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EFFECT OF OXIDATIVE STRESS ON ACUTE EXPOSURE ENDOSULPAN OF POISONING IN THE COMMUNITY

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ABSTRACT

Endosulfan compounds have been widely used for a few decades in agriculture for crop protection and pests control. Thousands of these compounds have been screened and over one hundred of them have been marketed for these purposes. The present study is focused on the effects of oxidative stress on acute exposure of endosulfan poison. The study was conducted at the emergency care department of a tertiary care teaching hospital, which is a 1200 bedded multidisciplinary super specialities government hospital. The study period was eight months from Jan 2011 to Aug 2011. The patients included in the study were those who had ingested endosulfan compound available as household or agricultural pesticide. Among 90 patients, 59 males and 31 were females. The mean age was 31.16±2.67 years with a range of 18-60 years. A control group consisting of 56 unexposed pesticides, were age-matched, who never had any exposure to pesticides as control group. The study highlighted 18-30 years age group patient who were most commonly ingested endosulfan compound. Whereas, MDA levels was found to be 19.887±1805 nM/ml, which is significantly (P => 0.0001) increased compared to healthy subjects. Whereas, glutathione levels was found to be 4.924±8991µmol/ml which is significantly lower (P = >0.0001) compared to healthy subjects and similarly, total antioxidant level was found to be 13.52±2.469 µmol/ml which is significantly lower (P = >0.0001) compared to healthy subjects. Our results elaborate the significance of oxidative stress in endosulfan poisoning and emphasis clinicians to prescribed antioxidants. However, in future pharmacological, histopathological studies necessary to explore toxicokinetics.

Key words: Antioxidant, Endosulfan, Glutathione, Morbidity, Mortality.

INTRODUCTION

Endosulfan (6,7,8,7,10-hexachloro-15,5a,6,9,9a hexahydro 6,9a hexahydro 6,9 methano 2,4,3 benzoicxanthiepin-3-oxide), a polycyclic chlorinated hydrocarbon of cyclodiene group is a widely used pesticide [1]. Pesticide induced oxidative stress as a possible mechanism of toxicity has been a focus of toxicological research [2]. Endosulfan is an important environmental pollutant, which is a pesticide of organochlorine. Primary site of organochlorine storage in the body is adipose tissue. It is metabolized in the liver as a lipophilic xenobiotic to hepatotoxic intermediates by monooxygenase systems which cause oxidative stress [3]. Free radicals generated during oxidative stress cause lipid peroxidation of cell membranes which is in turn prevented by antioxidant enzymes [4]. Endosulfan alters the activities of lactic dehydrogenase, glucose-6-phosphate dehydrogenase and alkaline phosphatase, and decreases mitochondrial energy production [5]. Lactic dehydrogenase is a hydrogen transferring enzyme that catalyzes the oxidation of L-lactate to pyruvate with the mediation of NAD+ as hydrogen acceptor. LDH activity is present in all cells of the body and is invariably found only in the cytoplasm of the cells [6]. Neurotoxicity is one of the major issue of concern in acute endosulfan exposure [7]). Effect of endosulfan poisoning with suspected involvement of central nervous system has been reported [8]. Organo chlorine (OC) compounds impair nervous system function by depolarization of the nerve membranes. They facilitate synaptic transmission and inhibit the GABA–chloride channel complex [9]. These agents accumulate within

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lipid-rich tissues. They also cause sensitization of the myocardium to both endogenous as well as exogenous catecholamines and predispose to arrhythmias. Radicals initiate auto catalytic reactions where by molecules with which they react are themselves converted into free radicals to propagate the chain of damage. Even a healthy body produces free radicals, many of them being oxygen free radicals. They may form in response to expose to the sun, toxic chemicals, air pollution, or as part of various metabolic process. The present study is focused on the effects of oxidative stress on acute exposure of endosulfan poison.

MATERIALS AND METHODS

The present study was conducted at the Emergency Departments of a tertiary care teaching hospital, i.e., Mahatma Gandhi Memorial Hospital, Warangal, Andhra Pradesh, India, which is a 1200 bedded multidisciplinary super specialties government hospital. The study was carried out over a period of eight months. The patients included in the study were who had undergone exposure to endosulfan poison as agricultural pesticides.

In addition to that emergency department serves residents up to a 6 mile west of the middle town area. Sample / Data collection was performed according to hospital regulations after approval by the Hospital administration. The setting was the emergency department of an inner city level trauma centre with approximately 85,000 patient visits per year. Patients between 1 to 90 years old and exposed to endosulfan poisons were selected. The study population consisted of 90 endosulfan poisoning cases admitted between the months of January 2011 to August 2011. A control group consisting of 56 unexposed pesticides, who never had any exposure to OC pesticides was taken as a reference group.

All subjects diagnosed as case of endosulfan poisoning, on the basis of history of the victim or the attendant along with the clinical features like mydriasis, numbness, bed wetting, seizures, agitation, weakness and throat pain were included in our study [14]. All subjects attendee completed a detailed standardized questioners especially aimed the time and the quantity of consumption of the endosulfan compounds. The victims were also sorted for different epidemiological factors like age, gender, marital status, socio-economic status, representative area (rural / urban) and the mode of intake (suicidal / homicidal / accidental). All patients were observed in the emergency department of our hospital by specialists in emergency medicine. The treatment included specific antidotes like phenobarbitone and ampicillin and supportive treatment like IV fluids and an antibiotic as prophylaxis.

The outcome was compared with severity and time lapse between ingestion of compound and initiation of the therapy. If patients needed advanced treatment they were sent either to other service or the ICU in our hospital, like RICU (Respiratory Intensive Care Unit) where emergency care observations and mechanical ventilator supports are available at bedside. The Protocol of the study was submitted to the Superintendent of our hospital and to Kakatiya Medical College to obtain the Ethical Committee approval. The study began after the approval was granted.

The literature supporting the study was collected and analyzed. The different sources used to collect the literature were Micromedex drug information databases, various websites like pub med, science direct, DOAJ, Dove press, Medline, etc.

Venous blood samples were collected from the patients after obtaining Inform consent from the patient or the attendee. The samples were collected in 5 ml heparinised vials (for plasma) and 5ml plain tubes (for serum). The samples were immediately centrifuged at 3000 rpm for 30 minutes and supernatant layer separated in labelled eppendroff's tubes and kept at 40ºC till biochemical analysis.

At the baseline all the patients had a complete history and physical examination. Pulse rate, blood pressure and ECG recordings were taken on arrival in the Emergency department. ECG analysis included the calculations of PR interval, QRS duration, RR interval, QT and QTc interval. QT interval was measured from the first deflection of QRS complex to the point of 'T' wave offset, defined as the return of the 'T' wave to the baseline of the ECG. The QTc interval was calculated using Bazett's formula [12] ludomirsky A *et al;* (1983). QTc was considered to be prolonged when it was longer than 0.41 sec for males and 0.42 sec for females.

1. Kidney function tests - Serum creatinine and blood urea were estimated using the kits (Excel diagnostics ltd) with colorimetric methodology.

2. Oxidative stress

Estimation of lipid peroxides

The amount of lipid per oxidation products present in the serum samples were estimated by the Thiobarbituric acid reactive substances (TBARS) method [16].

Procedure: To 0.5 ml of plasma/serum 0.5 ml of 30% Trichloro acetic acid (TCA) was added to precipitate the proteins and vortexed for 30 sec. Clear supernatant was taken after centrifuge at 3000 rpm for 10 minutes. To the supernatant 100µl of 1%TBA solution was added and the solution was heated for 1hr at 98ºC. It was kept in ice for 10-15 minutes. Then the supernatant was collected. The absorbance of mixture which was pink in color was read at 532 nm. The MDA content was determined from standard graph by using standard 1, 1, 3, 3 – Tetra ethoxy propane (Sigma Aldrich) in different concentrations.

2. Antioxidant status

Glutathione

Glutathione forms a coloured complex with DTNB, which is measured spectrophotometrically [14].

Procedure: To 0.5 ml of citrated blood, 0.5 ml of 5% trichloroacetic acid (TCA) solution was added to precipitate the proteins and centrifuged at 3000 rpm for 20 minutes. To 0.1 ml of supernatant, 1 ml of sodium phosphate buffer and 0.5 ml of DTNB (Himedia Labs) reagent were added. The absorbance of the yellow colour developed was measured at 412 nm. The glutathione content was determined from standard graph by using pure glutathione (Himedia Labs).

Estimation of Total Antioxidant levels

For the estimation of total antioxidant status, we used a stable free radical 2, 2 – diphenyl – picryl hydrazyl (DPPH – Sigma Aldrich) at the concentration of 0.2mM in methanol [15].

Procedure: 0.1ml of plasma was deproteinted by the addition of 1ml of methanol, vortexed for 30sec. Then centrifuge at 3000 rpm for 30 minutes to separate the proteins. To the clear supernatant 1.5ml of methanol and 0.5ml of DPPH solution were added, mixed thoroughly and absorbance was read at 517nm against blank, prepared in an identical way but without the addition of serum / plasma. Ascorbic acid (Finar Chemicals) was used as a reference standard. The standard graph was plotted using different concentrations of ascorbic acid and the antioxidant status values were expressed in terms of nM of ascorbic acid.

Statistical Analysis

The data were analyzed with unpaired 't' test, using the software Graph pad prism version-5. P values < 0.05 were considered significant.

RESULTS

Among 90 patients 59 were males and 31 females. We collected blood sample from 56 before initiation of therapy to the patients. Whereas, mean age was 31.16±2.67 years with a range of 18 to 60 years. There was no significant difference in the mean age between gender. Majority 41 (45%) of the patients were in the 21-30 age groups. The mean hospital stay was 7 days. Whereas, 34 (38%) patient was died because they was admitted to the hospital after 6 hours. It has been shown in table 1.

However, during our study period of 90 patients, there were, 14 (15%) students, 28 (31%) farmers, 17 (19%) housewives, labour 18 (20%) and 13 (14%) service holders. A majority of patients had suicidal intention. In this context, 6 patients had a history of previous suicidal attempt. The patients admitted as early as 30 minutes to as long as 12 hours after ingestion of the poison with 90% of the patients admitted to hospital within 2 hours after ingestion, with the mean time interval of about 2 hour 10 min. In addition to that, electrocardiographical manifestations of acute endosulfan poisoning found to be QTc prolongation, tachycardia, bradycardia (in few cases), ST-T changes (slight ST elevation & T wave depression). PR interval was found to be normal in our study. The MDA level found was 19.887±1805 nM/ml which is significantly increased compared controls (16.44±1.480). The levels of glutathione were 4.924±8991µmol/ml, which is significantly lower compared to controls (12.15±0.478) µmol/ml. The Total Antioxidant levels in those patients were found to be 13.52±2.469µmol/ml, which are significantly lower compared controls (32.84±2.705) µmol/ml. It has been depicted in table 2.

Table 1. Metabolic Markers in Endosulfan Poisoning Male Vs Female

Variable	Standard Deviation		'P' value	
	Male	Female	Male	Female
Age	9.501±2.02	8.99±3.18	>.0001	>.0001
BMI	1.922±.4098	2.22±.7869		
Systolic BP	16.22±3.459	20±7.071		
Diastolic BP	11.10±2.366	14.88±5.261		
MAP	12.90±2.602	16.05±5.678		
Blood urea	14.18±3.026	4.749±1.679		
Blood glucose	44.15±9.413	36.13±12.78		
Serum Creatinine	0.1738±.0370	0.1069±.0404		

"BMI- Body Mass Index; MAP- Mean Arterial Pressure"

Table 2. Oxidative Stress and Antioxidant Status in Control Versus Endosulfan Poisoning Patients

Variable	Control n=56	Endosulfan patients n=56	'P' Value
MDA (nM/ml)	16.44±1.48	19.887±1.805	>.0001
GSH (µmol/ml)	12.15±0.55	4.924±0.8991	>.0001
TAS (µmol/ml)	32.84±2.705	13.52±2.469	>.0001

"MDA- Malondialdehyde; GSH- Glutathione; TAS- Total Antioxidant Status"

DISCUSSION

Venkateshwarlu *et al* reported that Endosulfan poisoning may present with clinical or laboratory evidence of organ dysfunction to liver, kidney and muscles. Previously reported several cases, acute pathologic change of lungs, severe dynamic instability, elevated ALT and AST activities, acute tubular necrosis, haematological effects such as leucocytosis and thrombocytopenia and hyperglycaemia were observed in patients following injection of lethal quantity of endosulfan [10]. There are several pathways by which pesticide are thought to induce oxidative stress. It inhibits the mitochondrial electro-transfer chain reaction, leading to accumulation of semi ubiquitous, which enables it to transfer one electron to molecular oxygen to form superoxide radicals. Further it may also interference with cellular anti-oxidant defense system via alteration in activities of antioxidant enzymes. Endosulfan acts as a catalyst in the oxidative deterioration of biological macro molecules and this effect could be minimized by treatment with anti-oxidants. These indirectly suggest an increased production of oxygen free radicals in erythrocytes. Highly reactive oxygen metabolites, special hydroxyl radicals, act on unsaturated fatty acids of phospholipids components of membranes to produce MDA, a lipid peroxidation product. However, an antioxidant enzymes GSH, TAS, limit the effect of antioxidant molecule on tissues and are active in the defense against oxidative cell injury by means of their being free radical scavengers. These enzymes work together to eliminate active oxygen species and small deviation in physiological concentrations may have a dramatic effect on the resistance of cellular lipids, proteins and DNA to oxidative damage. Endosulfan is a chlorinated hydrocarbon insecticide and acaricide of the cyclodiene subgroup, which act as contact poison to a wide variety of insects and mites [11]. Clinical effects from endosulfan exposure are usually noted within 0.5 to 6 hours [10]. These rapid onset of action attributed endosulfan rapid absorption and distribution to lipophilic depots including CNS. Clinical signs following acute exposure are indicative of CNS disturbance or over stimulation. In our study, GTCS, pupil dilation, nausea and vomiting were common. However, it is unclear whether nausea and vomiting were the result of GI irritation or medication. In the present study nausea and vomiting began within 1 hour after ingestion and progressively subsides within several hours [13]. Our study reviews that endosulfan cause significantly increase GSH activity in human erythrocyte compared to the control value. There are several pathways by which pesticide is thought to induce oxidative stress. It inhibits the mitochondrial electron-transfer chain reaction, leading to accumulation of semi ubiquitous, which enables it to transfer one electron (e-) to molecular oxygen to form superoxide radicals [13]. Endosulfan acts as a catalyst in the oxidative deterioration of biological macromolecules and this effect could be minimized by treatment with antioxidants. These indirectly suggest an increased production of oxygen free radicals in erythrocytes. Highly reactive oxygen metabolites, especially hydroxyl radicals, act on unsaturated fatty acid of phospholipid components of membranes to produce malondialdehyde, a lipid peroxidation product. Endosulfan has been reported to induce oxidative stress, as shown by enhanced MDA production [2]. The use of vitamin E in conjunction with endosulfan affected such elevation in the level of MDA; bringing it within the normal limits ($p<0.05$). The normalization of LPO following vitamin E treatment is very likely due to its antioxidant properties, as has been shown previously [17]. However, the antioxidant enzymes GSH and TAS limit the effects of oxidant molecules on tissues and are active in the defence against oxidative cell injury by means of their being free radical scavengers [18]. These enzymes work together to eliminate active oxygen species and small deviations in physiological concentrations may have a dramatic effect on the resistance of cellular lipids, proteins and DNA to oxidative damage. The extent of the MDA was found significantly enhanced as evidenced by the increased serum TBARS activity levels ($p<0.001$) and reduced level of TAS and GSH ($p<.01$) as compared to control. The activity of antioxidant enzyme GSH and TAS in the red blood cells were found significantly decreased compared to the controls ($p<0.001$).

CONCLUSION

It can be concluded that endosulfan insecticides induced oxidative stress and lipid per oxidation level in human erythrocytes has ameliorated these effects. However, endosulfan compounds can produce significant ECG abnormalities such as QT prolongation and non-specific ST-T changes. The prolongation of QTc can process lethal arrhythmia, so that special care should be provided to patients exposed to endosulfan poisoning to the appropriate medical treatment.

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