may be involved in the pathogenesis of systemic toxic effect of fipronil, and all could be attributed to lipid peroxidation and increased free radicals formation which are responsible for the deleterious tissue damage in different

organs. This can be confirmed by a significant elevation of serum MDA levels as well as a significant decrease in serum SOD &GPX levels, which is an indicator that FPN induced oxidative stress. Fipronil is a genotoxic substance as it caused significant DNA damage in liver cells evidenced by the damaged nuclei in comet assay. Zinc plays an important role in protection against fipronil liver toxicity evidenced by improving the biochemical, histopathological and comet assay results in groups treated with zinc and fipronil

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