Pathological Effects of Pesticide Indoxacarb (Avaunt®) on Kidney of Female Mice

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Abstract

Exposure to pesticides has harmful impact on human and animal health. Indoxacarb is a relatively new insecticide extensively used in agriculture and veterinary to control pests. The present study was designed to examine the effect of indoxacarb (Avaunt®) on body weight, kidney function and histological changes in female albino mice. The mice were divided into three groups of six animals each. The first group was served as control group received distilled water intraperitoneally, while the other two groups: T1 and T2 were daily intraperitoneal injected with indoxacarb at a dose of 90 and 120 mg/kg body weight respectively for a period of 10 days. Biochemical parameters and histopathological studies were examined. The results of this study showed that indoxacarb induced a considerable increase in the level of creatinine at both doses but there was no significant change in the level of urea at both doses compared to the control. The histopathological study of mice kidney showed that renal corpuscles and associated tubules showed a pronounced damage. The histopathological changes included mild degeneration in the epithelial cells lining renal tubules, atrophy of the corpuscles, necrosis, enlarged and congested renal vein. Therefore, administration of indoxacarb intraperitoneally caused harmful effects in the kidney structure and function of female mice. Hence, indoxacarb could cause dangerous effects particularly at high doses to human and animals.

Key words: Indoxacarb, Pesticide, kidney function, histopathology, Mice.

Introduction

Pesticides are toxic chemical compounds aimed to control pests that decrease the quantity and quality of crop yield (EL-Deep et al., 2007). The severity of pesticides health risks are much obvious in developing countries as a result of mishandling and misuse of pesticides by farmers, farm workers, and employee in pest management as well as the lack of national controlling plans (Mansour, 2004). World Health Organization reported that about 3 million cases of pesticide toxicity happen yearly causing death to 250,000 people or more (Moqbel et al., 2017).

Indoxacarb (DPX-MP062) show potent insecticidal activity and low toxicity to non target organisms. DPX-MP062 is a mixture of two enantiomers: S (active)and R (inactive) in the ratio of 75:25. The activity of this insecticide is mediated through its metabolite DCJW (decarbomethoxylated JW062) (Dinter and Wiles, 2000; Lapied et al., 2001; Narahashi, 2001; Chemical fact sheet, 2012). Indoxacarb has broad spectrum activity, used extensively to manage sucking and chewing insects and is particularly efficient on Lepidoptera (Dias, 2006). Indoxacarb cause toxicity through inhibiting sodium ion access into nerve cells, leading to paralysis and death of the target insect (Dinter and Wiles, 2000; Narahashi, 2001; Dias, 2006; European Medicines Agency, 2010; Chemical fact sheet, 2012; Nassar, 2016).

EPA (2000) has reported low to moderate mammalian toxicity to indoxacarb exposure; however, functional and histological changes in some tissues of experimental animals have been recorded. Oral administration of indoxacarb to rats (Kumar, 2016) and intraperitoneal injection of indoxacarb to mice (EL Mabrouk et al., 2016) caused significant increase in liver enzyme alanine aminotransferase and a considerable decrease in total protein and albumin, in addition to a remarkable histological liver damage. Studies on the effects of indoxacarb on the histopathology and function of kidney in mammals are scarce (Goyal and Sandhu, 2009; Kumar, 2016). Therefore, the current study was carried out to examine the effects of indoxacarb on body weight, biochemical analysis and histological changes of the kidney in female albino mice.

Material and methods

Indoxacarb 200 g/L (75:25) was purchased from a local pesticide store. It was suspension concentrate diluted to the required doses using distilled water and administered to animals via intraperitoneal route at a dose of 90 and 120 mg/kg/day body weight. Female albino mice 6-8 weeks old, weighing between 26-30 g were sustained at the Animal House in the Department of Zoology, Faculty of Science, Tripoli University in plastic cages under standard conditions of temperature and natural light/dark cycle. They were fed with a standard pellet and ad libitum tap water for drinking.

The mice were divided equally into three groups, each including six animals. The control group was injected with distilled water intraperitoneally for 10 days. The treated groups (T) were administered indoxacarb intraperitoneally of 90 mg/kg (T1) and 120 mg/kg (T2) daily for 10 days. The initial and final body weights of each group was recorded. At the end of the treatment period, blood samples were collected from each mouse from jugular vein. Serum was separated after centrifugation (3000 rpm for 15 min) and used to determine the level of creatinine and urea in the blood.

The kidney tissues were fixed in a 10% neutral buffered formalin solution, and then dehydrated in graded series of ethanol, cleared in xylene and embedded in paraffin wax. The 5 µm thick sections were cut serially followed by rehydration in descending series of ethanol to water; the sections were stained with haematoxylin and eosin.

The results attained in the current study are represented as means ± standard deviation (SD). Data was statistically calculated by using one way analysis of variance (ANOVA) followed by a post-hoc test for multiple comparisons within SPSS software package version 16. The level of significance considered as $P < 0.05$.

Result

Intraperitoneal injection of indoxacarb demonstrated apparent effect on the body weight of female albino mice as showed in (Table 1). There was a significant decrease in body weight of mice at both treated groups compared to the control in concert with significant signs of toxicity (such as tremor, tilted head, diarrhea and vigorous rolling) especially at higher dose (120mg/kg/day).

Table 1. Effect of indoxacarb treatment on mean value of mice body weight (g).

Group	Weight before treatment	Weight at the end of treatment
Control	28.5±0.76[a]	29.5±1.07[a]
T1 (90mg/kg)	27.5±2.422[a]	22.83±1.94[b]
T2 (120mg/kg)	27±1.242[a]	20.50±1.97[c]

Values are presented as means ± SD (n = 6). The mean difference is significant at the $P \leq 0.05$ level. a, b, c, ($P \leq 0.05$) compared with the control group.

The current investigation revealed that treatment with indoxacarb resulted in non significant change in the level of urea in both treated groups compared to the control group. Conversely, creatinine level was increased significantly in the treated groups compared with the control group (Table 2).

Table 2. Effect of indoxacarb treatment on some biochemical parameters of kidney function in mice.

Group	**Urea**	**Creatinine**
Control	23.20±2.59[a]	0.62±0.15[b]
T1 (90mg/kg)	22.20±4.99[a]	0.85±0.10[a]
T2 (120mg/kg)	26.30±4.83[a]	0.90±0.18[a]0

Values are presented as means ± SD (n = 6). a, b ($p \leq 0.05$) compared with the control group.

Histological examination of the control mice kidney showed normal architecture of compact renal mass and renal tubules. The normal renal cortex contains glomeruli surrounded by narrow spaces called Bowman. Bowman's capsules consisted of visceral layer covering the glomerulus and parietal layer become continuous with the wall of the proximal convoluted tubule, distal convoluted tubules and interstitium as presented in figure 1 (A, B).

On the other hand, mice exposed to 90 mg/kg/day indoxacarb (T1) showed many histological abnormalities in the structure of kidney compared to the control group. Generally the areas of renal cortex containing renal corpuscles and associated tubules expressed a pronounced lesion in the kidney tissues, the changes characterized by mild degeneration and shrinkage in some of renal corpuscles, atrophy of the corpuscle and the Bowman's space was increased (Fig. 2A, B), also there was a dilation of capillaries tuft (Fig. 2B, C). Moreover degeneration of epithelial cells of renal tubules, narrowing of tubules lumen due to cloudy hypertrophied and edema of renal tubular cells and vacuolization (Fig. 2A, C), and there was widening in the interstitial space (Fig. 2D). Increasing the dose of indoxacarb to 120 mg/kg resulted in more degenerative changes including shrinkage of glomeruli with large Bowman's

space, congested renal vein with intertubular fibrosis as presented in figure 3 (A, B). Additionally, large space and inflammation between convoluted tubules with infiltrations of mononuclear cells (Fig. 3C), and widening of medulla renal tubules (Fig. 3D).

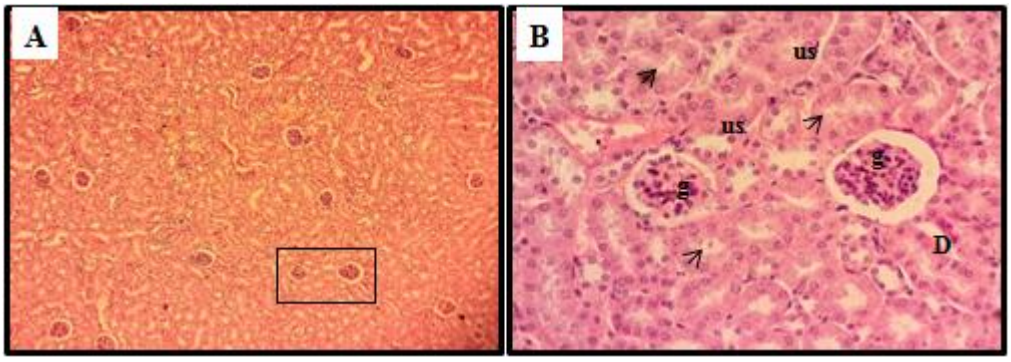

Fig. 1(**A** and **B**): Photomicrograph of mice kidney section of control group showing normal architecture of renal glomeruli (g), Bowman's capsule lined by squamous epithelium, distinct urinary space (us), the proximal tubule are lined by cuboidal epithelium with brush border (arrows) and distal tubules (D) are lined with low cuboidal epithelium. H & E. stain X200 (A) and X400 (B).

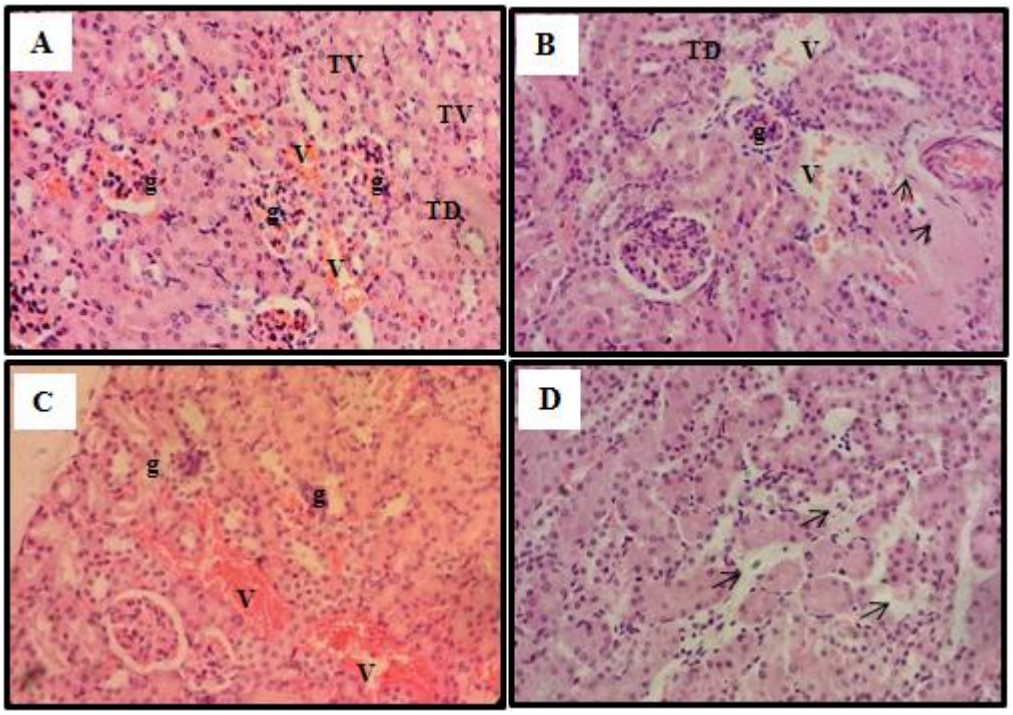

Fig. 2: Photomicrograph of mice kidney sections of treated group (T1), showing congested and atrophy of the glomeruli (g), vacuolization within the tubular cells (TV), degeneration epithelial cells of renal tubules (TD) and intertubular fibrosis (arrow) (**A, B**). Enlarged and congested renal vein (V) (**C**). Inter-tubular edema (arrow) (**D**). H. & E. stain X400.

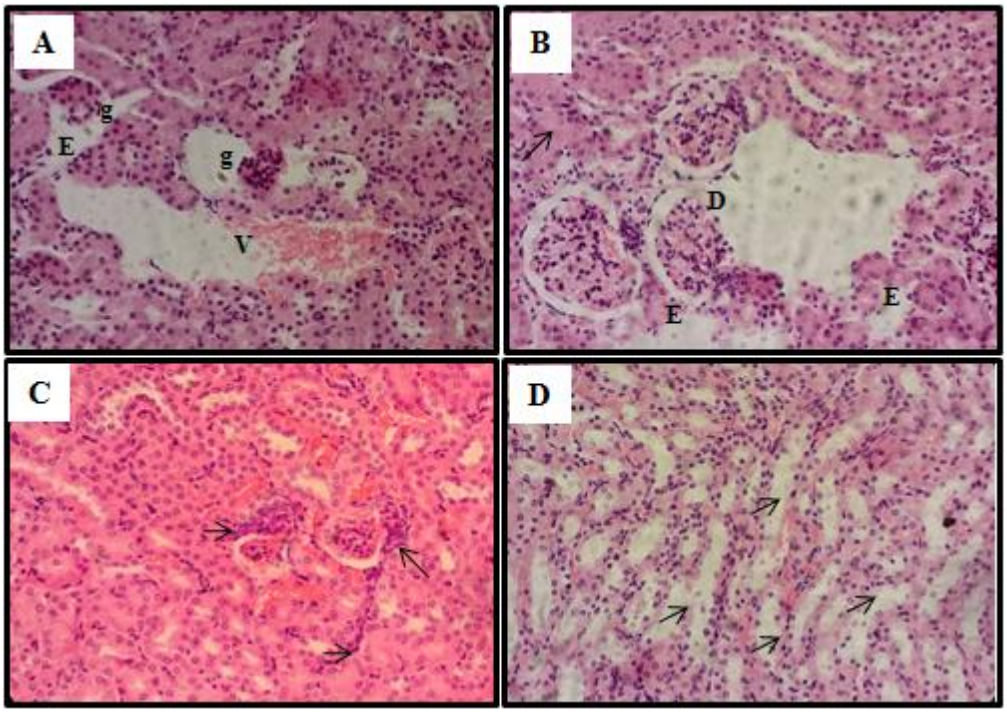

Fig. 3: Photomicrograph of mice kidney sections of treated group (T2); showing congestion and atrophy of the glomeruli (g), with degeneration (D) in the lining epithelial cells of Bowman's capsule, eroded (E), intertubular fibrosis (arrow), enlarged and congested renal vein (V) (**A** and **B**). Infiltration of mononuclear cells (arrow) (**C**). Widening of medulla renal tubules (arrow) (**D**). H. & E. stain X400.

Discussion

Indiscriminate use of pesticides in agriculture to control pests leads to toxic effects to humans, animals and environment. The insecticide indoxacarb is widely used to control insect pests. The toxicity of indoxacarb has not been fully studied; therefore, the objective of this study was to investigate the effects of indoxacarb on kidney function and histopathology in albino female mice. The results of the present study illustrated that there was a highly significant decline in the body weight of both treated groups as compared to the control group. The significant suppression of body weight in the treated groups may be related to reduction of food intake as a result of indoxacarb toxicity. This result was in accord with other studies (Hamed and Abdel-Razik, 2015; Moqbel et al., 2017) who reported that the body weight of rats exposed to the insecticide abamectin decreased considerably compared to the control group. Also, this result is in agreement with findings from previous study in male mice treated with indoxacarb (EL Mabrouk et al., 2016).

The biochemical parameters of kidney function (creatinine, urea) analyzed in this study showed significant increase in creatinine levels and non-significant changes in the level of urea in both treated groups as compared to control group. Similar results by Kumar (2016) revealed that administration of indoxacarb to rats resulted in a significant increase in creatinine level at a dose of 6.2 mg/kg for 28 days. Exposure to various pesticides cause increased creatinine level in experimental animals. For example, exposure to pesticides diazinon in rabbits (Sarhan and Al-Sahhaf, 2011), insecticide fenthion (Kerem et al., 2007) and fenitrothion (Elhalwagy et al., 2008) in rats caused marked increase in creatinine level in a dose-dependent manner. The increase in creatinine levels may be attributed to impaired kidney function. While, non-significant changes in the level of urea have been documented by Nishi and Hundal (2015) who reported that treatment of rats with chlorpyrifos resulted in non-significant change in blood urea, and Goyal and Sandhu (2009) who illustrated that administration of indoxacarb to buffalo calves for 90 days did not produce any significant changes in the level of urea at a dose of 1 mg/kg/day. However, other studies have reported considerable increase in the level of urea in rats after exposure to fenthion (Kerem et al., 2007) and fenitrothion (Elhalwagy et al., 2008). This conflicting variation could probably be attributed to animal species, type of pesticide, route of administration and duration of treatment.

Changes in function and structure of mice kidney treated with indoxacarb was demonstrated in this study. Sections from kidney tissue of treated groups showed shrinkage of glomeruli, necrosis of renal convoluted tubules, congested and dilated blood vessels, eroded Bowman's capsule, marked congestion of renal tubules, haemorrhage and infiltration of mononuclear cells. Similar results have shown that exposure to pesticides leads to histological disturbances in kidney of experimental animals (Yehia et al., 2007; Sarhan and Al-Sahhaf, 2011; Rekha et al., 2013; Tsitsimpikou et al., 2013). Yehia et al. (2007), Sarhan & Al-Sahhaf (2011), and Cakici & Akat (2013) reported that rabbits treated with diazinon resulted in hypertrophied glomeruli, destruction of epithelial renal tubules, and congestion of renal blood vessels. Afshar et al. (2008) illustrated that rats treated with 100 mg/kg of fenitrothion showed marked tubular hydropic degeneration in tubular epithelial, moderate congestion and haemorrhage. Furthermore, mice treated with cypermethrin

(Mamun et al., 2014) and dimethoate (Alarami, 2015) showed abnormalities in the structure of kidney tissue such as necrosis, changes in renal tubules, dilated blood vessels, severe congestion and haemorrhage. The reported histopathological lesions in kidney induced by exposure to pesticides are in agreement with the findings in this study.

However, the precise mechanism through which indoxacarb induces these histopathological changes in the renal tissue is unknown and needs more studies. Never the less, the results of this study suggest that the appearance of mononuclear cells in the kidney tissue of indoxacarb treated groups indicate that indoxacarb might interfer with the antioxidant enzymes leading to the production of reactive oxygen species (ROS). Decreased activity of antioxidant enzymes leads to oxidative stress, which causes damage to the cellular components through protein denaturation, lipid peroxidation and nucleic acids breakage, hence produce damage to cells, tissues and organs (Mohany et al., 2011). This suggestion is supported by previous studies (Mudaraddi et al., 2012; Choudhary and Singh, 2017), who reported that oral administration of indoxacarb to mice (Mudaraddi et al., 2012) and cockerels (Choudhary and Singh, 2017) induce oxidative stress as indicated by increasing lipid peroxidation. Elevation of ROS production and the diminished antioxidant enzymes lead to increased activity of ROS and subsequently lead to tissue damage (Ozbek, 2012; Al-Gubory, 2014). Moreover, Mudaraddi et al (2012) suggested that elevated level of thiobarbaturic acid in the liver of mice exposed to indoxacarb could be caused by association of indoxacarb or its metabolites with polyunsaturated fatty acids or through generation of ROS which interacts with polyunsaturated fatty acids. Previous studies have shown that several pesticides induce oxidative stress as indicated by increasing oxidative stress markers such as malondialdehyde (Elhalwagy et al., 2008; Kapoor et al., 2010; Sarhan and Al-Sahhaf, 2011) and decreasing activity of antioxidant enzymes superoxide dismutase (Kapoor et al., 2010; Sarhan and Al-Sahhaf, 2011) and catalase (Sarhan and Al-Sahhaf, 2011).

Furthermore, the process of indoxacarb metabolism in the body may lead to deprivation of tissues from oxygen (ischemia) which consequently causes tissue damage. Several reports suggested that methemoglobinemia occurs after indoxacarb

ingestion (accident or a suicide) which lead to systemic toxicity such as acute renal failure due to hypoxia (Wu et al., 2010; Park et al., 2011; Yen et al., 2017).

Conclusion

The findings of this study demonstrated that administration of indoxacarb intraperitoneally to albino female mice caused moderate damage in kidney structure and impairment of kidney function; therefore, strict cautious instructions and great care should be taken into account when handling the pesticide indoxacarb.

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