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EFFECT OF PROFENOFOS ON THIOBARBITURIC ACID REACTIVE SUBSTANCES, SCAVENGING ENZYMES AND GLUTATHIONE IN THE BRAIN OF *ALBINO RAT*

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ABSTRACT

By late the development and use of organophosphate (OP) compounds has gained huge mileage than before and this trend is like to continue in the years ahead. This is because new applications for many OPs have been discovered. Nevertheless, indiscriminate and excessive use of these pesticides is causing havoc to non-target organisms. Oxidative stress has recently been implicated as a factor in the mortality and morbidity induced by OP compound poisoning. Hence oxidative stress was studied in the *Albino rats* exposed to Profenofos. They were evaluated for oxidative stress markers MDA (end product of lipid peroxidation) and reduced glutathione (GSH). The effect of $1/10^{th}$ of LD_{50} of Profenofos (*i.e.* 39.5mg/kg body weight) was studied in different regions of the brain of *Albino rat* after exposure to 10, 20 and 30 days respectively. All the animals were administered with Profenofos on alternate days. From the results it is clear that xanthine oxidase, superoxide dismutase, catalase and lipid peroxidation activities showed a steady increase in all the regions of the brain with maximum increase in 30 days exposed animals clearly indicating that the effect of Profenofos is more when the animals are repeatedly exposed to this chemical. Glutathione peroxidase and glutathione reductase activities showed a sharp decline in all the regions of the brain in a time dependent manner. It was concluded on the basis of biochemical analysis that profenofos exposed rats are prone to more oxidative stress as evidenced by the changes in antioxidant status. The measurement of these substances in non-target organisms and agricultural workers who spray OPs could be a good biomonitoring factor and is recommended to be performed on a regular basis. The present study clearly indicates that Profenofos has serious impact on the non-target organisms and should be used with great caution.

KEY WORDS :Organophosphates, Profenofos, *Albino rat*, Brain, Toxicity

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INTRODUCTION

OP compounds have been used widely for several decades in agriculture for crop protection and pest control, thousands of these compounds have been screened and more than one hundred of them have been marketed for these purposes[1].OP compounds constitute a heterogeneous category of chemicals specifically designed for the control of pests, weeds or plant diseases. Currently the development and use of OP compounds has gained more importance than before and this trend is likely to continue in the years ahead, because new applications of these compounds have been discovered. Indiscriminate and excessive use of these pesticides is a major concern to non-target organisms, thus the use of pesticides has become a great concern worldwide[2].The majority of OP insecticides can be grouped according to their chemical structure as a diethoxy OP [with two $O–C_2H_5$ groups attached to the phosphorus that binds to and inhibits acetyl cholinesterase] or a dimethoxy OP (with two $O–CH_3$ groups). The identity of these alkyl groups has fundamental effects on the pharmacodynamics of poisoning and treatment, determining to a large extent whether oximes effectively reactivate OP-inhibited AChE[3]. However, a few OP insecticides do not fit into these categories, including profenofos and prothiofos. Both are highly lipid soluble, moderately toxic OP insecticides. Of note, they have an S-alkyl ($S–C_3H_7$) group attached to the phosphorus, in addition to the more typical $O–C_2H_5$ group. The consequence of this structure is not clearly understood. We therefore studied the sub-lethal effect of profenofos on antioxidant enzymes in the *Albino rat*. Profenofos (O-(4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate) is a moderately hazardous (class II) spectrum insecticide extensively used in agricultural and household applications and causes severe environmental pollution[4].Effect of Profenofos on animal occurs through food and water. Profenofos is a potentially ground water contaminating insecticide, slightly soluble in water and readily miscible in organic solvents.The substance is hydrolyzed with increasing pH and moreover the half-life of profenofos in soil is about one week. This property of Profenofos makes it a better choice for spray as compared to organochlorines, which are more persistent. Profenofos is highly toxic to aquatic organisms, zooplankton, crustaceans and insects. It is moderately toxic to birds and less toxic for mammals[5]. The toxicity caused by profenofos appears fatal even at a fairly low plasma concentration as recorded in a case of fatal human poisoning where high concentrations of metabolites were detected suggesting that profenofos is rapidly metabolized[6]. Profenofos is bioactivated by phosphorothioate oxidation with microsomal enzyme and NADPH. Profenofos induces apoptosis and necrosis in cultured human peripheral blood lymphocytes in *in vitro* conditions[7]. Generation of free radicals and reactive oxygen stress are considered as diagnostic index in profenofos poisoning[4]. The acute toxic action of profenofos is the inhibition of the AChE activity[8]. However, it has been reported that profenofos like other phosphorothiolate insecticides, is activated to desthiopropyl profenofos. Moreover profenofos and its metabolites have been determined in cases of fatal poisoning [6]. Quistad[9] reported that profenofos is an active inhibitor of mouse brain and blood platelet-activating factor acetylhydrolase presumably following the oxidative bioactivation. Profenofos, has been in agricultural use over the last two decades of controlling various pests. It is a non-systemic insecticide and acaricide with both contact and stomach action. The studies on the profenofos biotransformations are vital to explore the impact of profenofos, hence we have studied the effect of sub-lethal dose of profenofos in cholinergic mechanisms in different regions of the brain in *Albino rat.* Oxidative stress has recently been implicated as a factor in the mortality and morbidity induced by OP compound poisoning. Oxidative stress results when pro-oxidant are insufficiently balanced by antioxidants, resulting in cellular damage. Measurement of lipid peroxidation products, e.g.malondialdehyde(MDA) and endogenous oxygen free radical (OFR) scavengers such as SOD, CAT and GPx are effective markers to study OFR effects. Lipid peroxidation is

increased in many disease states as well as in tissues poisoned by toxins. One of the molecular mechanisms of the toxicity of the pesticides seems to be lipid peroxidation; as a consequence these compounds can disturb the biochemical and physiological functions of the brain, hence the study was planned to assess the oxidative damage in the different regions of the brain of *Albino rat* in acute profenofos poisoning.

MATERIALS AND METHODS

Profenofos (98.0% purity) was obtained from Nagarjuna Fertilizers, Hyderabad, A.P., India.

ANIMAL AND EXPERIMENTAL DESIGN

The protocol was approved by Institutional Animal Ethics Committee, S.V. University (ResolutionNo.08/20122013/(i)a/CPCSEA/IAEC/SVU/PJD-VUK/dt.1-2-2012). Male adult *Albino rats* of 7 weeks old and weighing 200 ± 10 g. were obtained from Indian Institute of Science(I.I.Sc.),Bangalore.Theywere housed in an ambient temperature 28 ± 2°C in a 12-h light/dark cycle and a minimum humidity of 40%.The animals had free access to commercial pellet diet supplied by Sai Durga Feeds and Foods, Bangalore, India and water *ad libitium*.All the male healthy adult *Albino rats* were randomly divided into four groups having with six rats per group. The first group animals were considered as control animals. Second group of animals were treated with profenofos via oral gavage (39.5 mg/kg body weight) for 10 days, third and fourth groups of animals were administered for 20 and 30 days with an interval of 48h respectively.

DETERMINATION OF ANTIOXIDANT ENZYMES

XANTHINE OXIDASE (XOD) ACTIVITY

XOD activity was estimated by the dye reduction method[10].The essay mixture contained 100 mM sodium phosphate buffer (pH 7.4), 50µM of INT and the enzyme source. The reaction was initiated by the addition of enzyme source and incubated at 37^{o}C for 30 minutes. The reaction was stopped by the addition of 5 ml of glacial acetic acid and the formazon formed overnight was extracted in toluene and read at 495 nm against toluene blank. The activity was expressed as µM of formazon formed/mg protein/hour.

SOD (SUPER OXIDE DISMUTASE ACTIVITY)

The activity of SOD was assayed by the reduction of nitro blue tetrazolium. Here the superoxide was produced by riboflavin mediated photochemical reaction system. Superoxide dismutase activity was determined according to the method of Beachamp[11].The brain tissue was homogenized in ice cold 50mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA to give 5% homogenate (w/v).The homogenate were centrifuged at 10,000 rpm for 10 minutes at 0ºC in cold centrifuge. The supernatant was separated and used for enzyme assay.The reaction mixture contained 1.7 ml of phosphate buffer (pH 7.8), 150 ml EDTA (10 mM), 600 ml methionine (130 mM), 300 ml nitro blue tetrazolium (750mM) and the enzyme source. The reaction was initiated by the addition of riboflavin and the samples were placed under 15 watts fluorescence bulb for 30 minutes and the absorbance was taken at 560 nm against reagent blank kept in a dark place. A system, devoid of any superoxide radical scavenger was used as a positive control to compare the results. The activity of the enzyme was expressed as units/mg protein.

CATALASE ACTIVITY

Catalase activity was measured by method of Aebi[12]. The brain tissue was homogenized in ice-cold 50 mM phosphate buffer (pH 7.0) containing 0.1mM EDTA to give 5% homogenate (w/v). The homogenates were centrifuged at 10,000 rpm for 10 minutes at 0ºC in cold centrifuge.The resulting supernatant was used as an enzyme source. 10µl of 100% ethyl alcohol was added to 100 µl tissue extract and then placed in an ice bath for 30 min. After 30 min the tubes were kept at room temperature followed by the addition of 100 µl of Triton X- 100 RS. In a cuvette containing 200 µl of phosphate buffer, 50µl of tissue extract and 250 µl of 0.066M H_2O_2 (in phosphate buffer) was added and decrease in optical density was measured at 240 nm for 60 seconds in a UV spectrophotometer.The molar extinction coefficient of 43.6µcm^{-1} was used to determine Catalase activity. One unit

of activity is equal to the moles of H_2O_2 degraded /mg protein/ min.

GLUTATHIONE PEROXIDASE ACTIVITY

Se-Dependent Glutathione Peroxidase was determined by a modified version Flohe[13]at 37˚C. 5% (w/v) of brain tissue homogenate was prepared in 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA. The homogenates were centrifuged at 10,000 rpm for 10 min at 0˚C in cold centrifuge. The resulting supernatant was used as enzyme source. The reaction mixture consisted of 500µl of phosphate buffer, 100µml of 0.01M GSH (reduced form), 100µl of 1.5mM NADPH and 100µl of GR (0.24 units).The 100µl of tissue extract was added to the reaction mixture and incubated at 37˚Cfor 10 min.Then 50 µl of 12m M-t-butyl hydroperoxide was added to 450µl of tissue reaction mixture and measured at 340nm for 180s. The molar extinction co-efficient of 6.22 x 10^3 M cm^{-1} was used to determine the activity. One unit of activity is equal to the mM of NADPH oxidized / mg protein / min. The enzyme activity was expressed in µ moles of NADPH oxidized / mg protein / min.

GLUTATHIONE REDUCTASE (GR) ACTIVITY

GR activity was determined by a slightly modified method of Carlberg[14] at 37˚C. The brain tissue was homogenized (5% - w/v) in to mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA. The homogenates were centrifuged at 10,000 rpm for 10 min at 0˚C in cold centrifuge.The separated supernatant part was used as enzyme source. NADPH(50µl, 2mM) in 10 mM tris buffer (pH 7.0) was added to the curette 50µl of GSSG (20mM) in phosphate buffer (0.5M, pH. 7.0, containing 0.1 mM EDTA) and 800 µl of phosphate buffer. The tissue extract was (100µl) added to the NADPH– GSSG buffered solution and measured at 340 nm for 3min. The molar extinction co-efficient of 6.22 x 10^3 M cm^{-1} was used to determine GR activity.The enzyme activity was expressed in µ moles of NADPH oxidized/ mg protein / min.

LIPID PEROXIDATION ACTIVITY

As a measure of malondialdehyde (MDA) formation,levels of thiobarbituric acid reactive substances (TBARS) were estimated following the method of Ohkawa[15]. Briefly, homogenate of brain regions in 0.1 M phosphate buffered saline (10% w/v) was incubated with 8.1% sodium dodecyl sulfate (SDS,w/v) for 10 min at room temperature followed by the addition of 20% acetic acid. Thiobarbituric acid (TBA,0.8%,w/v) was added in the reaction mixture after vortexing the contents of the tube.The tubes were kept in a boiling water bath for 1 h and the intensity of pink color (chromogen) formed during the reaction was read at 532 nm. The amount of TBARS was calculated using a molar extinction coefficient of 1.56 ×10^5 cm^{-1} M^{-1}.

STATISTICAL TREATMENT

The data was subjected to statistical treatment. One way analysis of variance (ANOVA), two way ANOVA and S-N-K tests were performed using SPSS (ver. 20) in the personal computer and $p<0.01$ was considered as statistically significant.

RESULTS

The results of the present study are presented in the tables (1-6). From the results it is clear that XOD and lipid peroxidaton activities showed a steady increase in all the regions of the brain with maximum increase in 30 days exposed animals, while SOD, catalase, glutathione peroxidase and glutathione reductase showed a steady decline, clearly indicating that the effect of profenofos was more when the animals are repeatedly exposed to this chemical.

DISCUSSION

Poisoning of OP compounds is primarily a problem of developing countries like India. Profenofos, a moderately hazardous (class II) pesticide, is extensively used in agricultural and household applications and causes severe environmental pollution[4]. Several authors have shown that oxidative stress could be an important component of the mechanism of toxicity of OP insecticides. OP insecticides may induce oxidative stress leading to a generation of free radicals and alterations in antioxidants or reactive oxygen species scavenging enzymes[16]. Pesticides

alter malondialdehyde level and antioxidant enzyme activities in gut tissue of insects[17, 18]. In the present study a change in SOD, CAT, GPx activities and MDA level was found in all the regions of the brain and this change was time and dose dependent suggesting that profenofos caused oxidative damage to the brain of the experimental animals. Several authors have reported that OP pesticides caused lipid peroxidation and the alterations in the antioxidant defense enzymes of insect[19,20].MDA, a major oxidation product of peroxidized polyunsaturated fatty acids, has been used to determine the degree of lipid peroxidation and as a biological marker of oxidative stress[21, 22]. It has been reported that pesticides increase MDA level in human erythrocytes and insects[23,24]. In our study, MDA level was increased in all the regions of the brain in the profenofos exposed animals, which suggest that MDA levels could be used as a marker of profenofos injury. Under physiological conditions, intracellular antioxidant enzymes, such as SOD, CAT, and GPx, eliminate ROS, thereby playing an integral role in the oxidative stress defenses of the cell [25].The antioxidant system has been found to be susceptible to damage by OP pesticides[26] and these pesticides have potential to generate free radicals in biological system. Hazarika[27] reported that oxidative stress induces an efflux of GSSG from erythrocytes, which may decrease the red blood cell GSH.It is possible that, the decreased GSH level observed in this study was not sufficient to combat with the enhanced production of MDA during increased lipid-peroxidation as a result of pesticides poisoning[28]. It has also been advocated that the cysteinyl residue of GSH offers a nucleophilic thiol which is important in the detoxification of electrophilic metabolites and metabolically produced oxidizing agents. SOD plays an important role as an antioxidant enzyme and the alteration in the SOD activity suggests that profenofos induces the superoxide radical. In the present investigation SOD activity showed a steady decline with increase in time and dose suggesting that SOD was stimulated by scavenging superoxide radical to protect the animals from profenofos stress. It has been reported that a decrease in SOD activity is probably a response towards increased ROS generation[29]. The decreased activity of CAT is seen in the poisoning cases coupled with an increase in the lipid peroxidation level (MDA) suggesting an insufficient antioxidant defense in the animals. CAT, an enzyme that transforms hydrogen peroxide into hydrogen and oxygen, plays an antioxidant role and its activity is decreased in acute poisoning which indicates that the animal is submitted to severe oxidative stress. In present study, CAT activity significantly decreased in all the regions of the brain in profenofos exposed animals. CAT is perfectly suited for reducing the high amount of H_2O_2 and regulated by the concentration of H_2O_2.The alteration in the SOD activity would result in an increased H_2O_2 concentration and consequently a further alteration in CAT activity. Previous studies have shown that CAT can protect against oxidative stress and extend the lifespan of insects. GSH is a substrate of enzymes namely GPx and Glutathione-S-transferase. GSH/GSSG ratio is a very important indicator of the redox status of the cell. Pesticides poisoning disturbs this ratio, again confirming the presence of oxidative stress. The results of the present study suggest that pesticide poisoning induces oxidative stress by depleting intracellular GSH and increasing ROS production. GSH plays a key role in regulating intracellular levels of ROS by scavenging free radicals to maintain the intracellular redox status. Profenofos appears to disturb this key cellular pathway by disrupting mitochondria metabolism. GPx catalyzes the glutathione-dependent reduction of lipid hydroperoxides and hydrogen peroxide for detoxification. In this study, GPx activity significantly decreased in experimental animals.In present study, we observed that $1/10^{th}$ LD_{50} dose of profenofos caused significant increase in lipid peroxidation and XOD in a time and dose dependent manner indicating an enormous oxidative stress in non-target organisms. Decreased levels of CAT, SOD, GPx and GR suggest insufficient antioxidant defense in these animals. The results of the present study clearly demonstrate that chemical insecticides affect the antioxidant defense system in non-target animals.

Table 1
Changes in the Xanthine Oxidase activity in different regions of the brain of Albino rats exposed to sub-lethal dose of Profenofos

Tissue	Control	10 days	20 days	30 days	F ratio
Cerebral Cortex	0.478	0.536	0.637	0.683	19.470*
± SD	0.039	0.057	0.056	0.023	
(% Change)		(12.26)	(33.32)	(43.05)	
Hippocampus	0.507	0.583	0.650	0.743	26.506*
± SD	0.066	0.035	0.042	0.067	
(% Change)		(14.90)	(28.22)	(46.45)	
Cerebellum	0.805	0.855	0.924	1.083	23.716*
± SD	0.062	0.038	0.030	0.075	
(% Change)		(6.30)	(14.85)	(34.55)	
Medulla Oblongata	0.797	0.899	0.988	1.260	63.849*
± SD	0.078	0.029	0.819	0.059	
(% Change)		(12.78)	(23.93)	(58.08)	

TWO WAY ANOVA

Source of Variation	df	SS	MS	F
Sample	3	1510.734	503.578	11301.27*
Columns	3	23.66138	7.887125	177.0025*
Interaction	9	48.01684	5.335204	119.7324*
Within	80	3.564753	0.044559	
Total	**95**	**1585.977**		

Values expressed in µmoles of formazon formed/mg protein/hr. are Mean ± SD of six individual observations. Values in the parenthesis indicate % change over control. Mean values with the same superscript do not differ among themselves through S-N-K test. *P<0.001

Table 2
Changes in the Superoxide Dismutase activity in different regions of the brain of Albino rats exposed to sub-lethal dose of Profenofos

Tissue	Control	10 days	20 days	30 days	F ratio
Cerebral Cortex	0.683	0.637	0.536	0.478	19.470*
± SD	0.023	0.056	0.057	0.039	
(% Change)		(-6.73)	(-21.52)	(-30.01)	
Hippocampus	0.743	0.650	0.583	0.507	26.506*
± SD	0.067	0.042	0.035	0.066	
(% Change)		(-12.51)	(-21.53)	(-31.76)	
Cerebellum	1.083	0.924	0.855[a]	0.805[a]	23.716*
± SD	0.075	0.030	0.038	0.062	
(% Change)		(-14.68)	(-21.05)	(-25.67)	
Medulla Oblongata	1.260	0.988	0.899	0.797	63.849*
± SD	0.059	0.819	0.029	0.078	
(% Change)		(-21.58)	(-28.65)	(-36.74)	

TWO WAY ANOVA

Source of Variation	df	SS	MS	F
Sample	3	1510.734	503.578	11301.27*
Columns	3	23.66138	7.887125	177.0025*
Interaction	9	48.01684	5.335204	119.7324*
Within	80	3.564753	0.044559	
Total	**95**	**1585.977**		

Values expressed in µmoles of formazon formed/mg protein/hr. are Mean ± SD of six individual observations. Values in the parenthesis indicate % change over control. Mean values with the same superscript do not differ among themselves through S-N-K test. *P<0.001

Table 3
Changes in the Catalase activity in different regions of the brain of Albino rats exposed to sub-lethal dose of Profenofos

Tissue	Control	10 days	20 days	30 days	F ratio
Cerebral Cortex	0.151	0.139	0.120	0.101	19.841*
± SD	0.015	0.005	0.013	0.009	
(% Change)		(-8.20)	(-20.88)	(-33.39)	
Hippocampus	0.395	0.361	0.316	0.276	80.977*
± SD	0.019	0.057	0.013	0.01	
(% Change)		(-9.34)	(-28.34)	(-42.94)	
Cerebellum	0.244	0.217	0.178	0.155	19.121*
± SD	0.030	0.017	0.016	0.021	
(% Change)		(-10.78)	(-26.77)	(-57.23)	
Medulla Oblongata	0.519	0.431	0.366	0.313	15.465*
± SD	0.079	0.023	0.015	0.041	
(% Change)		(-16.91)	(-29.51)	(-39.65)	

TWO WAY ANOVA

Source of Variation	df	SS	MS	F
Sample	3	1.19959	0.399863	472.3865*
Columns	3	0.170845	0.056948	67.27712*
Interaction	9	0.037293	0.004144	4.895141*
Within	80	0.067718	0.000846	
Total	**95**	**1.475446**		

Values expressed in H_2O_2 degraded/ mg protein/ min are Mean ± SD of six individual observations. Values in the parenthesis indicate % change over control. Mean values with the Same Super script do not differ among themselves through S-N-K test. *P<0.001

Table 4
Changes in the Glutathione peroxidase activity in different regions of the brain of Albino rats exposed to sub-lethal dose of Profenofos

Tissue	Control	10 days	20 days	30 days	F ratio
Cerebral Cortex	1.984	1.796	1.552	1.336	21.220*
± SD	0.085	0.152	0.124	0.138	
(% Change)		(-9.46)	(-21.75)	(-32.64)	
Hippocampus	1.966	1.725	1.370	1.156	23.803*
± SD	0.172	0.055	0.205	0.162	
(% Change)		(-12.29)	(-30.32)	(-41.23)	
Cerebellum	2.785	2.571	2.275	1.953	43.076*
± SD	0.096	0.080	0.153	0.293	
(% Change)		(-7.65)	(-18.29)	(-29.86)	
Medulla Oblongata	2.275	2.068	1.867	1.653	28.125*
± SD	0.138	0.101	0.102	0.067	
(% Change)		(-9.07)	(-17.92)	(-27.31)	

TWO WAY ANOVA

Source of Variation	df	SS	MS	F
Sample	3	10.1809	3.393634	162.2963*
Columns	3	7.262772	2.420924	115.7777*
Interaction	9	0.160633	0.017848	0.853564
Within	80	1.672809	0.02091	
Total	**95**	19.27711		

Values expressed in µ moles of NADPH oxidized/mg protein/min are Mean ± SD of six individual observations. Values in the parenthesis indicate % change over control. Mean values with the same super script do not differ among themselves through S-N-K test. *P<0.001

Table 5
Changes in the Glutathione reductase activity in different regions of the brain of Albino rats exposed to sub-lethal dose of Profenofos

Tissue	Control	10 days	20 days	30 days	F ratio
Cerebral Cortex	1.720	1.437	1.306	1.134	22.185*
± SD	0.226	0.187	0.108	0.073	
(% Change)		(-16.45)	(-24.07)	(-34.06)	
Hippocampus	0.949	0.842	0.649	0.523	15.955*
± SD	0.128	0.058	0.111	0.083	
(% Change)		(-11.35)	(-31.68)	(-44.95)	
Cerebellum	1.529	1.298	1.156	0.984	17.776*
± SD	0.130	0.105	0.060	0.144	
(% Change)		(-15.14)	(-24.42)	(-35.63)	
Medulla Oblongata	1.014	0.865	0.795	0.483	49.804*
± SD	0.045	0.086	0.072	0.043	
(% Change)		(-14.68)	(-21.61)	(-52.42)	

TWO WAY ANOVA

Source of Variation	df	SS	MS	F
Sample	3	7.73315	2.577717	195.5847*
Columns	3	0.687445	0.229148	17.38668*
Interaction	9	2.93088	0.325653	24.70901*
Within	80	1.054363	0.01318	
Total	95	12.40584		

Values expressed in µ moles of NADPH oxidized/mg protein/minare Mean ± SD of six individual observations.Values in the parenthesis indicate % change over control. Mean values with the same superscript do not differ among themselves through S-N-K test. *P<0.00

Table 6
Changes in the lipid peroxidation activity in different regions of the brain of Albino rats exposed to sub-lethal dose of Profenofos

Tissue	Control	10 days	20 days	30 days	F ratio
Cerebral Cortex	11.563	13.184	14.827	17.104	22.153*
± SD	0.890	1.148	1.281	1.231	
(% Change)		(14.01)	(28.23)	(47.92)	
Hippocampus	16.735	19.339	20.819	26.558	37.941*
± SD	1.085	1.435	0.771	1.925	
(% Change)		(15.56)	(24.40)	(58.69)	
Cerebellum	23.841	26.228	30.064	35.164	42.255*
± SD	1.734	1.712	1.504	1.536	
(% Change)		(10.26)	(26.10)	(47.49)	
Medulla Oblongata	34.450	37.909	49.304	52.732	46.130*
± SD	1.463	2.530	2.788	4.160	
(% Change)		(10.04)	(43.11)	(53.07)	

TWO WAY ANOVA

Source of Variation	df	SS	MS	F
Sample	3	11546.7645	3848.9215	1080.775289*
Columns	3	1783.18115	594.39372	166.9054664*
Interaction	9	458.750144	50.972238	14.31297971*
Within	80	284.900778	3.5612597	
Total	95	14073.5966		

Values expressed in µmoles of formazon formed/mg protein/hr. are Mean ± SD of six individual observations. Values in the parenthesis indicate % change over control. Mean values with the same superscript do not differ among themselves through S-N-K test. *P<0.001

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