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Effects of Shiga-Toxin Producing Strains of *Escherichia coli* (STEC), Lead and Bisphenol A Mixture on Kidney and Spleen of Whistler Mice

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Abstract: Human exposure to shiga toxin producing strains of *Escherichia coli* (STEC), and environmental contaminants such as lead and bisphenol A (BPA) are on the increase globally. This research work was aimed at investigating the effect of these contaminants on the kidney and spleen of Whistler mice when acting singly and in combination. A total of 24 mice (6 groups of 4 mice each) were obtained and feed with STEC, lead and BPA, either singly or in combination, using oral-gastric gavage at a concentration of 5.9×10^5 CFU/ml/bw/day, 60 µg/kg/bw/day and 10 µg/kg/bw/day respectively, for 7 days. After treatment, the mice were sacrificed, and the kidney and spleen observed microscopically by established methods. The results of the current study shows that mice, when administered only STEC, developed interstitial infiltration and congestion of the kidney and spleen respectively, while BPA and Lead, when acting independently, caused durable damages to both organs. Upon administration of two or more of these contaminants together, a somewhat different outcome was recorded, mild damage on both the kidney and spleen. The result therefore shows that exposure to microbial and chemical contaminants singly or together could have grave consequences, and efforts should be made to reduce exposure to these contaminants.

Keyword: Endocrine disrupting chemical; Public health; Pathogenicity; Heavy metal; Toxin

1. Introduction

Human exposure to shiga-toxin producing strains of *Escherichia coli* (STEC) are on the increase globally, but could be worst in developing countries were early detection remain a serious challenge. Although STEC have been reported in different food items, particularly in fresh vegetables, milk and milk products as well as meat products from different parts of Africa [1,2,3,4] outbreaks are however, yet to be reported. Seemingly, as in most developing countries, global cases of STEC are linked with the consumption of contaminated cheese, yogurt, lettuce, potatoes, seed sprouts, cooked maize, melon and fresh-pressed apple juice [5]. Secondary transmission of STEC, involving direct hand-to-hand contact (for example, among children in day care centers) or as indirect contaminant (via contaminated swimming pool, involving asymptomatic carriers) have also been reported. Outbreaks are also likely to occur in the food-chain of fast-food restaurants, particularly those with a common source of ground-beef patties, hamburgers/salad and sub-optimal cooking and handling procedures.

Since the identification of STEC as a human pathogen in 1982, more than 200 different serotypes have been reported from different sources, particularly of food and animal origin [6], with *E. coli* 0157:H7 being the main serotype responsible for sporadic cases and outbreaks of hemorrhagic colitis and hemolytic uremic syndrome (HUS). However, in recent times, non-0157:H7 serotypes are reported to be gaining lots of global attention, and becoming of public health concern. For example, non-0157:H7 serotypes cause an estimated 36,000 illnesses in the United States alone [7]. Similarly, a non-0157 serotype was reported as the causative agent from a recent outbreak in Germany [8]. In the European Union alone, there were 1930 cases of non-0157 serotype of STEC infection (ranging from 0 to 6.8 cases per country) in 2011, a 159.4% increase in the number of cases from 2010, and apparently due to the *E. coli* 0104:H4 outbreak that affected nearly 4,000 people [9].

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Human exposure to chemicals such as lead and bisphenol A (BPA) occur at various stages of human development, and could pose significant health risk at a later time in life [10]. In particular, lead has long been reported as a metal of public health concern. Its continuous usage in the past 5000 years is attributed to its low melting point, softness, malleability, ductility and resistance to corrosion [10]. Lead is used for the manufacture of kitchen utensils, plumbing materials, pipes, pigments and paints, construction materials, glass, ceramics etc. and could be persistent in the environment, because it is not biodegradable. Lead has been reported to impact negatively on both the central nervous system (CNS) and peripheral nervous system (PNS) [11], with the effect on the first being more deleterious in children, and the later in adults [12,13]. Lead is also reported to affect the hematopoietic system by inhibiting the synthesis of haemoglobin, interfere with the reproductive systems [14], as well as causes acute and chronic nephropathies [15].

On the other hand, the production of bisphenol A (BPA) – a highly ubiquitous synthetic chemical compound, is on the increase, and is mostly used in the production of polycarbonated plastics, epoxy resins, water bottles, water piping, lining of tin cans, toys and thermal receipt papers. BPA has been reported in effluents, soil leachates, river water, food samples, drinking water and consumer products [16,17,18] and in subjects working in plastic industry [19].

The extensive use of BPA and its continuous presence in food and the environment is worrisome with respect to its endocrine disrupting potential. BPA is reported to have neurological effect [20], estrogenic effect, effect on adipose tissues as well as damage mice liver etc.

While the toxicological effect of these individual chemicals and microbial toxin are well documented, there is however, no report on the effect of a mixture of these chemicals. The current study was therefore aimed at investigating the possible effect on the kidney and spleen of Whistler mice to different compounds (STEC, lead and BPA), both individually and in combination.

2. Materials and Methods

Isolation of STEC

Pure culture of STEC was obtained as previously described[2]. Briefly, 25g or ready-to-eat salad was weighed in 225ml of sterile peptone water to form a stock. Serial dilution was then made from the stock, and 1ml of appropriate dilution was inoculated on eosin methylene blue agar plate by the spread plate method, and then incubated at 37°C for 24hrs. Clearly distinct colonies exhibiting typically dark colonies with green metallic sheen were further identified based on their cultural, morphological and biochemical characteristics, principally characteristic of the Enterobacteriaceae family. Pure cultures were sub-cultured and re-plated on CHROMagar STEC base (CHROMagar, Paris, France).

E. coli strains habouring the shiga-toxin gene were noted for the mauve colouration on the chromogenic medium. The isolate was stored at -80°C before use.

Animal experiment/*in vivo* studies

Twenty four female mice (6 groups with 4 mice each) weighing between 22-32 grams were purchased from the animal house of the Department of Pharmacology, University of Benin, Benin city, Edo state, Nigeria and were acclimatized within the experimental animal handling facility of the Department of Biological Sciences, Benson Idahosa University, Benin city, Edo State, Nigeria at a suitable temperature and humidity, at a 12hr light/darkness cycle for 7days. The rats were fed twice daily on standard rodent chow and clean tap water during the 7 day period. Animal experiment was done with the kind approval of Benson Idahosa University's Animal Ethics Committee.

Exposure of mice to STEC, BPA and lead

After a 7 day acclimatization period, mice were allocated randomly into groups. Mice were given the bacterium and compounds orally, depending on the grouping; group 1 (STEC); group 2 (BPA); group 3 (lead); group 4 (STEC + BPA); group 5 (STEC + lead); group 6 (STEC + BPA + lead), using an oral-gastric gavage, according to the equivalent dose (in reference to the weight of the mice).

Dosage regimen

Aliquot (0.2ml) of STEC was administered once daily to the mice. The dosage for lead was 60µg/kg body weight/day, while that of bisphenol A was 10µg/kg body weight/day.

Histological Study

Mice were sacrificed by cervical vertebra dislocation; the kidney and spleen were removed and fixed in 4% formalin overnight at 4°C. Following overnight treatment, the organs (kidney and spleen) were run through alcohol-xylene for dehydration and clearing, and further embedded in paraffin. A thin section of the organs were cut, de-paraffinised and hydrated before being stained with hematoxylin-eosin (HE) for microscopic observation. Slides were prepared and examined under a microscope. Photomicrographs of the specimens were obtained using digital research photographic microscope in the University of Benin Teaching Hospital laboratory. Triplicate samples from different mice were obtained from each group, to confirm consistent/uniform effect.

3. Results and Discussion

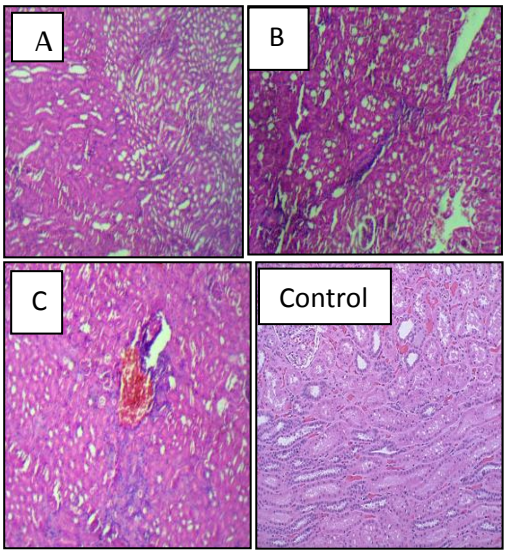
Humans are continually exposed to a mixture of compounds such as heavy metals, estrogens etc. as well as pathogenic microorganisms and their toxins. Unfortunately, most studies are focused on the effects of these compounds singly, without putting into cognizance, the situation in a "real life scenario", which is the possibility of exposure to a mixture of different chemicals and/or microbial toxin or pathogen at the same time. The current research therefore gives insight into the possible pathogenic effect of STEC singly and/or in combination with other compounds on the kidney and spleen.

The results of the current study shows that when administered independently, STEC was observed to induce interstitial infiltration, lead induced focal necrosis, while bisphenol A had mild necrotic effect on the kidney (Fig. 1).

Meanwhile, a combination of STEC and bisphenol A, caused severe inflammation of the kidney, STEC and lead together caused interstitial nephritis while STEC and bisphenol A caused interstitial inflammation (Fig. 2). Meanwhile, a combination of STEC, lead and bisphenol A yielded an unexpected outcome, with no observable effect on the kidney (Fig. 2).

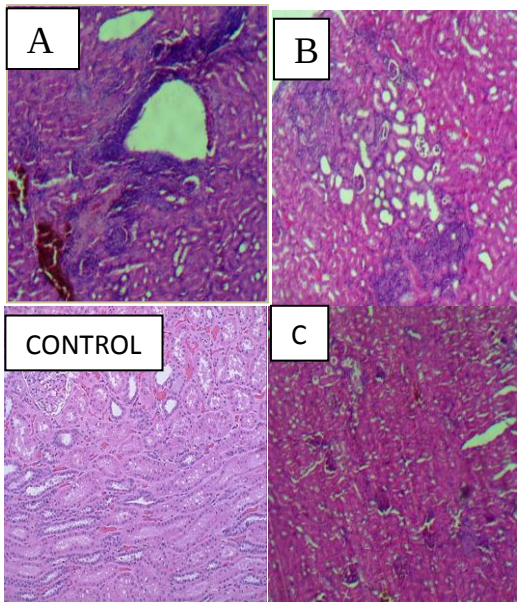
On the other hand, STEC, lead and bisphenol A, when acting singly caused congestion of the spleen. Meanwhile, the damaging effect caused by STEC was slightly mild compared with the effect of lead, which produced a more severe damage. The least damaging effect was however reported with bisphenol A (Fig. 3).

STEC administered to mice, in combination with lead caused a histiocytic reaction in the spleen, and was also the case with STEC when given, in combination with bisphenol A (Fig. 4). Surprisingly, STEC, lead and bisphenol A, when given together, did not result in any damaging effect on the spleen (Fig. 4).



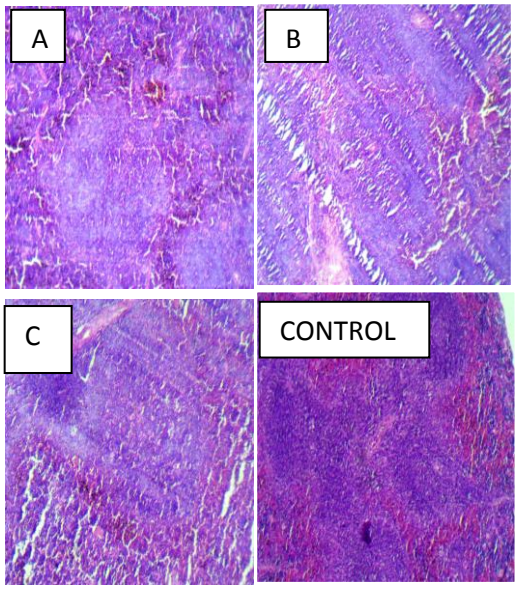
Key: A: Bisphenol A; B: Lead; C: STEC

Fig. 1: Effect of different compounds on the kidney *in vivo*.



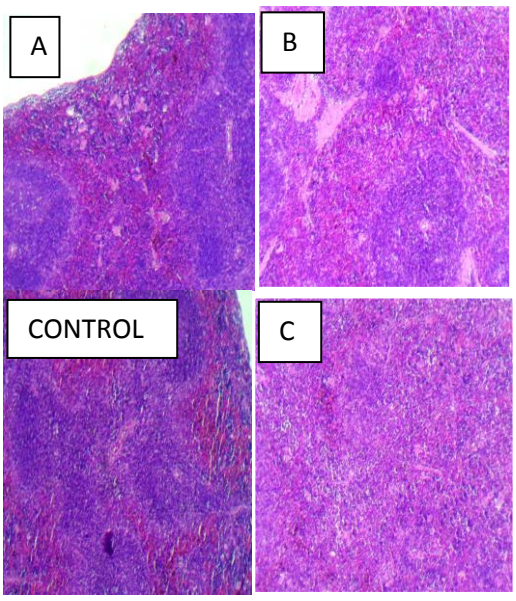
Key: A: STEC and bisphenol A; B: STEC and lead; C: STEC, lead and Bisphenol A.

Fig. 2: Effect of a combination of two compounds on the kidney *in vivo.*



Key: A: Bisphenol A; B: lead; C: STEC.

Fig. 3: Effects of bisphenol A, lead and STEC on the spleen *in vivo.*



Key: A: STEC and Bisphenol A; B: STEC and lead; C: STEC + lead + bisphenol A

Fig. 4: Effect of the combination of lead combined with bisphenol A and STEC combined with lead on the spleen *in vivo.*

Although most *E. coli* are considered harmless, certain strains can cause severe illness in humans, particularly the Shiga toxin producing strains of *E. coli* (STEC). Beside the pathogenic *E. coli* 0157:H7, non-0157:H7 strains have also been reported to cause an estimated 36,000 illnesses in the United States alone [7]. Also, a non-0157 strain was reported as the causative agent in a recent outbreak in Germany [8]. In the European Union, there were 1930 cases of STEC infection (ranging from 0 to 6.8 cases per countries), a 159.4% increase in the number of cases from 2010, partially due to the *E. coli* 0104:H4 outbreak that affected nearly 4,000 people [9].

STEC is mainly reported to cause hemolytic-uremic syndrome (HUS), with a complicated dose response relationship. Its health risk has also been reported to be difficult, due to the presence of putative virulence factors in some serotypes of STEC, whose pathogenesis is considered uncertain [21]. However, Haas *et al* [22] used data from an animal study undertaken by Pai *et al* [23] and validated their model by comparing their data with that of two human outbreaks (foodborne and waterborne) that occurred in the US. This model estimated that the dose required for 50% of the exposed population to

become ill was 5.9×10^5 Cfu/ml. Interestingly, this was also the dose used in the current study, to determine the pathogenic effect of STEC singly, and in combination with other compounds.

As observed in the current study, STEC was found to have a profound damaging effect on the kidney and spleen of mice, in most cases when acting singly and when administered in combination with other compounds (bisphenol A or lead). The obvious reason for this combination was to mimic a real life situation where humans are exposed to a mixture of more than one compound/chemical at a time.

Shiga-like toxin (SLT) or shiga-toxin (Stx) (the primary virulence factor expressed by STEC) is an AB_5 toxin with two antigenically distinct forms; Stx1a and Stx2a [24]. Although both toxins have similar biological effects, Stx2a have been reported to be more frequently produced by STEC strains that cause HUS than is Stx1a [24]. Based on published evidence, we would be right to allege that the toxin produced by STEC fed to mice in the current study was Stx1a. In a similar study, Wadolkowski *et al* [25] reported a revertant strain of *E. coli* 0157:H7 designated 933-rev to kill 100% of mice it was feed singly with. In that study, the death of the mice infected with STEC, were further revealed to be solely due to SLT2a production by the organisms. A further histological study revealed that the death was due to acute renal cortical tubular necrosis which was consistent with toxic renal damage. More recently, Russo *et al*. [24] reported Stx2a to show 50% lethal dose (LD_{50}) when feed orally (at a concentration of 2.9µg) to mice. Meanwhile, Stx1a did not show any form of morbidity even at a dose of 157µg. This finding by Russo *et al*. [24] was corroborated by an increase in serum creatinine and blood urea nitrogen, indicative of kidney damage. Elsewhere, Stx1a was reported to induce only detectable damage in the renal cortical tubule of epithelial cells [26]. The differences in the toxicity of SLT-1/Stx1a and SLT-2/Stx2a have been attributed to their structural/functional dissimilarities [26], possibly involving holotoxin stability and/or receptor affinity.

The toxicity of lead on the kidney and spleen of both humans and animals is well documented [27,28,29,30]. Lead acetate produces deleterious effects (moderate cortical tubular atrophy, desquamated epithelium with degenerated nuclei in proximal and distal tubules) on the developing kidney in mice [27]. In Swiss-albino mice exposed to lead acetate for 1, 40 and 80 days, significant decrease in kidney antioxidant enzymes (SOD and CAT) and an increase in kidney lipid peroxidase was reported [31]. The kidney antioxidant enzymes rely on essential trace elements and prosthetic groups for proper molecular organization and their enzymatic reaction. Meanwhile, lead being bivalently charged in its atomic form, displaces other bivalent micromolecules such as Zn^{2+}, Cu^{2+} and Fe^{2+}.

In a similar study, Aldahmash *et al*. [32] reported lead intake to cause severe alterations in the kidney and spleen, which was manifested by hepatocytes degeneration, leukocytic infiltration, ill-defined architecture of the spleen, presence of large macrophages and lymphoid necrosis. Hypocellular white pulp, enlargement of venous sinusoids, clustering of heterochromatin in the nucleus, vacuolation in the cytoplasm, swelling of mitochondria and complete distortion of rough endoplasmic reticulum cisterns are also some of the effects of lead [33,34].

Bisphenol A, a highly permeating chemical, is well known for its possible estrogenic effect, and has been reported to deplete lymphocytes, cause multiple focal necrosis and hyalinosis of the tunica media of the central arterioles of the spleen [35] as well as impair mitochondrial functions in the spleen [36].

Meanwhile, the administration of two compounds, when given together (STEC + bisphenol A; STEC + lead), yielded an obvious damage on kidney and spleen, but not as envisaged. Studies of Peng *et al*. [37] suggests that *E. coli* is inhibited in the presence of lead by a mechanism in which lead causes a permanent damage to the bacterial cell's outer membrane.

Also, it has been reported that bisphenol A possess antimicrobial and inhibitory effect on *E. coli* [38] by the inhibition of lipid synthesis, and disruption of its cell membrane activities. Similarly, heavy metals are reported to inhibit the growth of microorganisms [39].

Lead is one of the toxic metal pollutants of global concern, with the increased widespread of leaded gasoline, paints etc especially in developing countries becoming the major

focus in recent times. Exposure occurs mainly through the respiratory and gastrointestinal systems, and the ingested and absorbed lead is stored primarily in soft tissues. Meanwhile, autopsy studies of lead-exposed subject have shown large amount (approximately 33 %) of the absorbed lead in soft tissue stored in different organ [40]. In a similar study, Muselin *et al*. [41] reported the pathogenic effect of lead on the kidney of Whistler mice, following 6 months exposure, where similar effects such as corpuscular necrosis and amyloidosis were reported in the kidney and spleen respectively.

The report of the current study in as indication that exposure to a mixture of chemicals at the same time is rather, a biological phenomenal. For example, lead could inhibit bacterial cell wall, thereby preventing the cell from producing toxins, or could attach and/or damage the substrate the bacterium require for optimal growth in the host.

4. Conclusion

In conclusion, human exposure to known microbial and chemical contaminants independently could have devastating effect on the internal organs *in vivo*. However, a mixture of these contaminants, especially microbial and chemical could produce a rather unprecedented effect.

Conflict of interest

The authors declare that there are no potential conflicts of interest.

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