The Protective Effect Of *Curcuma Longa* Rhizome On The Toxicity Of Fentromethrin In Mature Male Albino Rats

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ABSTRACT

This study was carried out to investigate the chemoprotective effect of ground rhizome of *Curcuma longa* plant on the possible toxicity induced by the insecticide fentromethrin (organophosphorous insecticide fenitrothion and the type II pyrethroid deltamethrin). One hundred and fifty two mature male albino Sprague Dawley rats were used in this study. Seventy two males were assigned for determination of medium lethal dose (LD_{50}) which was found to be 30.12mg/kg B.wt. Other eighty male albino rats were divided into four groups, the first group was control, the second, third and fourth groups were orally dosed with *C.longa* rhizomal powder(100mg/kg B.wt); 1/20 LD_{50} of fentromethrin and both of the same compounds and doses were given to the fourth group every other day for sixty five days. All rats were sacrificed at the end of the experiment. Blood sera and tissue samples from liver and brain were used to detect enzymatic and non enzymatic antioxidants beside histochemical detection of DNA and RNA in tissues of liver and brain. The study showed significant increase of malondialdehyde, highly significant decrease of reduced glutathione and catalase, in addition to significant reduction of vitamin E in tissues of liver and brain, but reduced glutathione remain within normal in brain of rats dosed with fentromethrin. Superoxide-dismutase and glutathione peroxidase were highly significant decreased in serum of fentromethrin treated animals. All these parameters showed improvement toward normal when rats were received *C, longa* rhizomal powder with fentromethrin. Also rats dosed with *C.longa* rhizomal powder alone revealed enhanced activities of reduced glutathione, catalase (especially in liver), superoxide dismutase and glutathione peroxidase in serum. The histochemical study showed reduced staining affinity of DNA and RNA in hepatocytes, cerebral cortical and piamater cells. It was improved in rats dosed with both *C. longa* rhizomal powder and fentromethrin and it was strong in the group administered *C. longa* rhizomal powder only. This research demonstrates that *C.longa* rhizome has an effective role to salvage fentromethrin toxicity effects on Sprague Dawley rats.

INTRODUCTION

Since 1970s, pyrethroids have been widely used as insecticide to control insect and pests in agriculture and in the home so many significant exposure among the general population was reported *(1).* Organophosphorous insecticides are used in combination with pyrethroid insecticides to enhance the later toxic effects on target insects, especially those developed pyrethroid resistance *(2).* Many cases of acute poisoning resulted from exposure to organophosphate-pyrethroid mixtures reported in Chinese medical journals in the late 1990s was about 4 times greater than the cases reported in the late 1980s *(3).*Deltamethrin is α-cyano pyrethroid, used to protect stored cereals, grains, coffee beans and dry beans, in forestry, and in public health. It is also used against cattle infestation *(4).* Fenitrothion is an organophosphorous insecticide widely used, to protect fruits, vegetables and grain crops, and for malaria control, but lead to high exposure potential to humans, livestock, and poultry, in both rural and residential environment *(5).* Fenitrothion is present in the air, soil and water in many countries *(6).*The toxicity of pesticides may be due to their free radical initiating capability *(7).* Over the past few years; there has been increasing interest in turmeric due to its medicinal properties. Turmeric is derived from

rhizomes of *Curcuma longa* plant, a member of the family zingaberaceae; it is widely used as a food colorant and is one of the principal ingredients in curry powder. Components of turmeric are named curcuminoids which include mainly curcumin (diferuloylmethane), demethoxycurcumin and bisdemethoxycurcumin *(8)*. Ayurvedic and Chinese medicine also traditional Indian medicine claim the use of curcumin powder against biliary disorders, coryza, cough, sinusitis, diabetic wounds, hepatic disorders, rheumatism as anti-inflammatory, digestive disorders, treatment of skin diseases and wound healing. Also reduce certain forms of cancer and render other protective biological effects in humans, which are attributed to its active constituent curcumin *(9,10)*. Oral administration of curcumin and turmeric over two years to rats at a dose above hundred times than that consumed by human not induced any toxicity *(11, 12)*. Curcumin has been reported to have several pharmacological effects *(13)*. It inhibits tumor metastases, pancreatitis, drug or alcohol-induced liver fibrosis, cystic fibrosis and Alzheimer's disease *(14)*. Since oxidative stress imposed by reactive oxygen species (ROS) is known to play a crucial role in the pathophysiology associated with neoplasia, atherosclerosis, and neurodegenerative diseases, the potential mechanism of the protective effects of phenolic compounds and flavonoids (where *C. longa* rhizome is rich in these constituents) were thought to be due to direct scavenging of free radicals *(15)*.

MATERIAL AND METHODS

Experimental animals

One hundred and fifty two mature male albino Sprague Dawley rats, weighing 120-150gm body weight, were purchased from laboratory animals breeding Unit, National Research Center, Dokki, Egypt. The rats were accommodated to laboratory conditions for two weeks before beginning the experiment.

Tested compounds

I- *Curcuma longa* Linn. rhizome powder

C. longa L. named as turmeric, botanically related to Zingiberaceae family, is a perennial branched rhizomes and brownish-yellow colour *(16)*. Curcumin, known as curcumin I, makes up approximately 90% of the curcuminoid content (curcumin, monodexmethoxycurcumin & bisdesmethoxycurcumin) in turmeric *(17)*.

II- Fentromethrin

It consists of a mixture fenitrothion (260gm) which is an organophosphorothioate insecticide and deltamethrin (26gm/liter) which is a type II pyrethroid.

Determination of the oral Medium Lethal Dose (LD_{50})

Thirty six male albino rats were divided into 6 groups each of 6 rats in separate cages for the pilot trial for determination of zero and 100% mortality of fentromethrin then another 36 male rats were divided into 6 groups each of 6 rats in separate cages for determination of LD_{50} of fentromethrin according to the previously described method *(18)*.

Study the effect of fentromethrin and *C. longa* rhizome powder on male albino rats

Eighty male albino rats were divided into four groups each contain twenty rats. The first group was served as control. The second, third and fourth groups were dosed by rat stomach tube every other day for 65days as the 2nd group administered *C. longa* rhizome powder (100mg/kg B.wt) suspended in distilled water, the 3rd group received 1/20 LD_{50} of fentromethrin diluted in distilled water and the 4th group received both of the previously doses of *C. longa* rhizomal powder and fentromethrin with at least 2 hrs apart. The rats were sacrificed at the end of the experiment, blood samples were centrifuged at 3000rpm for 15 minutes to obtain serum. The clear supernatant serum was transferred into dry sterile and labeled stoppered vials and stored at -20°C until used for estimation of superoxide dismutase *(19)* and glutathione peroxidase *(20)*.

The liver and brain were divided into two equal parts. The first parts were homogenized with ice-cooled saline, then divided into two aliquots, the first part of the homogenate was deproteinized with ice-cooled 12% trichloroacetic acid, centrifuged at 3000 rpm for 15min and the obtained supernatant was used for

the estimation of malondialdehdye (MDA) *(21)*, GSH *(22)*, catalase *(23)*, and both vitamins A and E *(24)* . The second aliquot was centrifuged and the supernatant was used for estimation of thiobarbituric acid reactive substances (TBARS).The second part of each brain and liver were dissected and preserved in Carnoy's fixative for histochemical detection of DNA and RNA *(25)*.

Statistical analysis: One way ANOVA& T-test model procedures of SAS were done *(26)*.

RESULTS

The zero and 100% mortality of albino rats were shown in Table 1. Mortalities and solution of the dose response in male albino rats were recorded in Table 2.

Table 1. Clarified the doses which not induce any mortality (Zero% mortality) also the doses which produced 100% mortality in the experimental trials for detection of LD_{50} of fentromethrin in male Sprague-Dawley rats

Groups	Dose(mg/kg B.wt)	No. of rats in each group	No. of dead rats	Mortality %
Control	0	6	0	0
2	17.39	6	0	0
3	20.4	6	0	0
4	24.8	6	2	30
5	37.2	6	6	100
6	52	6	6	100

Table 2. Determination of the acute LD_{50} of fentromethrin in Sprague- Dawley rats

Groups	Dose (mg/kg B.wt)	Log dose	Log dose interval	No. of rats in each group	No. of dead	Mortality %
control	0	0	0	6	0	0
2	17.39	1.24054	0.11	6	0	0
3	22.4	1.35054	0.11	6	0	0
4	28.8	1.46054	0.11	6	2	30
5	37.2	1.57054	0.11	6	6	100
6	47.9	1.68054	0.11	6	6	100
Total				36	14	

Solution of LD_{50} of fentromethrin in male albino rats *(18)*:
Using the following formula:
$m = X_i + ½d - dr_i/N$
Where: $m =$ log.LD_{50}. X_i= log- dose causing 100% mortality.
d= logarithmic intervals of doses.
r_i= Sum of the number of animals dead at each of the individual dose levels.
N= Number of animals on each of the dose levels.
LD_{50} with 95% limits of confidence = 30.12(27.13mg- 33.4) mg/kg B.wt.

Rats treated with fentromethrin showed significant increase of malondialdehyde (MDA) in liver and brain in comparison to control group respectively. Rats dosed with *C.longa* rhizomal powder and fentromethrin, MDA in liver and brain were improved and become very near to the control. The group treated with *C. longa* rhizomal powder revealed highly significant decrease of MDA in liver, non significant decrease in brain, when compared to control group (Table 3). Superoxide dismutase (SOD) and glutathione peroxidase (GPx) in serum of treated rats with fentromethrin revealed highly significant decrease (Table 4). Glutathione and

catalase levels were significantly decreased in liver and brain but glutathione level in brain remain within the normal limits comparing to the control group (Table 5). Vitamin E was significantly decreased in both liver and brain of rats treated with fentromethrin in comparsion to control. Rats orally dosed with *C. longa* rhizomal powder and fentromethrin showed non-significantly increased levels of SOD and GPx. Normal level of GSH were observed in brain but insignificantly decreased in liver and normal catalase level in liver while it was significantly decreased in brain. *C.longa* rhizomal powder treated group showed normal levels of GSH, increased catalase level than the control in brain, while in liver catalase was significantly increased and GSH was non- significantly increased (Table 4). Both SOD and GPx were significantly increased in serum of rats treated with *C. longa* rhizomal powder in comparison to control group (Table 3).The levels of vit A and E were within the normal levels in both liver and brain of all treated groups (Table 5).

Table 3. Showing the level of serum superoxide dismutase (SOD) (U/ml serum) and glutathione peroxidase (GPx) (nmol/min/ml serum) of rats treated with Fentromethrin (1/20 LD_{50}), Fentromethrin with *Curcuma longa* rhizomal powder (100mg/kg B.wt.) and *Curcuma longa* rhizomal powder only comparing with control group along two months.

Parameter / Group	Superoxide-dismutas (U/ml serum)	Glutathione peroxidase (nmol/min/ml serum)
Control	0.15 ± 0.005^{b}	110 ± 2.5^{b}
Fentromethrin treated	0.12 ± 0.006^{c}	86.7 ± 2.7^{c}
C. longa rhizome powder + Fentromethrin treated	0.16 ± 0.01^{ab}	115 ± 2.1^{ab}
C. longa rhizome powder treated	0.175 ± 0.006^{a}	122 ± 1.7^{a}

Means within the same column carrying different superscripts are significant at $p\leq 0.05$.

Table 4. Showing the level of Malondialdehyde (MDA) (µm/gm tissue); Reduced Glutathione (GSH) (µm/gm tissue) and Catalase (U/mg protein) in liver and brain of rats treated with Fentromethrin (1/20 LD_{50}), Fentromethrin and *Curcuma longa* rhizomal powder and *Curcuma longa* rhizomal powder (100mg/kg B.wt.) only comparing with control group along two months.

Parameter / Group	Malondialdehyde(µm/gm tissue)		GSH(µm/gm tissue)		Catalase(U/mg protein)	
	Liver	Brain	Liver	Brain	Liver	Brain
Control	0.51 ± 0.024^{b}	1.08 ± 0.055^{b}	465.3 ± 19^{ab}	516.7 ± 5.99^{a}	465.3 ± 19^{ab}	516.7 ± 5.99^{a}
Fentromethrin treated	0.68 ± 0.023^{a}	1.75 ± 0.036^{a}	327 ± 13.5^{c}	458.8 ± 24.4^{a}	327 ± 13.5^{c}	458.8 ± 24.4^{a}
C. longa rhizomal powder + Fentromethrin treated	0.48 ± 0.046^{b}	1.16 ± 0.074^{b}	410 ± 28^{b}	467.76 ± 19^{a}	410 ± 28^{b}	467.76 ± 19^{a}
C. longa rhizome powder treated	0.23 ± 0.022^{c}	1 ± 0.076^{b}	481 ± 10.7^{a}	499.75 ± 14.97^{a}	481 ± 10.7^{a}	499.75 ± 14.97^{a}

Means within the same column carrying different superscripts are significant at $p\leq 0.05$.

Table 5. Showing the level of Vitamin E (µg/gm) and Vitamin A in liver and brain of rats **treated with Fentromethrin (1/20 LD_{50}), Fentromethrin with *Curcuma longa* rhizomal powder (100mg/kg B.wt.) and *Curcuma longa* rhizomal powder only comparing with control group along two months.**

Parameter / Group	Vit E(µg/gm)		Vit A(µg/gm)	
	Liver	Brain	liver	Brain
Control	5.36±0.13[a]	7±0.35[a]	0.5 ± 0.03[a]	0.62 ± 0.05[a]
Fentromethrin treated group	4.4±0.19[b]	5.9± 0.13[b]	0.53 ± 0.07[a]	0.53 ± 0.05[d]
C. longa powder + Fentromethrin treated group	5.1±0.33[a]	6.9±0.06[a]	0.52 ± 0.05[a]	0.54 ± 0.07[a]
C. longa powder treated group	5.5± 0.09[a]	7.3±0.17[a]	0.55 ± 0.03[a]	0.63 ± 0.04[a]

Means within the same column carrying different superscripts are significant at $p \leq 0.05$.

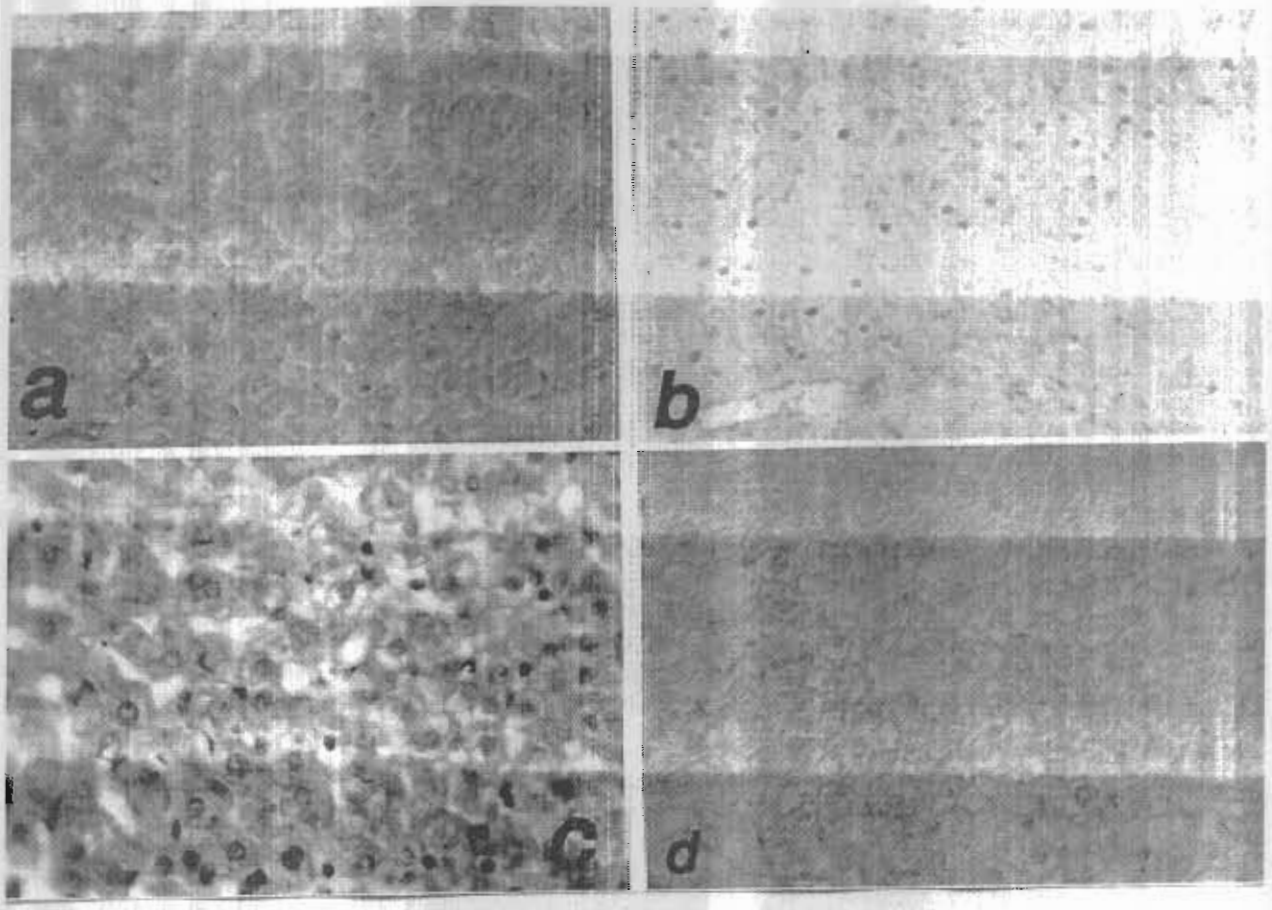

Fig. 1 Photomicrograph for a section in the liver of rats showing:
a) Normal distribution of RNA (in red) & and DNA (in green) through hepatocytes of control rats.
b) Low DNA level of the hepatocyte cells of rats administered 1/20$LD_{50}$of fentromethrin for 65days in comparsion to control, degenerated cells.The RNA is severely disrupted .
c) Slightly lesser reaction of both DNA and RNA in the hepatocytes of rats administered *Curcuma longa* rhizomal powder (100mg/kg B.wt) and fentromethrin in comparsion to control , but stronger than after administration of fentromethrin.
d) A better picture even than control liver in both DNA and RNA distribution through the hepatocytes of rats administered *Curcuma longa* rhizomal powder (100mg/kg B.wt).
Methyl green-pyronin method. X400.

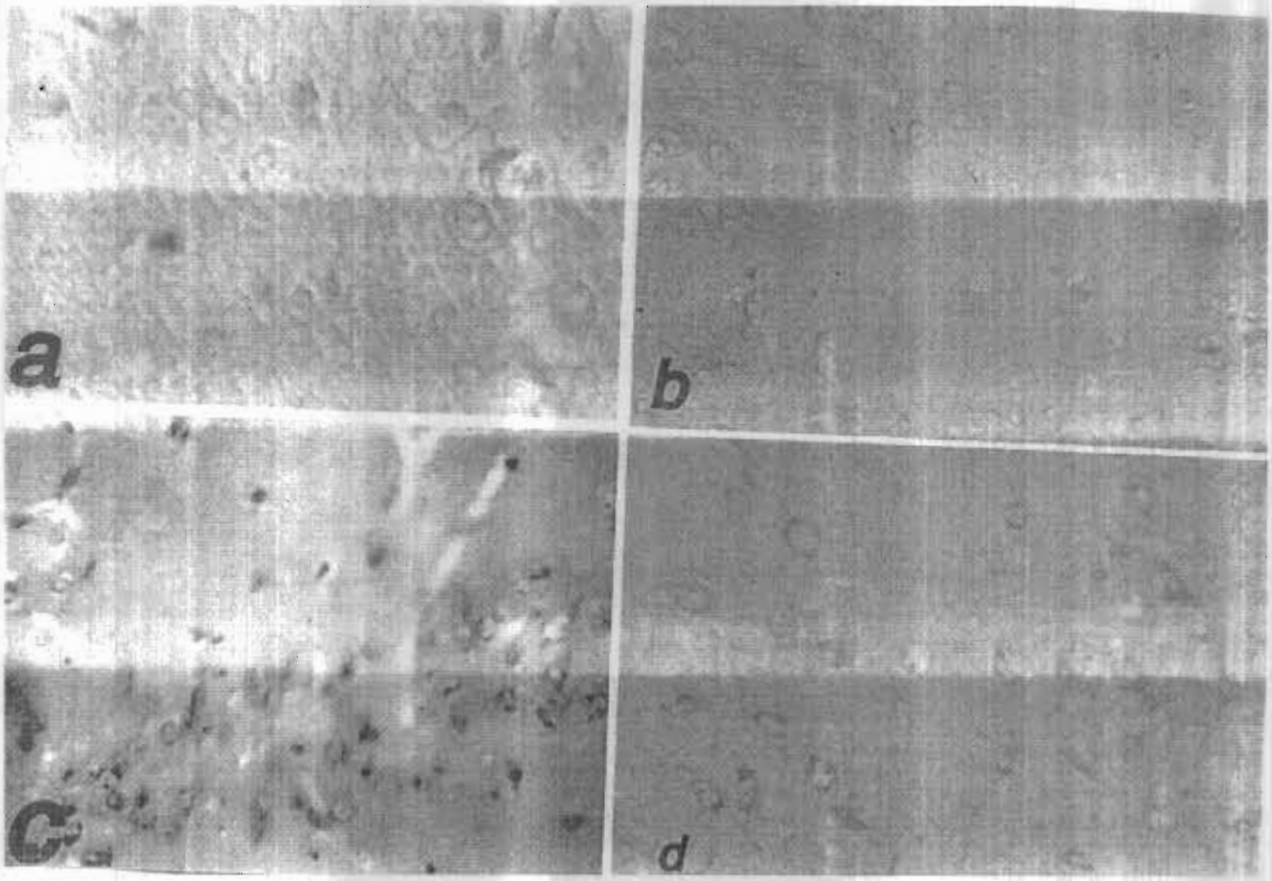

Fig. 2. Photomicrograph for a section in the brain of rats showing:

a) Normal distribution of RNA (in red) & and DNA (in green) through cerebral cortical, cerebral neurons (n), and piamater cells (p).

b) Cortical neurons and piamater cells after administration of 1/20 LD_{50} of fentromethrin for 2 months revealing more or less as in control.

c) Cerebral cortical neurons (n) and piamater supporting cells after administration of *Curcuma longa* rhizomal powder (100mg/kg B.wt) for 2 months revealing stronger reaction of DNA more than RNA.

d) Cerebral cortical neurons of rats administered *Curcuma longa* rhizomal powder (100mg/kg B.wt) and fentromethrin(1/20 LD_{50}) for 2 months revealing revealing stronger reaction of RNA(in red) in comparsion to those given fentromethrin alone but still the DNA is lower than control. Methyl green-pyronin method, X400.

The histochemical study revealed that DNA and RNA reaction is well apparent in nuclei and cytoplasm of hepatocytes of control group (Fig 1a).While in the brain the DNA reaction of control rats is indistinct in nuclei of cerebral cortical neuronal nuclei except strong reaction in piameter cell nuclei as supporting cells (Fig 2a).The DNA and RNA stainability is stronger than control after administration of *C.longa* rhizomal powder only as shown in hepatocytes (Fig 1d), cerebral neuronal cells and piamater supporting cells, but not RNA (Fig 2d).After administration of Fentromethrin, the picture of DNA staining affinity in the hepatocytes is some, what lesser than in control (Fig 1b), also the RNA is negative with many degenerated cells that lost their nuclei. The RNA is severely disrupted. In addition DNA and RNA reaction in brain become lesser than in control. The liver showed lesser reaction (Fig 1c) in both DNA and RNA in group treated with fentromethrin and *C. longa* rhizome powder than control but stronger than the liver of rats treated with fentromethrin alone. In brain there was an improved picture in cerebral neurons as the reaction to RNA was more apparent in cytoplasm than the reaction of DNA.

DISCUSSION

Although insecticides are economically important chemicals, their presence in food, water and the environment can present a real health risk to humans and animals *(27)*. Organophosphorus insecticides can induce oxidative stress and generate free radicals that altered antioxidant enzymes *(28)*.Also reactive oxygen species (ROS) are included in the toxicology of pyrethroids *(29)*.In the present work the first experiment done to evaluate the LD_{50} of fentromethrin. The obtained results clarified the LD_{50} was equal to 30.12mg/kgB.wt. No available data was found about the LD_{50} of fentromethrinin in previous studies, but information present about LD_{50} of deltamethrin alone, it is equal to 66.7mg/kg B.wt *(30)*. In the same line the LD_{50} of fenitrothion was found equal to 166.6 mg/kg B.wt *(31)* and range between 250-800 mg/kg **(32)**.Our results revealed that MDA was significantly increased in both liver and brain of male rats dosed with fentromethrin. Fenitrothion was very powerful inducer of lipid peroxidation and decreasing the enzymatic and non-enzymatic antioxidant *(33)*. In fact deltamethrin may induce cell damage due to its plasma membrane lipopholicity, as pyrethroids can bind to membrane lipid bi-layers and produce membrane depolarization in the rat synaptosome, toxicity by deltamethrin can cause perturbations in lipid- lipid and lipid- protein interactions, cause alterations of membrane permeability and mitochondrial enzyme activities *(34)*. Other group dosed with *C. longa* rhizome powder followed by fentromethrin revealed near normal levels of MDA in both liver and brain and it was greatly decreased in rats dosed with *C. longa* powder only which provides a convincing evidence for the involvement of reactive oxygen species (ROS) in fentromethrin toxicity and the possible role of *C. longa* in scavenging these radicals. Our results were in accordance with *(35)* who stated that curcumin has antilipid peroxidation effect in rat brain and *(36)* who revealed that curcumin inhibits lipid peroxides in rat liver microsomes, brain homogenates and erythrocyte membrane. Our results about the toxic effect of fenitrothion were convenient to previous informations *(37)*, but about deltamethrin confirmed earlier study *(27)*. Regarding GSH and catalase levels, our results demonstrated highly significant decrease in liver and brain but reduced glutathione remain within normal levels in brain of rats treated with fentromethrin, these results were indicative of oxidative stress and presence of free radicals produced by insecticides, and somewhat were ameliorated in rats treated with *C. longa* powder and fentromethrin. The ameliorative effect is suggested to be due to antioxidant property of *C. longa* possibly by increasing the endogenous defense to combat oxidative stress induced by fentromethrin as evidenced by more improved levels of reduced glutathione and catalase in rats dosed with *C. longa* powder only than control rats.GSH is involved in protection against free radicals, peroxides, singlet oxygen, hydroxyl radical, superoxide radical damage and other toxic components *(38)*.The fact that organophosphorus insecticides consume GSH through a detoxification reaction and/or that glutathione-s-transferase catalyzes the reaction between GSH and xenobiotics, so regulating possible harm *(39)*. As formation of xenobiotic-GSH complex lead to decrease of GSH content, also inhibition of catalase activity is suggestive of increased superoxide anion (O_2^-) synthesis. Regarding *C. longa*, these results were inconvenience with the effect on liver damage induced by alcohol *(40)*. In addition increased levels of reduced glutathione has been recorded in cerebral cortex and hippocampus of rats dosed with high dose of deltamethrin, but the author found unchanged catalase levels which disagreed with our obtained results *(41)*.

In concern to vitamins A and E, our study revealed significant decrease in vit E in liver and brain of group treated with fentromethrin, indicating low antioxidative defense; these altered vitamins showed modulation in rats dosed with powder of *C. longa* rhizome before dosing with fentromethrin which explained by the antioxidant effectiveness of the ground rhizome in addition to curcumin has been ten times more active as an antioxidant than vitamin E *(42)*. Both vitamins A and E are lipid soluble antioxidants and their decrease indicated oxidative stress due to lipid peroxidation. They

convert the peroxyl radical to the much less reactive hydroperoxide, so inhibit the propagation of peroxidative chain reactions. The Vit E radical is reduced by glutathione and/ or ascorbic acid to regenerate Vit E. In addition β-carotin (provitamin A) has the ability to inactivate singlet molecular oxygen ***(43).***

The present work showed highly significant decrease of both SOD and GPx in the serum of rats dosed with the insecticide fentromethrin, while they insignificantly increased in the group dosed firstly with powder of *C. longa* rhizome then with fentromethrin and significantly increased in rats dosed with the rhizome powder only. These results indicated that fentromethrin has harmful effect on SOD and GPx by decreasing their levels due to oxidative stress and the possible counteraction by *C. longa* rhizome's powder. Organophosphate pesticides caused a decrease in GSH -Px activity both *in vivo* and *in vitro* ***(44)***. Turmeric can lower lipid peroxidation by maintaining the activities of antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase at higher levels which play an important role in the regulation of lipid peroxidation; also it is capable of scavenging oxygen free radicals such as superoxide anions and hydroxyl radicals ***(45),*** peroxynitrite radicals ***(46),*** it inhibits hydrogen peroxide induced cell damage ***(47).*** Concerning the histochemical study, this study revealed that the reaction for RNA in the cytoplasm of hepatocytes was strong after co- treatment with fentromethrin and *C.longa* rhizomal powder and the strongest after *C.longa* rhizomal powder alone, so indicating the hepatoprotective effect of *C.longa* rhizomal powder. Superoxide radical ($O_2^{\bullet -}$) which may be produced as a result of oxidative stress due to fentromethrin toxicity, this radical is responsible for lipid peroxidation and also decrease the activity of other antioxidant defense system enzyme such as catalase (CAT) and glutathione peroxide (GPx), which causes damage of ribonucleotides which are required for DNA synthesis. Hydrogen peroxide (H_2O_2) is not a radical but behave the same way like superoxide($O_2^{\bullet -}$) by causing DNA damage, membrane disruption and release calcium ions within cell, resulting in calcium dependent proteolytic enzyme to be activated ***(48).*** Curcumin can protect against DNA strand breakage and DNA oxidative degradation ***(49).*** Hence, agreeing with the previous findings ***(50, 51)*** which showed that curcumin reduces hepatic tissue damage which may be induced by nicotine, iron, aflatoxins and hepatic carcinogenicity. Also curcumin protects DNA against oxidative damage induced by singlet oxygen ***(52)***. Moreover curcumin increases the total DNA content in cases of protein stress condition through the hepatocytes ***(50)***. It has been proposed that curcumin can protect lipids, hemoglobin and DNA against oxidative degradation due to its potent scavenging activity against a variety of reactive oxygen species, including superoxide anion, hydroxyl radical, singlet oxygen, nitric oxide and peroxy nitrite ***(53).*** ROS are considered to be endogenous mitogenic factors or apoptotic factors that can activate nuclear factor kabba B(NF-KB) and other transcription factors in the nucleus which determine the fate of cell such as proliferation, inflammation, carcinogenesis or apoptosis, curcumin block their sites and pathways ***(54)***. In general, the reaction for DNA and RNA with their different degrees in both nuclei and cytoplasm were the same in liver but with brain less reaction for DNA and RNA was noted that may be related to the larger nuclei of neurons which may withstand or inhibit the effect of fentromethrin.

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الملخص العربى

التأثير الواقى لجذور نبات الكوركوما لونجا على التسمم بالفنترومثرين فى ذكور الفئران البيضاء البالغة

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انصبت هذه الرسالة على دراسة التأثير الوقائى للكوركوما لونجا على الفئران البيضاء المتسممة بمبيد الفنترومثرين وهو عبارة عن خليط من المبيد الفوسفورى الفنتروثيون والمبيد البيروثرويدى الدلتامثرين حيث أجريت هذه الدراسة على مائة واثنان وخمسون من ذكور الفئران البيضاء، استخدم منهم اثنان وسبعون فأرا لحساب الجرعة النصف مميتة خلال مدة 96ساعة والتى قدرت ب 30.12مجم/كجم من وزن الفأر. تم إجراء التجربة الثانية على عدد 80 فأرا تم تقسيمهم إلى 4 مجموعات تحتوى كل مجموعة على 20 فأرا وتم تجريعهم عن طريق الفم يوم بعد يوم لمدة 65 يوما كالتالى:

المجموعة الأولى استخدمت كمجموعة ضابطة سلبية، المجموعة الثانية تم تجريعها بجذور الكوركوما لونجا المطحونة مضافة إلى الماء بجرعة(100مجم/ كجم من وزن الجسم)، المجموعة الثالثة تم تجريعها ب1 /20 من الجرعة النصف مميتة للفنترومثرين والتى تم تقديرها سابقا مخففا بالماء أما المجموعة الرابعة فقد تم تجريعها بالجرعة السابقة لمطحون جذور الكوركوما لونجا يليها الفنترومثرين بعد ساعتين من الجرعة الأولى. تم ذبح الفئران فى نهاية التجربة وأخذ عينات من الدم لفصل السيرم وعينات من الكبد والمخ لقياس الأنزيمات المضادة للأكسدة وأيضا تم إجراء الفحص الهستوكيميائى على الأنسجة السابقة لتقدير

محتواها من الحامض النووى الديوكسى ريبوسى(DNA) والحامض النووى الريبوسى(RNA) وقد أوضحت الدراســـة النتـائـج الآتيـــــة:

زيادة معنوية فى مستوى المالون داى ألدهيد بكبد ومخ الفئران المجرعة بمبيد الفنترومثرين بينما يقل ليكون قريبا من معدله الطبيعى فى المجموعة المجرعة بكلا من مطحون جذور الكوركوما لونجا والفنترومثرين ولكن سجل انخفاضا بالكبد والمخ فى الفئران المجرعة بمطحون جذور الكوركوما لونجا فقط مقارنة بالمجموعة الضابطة, ولقد سجل الجلوتاثيون المختزل انخفاض معنوى شديد بكبد الفئران المجرعة بمبيد الفنترومثرين ويظل منخفضا ولكن بما يقارب المعدل الطبيعى فى الفئران المجرعة بمطحون جذور الكوركوما لونجا ثم الفنترومثرين، بينما لم يحدث أى تغير بالجلوتاثيون المختزل بالمخ فى جميع المجموعات المجرعة وأيضا بكبد المجموعة المجرعة بمطحون جذور الكوركوما لونجا.أوضحت الدراسة وجود نقص معنوى شديد لمستوى الكاتاليز فى المجموعة المجرعة بالفنترومثرين بينما حدث تحسن للكاتاليز فى الفئران المجرعة بمطحون جذور الكوركوما لونجا والفنترومثرين و يظل فى معدله الطبيعى مع بعض التحسن فى كبد ومخ المجموعة المجرعة بمطحون جذور الكوركوما لونجا. وقد ظل معدل فيتامين(أ) وفيتامين (هـ) طبيعيا فى الكبد والمخ فى جميع المجموعات ماعدا وجود نقص معنوى لفيتامين (هـ) فى الفئران المجرعة بمبيد الفنترومثرين. أظهرت النتائج أيضا حدوث نقص معنوى شديد فى كلا من السوبر أوكسيد ديسميوتيز والجلوتاثيون بيرأوكسيديز فى مصل الفئران المجرعة بمبيد الفنترومثرين، بينما زاد كلا الإنزيمين زيادة غيرمعنوية تقارب المعدل الطبيعى لهما فى مصل الفئران المجرعة بمطحون جذور الكوركوما لونجا والفنترومثرين، أما الفئران المجرعة بمطحون جذور الكوركوما لونجا فقط فقد زادوا زيادة معنوية مقارنة بالمجموعة الضابطة.

بالنسبة للفحص الهستوكيميائى فقد لوحظ انخفاض نسبة (دى- إن- إيه)و (أر- إن- إيه) فى الخلايا الكبدية والقشرة المخية بصورة واضحة وانخفض معدلهما ولكن بصورة أقل عند تجريع الفئران بكلا من الكوركوما لونجا والفنترومثرين حيث أوضح (دى- إن- إيه)و (أر- إن- إيه) تفاعلاً أقوى مع صبغة البيرونين-ميثيل جرين بينما لوحظ تحسن فى تفاعل (دى- إن- إيه)و (أر- إن- إيه) فى الخلايا الكبدية والخلايا العصبية والخلايا الداعمة للأم الحانية مما يوضح التأثير الوقائى لجذور الكوركوما لونجا فى حال تجريعه للفئران.