NEUROGENOTOXICITY EFFECT OF DELTAMETHRIN AND AMELIORATIVE EFFECT OF VITAMIN E & GREEN TEA ON FEMALE RATS

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Abstract

Several studies have been implicated oxidative stress and genotoxicity as important mechanisms of toxic effects of pyrethriods in the present study. The beneficial effects of vitamin E and green tea extract were tested in protecting the deltamethrin (DM) induced oxidative stress, genotoxicity and alterations in some amino acid neurotransmitters in brain of adult female rats .Rats were exposed to 1mg/Kg b.wt .deltamethrin (1/10 LD_{50}) alone or in combination with vitamin E and/or green tea extract .The antioxidant such as catalse enzyme, reduced glutathione content, lipid peroxidation content and ChE activity were analyzed in cerebrum and cerebellum of brain excised at the end of experiment (30 days). Results showed that genotoxic effect of deltamethrin was proved by SSCP of HRas and P53 genes .Amino acids transmitters were estimated in brain of exposed DM rats . Exposure to deltamethrin resulted in a significant decrease in the activities of catalase, ChE and concentration of reduced glutathione, while the LPO levels were significantly increased in different brain regions .Also, DM exposure increases significant changes in brain amino acids. It is Interestingly that the supplementation with vitamin E or green tea extract or both resulted reversed in the deltamethrin induced antioxidant enzymes and effect on genotoxicity .Combination of both green tea and vit. E appeared to be more effective in protection than each one alone .

Keywords :Deltamethrin, Vitamin E, Green tea, Oxidative stress, Neurotoxicity, Genotoxicity

1 .INTRODUCTION

The widespread use of pesticides in public health protection and agricultural programs have caused severe environmental pollution and health hazards, particularly in developing countries, including cases of severe acute and chronic human and animal

poisoning as well as damage to other non–target organisms: The use of pyrethroid has been increased because of the restrictions placed on many of the organophosphorous insecticides (Assayed *et al.,* 2010).

The mechanism by which pyrethroids are thought to exert neurotoxicity is by prolonging the opening of Na^+channels The toxicity of pyrethroid insecticides to mammals has received much attention in recent years because of animals exposure to these insecticides showed changes in their physiological activities and pathological features.Pyrethroids are reported to generate free radicals through hydrolytic ester cleavage and oxidative pathway by the CYP450 enzymes Induction of oxidative stress has been reported with pyrethroids such as Cypermethrin, Deltamethrine and fenvalerate (Raina *et al.,* 2010).

A major contribution of non–enzymatic protection against lipid peroxidation is vitamin E (α–tocopherol) a fat soluble antioxidant is a powerful chain–breaking antioxidant which plays a major protective role against oxidative stress and prevents the production of lipid peroxides by scavenging free radicals in biological membranes (Hfaiedh *et al.,* 2012).

Polyphenols present abundantly in vegetables, fruits have been recognized as functionally active molecules, possessing antioxidant, anticancer and antimutagenic properties as well as, exerting protective effects against several diseases .Green tea extract (GTE)represents the richest source of natural polyphenols including catechins, aflavins and rubigin (Frei and Higdon, 2003).Polyphenols found in green tea show 20 times more powerful antioxidant activity than vitamin C .Green tea polyphenols (GTP) have demonstrated a protective effect against a spectrum of offensive oxidants, like superoxide and peroxy nitrite radicals. It was postulated that the supplementation of green tea attenuated the cyclosporine A and tamoxifen–induced oxidative stress and protected against the liver injury in rats .This study was planned to evaluate the role of Vit. E and/or green tea extract as protective agents against deltamethrin–induced oxidative stress, genotoxicity and alterations in some amino acids in brain of exposed rats

2 .MATERIALS AND METHODS

2.1 .Animals:

Fifty albino rats of 3 months age and weighing between 150–170 g were obtained from the breeding unit of the Toxicology and Forensic Medicine Department, Faculty of Veterinary Medicine, Cairo University .The animals were housed in plastic cages and fed a standard laboratory diet and water *ad libitum* .The animals were exposed to 12

hrs light/12 hrs dark cycle and temperature (25 ±2 °C) Rats were acclimatized for one week prior to the start of experiment. All animals experiments were carried out in accordance with the guide of the care and use of laboratory animals published by the national institute of health and approved by the animal experiments local ethics committee at the Cairo University.

2.2 .Chemicals:

Technical grade deltamethrin (DM, 98.8 %pure)was supplied by KZ pesticides company, Egypt .Green tea ; a Lipton green tea unilever brand, packed in the United Arab Emirates by unilever Gulf FZE, was dissolved in the drinking water at the concentration of 5 $mgml^{-1}$ .Tea was prepared freshly three times per week and stored at 4 °C until use .The control of drinking vessels was renewed every day .Green tea extract contains epigallocatechin gallate (33.7 mg L^{-1})epigallocatechin (268 mgL^{-1}), epicatechin (90 mgL^{-1})epicatechin gallate (60 mg L^{-1})and caffiec acid (35 mg L^{-1}) as determined by HPLC method (Maiani *et al.,* 1977) Vitamin E(α–tocopherol) was obtained from Sigma Aldrich chemicals, St .Louis, Mo, USA.Biodiagnostic Kits for determination of catalase activity, reduced glutathione, lipid peroxidation and acetyl cholinesterase activity were obtained from biodiagnostic company, Egypt.

2.3 .Estimation of Median Lethal Dose (LD_{50}).

Twenty four mature female rats were orally administered deltamethrin with different four concentration (6, 8, 10 and 12 mg/kg.) Six rats were kept as control group throughout the entire experimental period .Mortality was counted in the different groups .LD_{50} was calculated ad follows equation (LD_{50} =Largest dose – ΣaxB/N; where a :the mean of dead rats between two successive doses, b :dose difference between two successive doses, N :the total number of rat per group) according to Behrens and Karbers (1935).

2.4 .Animals and Treatment.

Fifty experimental mature female rats were randomly assigned into five equal groups; Group 1 :rats were received orally an equivalent volume of corn oil based on body weight .Group 2 :rats were orally administered $^1/_{10}$ LD_{50} of deltamethrin (1mg/Kg b.wt)dissolved in corn oil Group 3 :rats were orally administered deltamethrin in a dose level of 1mg/Kg b.wt beside 1ml of Green tea extract via gastric incubation.Group 4 : rats were orally administered the same dose of deltamethrin beside vit. E in dose level 200mg/Kg b.wt dissolved in corn oil via oral incubation.Group 5 :rats in this group were

orally administered deltamethrin in the same previous dose beside 1ml green tea extract and vit. E in the same dose .Deltamethrin was given to the experimental groups of rats followed by green tea extract or vit. E or both after $^1/_2$ hour for 30 days.

2.5 .Tissue preparation and Methods:

After the last treatment, rats were fasted for 6 hrs .then subjected to light anesthesia and sacrificed by cervical dislocation. Brain was immediately removed, washed using chilled saline solution then perfused with phosphate buffer saline (50 mM potassium phosphate, pH 7.4, containing 0.16 $mgml^{-1}$ heparin)to remove any red blood cells and clots .The tissue was homogenized in 5−10ml cold buffer (i.e 50mM potassium phosphate which composed of 9.4 ml of 1M mono basic solution and 40.6ml of 1M dibasic solution and complete to 1L by distilled water, pH 5.1, 1mM EDTA)per gram tissue using tissue homogenizer and centrifuged at 4000 rpm/15min at 4°C .The supernatant was washed and subjected to assay the activity of catalase (Aebi., 1984), reduced glutathione (Beutler *et al.,* 1963) and acetylcholinesterase (Ellman *et al.,* 1961)Lipid peroxidation (LPO) was measured by estimation of thiobarbituric acid reactive substance (TBARS)method of Ohkawa *et al.(*1979).

2.5.1 .Genomic DNA isolation:

Extraction of genomic DNA was done from cerebrum and cerebellum samples using QIAamp DNA Mini Kit (Qiagen)according to the manufacturer instructions.

2.5.2 .Polymerase chain reaction (PCR).

PCR reactions were performed in a 20 µl volume containing 1 µl DNA (approx 100 ng) as template, 2µl 10x reaction buffer (Promega, UK) 2µl 2mM dNTPs, magnesium chloride 1mM,0.2 µl of each primer pair (200 ngµl −1) forward GTG GTA CCG TAT GAG CCA CC and reverse CAA CCT GGC ACA CAG CTT CC) 1 unit Go Taq polymerase (Promega,UK) PCR cycling was as follows :94°C for 5 min, 35 cycles of 94°C for 1 min, :59°C for 1 min and 72°C for 1 min; with a final 10 min at 72°C.PCR was generated to amplify a 157 bp fragment flanking exon 7 as described by Gouda *et al*. (2008) and 72 bp of H Ras flanking exon 12 forward primer GGAGACCCTGTAGGAGGACCC and reverse primer TCTATAGTGGGGTCGTATTCGTCC Sakamoto *et al.* (2011).

2.5.3 .Single strand conformation polymorphism (SSCP).

Aliquots (2 µl)from the PCR reaction were mixed with 10 µl of SSCP dye)95 % formamide, 5 mM sodium hydroxide, 0.1 % bromophenol blue, 0.1 %xylene cyanol(heated to 95°C for 5 min, immediately chilled on ice and the samples were then electrophoresed in 15 %PAGE in 1x TBE buffer at 150 V for 5 min, then at 80 V at room

temperature until the blue dye reached the bottom of the gel (Liechti–Gallati *et al.,* 1999).The gel was stained with ethidium bromide (1 mg/ml)for 2 min and then destained in deionized water for 15 min .Gels were visualized under a UV transilluminator and photographed.

2.5.4 Brain amino acid concentrations:

Amino acid in cerebrum and cerebellum homogenates were estimated by HPLC using percolumn PTC derivatization technique according to Heinrikson and Meredith (1984).

2.6 .Statistical analysis.

The GRAPHPAD (ISI Software, Philadelphia, PA, USA) computer program was used to conduct regression analysis and to plot the collected data .Data were expressed as means standard error of means) SE . (Assessment of the results was performed using one–way analysis of variance (ANOVA)procedure followed by Tukey–Kramer multiple comparison post–tests .Statistical analyses were performed using Software GRAPHPAD INSTAT (Version 2)The 0.05 level of probability was used as the criterion for significance.

3 .RESULTS AND DISCUSSION

3.1 .Effect of deltamethrin on brain biochemical parameters:

The activity of catalase (CAT) concentration of reduced glutathione (GSH)and lipid peroxidation (LPO)expressed as (MDA)and activity of Acetylcholinesterase (ChE)in cerebrum and cerebellum of experimental rats were recorded in tables (1 and 2)Treatment with deltamethrin for 30 days resulted in a significant (P≤0.05)decrease in the activities of catalase and Acetylcholinestrase and concentration of reduced glutathione, while the level of lipid peroxidation showed significant (P≤0.05)increase in the two regions of exposed rats brain in comparison with the control group .The co–administration of green tea extract or vitamin E alone or in combination with deltamethrin normalized the elevation of lipid peroxidation and reduction in the level of GSH content, activities of CAT and ChE in brain of exposed rats in addition to decrease of LPO level in comparison to deltamethrin treated group in both cerebrum and cerebellum.

Deltamethrin is widely used in agriculture and public health preservation programs for farm animals and pests .It is one of the environmental pollutants which showed a broad spectrum toxicological effects and biochemical dysfunction constituting serious

hazards to health. Reactive oxygen species (ROS) including free radicals and other highly reactive form of oxygen are produced in cells during normal metabolic processes involving oxygen. ROS are released during cellular respiration, processes of biosynthesis and biodegradation, biotransformation of xenobiotics and phagocyte activation (Yaduvanshi *et al.,* 2010) However, the levels of ROS may be significantly increased by exposure to different environmental toxins including pesticides .The reaction of free radicals and oxidants with lipid, protein and DNA produces potentially harmful effects . Results of the present study clearly reveal that Deltamethrin exposure causes increase in the level of lipid peroxidation in brain tissue of rats At the same time, deltamethrin exposure induced a significant decrease in the activity of catalase enzyme and the level of reduced glutathione in brain tissue of exposed rats .Deltamethrin induced an increase in the level of lipid peroxidation which is indicative of involvement of free radical mediated mechanism in its toxicity.

The elevation in lipid peroxidation and the reduction in the antioxidant enzyme (CAT) and glutathione content in rat brain might be attributed to the metabolic activation of Deltamethrin, which is considered as a major mechanism of its toxicity . Deltamethrin caused significant oxidative stress in brain tissue of exposed rats as was evident by the elevation of lipid peroxidation level and reduced level of total glutathione .Catalase is considered as one of the most important defense mechanism against toxic effects of oxygen metabolism .CAT helps in the removal of H_2O_2 formed during the reaction catalized by SOD enzyme .Many by–products of oxygen metabolism initiate different outcomes at the subcellular level .The superoxide radical has been shown to inhibit the activity of GP_x and CAT activity .Enzymes that scavenge oxygen free radicals like CAT and SOD decreased by 50 %upon pesticide exposure (Gabbianelli *et al.,* 2002).

Glutathione is an important antioxidant agent in most cells .It plays a key role as a cofactor with variety of enzymes including GP_x .Glutathione depletion has been shown to intensify lipid peroxidation and predispose cells to oxidant damage .A significant depletion of glutathione along with a concomitant decrease in the activity of CAT was noted in the present study .In addition, GSH also participates in the detoxification of xenobiotics as a substrate for the enzyme glutathione–S–transferase (Kale *et al.,* 1999).

The marked neurotoxic effect of deltamethrin may be attributed to that central nervous system is particularly susceptible to toxic effects of ROS due to low levels of antioxidant enzymes and glutathione, high concentration of iron and readily oxidizable

substances such as polyunsaturated fatty acids and catecholamine, high rate of oxidative metabolic activity and highest accumulation of thiobarbituric acid reactive substance (TBARS.) In the present research, data collected on brain ChE activity proved that deltamethrin is an effective anticholinesterases agent, since the enzyme activity was inhibited in brain tissue in deltamethrin–treated group .This finding runs in parallel with the previous studies on pyrethroid insecticides (Hussien *et al.,* 2013)Several investigators have attributed this inhibition to attachment of pyrethroids to the ends of cholinergic nerve fibers that inhibit release of actylecholinestrase .This effect might also be due to inhibited AChE synthesis and/or increased degradation in brain tissue by deltamethrin intoxication.

Many insecticides are hydrophobic molecules that bind extensively to biological membranes, especially phospholipid bilayers and they may damage the membranes by inducing lipid peroxidation. Since Vit. E is known to be antioxidant, a number of studies have been performed to determine whether they can ameliorate the toxic effects of pesticides .In the present study coadminist ration of Vit. E with deltamethrin induced marked protective effect against the neurotoxicity of deltamethrin in exposed rats .This protective effect was manifested by the ameliorative brain damage besides improving the level of antioxidants and reduced level of lipid peroxidation .Vit. E (α–Tocopherol) is the major lipid–soluble antioxidant and is known to protect cellular membranes and lipoproteins from peroxidation (Yavuz *et al.,* 2004)may effectively minimize lipid peroxidation in biological systems .Vit. E allows free radicals to abstract a hydrogen atom from the antioxidant molecule rather than from polyunsaturated fatty acids, thus breaking the chain of free radicals reaction .Our results regarding the protective effect of Vit. E against the neurotoxicity of deltamethrin in rats were in accordance with data performed by Ali (2012).

The results from this study suggest that green tea extract (GTE) exhibits antioxidant effects against Deltamethrin–induced oxidative stress by preventing not only elevated MDA levels but also depleted GSH levels and by attenuating the depletion of antioxidant enzyme activities (CAT) It has been reported that phenolic compounds, such as catechins of GTE can react with superoxide radicals via one electron transfer mechanism or by a hydrogen mechanism to form corresponding semi-quinon .In addition, polyphenols can inhibit xanthine oxidase. This is possible due to its content of polyphenols that are characterized by their ability to scavenge free radicals produced during the aging process as well as ethanol metabolism .Efficacy of their activity in

other tissues such as liver and blood has already been demonstrated (Dobrzynska *et al.,* 2005).

Tea catechins are strong scavengers against superoxide hydrogen peroxide, hydrogen radicals and nitric oxide produced by various chemicals (Khalaf *et al.,* 2012) .The most abundant polyphenols such as epigallocatechingallate and epicatechingallate contained in green tea scavenge a wide range of free radicals including the most active hydroxyl radical, which may initiate lipid and protein oxidative modification . Furthermore, the green tea polyphenols have been demonstrated to inhibit iron–induced oxidation of synaptosomes by scavenging hydroxyl radicals generated in the lecithin/lipooxidasesystem

Table 1. Effect of deltamethrin on Cerebrum biochemical parameters.

Groups	Control	Deltamethrin	Deltamethrin +Green Tea	Deltamethrin +Vit E	Deltamethrin + Green tea +Vit E
CAT (**U**/**g** .**Tissue**)	354.05 ± 23.80	265.28 ± 15.26 [a]	335.91 ± 13.95 [b]	346.69 ± 15.18 [b]	345.65 ± 8.84 [b]
GSH(**mg**/**g** .**Tissue**)	18.98 ± 8.49	14.25 ± 6.37 [a]	23.52 ± 10.52 [a,b]	24.62 ± 11.01 [a,b]	25.43 ± 11.37 [a,b]
LPO(**nmole**/**g** .**tissue**)	15.97 ± 0.73	22.41 ± 1.49 [a]	16.69 ± 0.34	17.29 ± 1.83	16.01 ± 0.80 [b]
ChE(**U**/**g** .**tissue**)	3.88 ± 0.07	3.02 ± 0.02 [a]	3.50 ± 0.06[a,b]	3.57 ± 0.08 [a,b]	3.51 ± 0.02[a,b]

Data expressed as mean ± S.E(.n =5 animals).

(a)Significant different from corresponding control group by one–way ANOVA at P≤0.05 .

(b)Significant different from corresponding deltamethrin group by one–way ANOVA at P≤0.05 .

Table 2 . Effect of deltamethrin on some Cerebellum biochemical parameters.

Groups	Control	Deltamethrin	Deltamethrin +Green Tea	Deltamethrin +Vit. E	Deltamethrin +Green tea +Vit. E
CAT (**U**/**g** .**Tissue**)	378.45 ± 20.50	284.79 ± 12.19 [a]	352.75 ± 10.38 [b]	351.09 ± 15.00 [b]	366.83 ± 12.93 [b]
GSH(**mg**/**g** .**Tissue**)	20.81 ± 0.72	15.51 ± 0.48 [a]	21.78 ± 1.54 [b]	20.03 ± 0.67	22.48 ± 1.05 [b]
LPO(**nmole**/**g** .**tissue**)	14.44 ± 0.22	22.29 ± 1.90 [a]	15.15 ± 0.31 [b]	15.62 ± 1.24 [b]	15.01 ± 0.51 [b]
ChE(**U**/**g** .**tissue**)	3.74 ± 0.07	2.85 ± 0.04 [a]	3.36 ± 0.03 [a,b]	3.42 ± 0.07 [a,b]	3.43 ± 0.05 [a,b]

Data expressed as mean ± S.E(.n =5 animals).

(a)Significant different from corresponding control group by one–way ANOVA at P≤0.05 .

(b)Significant different from corresponding deltamethrin group by one–way ANOVA at P≤0.05 .

3.2 .Genotoxicity of deltamethrin.

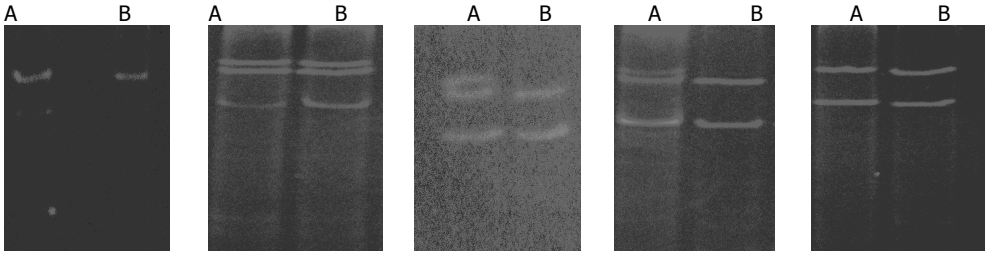
The PCR products of cerebrum and cerebellum DNA from tissue samples were analyzed by SSCP to identify DNA harboring mutations, which were identified as band shifts .Figures (1& 2) shows a representation of several samples that exhibited an abnormal band migration relative to normal tissue control.By PCR/SSCP, There are no mutational hotspots among the negative control group in p53gene .On the other hand, we observed 75 %frequency in the cerebrum and 55 %in the cerebellum of control positive groups which were treated by deltamethrin, a significant decrease was

observed in all treated groups. In the deltamethrin with Vit. E group, 34 %of cerebrum and 26 %of cerebellum samples exhibited abnormal band migration, while in the deltamethrin with green tea group, a mutation rate of 48 %in cerebrum and 32 %in cerebellum samples are reported .As for deltamethrin, green tea and Vit. E groups, 27 %of cerebrum and 26 %of cerebellum samples exhibited abnormal band migration.

*P*53 gene "the guardian of genome" is the most frequently mutated tumor suppressor gene identified in human cancer .*P*53 inactivation leads to decreased DNA repair, and increased genomic instability, furthermore, given the fact that *p*53 tumor suppressor gene mutations have been associated with progression towards more aggressive cancer or disease. Ras gene families consist of 3 members :N-ras, H-ras and K-ras, that encode for the highly homologous protein called *p*21 according to their molecular weights .The inactivation of ras genes by point mutation is the most frequent and well known genetic alteration associated with human cancer including brain tumors .Common mechanisms of inactivation of these genes include missense mutations at the well-known hot spots of codon 12,13 and 16 (Gomori *et al.,* 1999).

Molecular abnormalities associated with primary brain tumors include a wide variety of changes in tumor suppressor genes, proto-oncogenes and growth factors.Mutations in the p53and H-Ras genes can be detected by several different methods including SSCP .

The SSCP protocol that was employed in this research is inexpensive, easy, rapid, and gives no false positive results .Under non denaturing electrophoretic conditions, migration of single strand DNA is a function of its conformation, which depends on its sequence .Even single point mutations can be detected by variant migration patterns or by the presence of additional bands.



Negative control | Deltamethrin | Deltamethrin&Vit E | Deltamethrin& Green Tea | Deltamethrin, Vit . E& Green Tea

Fig. 1. SSCP analysis of exon (7)of p53 gene.

cerebrum and cerebellum sample containing mutation are shown for each group .

Samples that revealed mobility shift in their migration during SSCP screening mutation analysis are only shown .

(A)cerebrum, (B)cerebellum.

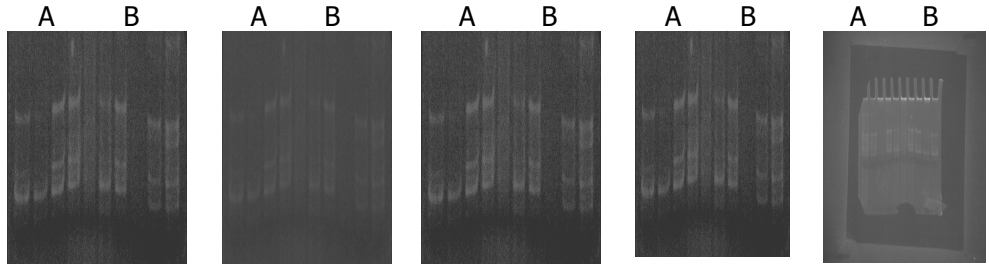
Fig. 2. SSCP analysis of exon (12) of H Ras gene.

Cerebrum and cerebellum sample containing mutation are shown for each group .

Samples that revealed mobility shift in their migration during SSCP screening mutation analysis are only shown .

(A) Cerebrum, (B)Cerebellum.

Fig. 3. The percentage of gene mutation in exon (7) of P53 gene.



Negative control | Deltamethrin | Deltamethrin&Vit E | Deltamethrin& Green Tea | Deltamethrin, Vit .E& Green Tea

Fig 4.The percentage of gene mutation in exon (12) of H Ras gene.

Results of H ras gene, Figures (3 & 4), showed no mutations among the negative control group, whereas, 55 %frequency in the cerebrum and 43 %of the cerebellum were recorded in the control positive groups treated by deltamethrin .In the deltamethrin with Vit. E group, 22 %of cerebrum and 14 %of cerebellum samples exhibited abnormal

band migration. While in the deltamethrin with green tea group, a mutation rate of 35 %in cerebrum and 22 %in cerebellum samples are reported .As for deltamethrin, green tea and Vit. E group, 21 %of cerebrum and19 %of cerebellum samples exhibited abnormal band migration.

3.3 Brain amino acids:

A .Cerebrum amino acid concentrations:

A.1 Aspartic acid level (ASP).

As Shown in table (3) subacute DM intoxication induced a significant increase in brain aspartic acid (excitatory amino acid)level among DM treated group versus the control group co administration of DM with vit. E or green tea extract or both revealed significant decrease in cerebral ASP concentration in comparison to that of DM treated group combination of green tea +vitamin E with DM give good improvement .

A–2 Glutamic acid level (Glu).

As shown in table (3) DM intoxication induced significant increase in cerebral Glu (excitatory amino acid)level in treated rats as compared to the control value .In contrast, a significant reduction in Glu levels were demonstrated in rat groups received DM in combination with vitamin E or green tea or its combination .The greatest reduction in glutamic acid levels was recorded in the last group (DM+Vit. E +green tea extract)

A3–Glycine (Gly)level.

Data presented in table (3) revealed a significant rise in cerebral (Gly)inhibitory amino acid (level in DM treated group versus the control group .Vit. E or green tea supplementation alone with DM failed to correct the significant rise in Gly level .While combination of both vitamin E +green tea extract together with DM induced marked and significant decrease in Gly level and the value near control value .

A–4 Taurine (Tau) level.

As shown in table (3) DM intoxication failed to induce significant changes in cerebral Tyr level in treated rats .Consequently co administration of DM with vitamin E or green tea extract or its combination together failed to induce any changes in cerebral Tyr level as compared to control value.

A–5 GABA.

The obtained data in table (3)revealed that intoxication of DM to rats induced marked and significant rise in cerebral GABA (inhibitory amino acid)level as compared

to that of control value.Also significant increase in GABA level was observed in both groups received DM with vitamin E or with green tea extract as compared to the control value but showed significant reduction than that of DM treated group .

In the same time, combination of vitamin E and green tea extract with DM induced significant decrease in GABA level in comparison to DM treated value.

B–cerebellum amino acids:

B–1 –Aspartic acid level (ASP).

Data presented in table (4)revealed a significant increase in cerebellum ASP level in DM treated rats as compared to the control value .Coadministration of Vit E or green tea alone or in combination to DM induced protective effect which reflected by significant decrease in ASP levels in comparison to DM treated group.

B–2 Glutamic acid level (Glu).

The obtained data in table (4)revealed that DM intoxication induced marked and significant increase in cerebellum Glu level in brain of DM treated rats as compared to the control group .In contrast supplementation of vit E or green tea extract alone or combination of both with DM revealed significant inhibition in cerebellum Glu levels as compared to DM treated group.

B–3 Glycine(Gly)levels.

An s shown in table (4)significant elevation was demonstrated in cerebellum Gly (inhibitory amino acid)among DM treated group .While in experimental groups received green tea extract or Vit. E or both in combination with DM, a significant inhibition in Gly levels were revealed as compared to that of DM treated group . Maximum inhibition in Gly levels was observed in the last group received combination of DM+green tea+Vit. E as compared to control value.

B–4 Taurine (Tau)level.

The present results in table (4)demonstrate significant elevation in tau level in cerebellum of DM treated rats as compared to control value .While in experimental groups received DM beside green tea or Vit E or both showed significant decrease in tau levels as compared to the DM treated value.

B–5–GABA levels.

As demonstrated in table (4) cerebellum GABA (inhibitory amino acid)level showed a marked and significant rise in DM treated group as compared to the control value. Also the obtained results revealed that green tea extract or Vit. E in combination with DM failed to induce protective effect and the values of GABA were significant rise

in compared to that of control value, but were significantly lower than that of DM treated value . While combination of green tea extract and Vit. E with DM induced significant inhibition in GABA levels as compared to DM treated group and the value nearly similar to that of control value. Amino acid concentration (Ug/g tissue)in cerebrum of rats exposed to deltamethrin alone of in combination with vitamin E or green tea extract or both.

Table 3. Amino acid concentration (Ug/g tissue) in cerebrum of rats exposed to deltamethrin alone of in combination with vitamin E and /or green tea extract or both.

Parameters	Control	Deltamethrin	DM +green tea	DM +Vit. E	DM+green tea +Vit E.
Aspartic acid	1.096± 0.11	1.183±0.12 a	1.110±0.12 b	1.15±0.11 b	1.023±0.12 b
Glutamic acid	1.389±0.12	1.496±0.13 a	1.399±0.13 b	1.335±0.12 b	1.328±0.12 b
Glycine	0.508±0.05	0.536±0.05 a	0.520±0.04	0.518±0.05	0.488±0.04 b
Taurine	0.414±0.04	0.436±0.03	0.417±0.03	0.413±0.03 a,b	0.403±0.04
GABA	1.85±0.13	3.81±0.24 a	2.65±0.17 a,b	2.55±0.15	1.8±0.04

Data expressed as mean ± S.E(.n =5 animals).

(a)Significant different from corresponding control group by one–way ANOVA at P≤0.05 .

(b)Significant different from corresponding deltamethrin group by one–way ANOVA at P≤0.05 .

Table 4. Amino acid concentration (Ug/g tissue) in cerebellum of rats exposed to deltamethrin alone of in combination with vitamin E and /or green tea extract or both.

Parameters	Control	Deltamethrin	DM +green tea	DM +Vit. E	DM+green tea +Vit. E
Aspartic acid	1.104± 0.11	1.341±0.12 a	1.127±0.09 b	1.084±0.11 b	0.980±0.081 b
Glutamic acid	1.478±0.12	1.933±0.13 a	1.541±0.13 b	1.410±0.13 b	1.361±0.12 b
Glycine	0.556±0.05	0.786±0.06 a	0.599±0.05 b	0.576±0.07 b	0.520±0.04 b
Taurine	0.486±0.03	0.588±0.04 a	0.482 ±0.03 b	0.485±0.04 b	0.385±0.03 b
GABA	2.16±0.31	5.79±0.52 a	3.86±0.23 a,b	.27±0.22 a,b	2.02±0.21 b

Data expressed as mean ± S.E(.n =5 animals).

(a)Significant different from corresponding control group by one–way ANOVA at P≤0.05 .

(b)Significant different from corresponding deltamethrin group by one–way ANOVA at P≤0.05 .

The effects of pyrethroids on the CNS are complex and may also involve antagonism of γ–aminobutyric acid (GABA) modulation of nicotinic transmission, enhancement of noradrenaline release, and direct actions on calcium or chloride ion

channels .Pyrethroids affect nervous system function by producing hyperexcitability in neurons (Murakami *et al.,* 1992).

Thus, the ability of pyrethroid insecticides to cause apoptosis may contribute to the potential for high–level exposures to contribute to neurodegeneration. However, the mechanism by which pyrethroids, and in particular deltamethrin, induces apoptosis has not been established .Further studies identified the role of calpain and the endoplasmic reticulum (ER)stress pathway as mediators of deltamethrin–induced apoptosis. The prominent role of apoptosis, calpain and the ER stress pathway in neurodegeneration, these data provide mechanistic information as to how high–level exposure to pyrethroids could result in neurodegeneration (Vosler *et al.,* 2008).

In conclusion, the sample percentage that revealed mobility shift in their migration during SSCP screening mutation analysis was significant among the control positive group such which deltamethrin caused high incidence of p53 mutations among cerebrum and cerebellum samples .The vitamin E and green tea are proved to protect against the mutational effect of deltamethrin .Vitamin E showed more protection compared to green tea whereas, the usage of both of them provided the most protection .The cerebrum samples are more affected by the deltamethrin than the cerebellum samples.

The obtained data revealed that exposure of rats to deltamethrin induced significant changes in the brain levels of some amino acids in cerebrum and cerebellum of intoxicated rats .It have been shown that DM induced significant elevation in ASP, Glu, Gly and GABA concentration in cerebellum of intoxicated rats.

The amino acids (AAs)such aspartic acid, glutamic acid, amino butyric acid (GABA)and 3,4dihydroxy phenylalanine (DOPA)is known to act as neurotransmitters in the central nervous system. They play an important role in response to neurodegenerative conditions. Neurotransmitter systems alterations can be implicated in seizures due to an increase in their oxidative metabolism or a decrease in their synthesis and or release and disturbance of glutamate and GABA metabolism showed to play an important role in the development of pyrethroid neurotoxicity .Excessive activation of glutamate receptors by excitatory amino acids leads to a number of deleterious consequences, including impairment of calcium buffering, generation of free radicals, activation of the mitochondrial permeability transition and secondary excitatory (KanunnKova, 2012).

The obtained results also explored remarkable increase in the brain levels of Gly in DM treated rats and in both examined parts (cerebrum and cerebellum)Glycine is an

inhibitory glucogenicnon essential amino acid that can be synthesized from threonine and serine by a reversible reaction catalyzed by the enzyme serine trans hyroxymethylase Gly seen to be correlated with stimulated synthetic pathway and /or release of this neurotransmitter amino acid by subacute administration of DM(.Ahmed *et al.,* 1992).

The present investigation has also revealed significant increase in Tau level in cerebellum of DM treated rats .This effect of DM is considered stimulatory effect on brain Tau .In general, taurine is an inhibitory neurotransmitter that initiates membrane polarization which results in decreased neuronal firing Also Taurine is also known to decrease K stimulated release of epinephrine and acetylcholine, which in turn support the present finding .Rise of taurine levels in the Bain seems to be correlated with damage be induced by DM, it might also be correlated to the stimulatory effect of DM on the synthesis and /or release of this amino acid neurotransmitter (Oja and Kontra, 1983).

The present investigation revealed that coadministration of Vit. E or green tea extract with deltamethrin DM or their combination attenuated the toxic effect of an evaluated brain amino acids. The data revealed that green tea extract induce protective effect more than that of Vit. E .in the same time combination of vit. E & green tea extract produce more protective effect against DM neurotoxicity than each alone .Little is known about the effects of antioxidant compounds in amino acid concentration in brain of adult rats exposed to pesticide toxicity

Green tea catechins have a wide spectrum of neuroprotective cellular mechanisms such as in chelation, scavenging free radicals, regulation of mitochondrial function and activation of survival genes and cell signaling pathways .EGCG is a powerful antioxidant and is 20 times than Vit. E in protecting essential brain lipids .It acts as an iron chelator in the brain, preventing iron from contributing to the production of EGCG also increases the activity of superoxide dismutase and catalase; antioxidant enzymes that help decrease free radical damage.They investigate the effect of EGCG on excitotoxic neuronal damage in a culture system .EGCG reduced excitotoxin induced MDA production and neuronal damage in the culture system .L−threonine is a unique amino acid present almost exclusively in the tea plant .It appears the amine competively inhibits glutamate transport into tumor cells, which causes decreased intracellular glutathione levels Regarding the protective effect of Vit. E or green tea extract against neurotoxic effect of DM

In conclusion, exposure of rats to 1/10 LD_{50} of deltamethrin for 1 month induced oxidative damage in cerebrum and cerebellum of treated rats .This damage represented by reduction in CAT, GSH and ChE levels .Also significant increase in MDA was recorded. Genotoxicity was detected by SSCP of HRas and P53 genes .Also DM induced significant alterations in the levels of some brain amino acid .Co administration of green tea extract of Vit. E alone or in combination with DM induced significant protective effect against the neurotoxicity of DM .combination of green tea extract +Vit E induce proper protective effect than each alone.

4 .REFERENCES

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تأثير السميه العصبيه والوراثيه للدلتاميثرين والتأثير الوقائي لفيتامين هـــ والشاي الأخضرعلي إناث الفئران

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العديد من الدراسات تؤكد أهمية الأجهاد التأكسدي والسميه الوراثيه كدليل للتأثير السام لمركبات البيروثرويد لذلك في هذه الدراسه يتم إختبار التأثيرات المفيده والنافعه لفيتامين هـــ ومستخلص الشاي الاخضر كمركبات واقيه لتأثير الدلتاميثرين علي الاجهاد التأكسدي والسميه الوراثيه وكذلك بعض الخلل في الاحماض الامينيه الناقله للتيار العصبي في المخ لاناث القئران البالغه. يتم إعطاء الفئران 1 ملجم /كجم وزن جسم من مبيد الدلتاميثرين (1/10 LD_{50}) فقط او مع فيتامين هـــ او مع مستخلص الشاي الاخضر . ويتم قياس كل من إنزيم الكتاليز والجلوتاثيون المختزل وتقدير الدهون المتأكسده والكولين إستيراز بالاضافه إلي تأثيره علي السميه الوراثيه من خلال قياس جين الـــ P53 وقياس الاحماض الامينيه الناقله للتيار العصبي في كل من المخ والمخيخ وفي نهاية التجربه (30 يوم) اوضحت النتائج إنخفاض معنوي ملحوظ في كل من انزيم الكتاليز والكولين إستيراز والجلوتاثيون المختزل بينما لوحظ زياده معنويه للدهون المتأكسده والاحماض الامينيه في أنسجة المخ والمخيخ .

الخلاصه: تبين من خلال الدراسه الدور الواقي الذي قد يلعبه فيتامين هـــ والشاي الاخضر من تأثير الدلتاميثرين سواء علي المستوي العصبي او الوراثي لكل المعاملات التي تم تحليلها بما فيها جميع المعايير الكيميائيه الحيويه والاجهاد التأكسدي.