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Endosulfan-Induces Renal Toxicity Independent of the Route of Exposure in Rats

[1]F.E. Uboh, [1]E.N. Asuquo, [1]M.U. Eteng and [2]E.O. Akpanyung

[1]Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Calabar, P.M.B. 1115, Calabar, Nigeria

[2]Department of Biochemistry, Faculty of Health Sciences, University of Uyo, Uyo, Nigeria

Corresponding Author: F.E. Uboh, Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Calabar, P.M.B. 1115, Calabar, Nigeria

ABSTRACT

Endosulfan is known to be one of the highly toxic agricultural pesticides commonly used in our societies. With the widespread use of endosulfan in agriculture, human beings are most likely to be exposed to it, either orally by eating endosulfan-contaminated foods or by nose and whole body inhalation in the farms during its application. In this study, we assessed the route-of-exposure-dependent effect of endosulfan on renal functions in male rats, using serum creatinine, urea, Blood Urea Nitrogen (BUN), uric acid, Na^+, Cl^- and K^+, as well as histopathological assay. Eighteen mature male albino Wistar rats (200±20 g), divided into three groups of six rats each, were used in this study. The 20 mg kg^{-1} body weight of endosulfan was daily administered orally to one group of rats while another group was exposed to ungraded concentration of endosulfan by nose and whole body inhalation exposure method, (4 h daily, 6 days per week), for 30 days. It was observed from the results of this study that exposure to endosulfan by both oral and inhalation routes produced a significant increase ($p<0.05$) in serum creatinine, urea, BUN, uric acid and K^+ and a significant decrease ($p<0.05$) in serum Na^+ and Cl^- levels, compared, respectively to the control. Also, microscopic examinations showed that both routes of exposure to endosulfan exhibited severe a histopathological damage to the renal tissues. These observations indicated that exposure to endosulfan may be a risk factor for nephrotoxicity in rats, independent of the route of exposure.

Key words: Endosulfan, creatinine, urea, BUN, electrolytes, nephrotoxicity

INTRODUCTION

Endosulfan is one of the synthetic organochlorine compounds widely used as agricultural insecticides. The chemical is sometimes found to affect non-target organisms including humans, in the course of its application. Because of the serious environmental problems resulting from the use of pesticides in the agricultural sector, several governments are seeking to employ biological and other nonpolluting methods for combating pests. Several biocides and/or their metabolites are suggested to be prior mutagenic and/or teratogenic compounds (El-Sharkawy *et al*., 1994). Although endosulfan is reported to be one of the most toxic pesticides in the market today, responsible for many fatal pesticide poisoning incident around the world; it is still used in many tropical developing societies (Extension Toxicology Network, 2000). Particularly, endosulfan is known to be an endocrine disruptor, being highly toxic at acute exposure (Chitra *et al*., 1999;

Rose *et al.*, 1999; Fulia *et al.*, 2011). Like DDT and other pesticides that have been found to cause irreparable damage to human and environmental health, endosulfan may pose a greater risk than the product label would lead one to believe (Extension Toxicology Network, 2000). Typically, it has been shown to be neurotoxic, implicated in hyperactivity tremors, convulsion, lack of coordination, staggering, difficulty in breathing, nusea and vomiting, diarrhea and severe cases, unconsciousness and even permanent brain damage (USEPA, 2002; Dutta and Arends, 2003; Yavuz *et al.*, 2007). Exposure to endosulfan may therefore, cause myriad negative health effects in human and animal studies, damaging nontarget organisms (Chitra *et al.*, 1999; Rose *et al.*, 1999; Sharma and Chauhan, 2009; Fulia *et al.*, 2011).

Endosulfan can be absorbed into the body either through ingestion, inhalation and/or skin contact. The LD_{50} of endosulfan varied widely depending on the route of administration, species, vehicle and sex of the animal (Boereboom *et al.*, 1998; Rezg *et al.*, 2006). According to Boereboom *et al.* (1998), the oral LD_{50} of endosulfan in rats ranges from 18-35 mg kg^{-1} body weight. 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 (Rezg *et al.*, 2006). Hence, chemical pesticides like endosulfan and their metabolites may induce metabolic changes which are indicators of toxicity, in liver and other tissues. Several underlying mechanisms have been highlighted to explain the nature of changes in liver under given conditions of pesticidal exposure and dosage (Rezg *et al.*, 2007; Saadi *et al.*, 2008). Literature shows that male rats given a single oral dose of endosulfan at 40 mg kg^{-1} body weight displayed neurotoxic manifestations and showed a significant increase in blood glucose, blood ascorbic acid and blood and brain glutathione, as well as decrease in plasma calcium levels (Garg *et al.*, 1980). Studies have also shown that endosulfan toxicity resulted in increased lipid peroxidation in liver, kidney and gills of a fish (Kurutas *et al.*, 2006). Endosulfan is reported to possess the ability to reduce erythrocyte, lung and liver Superoxide Dismutase (SOD) activity as well as lung and liver Glutathione (GSH) levels in rat (Bebe and Panemangalore, 2003). Among the suggested mechanisms of endosulfan induced toxicities in biological systems include the generation of Reactive Oxygen Species (ROS) and other free radicals into the system during the process of detoxification of the pesticide. The generated ROS may then provoke certain unwanted reactions in the cell and lead to membrane damage, alterations in metabolic activity, necrosis and cell death. The action of these reactive species on the cell membranes alters the permeability of the membrane, impairs the functionality of the plasma membrane, causing the intracellular constituents to leak out into the extracellular compartment (Banerjee *et al.*, 1999; Etemadi-Aleagha *et al.*, 2002). The leakage of the intracellular constituents, including 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 (Hagen *et al.*, 1999).

Moreover, the interaction of the ROS and other reactive metabolites, generated from endosulfan metabolism, with the renal tissues may as well cause cellular injury, hence, damage to the tissues. Once the renal tissues are damaged, the overall functionality of the kidneys may be compromised. The kidney functions may be assessed from the level of some electrolytes (such as K^+, Na^+, Cl^-) and metabolites (such as creatinine, urea and blood urea nitrogen) in the plasma (Atangwho *et al.*, 2007; Uboh *et al.*, 2009). Renal dysfunction may be caused by several diseased conditions and exposure to certain reactive or toxic metabolites (Jimoh and Odutuga, 2004). Also, renal dysfunction of any kind affects all parts of the nephron to some extent, although sometimes, either glomerular or tubular dysfunction is predominant. The net effect of renal disease on plasma and

urine depends on the proportion of glomeruli to tubules affected and on the number of nephrons involved. Since endosulfan is a frequently used pesticide and the incidence of toxic injury to the liver in relation to its widespread use reported in the literature (Sharma and Chauhan, 2009), we considered it necessary to investigate whether exposure to endosulfan also induces renal injury. In this study, route of exposure dependent effect of endosulfan on renal functions was investigated in male rats. Rats were selected for this study because of their remarkable biochemical, physiological and indeed genetic systems and responses, similarities to those of the human systems.

MATERIALS AND METHODS

Animals experimental design: Eighteen mature albino Wistar rats, weighing between 180 to 2000 g were obtained from Biochemistry Department Experimental Research Animal House of the University of Calabar, Calabar, Nigeria. They were fed with a standard laboratory diet and tap water. Illumination was 12 h light/dark cycle and room temperature was 25±2°C. The animals were divided into experimental and control groups which consisted of apparently normal albino Wistar rats. The experimental group was further divided into two groups (Group A and B), of six rats each. Rats in Group A were exposed to endosulfan by oral administration (20 mg per kg body weight) daily for 30 days while the rats in Group B were exposed to ungraded concentrations of endosulfan by 4 h daily nose and whole body exposure method for a period of 30 days. Each group was made up of six rats. The endosulfan fraction administered orally was solubilized in Goya Olive oil as a vehicle. In this study, all animal experiment followed the Guide for the care and use of laboratory animals obtained from the Institutional Animal Ethics Committee. This work was carried in October/November, 2010.

Nose and whole body inhalation exposure to endosulfan: The liquid endosulfan (Thionex 35EC) used in this study was obtained from ABC Agrochemical shop in Watt Market, Calabar, Nigeria. The animals in the test Group B were exposed to ungraded concentrations of endosulfan vapors in an exposure chamber. A modified nose and whole body inhalation exposure method previously described for gasoline vapors (Uboh *et al.*, 2008, 2009), was used to expose the animals in this group to ungraded concentrations of endosulfan vapors. In this method, endosulfan vapors were generated from 100 mL of liquid endosulfan pumped daily by a manual spraying machine into the exposure chamber (1.5×0.9×2.1 m), simulating an endosulfan polluted agricultural environment. The chamber compartment was fully saturated with the endosulfan vapors before the animals were transferred into it. These animals were then allowed to freely inhale the vapors in the chamber during the exposure period through nose and whole body route of exposure. At the end of each day's exposure period, the animals were transferred to a non-endosulfan-contaminated section of the animal house. An exposure period of 4 h (9.00 am to 1.00 pm) daily, 6 days per week, was adopted for 30 days.

Collection and handling of blood serum for biochemical assays: Twenty-four hours after last exposure, the animals were anaesthetized with chloroform vapour and dissected. Whole blood from each animal was collected by cardiac puncture into well labelled non-heparinized sample tubes and allowed to clot for 3 h in iced water. The serum was separated from the clots after centrifuging at 10,000 rpm for 5 min into well-labeled plain sample bottles and used for biochemical assays.

Serum urea and blood urea nitrogen: Urea in serum was estimated by the endpoint colorimetric method using Dialab reagent kits (Searcy *et al.*, 1967). In this method, urease enzyme hydrolyses

urea to ammonia and carbon dioxide. The ammonia so formed reacts with alkaline hypochloride and sodium salicylate in the presence of sodium nitroprusside to form a colored chromophore which was measured with DREL 3000 HACH (England) model spectrophotometer.

Serum creatinine: The concentration of serum creatinine was assayed based on the reaction of creatinine with an alkaline solution of sodium picrate to form a red complex (Newman and Price, 1999). The red coloured complex which is proportional to the concentration of creatinine in the sample was measured spectrophotometrically.

Serum potassium: Potassium in serum was determined by photometric turbidemetric test using TECO analytical reagent kits (Tietz, 1976). Potassium ions in a protein-free alkaline medium react with sodium tetraphenylboron to produce a finely dispersed turbid suspension of potassium tetraphenyboron, whose turbidity is in proportion to the potassium concentration originally in the sample.

Serum sodium: Serum sodium concentration was estimated using Mg-Uranylacetate reaction method described in Dialab diagnostic kits (Trinder, 1957). Sodium in serum is precipitated with Mg-Uranylacetate, the remaining uranyl ions form a yellow-brown complex with thioglycolic acid. The difference between reagent blank analyses is proportional to the sodium chloride.

Serum chloride: Chloride in serum was determined using mercuric thiocyanate reaction method described in Dialab diagnostic kits (Tietz, 1976). Chloride ions in the sample react with mercuric thiocyanate displacing the thiocyanate ions. The displaced thiocyanate ions react with ferric ions producing a coloured complex.

Histopathological examination: For light microscopic examination, liver and kidney tissues from each groups were fixed with 10% buffered formalin, embedded with paraffin. After routine processing, paraffin sections of each tissue were cut into 4 μm thickness and stained with haematoxylin and eosin.

Statistical analysis: The data for the biochemical assay results were presented as Mean±SEM. These results were analyzed using the Statistical Package for Social Sciences (SPSS for windows, version 11.0). Comparison were made between experimental groups using one-way Analysis of Variance (ANOVA) followed by Student's t-test. Values of less than 0.05 (i.e., $p = 0.05$) were regarded as statistically significant.

RESULTS

Changes in the levels of serum creatinine, urea, Blood Urea Nitrogen (BUN), uric acid, total protein, albumin, sodium, potassium and chloride ions (that is Na^+, K^+ and Cl^-, respectively) as well as the histopathology of the kidney tissues were used to assess the renal function impairment effect of oral and inhalation exposure to endosulfan in rats. The results of this study showed that the levels of serum creatinine, urea, BUN, uric acid and serum K^+ increased significantly ($p<0.05$) within and among the groups of rats exposed to endosulfan by both oral and inhalation routes of exposure, compared with the rats in the control group (Table 1). Whereas the levels of total serum proteins, albumin, Na^+ and Cl^- were decreased significantly ($p<0.05$) following oral and inhalation

Table 1: Effect of oral and inhalation exposure to endosulfan on some renal function test serum indices in rats

Serum indices	GroupI (Control)	Group II (Oral exposure)	Group III (Inhalation exposure)
Creatinine (μ moL L^{-1})	130.80±7.29	247.96±33.04[a]	249.60±0.02[a,b]
Urea (m moL L^{-1})	5.82±0.19	11.46±0.96[a]	11.52±1.12[a,b]
BUN (m moL L^{-1})	2.79±0.15	5.50±1.05[a]	5.53±1.10[a,b]
Uric acid (m moL L^{-1})	2.16±0.15	6.36±1.28[a]	6.80±1.92[a,b]
Total protein (g L^{-1})	47.78±4.03	38.02±4.88[a]	36.56±2.35[a,b]
Albumin (g L^{-1})	35.84±1.82	31.74±3.14[a]	30.78±1.64[a,b]
Sodium (m Eq L^{-1})	119.20±6.71	91.85±0.65[a]	74.67±3.53[a,b]
Potassium (m Eq L^{-1})	2.30±0.14	5.86±1.15[a]	6.08±1.28[a,b]
Chloride (m Eq L^{-1})	100.76±0.75	97.71±0.40[a]	89.90±1.62[a,b]

Value are presented as Means±SD; n = 6; [a]$p \leq 0.05$ compared with group I; [b]$p \geq 0.05$ compared with group II

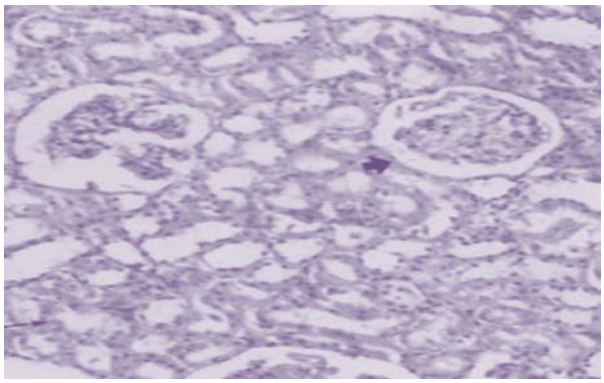

Fig. 1: Histology of the kidney from rat in the control group showing cortex with normal tubular (light arrow) and glomerular (heavy arrow) structures (Hematoxylin-Eosin, x 200)

exposure to endosulfan, in comparison with the levels obtained for the control group (Table 1). However, while the levels of serum creatinine, urea, Blood Urea Nitrogen (BUN), uric acid, total protein, albumin, sodium, potassium and chloride ions obtained for rats exposed to endosulfan both by oral and inhalation routes were significantly different ($p \leq 0.05$) compared, respectively to the levels obtained for rats in the control group; the levels of these respective parameters obtained for rats exposed to endosulfan by inhalation were not significantly different ($p = 0.05$) from the levels obtained for rats exposed orally.

The histological structures of the kidney tissues of rats in the control group, rats exposed to endosulfan orally and rats exposed to endosulfan by inhalation are shown in Fig. 1, 2 and 3, respectively. The renal cortex areas were selected for histological examination with the light microscope, because these areas which contain renal corpuscles and associated tubules, showed more pronounced histopathological changes in endosulfan-treated animals, compared with the control. These histopathological changes in the renal tissues of rats exposed to endosulfan by both inhalation and oral routes showed pronounced changes in the structure of renal corpuscle including swelling appearances, increasing of urinary spaces, highly degeneration of glomeruli, Bowman's capsules and associated tubules structure (Fig. 2, 3). However, no significant difference was observed between the histopathological changes of the renal tissues of rats exposed to endosulfan by inhalation and that of the rats exposed orally. Also, the result of the histology of the renal tissues obtained for the control rats showed normal renal corpuscle, consisting of a tuft of

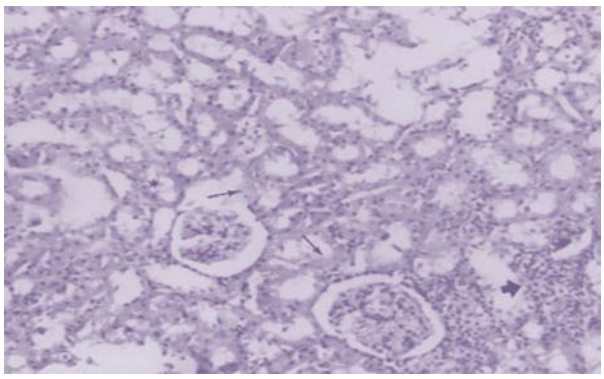

Fig. 2: Histopathology of the kidney from rat exposed to endosulfan by inhalation. The section shows tubules with degeneration (asterisk) and epithelial cell necrosis (light arrows) in the epithelial linings and mononuclear cell infiltration (heavy arrow) in the interstitium. (Hematoxylin-Eosin, x 200)

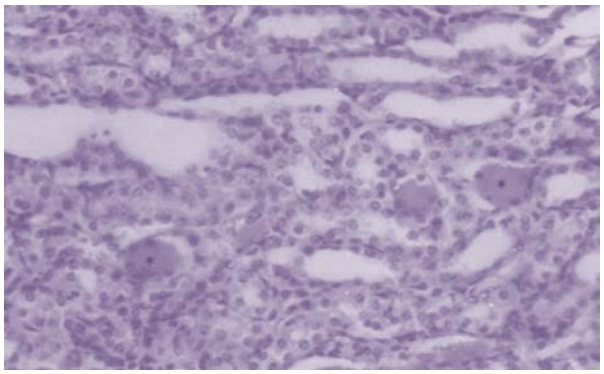

Fig. 3: Histopathology of the kidney from rat orally exposed to endosulfan. The section shows tubular lumen with eosinophilic secretion (asterisk) and epithelial cell necrosis (Hematoxylin-Eosin, x 200)

capillaries, the glomerulus, surrounded by a double walled epithelial capsule called Bowman's capsule (Fig. 1). These results showed that both oral and inhalation exposure to endosulfan induced abnormal histology of renal cortex areas, thereby causing impairment of the renal functions.

DISCUSSION

The kidney is one of the organs responsible for the maintenance of constant extracellular environment through its involvement in the excretion of such catabolites as urea and creatinine, as well as electrolyte balance. Abnormal concentration of these catabolites and some electrolytes in the plasma or serum is a clear indication of renal function impairment (Zanna *et al.*, 2008). Renal function impairment manifests in a variety of different clinical

presentations, some of which may be asymptomatic and can only be detected by routine laboratory examinations. Azotaemia, a clinical condition associated with renal function impairment is one of such presentations that can rightly be detected by laboratory findings. The condition is characterized by elevated levels of serum creatinine, urea and BUN (Cotran *et al*., 1999). A persistently increased serum creatinine is reported to be one of the risk factors for chronic kidney disease which may results in renal failure (Appel *et al*., 2003). Impairment of the renal functions may be caused by exposure to different nephrotoxic substances, in addition to certain diseased conditions.

The present investigation indicates that oral and inhalation exposure to endosulfan caused significant alterations in serum renal function biochemical parameters and histological integrity. The levels of creatinine, urea, BUN, uric acid and K^+ were significantly increased while the values of total protein, total albumin, Na^+ and Cl^- were statistically decreased. These results are in agreement with the research reports for malathion and other pesticides which indicated that the exposure to these pesticides led to induce severe physiological and biochemical disturbances in experimental animals (Appel *et al*., 2003; Yousef *et al*., 2003; Garg *et al*., 1980; Adeniran *et al*., 2006; Kerem *et al*., 2007; Attia and Nasr, 2009). Also, different other studies, supporting the results of this present study, showed that malathion and other pesticides-induced liver and kidney histopathological alterations in experimental animals (Farrag and Shalby, 2007; Afshar *et al*., 2008; Saadi *et al*., 2008; Attia and Nasr, 2009). Tos-Luty *et al*. (2003) showed that malathion intoxication led to severe effects on the structures of the kidney, covering parenchymatous degeneration of the cells of renal tubules and hyperemia of the cortical part of the kidney, especially of renal glomeruli, as well as infiltrations. The results of this present study showed that oral and inhalation exposure to endosulfan induced severe renal damages, as shown in the histopathological examinations coupled with significant changes in kidney functions assessment biochemical indices, including significantly increased levels of creatinine, urea, BUN, uric acid and K^+ as well as decreased levels of total protein, total albumin, Na^+ and Cl^- concentrations. Also, the ultrastructure of the cells of renal proximal tubules, vacuoles with damaged external membrane were observed, as well as swollen and pleomorphic mitochondria. The histopathological changes observed to be associated with oral and inhalation exposure to endosulfan therefore correlates with the results of the study reported by Tos-Luty *et al*. (2003) for malathion.

However, the specific mechanism(s) through which endosulfan induces significant changes in kidney functions assessment biochemical indices and histopathological changes in the renal tissues is not quite clear. It is suspected that the process of endosulfan metabolism in the body generate some Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) as the products of oxidative metabolism. High levels of ROS and RNS have been considered to potentially damage cellular macromolecules and have been implicated in the pathogenesis and progression of various chronic diseases.

CONCLUSION

The present findings suggested that endosulfan is a nephrotoxic chemical and that oral and inhalation exposure to produces significant nephrotoxicity effect, in a route-of-exposure-independent pattern, in rats. Hence, stringent regulation of the use of endosulfan in our environments should be highly recommended to the various environmental protection agencies.

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