World Journal of Fish and Marine Sciences 7 (4): 313-324, 2015
ISSN 2078-4589

DOI: 10.5829/idosi.wjfms.2015.7.4.96163

Vitamin E Attenuated the Oxidative Stress and Biochemical Changes Induced by Endosulfan in Female Catfish (*Clarias gariepinus*)

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Abstract: Endosulfan, a persistent organochlorine pesticide, is widely used in agriculture, causing negative effects on non target organisms, especially aquatic biota, including disruption of hormonal systems. However, the effect of endosulfan on fish fecundity has been hardly studied. The aims of the present study were: to determine LC_{50} of endosulfan, to investigate its propensity to induce oxidative stress, fecundity and biochemical disturbances in female catfish and to study the protective role of Vitamin E (Vit. E). The results indicated that LC_{50} of endosulfan at 96h was 0.89µg/l. The absolute fecundity showed significant decrease in total ripened egg number (T.R. Egg No.). The activity of acetylcholinesterase (AchE) and the level of 17ß-Estradiol hormone (E_2) were significantly decreased. However, endosulfan induced marked increase in testosterone hormone (T), glucose and cortisol levels. Moreover, endosulfan exposure was associated with oxidative damage in liver tissue as evidenced by the increase of lipid peroxidation (LPO), catalase (CAT) and superoxide dismutase (SOD) levels and the decrease in reduced glutathione (GSH). Vit.E was effective to alleviate these toxic effects on the studied parameters. It could be concluded that Vit. E has beneficial effects and was able to antagonize endosulfan toxicity in catfish.

Key words: Organochlorine pesticides · LC_{50} · Fish physiology · Fish Fecundity

INTRODUCTION

Organochlorine pesticides (OCPs) are persistent and ubiquitous pollutants. After their release to the environment, OCPs are subject to transport and transformation processes that, in addition to environmental parameters and physicochemical properties regulate their distribution and concentration in water, soil, sediments and biota [1]. The high molecular weight and the low water solubility of most OCPs lead to their bioaccumulation in the biota, mainly in fatty tissues and biomagnification in the food chain [2]. Endosulfan belongs to the cyclodiene group and represents the last OCPs under current use in many countries, as a broad spectrum insecticide control insects and mites in crops of high commercial value (soy, cotton, tea, coffee, maize, vegetables and fruits) [3]. Following application, endosulfan can reach non-target aquatic animals including fishes through groundwater, surface runoff and air drift from nearby agricultural fields [4]. Negative effects of endosulfan on fish have been documented including histological, physiological, hematological, neurological, behavioral, immunological and mutagenic disorders [5, 6]. Endosulfan is a known neurotoxin and is very highly toxic to fish (LC_{50} for aquatic species =100 g/L, 96-h LC_{50} median value of 2.6 g/L for teleost fish [7]. Also, endosulfan has been suggested as an endocrine disrupting chemical, capable of interfering with the normal functions of the endocrine system of animals [8]. Evidences for the action of endosulfan on the reproductive axis of fish; reducing gonadosomatic index in female zebrafish, *Danio rerio* (*D. rerio*) [9], lowering vitellogenin plasma levels in female *Clarias batrachus* (*C. batrachus*) [10] and regression in the ovary of *Lepomis macrochirus* (*L. macrochirus*) [11]. OCPs are known to cause inhibition of acetyl cholinesterase activity in the target tissues. The exposure to contaminants in aquatic ecosystems can enhance the intracellular formation of reactive species of oxygen, which induce oxidative damage to biological systems [12]. So, the understanding of the toxic response at sub individual level (biochemical, physiological) is useful for identifying

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early ''warning signals'' that can be used as biomarkers of exposure or effect to assess pollution-induced stress before environmental ''damage'' become irreversible [1]. In this study, the catfish, *Clarias gariepinus* (*C. gariepinus*) (Clariidae, Siluriformes) was chosen as it can withstand higher toxicity than other teleosts as evidenced from high LC_{50} values for endosulfan [13]. Vit. E is an indispensable nutrient which is supplemented in fish feed as α- tocopherol acetate to enhance fish growth, survival rate and fish flesh quality [14]. Vit.E is also considered as a stable antioxidant with respect to oxidation during feed processing and storage [15], which protects essential fatty acids from oxidation in the body cells as well as prevents breakdown of body tissues [16]. Likewise, dietary vit. E has shown to be an essential nutrient for fish reproduction with its deficiency resulting in immature gonads in freshwater crayfish and reduced hatching rates and fry survival [17]. Hence, the aim of the present study was to determine LC_{50} of endosulfan in female catfish, *C. gariepinus* and the chronic toxicity to assess various physiological and biochemical parameters representative of the exposed endosulfan fish and the ameliorative effect of Vit. E supplemented diet on the studied parameters of catfish.

MATERIALS AND METHODS

Chemicals: Endosulfan is a colorless crystal product of FMC Corporation Agricultural Chemicals Group, Philadelphia, PA 19103. The molecular weight of endosulfan is 406.96 and its chemical formula is (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzadioxathiepin 3-oxide). Vit. E (α-tocopherol acetate) was obtained from Merck (Germany). All biochemical kits (AchE, glucose, cortisol, E_2, T, LPO, SOD, CAT and GSH) were purchased from Bio-Diagnostic Co., (Dokki, Cairo, Egypt).

Fish and Rearing Conditions: A total number of 200 female *C. gariepinus* with average body weight 300±50 g. and body length 38.20±3.00 cm. were obtained from Abassa fish farm, El-Sharkya governorate, Egypt. Fish were transported to the laboratory in 150 litres well aerated fiberglass tanks. The fish were kept in identical glass aquaria aerated with air pumps, supplied with dechlorinated tap water and left for 15 days for acclimation. Physicochemical characteristics of the water samples were analyzed by procedures of Hack Method (Sigma Laboratory) according to the World Health Organization, 2001, dissolved oxygen 7.3±0.5 mg/l, pH 7.5±0.8, temperature 28±1 °C, alkalinity 122 mg/l and hardness 152 mg/l $CaCO_3$. Fish were fed diet containing 32% protein and transferred to a fresh volume of water every 24 h to minimize contamination from metabolic wastes.

Determination of 96 h LC_{50} of Endosulfan: The half lethal concentration (LC_{50}) was determined for endosulfan in female *C. gariepinus* using minimum of five concentrations (0.19, 0.38, 0.77, 1.54 and 3.08µg/l.), plus the control group, where eight fish were used for each concentration according to the method of Litchfield and Wilcoxon [18].

Experimental Design: Fish were divided into five groups (30 fish per group) in fifteen glass aquaria, 10 fish per glass aquarium (80 x 40 x 40 cm). The experimental period for all groups either fed a basal diet or Vit. E-supplemented diet was continued for 40 days. The first group, served as a control group, was fed basal diet. while, the second group was fed Vit. E-supplemented diet (480 mg/Kg diet). The third group was fed a basal diet and exposed to 1/8 LC_{50} of endosulfan for 40 days. The fourth and fifth groups were fed Vit. E-supplemented diet (240 and 480 mg /Kg diet), respectively and exposed to endosulfan at the same concentration and duration of that of the third group.

Growth Measurements and Morpho-anatomical Parameters: These parameters were calculated for each fish separately according to Coward and Bromage [19].

Female Fish Fecundity: Ovaries of each fish in every group were examined microscopically to count total egg number. Absolute and Relative fecundity were evaluated referring to Babiker and Ibrahim [20].

Serum Biochemical Analysis: Blood samples were collected from the caudal vessels after anesthetization with 0.02 % benzocaine solution using plastic syringes and transferred to clean dry centrifuge tubes, allowed to clot at room temperature and then centrifuged at 3000 rpm., 4°C for 15 minutes. Sera were separated and stored at -80°C until analysis. Serum AchE was determined according to the method described by Dietz *et al*. [21]. Glucose was measured by Trinder [22]. Cortisol, (E_2) and (T) hormones were determined by ELISA Reader using the method described by Foster and Dunn [23], Abraham [24] and Tietz [25], respectively.

Tissue Lipid Peroxidation and Antioxidant Biomarkers: Samples from liver tissues were homogenized in cold phosphate buffer saline (0.1M pH 7.4) using a Potter-Elvejhem glass/Teflon homogenizer. Then, this homogenate was filtered and centrifuged at 1600 rpm and 4°C for 10 min.; the supernatant was stored at -20 °C until analysis. This supernatant (20%) was used for the determination of lipid peroxidation was expressed as nmol (LPO)/g tissue according to the method of Ohkawa *et al*. [26]. The activity of SOD was assessed by the method described by Nishikimi *et al.* [27]. CAT activity was determined according to the method of Aebi [28]. The non-enzymatic antioxidant marker, GSH was measured according to Beutler *et al*. [29].

Statistical Analysis: Data were statistically analyzed and expressed as means ± SE using SPSS (14.0) software (2004). One way "ANOVA" and Duncan's multiple range test to evaluate comparison between means at $P< 0.05$.

RESULTSAND DISCUSSION

To study the toxic effects of endosulfan on female *C. gariepinus*, it was necessary to determine its half lethal concentration (LC_{50}) /96h. Results revealed that the (LC_{50})/96h of endosulfan was 0.89µg/l (Table 1). The result is nearly similar to those of Ezemonye and Ikpesu [30], Suneetha [31] who recorded that LC_{50} values of endosulfan for *C. gariepinus* and *Labeo rohita* (*L. rohita*) were 0.77 and 0.6876 µg/l, respectively. Our estimate is higher than the LC_{50} /96 h values of 2.6 µg/l and 3.6 µg/l for *Cichlasoma dimerus*(*C. dimerus*) and *Oreochromis mossambics (O. mossambics),* respectively [32, 33] and lower than the value of 0.070 ppm for crucian carp, *Carassius carassius* (*C. carassius*) [34]. The variation may be due to the difference and toughness of the test species and water quality parameters. The differences in results may be attributed to the toxic effects with respect to age, size, health and fish species [6, 34].

Biometric parameters are regarded as a general indicator of fish health and the quality of the aquatic environment. In the present study endosulfan during the chronic exposure showed marked decrease in body length (B.L.), body weight (B.Wt.), gonadal weight (W_G) and gonadosomatic index (GSI) and obvious increase in hepatic weight (W_H) and hepatosomatic index (HSI) (Table 2). Similar findings were recorded by Li *et al.* [35] working on Rainbow trout, *Oncorhynchus mykiss* (*O. mykiss*) using carbamazepine. The decrease in (GSI) is known to occur in reproductive female and male freshwater teleosts exposed to pesticides [36]. So the possible explanation for the observed reduction in (GSI) is a high demand for energy and reduced food consumption of fish exposed to pesticide [37]. Reduction in reproductive investment is associated with the real location of energy in response to natural and anthropogenic stressors [38, 39]. (HSI) is a non-particular biomarker, influenced by variables such sex, season, ailment and nourishing level [1]. Changes in liver weight is associated with vitellogenesis in both sexes of fish [40] and with increased detoxification of xenobiotics [41]. The observed increase in (HSI) following endosulfan exposure is most likely due to the enhanced activity of xenobiotic biotransformation.

Fecundity was defined by Gerking [42] as the number of ripening eggs found in the female just prior to spawning. Fecundity of individual fish is called absolute fecundity (T.R. Egg No.) which is the action of yolk material (vitellogenin) uptake by the perifollicular cells expressed as the formation of well matured ova filled with vitellogenin. Exposure of female *C. gariepinus* to endosulfan revealed highly significant drop in ovarian weight (W_G), absolute fecundity (T.R. Egg No.) and relative fecundity, which is {fecundity related to: body length (FBL), body weight (FBW) and ovarian weight (FOW)} (Table 2), since the production of eggs was the dominant function of an ovary, a close relationship could be expected between the weight of ovary and the number of ova produced. The results are in accordance with Tillitt *et al*. [43]. The reduction in egg production may be due to reduced numbers of spawning events, which was not predicted by routine physiological measurements of endocrine disruption (steroid hormones or gonado-somatic indices) [43]. Besides, the hepatic tissue being a marker for reabsorbing toxic compounds, it is also the vitellogenin (yolk protein) precursor. This explained the presence of a high number of empty ova which caused highly significant drop in (T.R. Egg No.). Supplementation with vit. E against the toxicity of endosulfan showed improvement in fish fecundity translated as significantly increase in (FOW), (T.R. Egg No.) and enhancement of the other growth parameters compared to the pesticide intoxicated group. This improvement was more pronounced in the fifth group received the high dose of vit.E (480 mg/kg) (Table 2). The results are in accordance with Barim [15] who recorded a marked increase in the ovarian eggs number of the freshwater crayfish, *Astacus leptodactylus* (*A. leptodactylus*) fed diet supplemented with vit.E for 72 days, which indicated that vit. E had a positive impact on the breeding performance of female catfish [44].

Table 1: Half lethal concentration (LC_{50})/96h of endosulfan for female*C. gariepinus*

Endosulfan Conc. (µg/l)	No. of fish for every concentration	No. of alive fish	No. of dead fish	a	b	a x b
0.00	8	8	0	0	0	0
0.19	8	6	2	0.19	1	0.19
0.38	8	6	2	0.19	2	0.38
0.77	8	4	4	0.39	3	1.17
1.54	8	1	7	0.77	5.5	4.23
3.08	8	0	8	1.54	7.5	11.55
						Σa x b =17.52

Halflethal concentration of endosulfan = Highest concn. - Σa x b /n

LC_{50} = 3.08 - 17.52/8= 0.89µg/l Where: a: Constant factor of difference between groups.

b: Mean value of dead fish between each two successivegroups. n: Number of fish in each group.

Table 2: Comparative reproductive parameters of female *C. gariepinus* exposed to 1/8 LC_{50} of endosulfan and treated with (240 and 480 mg vit.E/kg) for 40 days

Reproductive Parameters		Groups				
		1st group	2nd group	3rd group	4th group	5th group
Growth Measurement	B.L.(cm)	39.68±0.81^{a}	39.03±0.92^{a}	30.61±0.20^{d}	33.25±0.24^{c}	36.13±0.74^{b}
	B.Wt.(g.)	347.45±0.94^{a}	345.64±1.27^{a}	303.21±4.28^{d}	323.23±1.97^{c}	337.71±1.19^{b}
	W_G. (g.)	19.96±0.08^{a}	20.33±0.23^{a}	13.51±0.68^{d}	16.08±0.14^{c}	18.48±0.27^{b}
	W_H (g.)	1.87±0.02^{c}	1.90±0.02^{c}	4.7±0.17^{a}	3.00±0.03^{b}	2.06±0.03^{c}
Morpho- anatomical Parameters	GSI	6.09±0.02^{ab}	6.25±0.09^{a}	4.66±0.18^{d}	5.23±0.06^{c}	5.79±0.09^{b}
	HSI	0.54±0.01^{c}	0.55±0.01^{c}	1.58±0.08^{a}	0.94±0.01^{b}	0.62±0.01^{c}
Relative Fecundity	FBL	4855.87±19.68^{a}	4699.68±22.06^{a}	2875.24±38.46^{d}	3397.41±49.09^{c}	4019.42±16.22^{b}
	FBW	2071.59±4.66^{a}	2062.62±6.28^{a}	1850.14±21.64^{d}	1951.00±9.88^{c}	2023.26±5.90^{b}
	FOW	4451.84±15.45^{a}	4526.64±45.92^{a}	3136.04±39.66^{d}	3659.64±27.63^{c}	4149.24±54.79^{b}
Absolute Fecundity	T.R. Egg No.	12413.00±32.79^{a}	12481.67±25.66^{a}	7437.67±49.84^{c}	9656.67±52.63^{b}	11922.67±17.33^{a}

Data are represented as means ± SE (n = 10). Values with different superscript letter in the same row for each parameter are significantly different ($P < 0.05$).

-1st group: control group, was fed basal diet.

-2nd group was fed Vit.E-supplemented diet (480 mg/Kg diet).

-3rd group was fed a basal diet and exposed to 1/8 LC_{50} of endosulfan for 40 days.

-4th group was fed Vit.E-supplemented diet (240 mg /Kg diet) and exposed to 1/8 LC_{50} of endosulfan for 40 days.

-5th group was fed Vit.E-supplemented diet (480mg/Kg diet) and exposed to 1/8 LC_{50} of endosulfan for 40 days.

One of the most frequently used biomarkers of exposure to pesticides in fish, in particular organophosphorus and organochlorines, is the activity of the enzyme (AChE). In the central nervous system of vertebrates, AChE degrades the neurotransmitter acetylcholine (ACh) into choline and acetic acid causing the termination of the synaptic transmission. Its inhibition by toxicants can lead to an excess of ACh levels resulting in neuromuscular paralysis and eventually death [45, 46]. Serum AchE level revealed a significant inhibition in the exposed fish during the chronic exposure in comparison to the control group (Fig. 1). As, when AchE decreased, Ach is not broken and accumulated within synapses, causing overall decline in neural and muscular control [47]. Our results are consistent with several reports that revealed a reduction in plasma AChE activity under various concentrations of endosulfan toxicity in different fishes such as *O. mossambicus* [33], *L. rohita* fish fingerlings [48], zebrafish, *D. rerio* [49]. On the other hand, administration of vit. E restores the activity of AChE to nearly normal level (Fig. 1), additionally, the enhancement in AchE was more obvious at the higher dose of vit.E. These findings are in agreement with previous investigators [50, 51]. In the present study, the blood glucose level was markedly influenced by the endosulfan exposure (Fig. 2). The glucose level increased significantly ($P<0.05$) compared to the control. The rise in blood glucose reflects a generalized stress response to a variety of environmental conditions. Thus, blood glucose is considered as an indicator for stress response in fish [52] . Similar results have been reported by Bakhshwan *et al*. [53], Bacchetta *et al*. [54], Yekeen and Fawole [55], Marzouk *et al*. [56], Hamed [57]. The hyperglycemia is generally interceded by the activity of cortisol which empowersliver gluconeogenesis and also halts peripheral sugar uptake [58]. However, the increase in blood glucose may be attributed to a different mechanism not linked to cortisol [59]. To this respect,

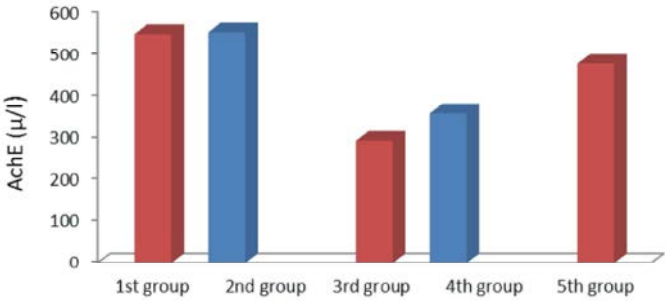

Fig. 1: Showing the variation in AchE activity (µ/l) of female *C. gariepinus* exposed to 1/8 LC_{50} of endosulfan and treated with (240 and 480 mg vit.E/kg) for 40 days.
-1st group: control group, was fed basal diet.
-2nd group was fed Vit.E-supplemented diet (480 mg/Kg diet).
-3rd group was fed a basal diet and exposed to 1/8 LC_{50} of endosulfan for 40 days.
-4th group was fed Vit.E-supplemented diet (240 mg /Kg diet) and exposed to 1/8 LC_{50} of endosulfan for 40 days.
-5th group was fed Vit.E-supplemented diet (480mg/Kg diet) and exposed to 1/8 LC_{50} of endosulfan for 40 days.

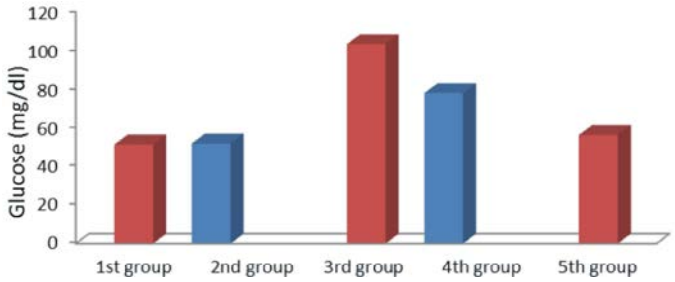

Fig. 2: Showing the variation in glucose level (mg/dl) of female *C. gariepinus* exposed to 1/8 LC_{50} of endosulfan and treated with (240 and 480 mg vit.E/kg) for 40 days.
-1st group: control group, was fed basal diet.
-2nd group was fed Vit.E-supplemented diet (480 mg/Kg diet).
-3rd group was fed a basal diet and exposed to 1/8 LC_{50} of endosulfan for 40 days.
-4th group was fed Vit.E-supplemented diet (240 mg /Kg diet) and exposed to 1/8 LC_{50} of endosulfan for 40 days.
-5th group was fed Vit.E-supplemented diet (480mg/Kg diet) and exposed to 1/8 LC_{50} of endosulfan for 40 days.

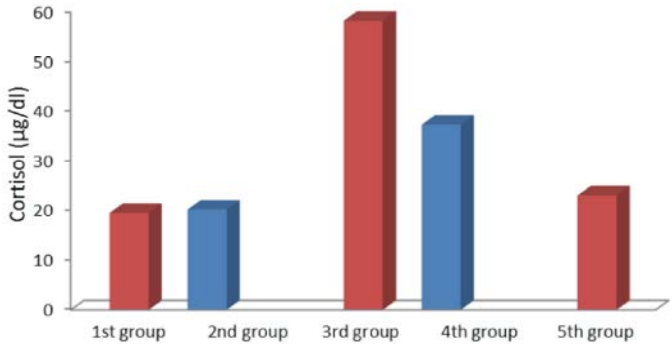

Fig. 3: Showing the variation in cortisol level (µg/dl) of female *C. gariepinus* exposed to 1/8 LC_{50} of endosulfan and treated with (240 and 480 mg vit.E/kg) for 40 days.
-1st group: control group, was fed basal diet.
-2nd group was fed Vit.E-supplemented diet (480 mg/Kg diet).
-3rd group was fed a basal diet and exposed to 1/8 LC_{50} of endosulfan for 40 days.
-4th group was fed Vit.E-supplemented diet (240 mg /Kg diet) and exposed to 1/8 LC_{50} of endosulfan for 40 days.
-5th group was fed Vit.E-supplemented diet (480mg/Kg diet) and exposed to 1/8 LC_{50} of endosulfan for 40 days.

it has been proved that catecholamines itself can increase sugar levels [60]. On the other hand, dietary vit.E along with endosulfan in the fourth and fifth groups resulted in statistically significant reduction in serum glucose level (Fig. 2), which was more evident in the fifth group received vit.E at dose (480mg/kg). Cortisol is the most active and abundant corticosteroid in fish blood and its structure has been highly conserved in all of the vertebrate species in which it is found [61]. The cortisol level of endosulfan exposed fish was significantly increased ($P<0.05$) during the long term of exposure compared to the control fish (Fig. 3). Similar results were verified in *O. mossambicus* [33], *C. gariepinus* [53], *Oreochromis niloticus* (*O. niloticus*) [57]. This may be attributed to the activation of hypothalamo-pituitary-inter renal axis with the release of steroid cortisol in blood stream due to stress [62]. Also, increased cortisol level may be due to the increase in osmotic water- influx, which may cause a cortisol elevation, to restore the hydromineral balance. This osmoregulatory dysfunction might be harmful perse; and owing to sustained high cortisol level which may cause several deleterious physiological changes, affecting the immuno-competence, health status and survival of the fishes [63]. However, our results expressed a remarkable disagreement with those demonstrated by Ezemonye and Ikpesu [30] who recorded significant reduction in plasma cortisol level of *C. gariepinus* exposed to endosulfan . While, dietary of vit.E along with endosulfan improved serum cortisol level. The improvement in cortisol was noticeable in the fifth group which received the high dose of vit.E (Fig. 3). This view was in agreement with Kadry *et al.* [50] who recorded a marked decline in cortisol level of female catfish exposed to atrazine and supplemented with 240mg vit.E /kg.

Spawning season, a period of steroidal superiority in fish gonads where by the action of piturity gonadotropin hormones, the levels of E_2 and T should reach highest peak in females and males, respectively to perform their role as a biomarkers to identify gender of adult fish till ovulation or spermitation process took place [64]. Steroid hormones E_2 and T of female catfish exposed to endosulfan (Figs. 4 & 5), exhibited a highly significant reduction in E_2 level (Fig. 4). Oruç [65] reported that E_2 level decreased significantly in *O. niloticus* exposed to chlorpyrifos.The depletion in E_2 hormone may be indicative of a change in the biosynthetic capacity of the gonad, either directly by the inhibition of aromatization activity which is responsible for estrogen synthesis or in directly by suppressing the secretion of gonadotropin [65], whereas, the level of T of endosulfan exposed fish showed a marked increase ($P<0.05$) in comparison to the control ones (Fig. 5). These data are corroborated by previous report [66] of gamma-hexachlorocyclohexane pesticide which induced T activity of the freshwater catfish, *Heteropneustes fossilis* (*H. fossilis*). The elevation of T level may be due to endosulfan, which competes with androgen and irreversibly blocks androgen receptor thereby increasing the plasma level of T [67]. Also, the incease of T level could be resulted either from the hypothalamo-hypophyseal-gonadal axis or from direct action on hepatic and steroidogenic enzymes causing reproductive endocrine disruption [66]. Interestingly, vit.E supplementation at both used doses tends to normalize the levels of E_2 and T, particularly, the fifth group which received the higher dose of vit. E (Figs. 4&5). This finding is consistent with Kadry *et al*. [50] who found that dietary vit.E improved the activity of E_2 in atrazine exposed female *C. gariepinus* for 6 weeks.

Oxidative stress is caused by many xenobiotics through the generation of ROS and can alter the free oxygen radical scavenging enzyme systems in aquatic organisms. ROS can react with susceptible biological macromolecules and cause LPO, which in turn leads to nucleic acid damage and protein oxidation [68]. LPO is a complex process in which polyunsaturated fatty acids in the biological membrane system undergo changes by chain reactions and form lipid hydroperoxides, which dissociate double bonds of unsaturated fatty acids and interrupt membrane lipid [69]. The hepatic LPO level in the third group exposed to 1/8 LC_{50} of endosulfan increased significantly all over the experimental period than the other groups (Table 3). The results are consistent with the previous literature [6, 33, 34, 70, 71]. This increase in TBARS levels can be a result of the impairment in antioxidant enzymes due to ROS formation that attacks the cell membrane, with direct consequences on cell integrity and cell function [34]. Vit. E neutralizes lipid peroxidation and unsaturated membrane lipids because of its oxygen scavenging effect [72]. Consequently, administration of vit. E along with endosulfan exposure decreased the peroxidation of unsaturated fatty acids and thus protected the cell membranes. The reduction in liver LPO level was obvious in the group received (480 mg vit.E/kg) (Table 3). It has been reported that the hepatic LPO was prevented by vit. E [50, 73]. SODs are a group of metallo-enzymes that play a crucial antioxidant role and constitute a first line defense system against the natural or chemically induced production of ROS [74].

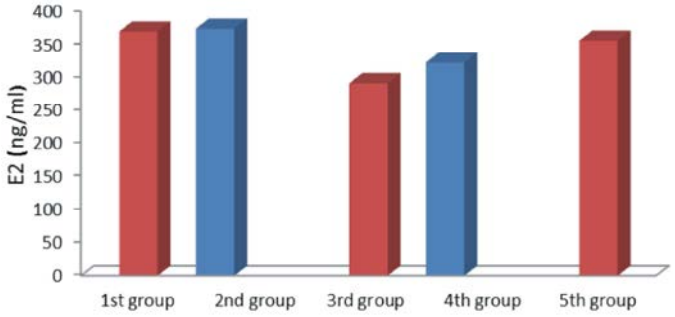

Fig. 4: Showing the variation in E_2 level (ng/ml) of female *C.gariepinus* exposed to 1/8 LC_{50} of endosulfan and treated with (240 and 480 mg vit.E/kg) for 40 days.
-1st group: control group, was fed basal diet.
-2nd group was fed Vit.E-supplemented diet (480 mg/Kg diet).
-3rd group was fed a basal diet and exposed to 1/8 LC_{50} of endosulfan for 40 days.
-4th group was fed Vit.E-supplemented diet (240 mg /Kg diet) and exposed to 1/8 LC_{50} of endosulfan for 40 days.
-5th group was fed Vit.E-supplemented diet (480mg/Kg diet) and exposed to 1/8 LC_{50} of endosulfan for 40 days.

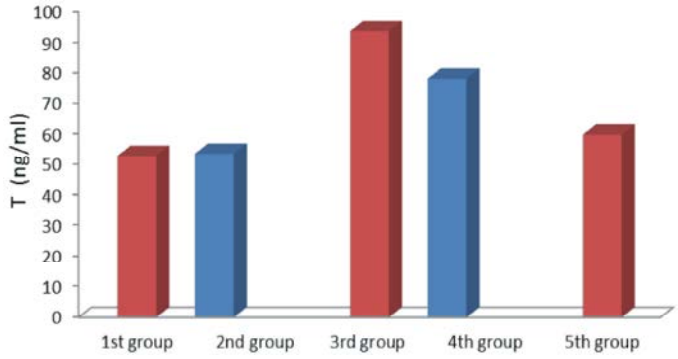

Fig. 5: Showing the variation in T level (ng/ml) of female *C.gariepinus* exposed to 1/8 LC_{50} of endosulfan and treated with (240 and 480 mg vit.E/kg) for 40 days.
-1st group: control group, was fed basal diet.
-2nd group was fed Vit.E-supplemented diet (480 mg/Kg diet).
-3rd group was fed a basal diet and exposed to 1/8 LC_{50} of endosulfan for 40 days.
-4th group was fed Vit.E-supplemented diet (240 mg /Kg diet) and exposed to 1/8 LC_{50} of endosulfan for 40 days.
-5th group was fed Vit.E-supplemented diet (480mg/Kg diet) and exposed to 1/8 LC_{50} of endosulfan for 40 days.

Table 3: Effect of endosulfan exposure and treatment with (240 and 480 mg vit.E/kg) in LPO and antioxidant biomarkers of female *C. gariepinus* for 40 days

	Parameters			
Groups	LPO (nmol/g tissue)	SOD (µg/g tissue)	CAT (µg/g tissue)	GSH (mg/g tissue)
1st group	32.67±0.55^{d}	1317.29±1.28^{d}	119.31±1.13^{c}	63.76±0.91^{a}
2nd group	30.88±1.09^{d}	1319.77±0.78^{cd}	116.49±01.05^{c}	60.72±0.65^{a}
3rd group	101.45±4.51^{a}	1357.83±1.61^{a}	168.08±1.85^{a}	34.88±0.36^{c}
4th group	77.47±2.55^{b}	1333.11±1.90^{b}	152.22±1.38^{b}	45.63±0.61^{b}
5th group	43.21±0.95^{c}	1321.75±0.77^{c}	120.23±0.23^{c}	60.96±0.92^{a}

Data are represented as means ± SE (n = 10). Values with different superscript letter in the same column for each parameter are significantly different ($P < 0.05$).
-1st group: control group, was fed basal diet.
-2nd group was fed Vit.E-supplemented diet (480 mg/Kg diet).
-3rd group was fed a basal diet and exposed to 1/8 LC_{50} of endosulfan for 40 days.
-4th group was fed Vit.E-supplemented diet (240 mg /Kg diet) and exposed to 1/8 LC_{50} of endosulfan for 40 days.
-5th group was fed Vit.E-supplemented diet (480mg/Kg diet) and exposed to 1/8 LC_{50} of endosulfan for 40 days.

The hepatic SOD of endosulfan exposed fish increased significantly ($p < 0.05$) when compared to the control fish (Table 3). A significant increase in SOD activity has been observed in *O. mossambicus* [33] and in *L. rohita* [31, 48] exposed to endosulfan. The increase in hepatic SOD level may be attributed to the elimination of ROS from the cell induced by pesticide exposure [75]. As, SOD plays an important role in the defense against the toxic effects of ROS, which can clean up O_2^- to protect cells from lesions and maintain the balance between oxidant and antioxidant [76]. Supplementation with vit.E at both 240 and 480 mg/kg could down regulate SOD level, while the high dose of vit.E significantly ($P<0.05$) decreased SOD level compared to endosulfan intoxicated group (Table 3). This finding is consistent with Kadry *et al.* [50] who found that dietary of vit.E resulted in depletion in the level of hepatic SOD in female catfish. Likewise, The CAT activity in liver tissue of female *C. gariepinus* increased significantly ($p<0.05$) in response to endosulfan exposure alone when compared to the control (Table 3), Similar increase in hepatic CAT enzyme was also observed in *O. mossambicus* upon exposure to endosulfan [33], female *C. gariepinus* upon exposure to atrazine for 6 weeks [50]. The increase in the activity of CAT enzyme could be contributed to the action of pesticide which induce the removal of ROS from the cell and altering H_2O_2 into H_2O and O_2 [50, 75]. In contrast to the aforementioned values, Ballesteros *et al*. [70] recorded significant reduction in CAT activity of *Jenynsia multidentata* (*J. multidentata*) exposed to endosulfan. However, administration of vit.E at the two doses reversed this marker level in both the fourth and fifth groups, but it was more apparent in the fifth group received vit.E at dose (480 mg/kg) (Table 3). These results are matched with the results obtained by Kadry *et al.* [50] who noticed improvement in CAT activity of female catfish exposed to atrazine and supplemented with vit.E. Hepatic GSH content showed a marked depletion in endosulfan exposed fish throughout the period of study (Table 3) compared to the control ones. Our results are in close agreement with the findings of Dar *et al*. [6, 34] who recorded reduction in GSH content of *C. carassius* exposed to endosulfan. A considerable decline in GSH content in the current study may be due to its utilization to challenge the dominant oxidative stress under the influence of ROS generated from endosulfan exposure. Reduced GSH and its metabolizing enzymes provide the foremost defense against ROS-induced cellular damage [77]. On the other hand, supplementation with vit.E along with endosulfan exposure showed significant ($p<0.05$) improvement in the hepatic GSH content. This improvement was more pronounced in the fifth group received the high dose of vit. E (Table 3). Shakun and Koval'Chuk [78] found that administration of vit.E (α-tocopherol acetate) prevents depletion of the GSH content. These results are consistent with the previous investigation [50].

CONCLUSION

This study concluded that endosulfan exhibits an endocrine disruptive action in female catfish and caused oxidative damage. The incorporation of vit. E in the diet of catfish could minimize the toxicity of endosulfan. Hence, improving their physiological status and in turn raising their resistance.

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