

## Effects of $\alpha$ -Tocopherol on Liver Biochemistry of Endosulfan Intoxicated Mice: A Preliminary Study



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**Abstract :** The present study was designed to evaluate the protective role of  $\alpha$ -tocopherol (vit.E) against the toxic effects of chlorinated insecticide endosulfan. Forty male albino mice were used as mammalian model in this study. Animals were divided into 4 groups (ten animals each) on the basis of Vitamin-E treatment and endosulfan exposure, Vitamin treatment was started 15 days prior to 1st exposure to endosulfan. Animals were exposed to endosulfan @ 5mg/kg body weight by intramuscular route and vitamin-E @ ---mg/kg body weight, was administered by forced drinking. Animals were sacrificed after 15 and 30 days of first exposure to endosulfan and the role of Vitamin-E in reducing Endosulfan induced toxicity was evaluated by using liver tissue biochemistry. Comparisons were made with respective control groups (table 1 & 2)

A significant inhibition was found in (1) Alkaline phosphatase in both vitamin non-treated and vitamin treated exposed groups at 30 days, (2) protein, and body weight in only vitamin non-treated exposed groups after 15 and 30 days. In contrast a significant elevation was observed in (1) GOT and RNA at 15 and 30 days and (2) LDH at 30 days in both vitamin non-treated and vitamin treated exposed groups. The comparison was also made between vitamin treated and non treated exposed groups (table 3 and 4), it showed a pronounced elevation in, LDH (15 & 30 days), RNA (15 days), DNA & GOT (30 days) in vitamin non treated exposed group. It can be concluded from present study that Endosulfan alters various biochemical parameters (AP, GOT, LDH, RNA and protein) and  $\alpha$ -tocopherol may play a protective role in reducing toxicity of endosulfan.

**Key words :**  $\alpha$ -tocopherol, Liver Biochemistry, Endosulfan Intoxicated Mice

### Introduction

Pest control chemicals are poisons and they may present immediate danger to user if used improperly. Some of these are highly toxic and may cause serious metabolic disorders and even death if inhaled or ingested through oral route (Frank and Brawn, 1984; Zhou and Hu, 1984). Important among these are organochlorines, which have been used widely during previous

years (Lodha and Saxena, 1991). Extensive use and limited biodegradation are the two major factors involved in their worldwide contamination and biomagnifications (Hargrave *et al.*, 1992; Fossi *et al.*, 1995; Nichols *et al.*, 1995).

Endosulfan is one of the organochlorine (OC) compounds used extensively for the control of agricultural pests. Its metabolites have strong tendencies to get accumulated in

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different organs and tissues of the body *e.g.*, adipose tissue, liver and food items (Winter and Street, 1992; Thao *et al.*, 1993). They induce metabolic changes in liver, which are indicators of toxicity. A positive correlation between changes in liver structure and biochemical constituents of the liver and serum has been shown in a number of studies on different mammals exposed to various pesticides (Boulechbache and Spries, 1974; Gertig and Nowakzyk, 1975; Ali and Shakoori, 1990, 1996 and 1999). However, a considerable variation in nature, magnitude and direction of changes in response to pesticide exposure are evident from these studies.

Several underlying mechanisms have been invoked in past to explain the nature, of changes in liver under given conditions of pesticidal exposure and dosage (Kimbrough *et al.*, 1971; Meany and Pocker, 1979). The suggested mechanisms include elevated biosynthetic activity associated with parallel regeneration of liver tissue, concomitant curtailment of leakage of enzymes from it and elevated synthesis of liver enzymes. This leakage of enzymes is obviously due to impaired functions of plasma membrane and it has been reported that administration of lindane significantly decreases the brush border sialic acid content of the membrane, which alters membrane permeability (Labana *et al.*, 1997). It is believed that the loss of plasma membrane permeability is due to the production of reactive oxygen species (ROS) during the process of detoxification of pesticides. ROS provoke unwanted reactions in cell and lead to membrane damage, alterations in metabolic activity, necrosis and cell death. The altered concentration of antioxidants along with elevated activity of antioxidant enzymes has

been reported in sprayer population (Parakasam *et al.*, 2001) indicating their role in detoxification of pesticides.

Keeping in view the deleterious effects of organochlorines and protective role of vit. E in various conditions, the present study was planned to evaluate the protective role of Vitamin-E against the potential damages and abnormalities produced by insecticide exposure

## Materials and Methods

In order to evaluate any effect of  $\alpha$ -tocopherol (vitamin-E) on liver biochemistry of Endosulfan intoxicated animals. Forty mice were divided into 4 groups (I-IV), ten animals in each group, Group I was not exposed to any treatment, Group II was exposed to Vitamin-E, group III was exposed to endosulfan and Group IV was given Vitamin E as well as endosulfan. Vitamin treatment was started 15 days prior to 1<sup>st</sup> exposure to endosulfan. A rough estimate of LC-50 (through intramuscular route) was made prior to actual experimentation and a sub-lethal dose was selected for exposure to insecticide. Animals were exposed to endosulfan intramuscularly @ 5 mg/kg b.w./week (one injection/ wk) and vitamin-E was administered by force drinking @ 80 mg/kg b.w./day (group was administered a daily dose of Vitamin E that would have equaled 800 units in human subjects). Animals were sacrificed after 15 and 30 days of first exposure to endosulfan *i.e.* after 2 and 4 times exposure to endosulfan respectively. Liver was dissected out. The effects of Vitamin-E on liver tissue biochemistry were evaluated by using various parameters. Comparisons were made between groups (i) I vs III, (b) II vs IV, (c) IV vs III and (d) I vs II using Student's "t" test (Steel and Torrie, 1982).

Body weight and various parameters of

liver biochemistry were selected as indicators of toxicity. Saline extract was prepared by homogenizing a weighed piece of liver in measured quantity of 0.89% (ice cold) saline in a glass homogenizer. The homogenate was centrifuged at 4000 rpm for 15 minutes, to obtain clear supernatant. It was used for different enzymes and soluble protein estimations. Another portion of liver was weighed and processed for the extraction and estimation of nucleic acids (DNA and RNA) following Shakoori and Ahmad (1973).

Aqueous liver extract was used for the estimation of (i) Alkaline phosphatase (Ap), and Acid phosphatase (AcP) activity according to Kind and King (1954), (ii) Glutamate Oxaloacetate transaminase (GOT) and Glutamate pyruvate transaminase

(GPT) activity according to Reitman and Frankle (1957), (iii) Lactate dehydrogenase (LDH) activity according to Weisshaar, (1975) and soluble proteins were estimated using Biuret method (Henry *et al.*, 1974).

### Results and Discussion

Antioxidants are now being tried to ascertain that (1) pesticides behave as peroxidants and (2) To minimize losses due to ROS released during the process of detoxification at sublethal continuous exposure to various toxicants (Reuber, 1981; Zhang *et al.*, 2001; Aldana *et al.*, 2001). Present study has been designed to evaluate the role of antioxidant in reducing the toxicity of endosulfan. Tables 1-2 are showing effects of  $\alpha$ -tocopherol on various parameters in endosulfan intoxicated mice.

**Table 1 : Effects of  $\alpha$ -tocopherol on body weight and Hepatic Enzymes in Endosulfan Intoxicated Mice.**

Parameters	Duration of treatment	GROUPS (n = 5)			
		I	II	III	IV
Body Weight (g)	15	28.52±0.35a	26.25±0.95	22.87±0.15**b	28.52±1.23
	30	27.60±0.33	27.00±0.40	23.00±1.23*	25.50±0.50
AP (KAU/g)	15	0.61±0.003	0.58±0.008	0.48±0.001	0.32±0.006
	30	0.64±0.008	0.65±0.001	0.259±0.005*	0.255±0.001*
AcP (KAU/g)	15	2.29±0.05x	3.85±0.53	2.27±0.06	3.55±0.38
	30	4.43±0.13	4.34±0.31	4.61±0.16	3.218±0.25
GOT (IU/g)	15	38.52±4.21	37.85±3.21	52.62±2.59***	50.20±3.8**
	30	36.67±1.57	34.65±3.15	55.5±1.5***a	49.9±2.5**
GPT (IU/g)	15	40.23±2.57	37.15±5.21	38.53±4.75	33.25±4.28
	30	39.8±1.90	41.1±4.5	36.52±4.72	35.84±6.66
LDH (IU/g)	15	132.18±3.74	145.0±8.32	199.82±29.51*a	164.1±39.4
	30	160.0±17.31	150.0±10.50	211.0±5.37**a	192.0±12.3**

<sup>a</sup> Mean  $\pm$  SEM

Estarics on group III show significant differences from group I and on group IV show significant differences from group II \* P>0.05; \*\* P>0.01; \*\*\* P>0.001

Alphabets **a** and **b** on group III show significant differences from group IV., **a**=p>0.05, **b**=p>0.01

Alphabets **x** on group I show significant differences from group II., **x**=p>0.05

**Table 2 : Effects of Vit, E on Tissue Protein and Nucleic Acids in Endosulfan Intoxicated Mice**

GROUPS→ PARAMETERS↓		I (n = 3)	II (n = 3)	III (n = 3)	IV (n = 3)
Protein (x10 <sup>2</sup> mg/g)	15 days	2.67±0.04	3.12±0.04	1.47±0.02*** <sup>b</sup>	2.85±0.08
	30 days	2.40±0.04	3.10±0.05	1.30±0.01*** <sup>c</sup>	2.70±0.01
RNA (mg/g)	15 days	2.75±0.02	2.35±0.08	3.78±0.03*** <sup>b</sup>	2.42±0.05
	30 days	2.52±0.08	2.45±0.03	3.60±0.08* <sup>a</sup>	2.05±0.05
DNA (mg/g)	15 days	0.32±0.001	0.38±0.004	0.48±0.005	0.31±0.002
	30 days	0.31±0.008	0.34±0.006	0.56±0.004	0.31±0.003

<sup>a</sup> Mean SEM

Estarics on group III show significant differences from group I \* P>0.05; \*\* P>0.01; \*\*\* P>0.001  
Alphabets a - c on group III show significant differences from group IV., a = p>0.05, b=p>0.01, c=p0.001

Body weight is an indicator of protein and fat metabolism. Various authors have shown a negative correlation in body weight and dose of toxicants *i.e.*, decrease in body weight gain with increase in dose and time of insecticide treatment (Laborda and Delaperia, 1983; Ali and Shakoori, 1996, 1999). In present study, a significant reduction in body weight 19.8% and 16.6%, after 15 and 30 days respectively, was recorded in vitamin non-treated exposed group (group III) when compared with group I and 19.8% after 15 days when compared with group IV. The reduction in body weight may be due to high rate of protein breakdown, which might be needed to fulfill energy requirement during detoxification. At the same time, no variations could be recorded in any of vitamin treated exposed group (comparison in II vs IV). It might be due to some protective role of vitamin E. Cabral *et al.* (1982) did not report any adverse effect of Dichloro-diphenyl-trichloroethane on body weight up to 500 ppm dose. Alkaline Phosphatase (Ap) is widely distributed in the body. The

activity of alkaline phosphatase altered in present experiments. It reduced both in vitamin treated and non-treated intoxicated groups after 30 days of endosulfan treatment. The magnitude of deviation was 60.3% in group IV when compared with group II and 59.5% in group III with respect to group I vitamin non-treated group after 30 days of exposure to toxicant. This depletion might be due to low level of synthesis of the enzyme or change in permeability of hepatocyte membrane leading to its leakage from the cell. Similar depletion in alkaline phosphatase was also reported by Chitra *et al.* (1999) in testis of endosulfan treated rats, who considered it due to decreased metabolic activities. Acid Phosphatase (AcP) is an enzyme of lysosomal origin, but is also found in the endoplasmic reticulum and possibly in the hyaloplasm. AcP is used to estimate interference with catabolic and autophagic processes in the liver. No significant alterations could be recorded in AcP level in any experimental group except slight depletion in group I when compared with group II, but it seems to be some

experimental error or vitamin-E treatment might have helped in stabilizing the concentrations of various enzymes.

GOT is one of the enzymes, which gives valuable diagnostic information for a number of disease conditions. In present study a significant elevation in GOT was recorded in vitamin non-treated exposed groups (comparison between group I vs III). The magnitude of variation was 36.7% and 51.3% after 15 and 30 days respectively. The vitamin treated group also showed significant elevation in GOT level in endosulfan exposed groups (comparison between group II vs IV). The variations were 32.1 and 44.1% after 15 and 30 days respectively. The raised level may be (1) due to enzyme induction as a result of endosulfan stress or (2) endosulfan may have adversely affected oxidation by Krebs's cycle. Vitamin E succinate is known to protect hepatocytes against toxic effects of reactive oxygen species at mitochondrial complexes I and III (Zhang *et al.*, 2001). As elevated levels of GOT have also been observed in vitamin treated groups thus our findings are not in agreement with above-mentioned authors. Glutamate Pyruvate Transaminase (GPT) plays an important role in transporting amino group to liver in a non-toxic form via a pathway called Glucose alanine cycle. In various tissues of body that degrade amino acids for fuel, amino groups are collected in the form of glutamate by transamination. In present study, GPT has remained unaltered in all groups. There are evidences that GOT alters frequently as compared to GPT in various types of stress conditions (Nelson and Cox, 2001). Ali and Shakoori, (1996) have also reported similar results.

Lactate dehydrogenase catalyses the reversible oxidation of L. lactate to pyruvate.

It is also capable of oxidizing a number of  $\alpha$ -hydroxy acids. The enzyme has been found to be very sensitive to exposure to endosulfan. Just like GOT, its level has also been found significantly elevated in both vitamin treated and non-treated, exposed groups (comparisons Groups I vs III, II vs IV and IV vs III) but elevated level in Vit. treated group (IV) was observed only after 30 days of exposure to endosulfan, where as in vitamin non-treated exposed group (III), the higher level of LDH was found high throughout the experimentation. The higher levels of LDH may be due to elevated rate of enzyme synthesis it is probably reflecting enhanced rate of gluconeogenesis. Similar results have been reported in various studies on pesticide exposure (Story and Freedland, 1979). It is interesting to note that in vitamin treated group, the rise in LDH level was delayed. It is pointing towards some protective role of vitamin E (Zhang *et al.*, 2001).

Proteins are good indicators of metabolic activity of cell. Hepatocyte fluids contain both stimulatory and inhibitory factors that selectively alter the protein synthesis and secretions. A significant depletion (44.9% and 45.8%) in soluble protein was recorded in vitamin non-treated exposed groups (at 15 and 30 days) respectively, (comparison I vs III) a similar depletion in protein concentration was also observed while comparing group IV with group III but no such variation could be observed in vitamin treated exposed group (II vs IV). The depletion may either be due to low level of anabolic activity of cell or higher levels of degradative activities. The higher level of catabolic activity is also evident by raised levels of GOT which is involved in breakdown of proteins to amino

acids and routing of these amino acids to Krebs cycle for meeting higher energy requirements under endosulfan insult (Barros and Soliba, 1978; Zhou *et al.*, 1985). The depletion in soluble protein contents has been reported by various authors under insecticidal stress (Murty *et al.*, 1986; Chitra *et al.*, 1999).

Concentration of RNA in cell reflects the rate of transcription in the cell. Liver showed higher levels of RNA only in vitamin non-treated exposed group (I vs III, and IV vs III) whereas no such variation could be recorded in rest of the experimental groups. But at the same time, total soluble proteins were found depleted in the same group. It might be indicating that endosulfan has the potential to arrest protein synthesis at translation stage. At the same time no alteration in RNA level was observed in vitamin treated exposed group (II vs IV), which might be due to vitamin supplementations.

Deoxyribonucleic acid (DNA) is confined to the nucleus. Its variation in tissue is of clinical importance. In present study an increasing pattern of DNA in vitamin non-treated exposed group was observed in all experiments but the variation was not statistically significant. It might be due to slight hypertrophy of tissue under insecticidal stress. Depletion in total DNA contents in testis has been reported by Chitra (1999), whereas Ali and Shakoori (1996) have reported a raised level of DNA in liver of rats treated with chlorinated insecticide. No variation in vitamin treated exposed group might be reflecting a protective role of  $\alpha$ -tocopherol.

Following conclusion could be drawn from present study :

1. Some parameters (AP, GOT, LDH, RNA and protein).are more sensitive to toxicity as compared to others (AcP, GPT, DNA and body weight).

2.  $\alpha$ -tocopherol may play a protective role in reducing toxicity of endosulfan. The supplementation of other antioxidant along with vitamin E may give better results.. Chen et al. (2001) have reported that vitamin E along with ascorbic acid modulate activity of NADPH oxidase and superoxide dismutase. NAD is an important electron acceptor of Krebs's cycle.

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### **References**

- Aldana L., Tsutsumi V., Craigmill A., Silveira M.I. and Gonzalez De Mejia E. (2001) :  $\alpha$ -tocopherol modulates liver toxicity of the pyrethroid cypermethrin. *J. Toxicol cett.*, **125**: 107-116.
- Ali S.S. and Shakoori A.R. (1990) : Toxicity of aldrin in rats. *Punjab Univ. J. Zool.*, **5**: I-56.
- Ali S.S. and Shakoori, A.R. (1999) : Hepatotoxicity of Lindane (Gamma HCH) in albino rats: Short and long term studies. *Pakistan J. Zool.*, **31**: 175-185.
- Ali S.S. and Shakoori A.R. (1996) : Short and long term toxicity of DDT in albino rats: Biochemical effects in liver. *Punjab Univ. J. Zool.*, **11**: 7-24.
- Barros S.B.M. and Saliba M.A. (1978) : Toxicity of the hexachlorocyclohexane in rats. *Toxicology.*, **10**: 271-280.
- Berg B.M., Godbout J.P., Kelley K.W., Johnson R.W. (2004) :  $\alpha$ -tocopherol attenuates lipopolysaccharide-induced sickness behavior in mice. *Brain Behav Immun.* **18**:149-57.
- Boulecache H. and Spiess C. (1974) : Effect of lindane on trout fly (*Salmo irideus* Gibb).

- Changes in glycolytic enzymes. *Bull. Soc. Zool. F.R.*, **99**: 79-85.
- Bourdel-Marchasson I., Delmas-Beaurieux M.C., Peuchant E., Richard-Harston S., Decamps A., Reignier B., Emeriau J.P. and Rainfray M. (2001) : Antioxidant defenses and oxidative stress markers in erythrocytes and plasma from normally nourished elderly Alzheimer patients. *J. Age-ageing*, **30**: 235-241.
- Cabaud P.G. and Wroblewski F. (1985) : Colorimetric measurement of lactate dehydrogenase activity of body fluids. *Am. J. clin. Pathol.*, **30** : 234-236.
- Cabral J.R.P., Hall R.K., Rossi L., Bronczk S.A. and Shubik P. (1982) : Lack of carcinogenicity of DDT in hamsters. *Tamori*, **68**: 5-10.
- Chen X., Touyz R.M., Park J.B. and Schiffrin E.L. (2001) : Antioxidant effects of vitamin C and E are associated with altered activation of vascular NADPH oxidase and superoxide dismutase in stroke prone spontaneously hypertensive rats. *J. Hypertension.*, **38**: 606-611.
- Chitra K.C., Latchoumycandane C. and Mathur P.P. (1999) : Chronic effect of endosulfan on the testicular functions of rat. *Asian. J. Androl.*, **1**: 203-206.
- Chugh S.N., Dhawan R., Agrawal N., Mahajan S.K. (1998) : Endosulfan poisoning in Northern India: a report of 18 cases. *Int. J. Clin. Pharmacol. Ther.*, **36**: 474-477.
- Fossi M.C., Massi A., Lari L., Marsili L., Focardi S., Lionzio C. and Renzoni A. (1995) : Interspecific differences in mixed function oxidase activity in birds: Relationship between feeding habits, detoxication activity and organochlorine accumulation. *Environ. Pollut.*, **90**: 15-24.
- Frank R. and Braun H.E. (1984) : Lindane toxicity to 4 month-old calves. *Bull. Environ. Toxicol.*, **32**: 530-533.
- Gertig H. and Nowaczyk W. (1975) : The influence of lindane on enzymes activity in the tissues of rats on protein rich diet. *Pol. J. Pharmacol. Pharm.*, **27**: 529-537.
- Hargrave B.T., Harding G.C., Vass W.P., Erickson P.E., Fowler B.R. and Scott V. (1992) : Organochlorine pesticides and polychlorinated biphenyls in the Arctic ocean food web. *Arch. Environ. Toxicol.*, **22**: 41-54.
- Henry R.J., Cannon D.C. and Winkelman J.W. (1974) : Clinical chemistry: Principles and techniques, 2<sup>nd</sup> ed. Harper and Row, New York, pp.96-98.
- Kimbrough R.D., Gaines T.B. and Linder R.E. (1971) : The ultrastructure of livers of rats fed DDT and dieldrin. *Arch. Environ. Hlth.*, **22**: 460-467.
- Kind P.R.N. and King E.J. (1954) : Estimation of plasma phosphatases by determination of hydrolysed phenol with aminoantipyrine. *J. clin. Pathol.*, **7** : 322-326.
- Kolaja K.L., Xu Y., Walborg E.F. Jr., Stevenson D.E., Klaunig J.E. (1998) : Vitamin E modulation of dieldrin-induced hepatic focal lesion growth in mice. *J. Toxicol Environ Health A*. **53**: 479-92.
- Labana S., Bansal R.C. and Mahmood A. (1997) : Differential effects of Lindane on intestinal functions in normal-fed and malnourished rats. *J. Pestic. Biochem. Physiol.*, **57**: 192-199.
- Laborda E. and Delapena E. (1983) : Statistical evaluation of the toxic effects of the p,p'-DDT in NMRI mice. *Arch. Pharmacol. Toxicol.*, **9**: 59-64.
- Lodha R.M and Saxena V. (1991) : Pesticides and environmental pollution. *Himanshir publications Udaipur, India*. **1**: 87-90
- Maggi-Capeyron M.F., Cases J., Badia E., Cristol J.P., Rouanet J.M., Besancon P., Leger C.L. and Descomps B. (2002) : A diet high in cholesterol and deficient in vitamin E induces lipid peroxidation but does not enhance antioxidant enzyme expression in rat liver. *J. Nutr. Biochem.*, **13**: 296-301.
- Meany J.E. and Pocker Y. (1979) : The in vivo inactivation of lactate dehydrogenase by organochlorine insecticides. *Pestic. Biochem. Physiol.*, **11**: 232-242.
- Murthy B.N., Reddy M.S., Venkates Warli Y. and Rao K.V.R. (1986) : Lindane induced alterations

- in the protein breakdown and utilization in the selected tissues of freshwater fish, *Tilapia mossambicus* (Peters). *Natl. Acad. Sci. Lett. (India)*, **9**: 27-30.
- Nelsen D.L. and Cox M.M. (2001) : Lehinger Principles of Biochemistry, 3<sup>rd</sup> ed. MacMillan worth Publishers, pp.631-632.
- Nichols J.W., Larsen C.P., McDonald M.E., Niemi G.J. and Ankley G.T. (1995) : Bioenergetics-based model for accumulation of polychlorinated biphenyls by nesting tree swallow, *Tachycineta bicolor*. *Environ. Sci. Technol.*, **29**: 604-612.
- Nigam S.K., Thakore K.N., Kamik A.B. and Lakkad B.C. (1984) : Hepatic glycogen, iron distribution and histopathological alteration in mice exposed to hexachlorocyclohexane fed mice. *J. Arch. Environ. Hlth.*, **37**: 156-158.
- Parakasam A., Sethupathy S. and Lalitha S. (2001) : Plasma and RBCs antioxidant status in occupational male pesticide sprayers. *J. Clin. Chim. Acta.*, **310**: 107-112.
- Reitman S. and Frankel S. (1957) : A colorimetric method for the determination of serum glutamate oxaloacetate and glutamate pyruvate transaminase. *Am. J. clin. Pathol.*, **28** : 56-63.
- Reuber M.D. (1981) : Therole of toxicity in the carcinogenicity of endosulfan. *J. Sci. total. Environ.*, **29**: 23-47.
- Sai-Kato K., Umemura T., Takagi A., Hasegawa R., Tanimura A., Kurokawa Y. (1995) : Pentachlorophenol-induced oxidative DNA damage in mouse liver and protective effect of antioxidants. *Food Chem Toxicol* **33**:877-82.
- Shakoori A.R and Ahmad M. (1973) : Studies on the liver of chicken. *Gallus domesticus*. Liver growth and nucleic acid contents. *Pakistan J. zool.*, **5** : 111-117
- Soo C.C., Haqqani A.S., Hidiroglou N., Swanson J.E., Parker R.S., Birnboim H.C. (2004) : Dose-dependent effects of dietary alpha- and gamma-tocopherols on genetic instability in mouse Mutatect tumors. *J Natl Cancer Inst.* **96**:796-800.
- Steel R.G.D. and Torrie J.H. (1982) : Principles and procedures of statistics. A biometrical approach, 2<sup>nd</sup> ed. McGraw Hill, Kogalkusha., pp152.
- Story D.L. and Freedland R.A. (1979) : The effect of DDT feeding on gluconeogenesis in isolated hepatocytes from starved rats. *Toxicol. Appl. Pharmacol.*, **43**: 547-557.
- Thao V.U.D., Kawano M. and Tatsukawa R. (1993) : Persistent organochlorine residues in soils from tropical and sub-tropical Asian countries. *Environ. Pollut.*, **81**: 61-71.
- Thathoo A.K. and Prasad M.C. (1989) : Experimental malathion toxicity in lambs. A biochemical study. *Ind. J. Anim. Sci.*, **59**: 1237-1242.
- Tilak K.S., Janardhana Rao N.H. and Jhansi L. (1991) : Effect of pesticides mixed in different ratio to the fresh water, *Labeo rohita*. *J. Ecotoxicol. Environ. Monit.*, **1**: 49-52.
- Weisshaar H.D. et al (1975) Estimation of Lactate Dehydrogenase in serum/plasma. *Med. Welt.* **26**:387
- Winter S. and Street B. (1992) : Organochlorine compounds in the three step terrestrial food chain. *Chemosphere.*, **24**: 1766-1774.
- Zhang J.G., Nicholls Gremaskif., Trimenstein M.A. and Farris M.W. (2001) : Vitamin E succinate protects hepatocyte against the toxic effect of reactive oxygen species generated at mitochondrial complexes I and III by alkylating agents. *J. Chem. Biol. Interact.*, **138**: 267-284.
- Zhou Y. and Hu F. (1984) : Survey of the accumulation of BHC and DDT in body fat of the general population of Wuhan (China). *Wuhan Yixueyuan Xuepao.*, **41**: 344-346.
- Zhou Y., Huang L., Liu X., Yang L. and Liang X. (1985) : Experimental studies of the toxicity of chlorinated hydrocarbon insecticide hexachlorocyclohexane. *Zhonghua Yufungyixue Zazhl.*, **19**: 338-340.