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Antioxidative stimulation by di-and tri-organotin (IV) oximates against hepatic peroxidative stress in experimental animals

Vikram Singh and Dharmendra Singh

Abstract
The antioxidative response of di- and tri-organotin (IV) oximates was examined in contrast to alcohol-induced hepatic peroxidative stress in experimental animals. The 30% alcohol (3.0ml/150g b wt./day, PO, for 30 days) treated experimental animals have been found to be more susceptible to peroxidative stress as measured by thiobarbituric acid reactive species. After 30% alcohol induction to the experimental animals, the concentration of lipid peroxidation was significantly ($p \leq 0.001$) higher in their hepatic tissues and serum, along with a concomitant significant ($p \leq 0.001$) decrease in the levels of enzymatic antioxidants as SOD (superoxide dismutase), CAT (catalase), GPx (glutathione peroxidase), GR (glutathione reductase), GST (glutathione-s-transferase) in the hepatic tissues and, non-enzymatic antioxidants as GSH (reduced glutathione), Vit. C (ascorbic acid), Vit. E (tocopherol), ceruloplasmin, and β-carotene in the serum of treated animals as compared to normal controls. When these animals concomitantly received di- and tri-organotin (IV) oximates (compound-1, 2, 3, 4 & 5), at the dose level of 10mg/kg b wt./day, PO, for 30 days, peroxidative stress was significantly minimal in both the hepatic tissues and serum, along with effectively inducing the antioxidative stimulation in the alcohol-treated animals. In conclusion, these results suggest the increased peroxidative stress in the hepatic tissues is likely to be associated with alcohol induction pathology, which could be reduced by di- and tri-organotin (IV) oximates mediated through interference with free radical generation along with stimulation of antioxidative action. Therefore, some of the di- and tri-organotin (IV) oximates might be promising as artificial antioxidants for curing hepatic peroxidative stress.

Keywords: Antioxidant action, lipid peroxidation, oxidative stress, organotins

Introduction
There is extensive evidence to implicate free radicals in the development of degeneration diseases[1]. Free radicals have been implicated in the causation of ailments such as diabetes, liver cirrhosis, cardiovascular diseases, neurodegenerative diseases, etc. [1, 2] Lipid peroxidation mediated by free radicals is considered to be a primary mechanism of cell membrane destruction and DNA damage [3]. One of the most thoroughly investigated examples is the lipid peroxidation stimulated by the model hepatotoxin-alcohol [3,4]. There appears to be increasing evidence that alcohol toxicity may be associated with increased oxidative stress and free radical-associated injury [1, 4]. The generation of oxygen metabolites such as superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (●OH) is believed to be important in the pathogenesis of alcoholic liver injury [5]. To counteract these oxidants, cells have several antioxidant enzymes including SOD, CAT, GSH,GPx, etc. [2, 5]
Some potential antioxidative activities of N-oxides of tertiary amines have been carried out [6]. Antioxidant activity of butyl- and phenylstannoxanes derived from 2-,3- and 4-pyridine carboxylic acids have been also studied[7]. Souza and Giovani [8] demonstrated that the complexed flavonoids are much more effective free radical scavengers than the free flavonoids. Further, it has been argued that the higher antioxidant activity of the complexes is due to the acquisition of additional superoxide dismutating centers [9]. Boadi *et al*., [10] Have shown that the complexed flavonoid offered better protection than the single treatment and this may be attributed to the better radical scavenging capabilities of the combined treatments over a single treatment.
Although, the mechanisms involved in alcohol-induced peroxidative damage in the hepatic cells are poorly understood. Therefore, in the present study, we have evaluated the

antioxidative stimulation by di- and tri-organotin (IV) oximates against alcohol-induced hepatic peroxidative stress in the experimental animals.

Materials and Methods

Animals

Adult, male, Wistar strain, albino rats weighing 150-170g were used. The animals were housed in standard laboratory conditions and maintained on a rat diet (Lipton India Ltd., Bangalore, India) and tap water *ad libitum* under a natural light-dark cycle.

Syntheses of organotin (IV) oximates

The syntheses of di-/tri- organotin(IV) oximates (compound-1, 2, 3, 4 & 5) were accomplished by the reaction of corresponding organotin(IV) chlorides with sodium salt of 9,10-phenanthrenequinone monooxime in required molar ratios (1:1 or 1:2) (eq. 1)

$$R_{4-n}SnCl_n + mL^- Na^+ \xrightarrow[Reflux]{C_6H_5} R_{4-n}Sn(L)_m\, Cl_{(n-m)} + mNaCl \quad (1)$$

n = 1; m = 1; R = Me(1); R = Et(2); R = Ph (3)
n = 2; m = 1; R = Me (4); m = 2; R = Me (5)

L = 9,10-phenanthrenequinone monooxime

The structures of complexes 1-5 are given in Fig. 1, which have been unambiguously ascertained on the basis of detailed spectroscopic studies.

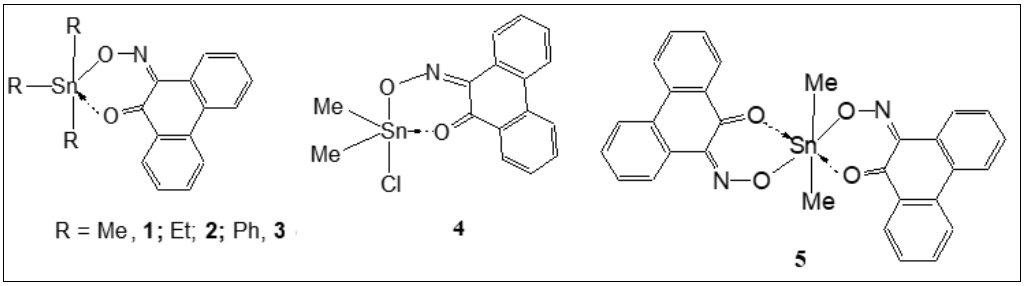

Fig 1: Proposed structures of organotin (IV) oximates

Optimum dose selection of di- and tri-organotin(IV) oximates

Rats were divided into five groups (ten animals/group) for each compound and given orally 10-160 mg/kg b wt./day di- and tri-organotin (IV) oximates with olive oil for 30 consecutive days and observed for any symptoms of mortality and behavioral toxicity for 30 days afterward, then autopsied for examining the hepatic peroxidative stress. The compounds were found to be practically non-toxic when given orally to rats and their LD^{50} values were found to be higher than 160 mg/kg body weight [data not shown]. The minimum dose level *viz*. 10 mg/kg body weight of each compound was used for oral administration to rats during the carrying out of all experiments [11].

Chemicals

All chemicals were analytical grade, and chemicals required for all biochemical assays were obtained from Sigma Chemicals Co., St. Louis, MO, USA.

Ethical Aspects

The study was approved by the ethical committee (Protocol No:1678/Go/a/12/CPCSEA/204) of the University Department of Zoology, Jaipur, India. Indian National Science Academy, New Delhi (INSA, 2000) guidelines were followed for the maintenance and use of the experimental animals.

Experimental protocol

After acclimatization of 15 days, the animals were divided into the following groups of 06 rats in each group:

Group I: Untreated rats were kept on a normal diet and served as control.

Group II: Rats were orally treated with 30% alcohol (3.0ml/twice a day) for 30 days.

Group III: Rats received orally compound (1) (10 mg/kg b wt./day, dissolved in olive oil) for 30 days and 30% alcohol as group II.

Group IV: Rats received orally compound (2) (10 mg/kg b wt./day, dissolved in olive oil) for 30 days and 30% alcohol as Group II.

Group V: Rats received orally compound (3) (10 mg/kg b wt./day, dissolved in olive oil) for 30 days and 30% alcohol as Group II.

Group VI: Rats received orally compound (4) (10 mg/kg b wt./day, dissolved in olive oil) for 30 days and 30% alcohol as Group II.

Group VII: Rats received orally compound (5) (10 mg/kg b wt./day, dissolved in olive oil) for 30 days and 30% alcohol as Group II.

At the end of the experimental period, all treated rats were kept in starvation conditions for 24hrs. Thereafter all rats were anesthetized, blood samples were collected by cardiac puncture, and serum was analyzed for various antioxidant markers such as reduced glutathione (GSH) [12], vitamin C [13], vitamin E [14], ceruloplasmin [15], and β-carotene [16]. Liver was frozen (-20 °C) for biochemical analysis of superoxide dismutase (SOD) [17], catalase (CAT) [18], glutathione peroxidase (GPx) [19], glutathione reductase (GR) [20], and glutathione-S-transferase (GST) [21], respectively. Simultaneously, lipid peroxidation (LPO) [22] was monitored in both serum and liver as an indicator of unsaturated fatty acid formation in the hepatic cells.

Statistical analysis

All values are expressed as mean ± SEM. Data were analyzed by Student '*t*' test. A probability value of $P \leq 0.05$ was considered significant.

Results

The results of biochemical parameters revealed that the administration of 30% alcohol to rats (Group II) caused significant ($P \leq 0.001$) hepatic peroxidative stress as evidenced by lipid peroxidation, enzymatic and non-enzymatic antioxidants through the liver and serum contents in comparison to the normal controls (Group I) following 30 days of the experiments represented in Table-1 and Figs-2, 3&4.

Rats treated with 30% alcohol (Group II), developed a statistically significant ($P \leq 0.001$) elevation of lipid peroxidation in both liver and serum contents in comparison to the normal controls (Group I) after 30 days of the experiment. In contrast, simultaneous treatment with di- and tri-organotin(IV) oximates (compound-1, 2, 3, 4 & 5) (10 mg/kg b wt./day) showed a significant ($P \leq 0.001$; $P \leq 0.01$; $P \leq 0.05$) lowering effect on 30% alcohol-induced elevation of lipid peroxidation (LPO) in both liver and serum contents in the Groups III to VII after 30 days of the treatment (Fig.-2).

Table-1 depicts that the activities of hepatic antioxidants such as SOD, CAT, GPx, GR, and GST declined significantly ($P \leq 0.001$) upon 30% alcohol administration alone to rats (Group II) as compared to group I (normal control). These decreased hepatic antioxidant marker enzymes were significantly ($P \leq 0.001$) brought towards normalization by di-ant tri-organotin(IV) oximates (Compound-1, 2,3, 4 & 5) in the Groups III to VII after 30 days of the experiments, respectively.

Activities of the non-enzymatic antioxidants like GSH, Ceruloplasmin, β−Carotene, Vitamin C, and Vitamin E in serum were significantly ($P \leq 0.001$) decreased in 30% alcohol-treated rats of Group II as compared to normal control Group I (Figs.-3&4). Simultaneously, oral treatment with di- and tri-organotin(IV) oximates (Compound-1, 2,3, 4 & 5), afforded a significant ($P \leq 0.001$; $P \leq 0.01$; $P \leq 0.05$) protection against a 30% alcohol-induced decrease in the levels of serum antioxidants in the Groups III to VII, respectively (Figs.-3&4).

Table 1: Showing antioxidative stimulation by di-and tri-organotin (IV) oximates through enzymatic antioxidants in liver tissue of the control and experimental rats

Groups	SOD(μ mole/mg protein)	CAT(μmol H_2O_2 consumed/min/mg protein)	GPx(n mole NADPH consumed/min/mg protein)	GR(n mole NADPH consumed/min/mg protein)	GST(μ mole CDNB-GSH conjugate formed/min/mg protein)
Normal(Vehicle treated) (Group I)	10.25±0.55	61.33±2.10	12.18±0.48	16.47±0.56	6.86±0.34
30% alcohol(3.0 ml/day, orally) (Group II)	4.15±0.08[a]	23.29±1.08[a]	4.76±0.22[a]	7.31±0.17[a]	2.55±0.08[a]
30% alcohol +Compound-1 (10 mg/kg.b.wt./day, orally) (Group III)	8.95±0.48[a]	56.78±1.49[a]	9.98±0.28[a]	13.26±0.34[a]	5.33±0.19[a]
30% alcohol +Compound-2 (10 mg/kg.b.wt./day, orally) (Group IV)	9.10±0.24[a]	58.28±1.24[a]	8.36±0.21[a]	13.95±0.29[a]	5.44±0.23[a]
30% alcohol +Compound-3 (10 mg/kg.b.wt./day, orally) (Group V)	9.13±0.51[a]	55.16±1.51[a]	10.12±0.29[a]	14.16±0.35[a]	5.93±0.20[a]
30% alcohol +Compound-4 (10 mg/kg.b.wt./day, orally) (Group VI)	6.30±0.09[a]	36.21±.1.10[a]	7.25±0.19[a]	10.51±0.18[a]	4.21±0.10[a]
30% alcohol +Compound-5 (10 mg/kg.b.wt./day, orally) (Group VII)	7.50±0.22[a]	45.35±1.15[a]	9.20±0.31[a]	12.13±0.12[a]	4.90±0.18[a]

Levels of significance: Data are mean ± SEM (n = 6)

a = $P \leq 0.001$ Group II compared with control (Group I); a = $P \leq 0.001$ Group III to VII compared with Group II

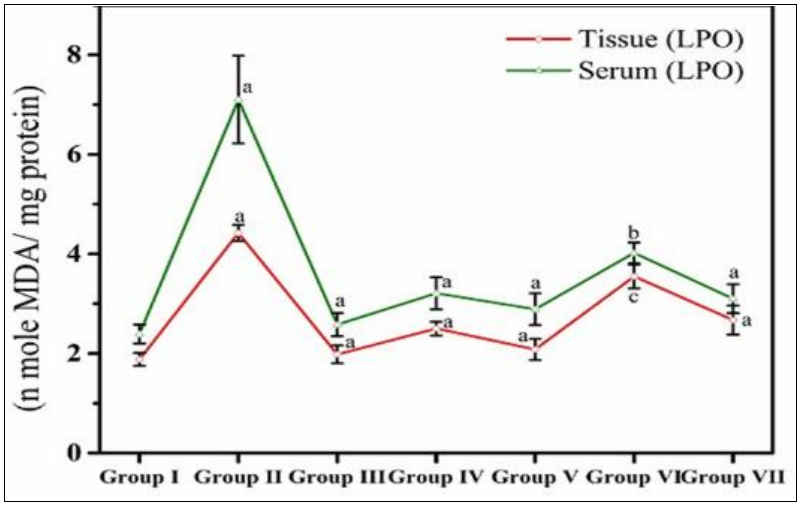

Fig 2: Illustrating antioxidative stimulation by di-and tri-organotin (IV) oximates through lipid peroxidation in serum and liver tissue of the control and experimental rats. Data points with different letter notations (a, b & c) are significantly different at a=$P \leq 0.001$; b=$P \leq 0.01$ & c=$P \leq 0.05$.

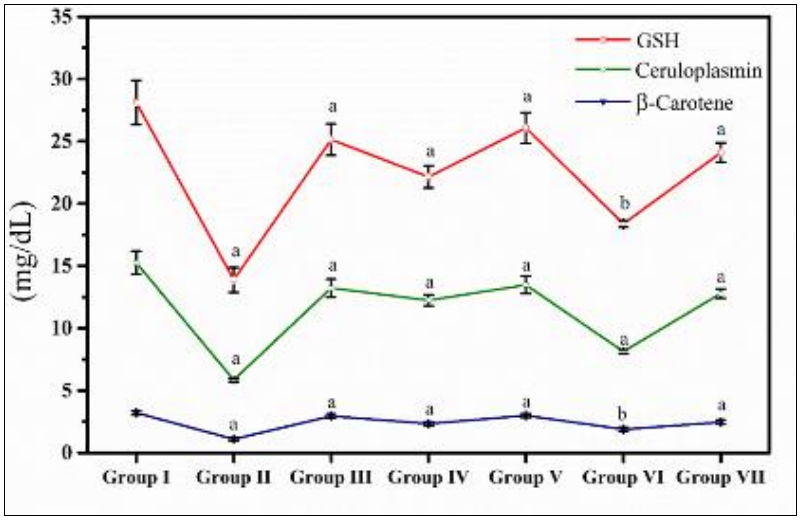

Fig 3: Illustrating antioxidative stimulation by di-and tri-organotin (IV) oximates through non-enzymatic antioxidants-GSH, Ceruloplasmin & β-Carotene in serum of the control and experimental rats. Data points with different letter notations (a & b) are significantly different at a=$P \leq 0.001$ & b=$P \leq 0.01$.

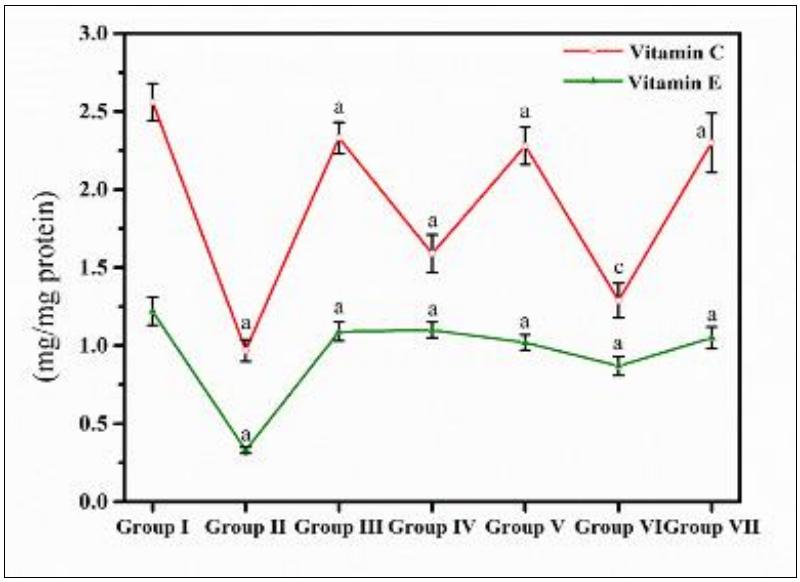

Fig. 4: Illustrating antioxidative stimulation by di-and tri-organotin (IV) oximates through antioxidative Vitamins C & E in serum of the control and experimental rats. Data points with different letter notations (a & c) are significantly different at a=$P \leq 0.001$ & c=$P \leq 0.05$.

Discussion

Alcohol promotes the generation of free radicals which interfere with normal defense mechanisms and damage the cellular macromolecules such as fats, proteins, or DNA through numerous processes, particularly in the liver. Because alcohol breakdown in the liver results in the formation of molecules whose further metabolism in the hepatic cell leads to oxidative stress [5]. Alcohol also stimulates the activity of enzymes called cytochrome P450s, which contribute to the generation of excess free radicals. The excess free radicals reduce the normal level of antioxidants. Therefore, the resulting state of the cell leads to its injury and oxidative stress in the hepatic cells [5, 23].

In the state of oxidative stressed hepatic cells, enhanced lipid peroxidation (LPO) is one of the most dangerous manifestations of chronic alcohol ingestion. Pieces of evidence have indicated that excessive alcohol consumption leads to overproduction of MDA in the liver due to lipid peroxidation, causing hepatic injury and apoptosis [23, 24]. Since, a number of free radicals such as hydroxyethyl radical, superoxide radical ($O_2^{\bullet -}$), hydroxyl radical (●OH), peroxyl radical (ROO•), and hydrogen peroxide (H_2O_2) are implicated in alcohol-induced lipid peroxidation [23]. These enhanced free radicals increase the degree of LPO in serum and liver tissue during alcohol ingestion [25].

In our present protocol, the measurement of LPO in the liver and serum is a convenient method to monitor hepatic peroxidative stress. Inhibition of elevated LPO has been observed in the organotin (IV) derivatives treated groups due to their ability to prevent free radicals or oxidative stress from oxidizing the hepatocellular membranes by donating electrons to scavenge free radicals. Similar results were presented in previous studies in support of the results of reduced MDA levels by antioxidant compounds in the alcoholic liver injury models [9, 11].

Generally, our body has an efficient antioxidant defense mechanism to neutralize the peroxidative damage by using some endogenous antioxidants like SOD, CAT, GPx, GR, GST, GSH, ceruloplasmin, β-carotene, vitamin C, vitamin E, etc. Since SOD catalyzes the dismutation of radical anions to H_2O_2 and $O_2^{\bullet -}$. A number of studies have shown the importance of SOD in protecting hepatic cells against oxidative stress [25, 26]. Our study has shown a decrease in SOD activity in the hepatic tissue during alcohol ingestion.

This decrease may be due to oxidative inactivation of enzyme protein by the excess generation of α-hydroxy ethyl radical which leads to inactivation of the enzyme [23]. CAT acts as a preventative antioxidant and plays an important role in the protection against deleterious effects of lipid peroxidation during alcohol ingestion. The inhibition of catalase activity is suggestive of enhanced synthesis of $O_2^{\bullet-}$ during the ingestion of alcohol since $O_2^{\bullet-}$ is a powerful inhibitor of CAT [23, 26]. GPx plays a well-established role in the protection of hepatic cells against peroxidative stress. Because GPx is non-specific for H_2O_2 and lack of this substrate specificity extends a range of substrates from H_2O_2 to organic hydroperoxides. Therefore, the excess H_2O_2 and lipid peroxides generated during alcohol ingestion are efficiently scavenged by GPx activity. The despair of this enzyme activity reflects perturbations in normal oxidative mechanisms during alcohol ingestion [5, 23].

Glutathione reductase (GR) is responsible for maintaining the supply of intracellular GSH by reducing GSSG in the presence of NADPH and FAD (flavine adenine dinucleotide) by the activation of GPx. After alcohol ingestion, the decreased GR activity may reflect the decline in the availability and production of GSH to conquer H_2O_2.This may be due to over production of H_2O_2 which can inactivate the GPx activity and finally it can lead to disturbing the GSH/GSSG ratio [25, 27]. GST is a soluble protein located in the cytosol and plays an essential role in the liver by eliminating toxic compounds by conjugating them with GSH and increasing the solubility of hydrophobic substances. During alcohol ingestion, high production of H_2O_2, and overuse for toxic compounds elimination, its activity was reflected below the normal level [25, 28].

GSH is a cysteine-containing peptide found in the majority of cells. It is not required in the diet and is instead synthesized in the cells from its constituent amino acids. GSH has antioxidant properties since the thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced [1, 5]. In the hepatic cells, GSH is maintained in the reduced form by the enzyme glutathione reductase and in turn, reduces other metabolites and enzyme systems as well as reacts directly with free radicals. Due to its high concentration and central role in maintaining the cell's redox state, glutathione is one of the most important cellular antioxidants [1, 27].

Ceruloplasmin is a glycoprotein made in the hepatic cells**.** It is a principal carrier of mineral- copper in plasma and involved in oxidative modification of lipoproteins and participates in the chronic/acute phase reaction to peroxidative stress. Therefore, ceruloplasmin is an effective antioxidant for a variety of free radicals and has a potent peroxidative activity to decompose H_2O_2 in the presence of GSH [28, 29]. β-Carotene is a fat-soluble member of the carotenoids which are considered pro-vitamins because they can be converted to active vitamin A (retinol), which is essential for vision. It is a strong antioxidant and is the best quencher of singlet oxygen and other free radicals during alcoholic peroxidative stress. Thereby, due to its excess use, it may reflect below the normal level [25, 28, 30].

Vitamin C is a monosaccharide antioxidant found in the cells. As it cannot be synthesized in humans and must be obtained from the diet but most other animals are able to produce this compound in their bodies and do not require it in their diets. In cells, it is maintained in its reduced form by reaction with GSH, which can be catalyzed by protein disulfide isomerase and glutaredoxins [1, 25]. Since vitamin C is a reducing agent thereby neutralizes reactive oxygen species such as H_2O_2 during the alcoholic peroxidative damage and altered from the normal levels [1, 28, 30].

Vitamin E is the collective name for a set of eight related tocopherols and tocotrienols, which are fat-soluble vitamins with antioxidant properties. Of these, α-tocopherol has been most studied as it has the highest bioavailability, with the body preferentially absorbing and metabolizing this form [1, 25]. It has been claimed that the α-tocopherol form is the most important lipid-soluble antioxidant and that it protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction during alcoholic peroxidative damage. This removes the free radical intermediates and prevents the propagation reaction from continuing. This reaction produces oxidized α-tocopheryl radicals that can be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol, or ubiquinol [1, 30].

The antioxidant defense mechanisms also have been suggestive of playing an important role in maintaining physiological levels of oxygen and hydrogen peroxide and eliminating peroxides generated from inadvertent exposure to alcohols and drugs [25, 28]. Any compound with antioxidant properties may help in maintaining health when continuously taken as components of dietary food or drugs [28]. In the present study, the significantly increased levels of antioxidant profiles *i.e*. SOD, CAT, GPx, GR, GST, GSH, ceruloplasmin, β-carotene, vitamin C, and vitamin E by the supplementation of organotin(IV) derivatives may be attributed to having biological significance in eliminating reactive free radicals that may affect the normal functioning of hepatic cells in 30% alcohol-treated rats [31].

Conclusion

It may be concluded that the biochemical alterations observed in hepatic stress seem to be mainly due to an oxy-radical-mediated mechanism, involving lipid peroxidation under conditions of reduced antioxidant levels that scavenge $O_2^{\bullet-}$, H_2O_2, and lipid peroxides. The results yet available are overwhelming, which suggests that the organotin(IV) derivatives are effective in their antioxidant properties and tri-organotin(IV) oximates are more active antioxidants than that di-organotin(IV) derivatives (R_3ML > $R_2M(L)$ Cl, R_2ML_2). Further, the activities of tri-organotin (IV) derivatives follow the order: Ph_3SnL > Me_3SnL ≃ Et_3SnL due to some relation between structure and bioactivity exhibited by organotin (IV) derivatives.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

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