Biochemical and histological changes in liver and kidney in male Wistar albino rats following exposure to Solignum®, a permethrin-containing wood preservative

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Abstract

The present investigation was aimed to determine the effect of sub-chronic exposure to Solignum®, a permethrin-containing wood preservative on biochemical and histological changes in liver and kidneys of male Wistar albino rats. Thirty-two male rats were randomly divided into four groups: control and three treatment concentrations containing 8 rats each. The treatment groups were exposed to Solignum® at dose rates of 100, 200 and 400 mg/kg body weight (BW) respectively per day orally for four weeks. Data obtained from the study showed a progressive increase in the body weight of rats in control whereas, rats treated with different concentrations (100, 200 and 400 mg/kg BW) of Solignum® decreased significantly (≤0.05) especially at the end of the second and fourth week when compared with control. On the other hand, there was a significant decrease in the relative liver weights of rats treated with 100 and 200 mg/kg BW Solignum® while rats treated with 400 mg/kg BW showed a significant increase when compared with control. The relative weight of kidneys in experimental groups increased significantly when compared with control. Biochemical analysis results illustrated that there was a significant increase in marker enzymes namely alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase activity at the end of the fourth week. Similarly, total bilirubin, serum urea, creatinine and electrolytes (Na⁺, K⁺ and Cl⁻) levels increased in a dose dependent manner in treated rats when compared with untreated control group. Serum total protein decreased significantly in experimental rats when compared with control. However, cholesterol and triglycerides showed no significant difference when compared with control. Histopathological examination of hepatocytes in treated rats was characterized by mild periportal inflammatory cells and cytoplasmic degeneration. Furthermore, histopathological examination of rat kidneys revealed inflammatory cells, congested vessel and interstitial hemorrhage in rats treated with Solignum®. Therefore, this present study is aimed to evaluate the hepatotoxic and nephrotoxic potentials associated with sub-chronic exposure to the commercial pesticide Solignum®.

Introduction

Solignum® is a brand of insecticide and wood preservative commonly used by carpenters, furniture makers and roof builders in general for wood preservation. Its main active constituent is permethrin, a type 1, broad spectrum, non-systemic, synthetic pyrethroid insecticide widely used in association with fungicidal agent providing protection against termites. Pyrethroids are used primarily to control household and agricultural insect pests as well as in industrial uses. It is used in greenhouses, home gardens and for termite control. Commonly used Synthetic pyrethroids include cypermethrin, permethrin and deltamethrin. permethrin-containing products are usually applied as powders in many household for the killing of nuisance insects, like cockroaches. In the tropics, insecticide nets are usually applied to prolong the closure time, with consequent reduction in action potential threshold and repetitive firing. Since there is little data available on the combined hepatotoxic and nephrotoxic effects of commonly used wood preservatives, the aim of this study was to determine the hepatotoxic and nephrotoxic potentials of sub-chronic exposure to Solignum®, a permethrin-containing wood preservative.

Materials and Methods

Sample collection

One gallon of commercial wood preservative - Solignum® was purchased from the Timber section of Mile 3 Market while Goya® Olive oil was purchased from Everyday Emporium Super Market all in Port Harcourt, Rivers State, Nigeria.

Animals

Male Wistar albino rats weighing between 110-120 g were purchased from the Animal House of the Department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria. They were housed in individual cages and allowed to acclimatize under laboratory conditions for one week prior to the start of the experiment.
conditions at room temperature for one week prior to commencement of the experiment. They were kept under hygienic and favorable conditions, and maintained under a 12 h light/12 h dark cycle, with pelletized rat feeds (UAC Vital Feeds®, Nigeria) and water available ad libitum.

Experimental design
A total number of thirty-two adult male albino rats were used in the current study. Reports have shown that the LD₅₀ for permethrin in rats ranges from 430-4000 mg/kg. Therefore, 1/10 of the upper range (400 mg/kg) was selected as the highest dose in this study. The animals were randomly divided into 4 groups of 8 animals per group. Group I (control) received 1 mL olive oil orally only. Group II: received 100 mg/kg body weight (BW) Solignum® orally daily dissolved in olive oil in a ratio of 1:1. Group III: received 200 mg/kg BW Solignum® orally daily dissolved in olive oil in a ratio of 1:1. Group IV: received 400 mg/kg BW Solignum® orally daily dissolved in olive oil in a ratio of 1:1. The experimental animals were maintained under normal diet and water ad libitum throughout the period of the experiment. During the four-week dosing period, all the animals were observed daily for clinical signs and mortality patterns once daily. The body weights of the animals were evaluated weekly and recorded using sensitive balance.

Sample collection
Twenty-four hours after the last treatment, the rats were fasted overnight then anaesthetized in a chloroform-saturated chamber after which the animals were sacrificed using cervical dislocation method. Blood samples were obtained by cardiac puncture from each rat by means of a 2 mL hypodermic syringe and needle. The blood samples were collected in an anticoagulant free bottle for serum biochemical parameters. Serum was separated by centrifugation at 2500 rpm for 10 min and stored in a refrigerator at 4°C until use. The levels of marker enzymes (alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP)) and biochemical parameters (total and direct bilirubin) were estimated using the Humazym MUV colorimetric test kits. Serum total protein was measured using the Humazym MUV colorimetric method. Serum total cholesterol was measured using the Biuret method while cholesterol level was estimated by enzymatic colorimetric method (Cholesterol oxidase-phenol + aminophenazone) using a spectrophotometer (Model AA200; Shimadzu, Tokyo, Japan). Blood urea was estimated by an enzymatic method while serum creatinine was determined according to the method described by Faulkner and King.

Relative organ weight
At the end of the fourth week, the liver and kidneys were carefully dissected out and weighed in grams (absolute organ weight). The relative organ weight of each animal was then calculated as follows:

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

Histopathological examination
Histopathological examination was carried out according to the methods of Galigher and Kayloff, modified and modified by Sarkar et al. Liver and kidneys were dissected out and fixed instantaneously in 10% buffered neutral formalin for 24 h. The specimens were washed in tap water, dehydrated in ascending grades of ethanol, treated in xylene and embedded in paraffin wax (melting point of 50-56°C). Paraffin sections were cut at 6 µm thicknesses using a rotary microtome (Model MR 60, Russian); the sections were stained with Harris hematoxylin and eosin. Observation was made using a light microscope (Zeiss Axioshot, Germany) and photographs were taken with an automatic photomicrographic camera.

Statistical analysis
The mean-standard error of the mean (S.E.M) was calculated for each parameter. Total variations present in a set of data were estimated by one-way analysis of variance (ANOVA), followed by Dunnet’s test. Significance was set at ≤0.05.

Results
Oral administration of Solignum® at doses of 100, 200 and 400 mg/kg BW for four weeks showed no mortality or physical changes in skin and fur, eyes and mucus membrane (nasal), respiratory rate, circulatory signs (heart rate and blood pressure), autonomic effects (salivation, piloerection, urinary incontinence and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) among treated rats. The effects of the oral administration of Solignum® on body weight of male Wistar albino rats are presented in Figure 1. Treatment of experimental rats with increasing concentrations of Solignum® (100, 200 and 400 mg/kg BW) showed a significant (≤0.05) decrease in body weight while rats in control showed a progressive increase in the body weight up till the fourth week (Figure 1). Furthermore, absolute and relative liver weight of rats treated with 100 and 200 mg/kg BW decreased significantly. Whereas absolute and relative liver weight of rats treated with 400 mg/kg BW increased significantly when compared with control (Table 1). On the other hand, there was a significant increase in both absolute and relative kidney weights in a dose dependent manner in rats treated with Solignum® when compared with control (Table 1). The results of the effects of oral administration of different concentrations of Solignum® on biochemical parameters in male Wistar albino rats are shown in Table 2. Data from the study showed that there was a significant increase in the levels of AST, ALT, ALP parameters (total and direct bilirubin) were estimated using the Humazym MUV colorimetric method. Serum total protein was measured using the Biuret method while cholesterol level was estimated by enzymatic colorimetric method (Cholesterol oxidase-phenol + aminophenazone) using a spectrophotometer (Model AA200; Shimadzu, Tokyo, Japan). Blood urea was estimated by an enzymatic method while serum creatinine was determined according to the method described by Faulkner and King.

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

Figure 1. Effect of oral administration of Solignum® on body weight changes in control and treated rats.
and total bilirubin in a dose-dependent manner with group treated with 400 mg/kg BW recording the highest value when compared with control (Table 2). On the other hand, the level of conjugated bilirubin was highest in the group treated with 200 mg/kg BW while protein was lowest in the group treated with 200 mg/kg BW when compared with control (Table 2). There was no significant difference in the level of cholesterol and triglyceride when compared with control (Table 2). There was a significant increase in the levels of sodium ion (Na+), potassium ion (K+), chloride ion (Cl-), urea and creatinine in experimental groups treated with Solignum® in a dose-dependent manner when compared with control (Table 3). Histopathological examination of the liver and kidneys are shown in Figure 2. Hepatocytes of control group showed normal architecture whereas rats treated with different concentrations of Solignum® were characterized by mild periportal inflammatory cells and cytoplasmic degeneration (Figure 2A-D). Kidneys of control rats also showed normal architecture whereas treatment with different concentrations of Solignum® showed the presence of inflammatory cells, congested vessel and interstitial hemorrhage (Figure 2E-H).

**Discussion and Conclusions**

Liver and kidney are important vital organs in the animal body as they are the sites of detoxification and elimination of toxic materials. A foreign body in form of a chemical stress is sufficient enough to cause severe hepatic and renal dysfunction. Permethrin, the active chemical compound in Solignum® is one of the most widely used insecticide active ingredient registered for use in agricultural and domestic uses. The common uses of permethrin-containing insecticides have resulted in different forms of diseases that are consistent with the observed effects in the present study. In the present study, the oral administration of the commercial wood preservative-Solignum®, a permethrin-containing insecticide showed no mortality or physical changes up to 1/10 of the higher range of the LD₅₀ for permethrin in rats. This corroborates earlier reports that the no-observed-adverse-effect-level is greater than 400 mg/kg BW. Changes in body weight gain and internal organ weights reflect toxicity after exposure to toxic substances. Therefore, the significant decrease in the body weight of rats treated with Solignum® at the end of the second and fourth week may be an indication of adverse effects of sub-chronic administration of Solignum® to experimental rats. Body weight changes have been used as markers of adverse effects of chemicals especially if the body weight loss is more than 10% of the initial...
body. A significant increase in the absolute and relative weight of liver and kidney of rats in the group treated with 400 mg/kg BW of Solignum® may be attributed to an increase in activity of inflammatory agents present in Solignum® that could have resulted to inflammation of liver and kidney tissues. In the present study, treatment with different concentrations of Solignum® caused a significant elevation of marker enzymes namely ALT, AST and ALP. Aminotransferases (ALT and AST) are sensitive indicators of liver cell damage for both acute and chronic hepatocellular injury. Therefore, serum levels of these enzymes reflect the state of hepatic function. The elevated levels of ALT indicate a possible hepatotoxicity that would have resulted in the leakage of the enzyme into the serum. Similarly, Shakoori et al. reported that the increase in the activity of ALT is mainly due to the leakage of this enzyme from the liver cytosol into the blood stream, which reflects liver damage and disruption of normal liver function. On the other hand, ALP is often employed to assess the integrity of the plasma membrane of the liver. The significant increase in serum activity in ALP following treatment with different concentrations of Solignum® may be due to disruption of the liver plasma membrane. Furthermore, the increased level of total bilirubin in treated rats maybe an indicator of hyper-bilirubinemia - a useful index for the severity of hepato cellular injury. Histological changes provide a less rapid means than serological analyses for detecting the effects of xenobiotics in various organs but provides stronger evidence of toxic damage in tissues and reduced health. The liver has been described as the major site of pyrethroid metabolism. The present study revealed histological changes in hepatocytes of rats exposed to permethrin, the active constituent in Solignum®. Generally, they were characterized by mild perportal inflammatory cells and cytoplasmic degeneration; whereas treatment with different concentrations of Solignum® showed inflammatory cells, congested vessel

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<th>Table 1. Toxicological effects of Solignum® on body weight, absolute and relative organ weights of rats (g/100g body weight) on the day of sacrifice.</th>
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<td>Description</td>
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<td>Initial body weight</td>
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<td>Final body weight (g)</td>
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<td>Absolute liver weight (g)</td>
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<td>Absolute kidney weight (g)</td>
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Data are expressed as mean±standard error mean, n=8. *Significant difference at □0.05.

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<th>Table 2. Toxicological effect of Solignum® on biochemical parameters of rats.</th>
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<td>Description</td>
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<td>AST (IU/L)</td>
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<td>ALT (IU/L)</td>
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<td>Total bilirubin (mmol/L)</td>
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<td>Protein (mmol/L)</td>
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<td>Cholesterol (mmol/L)</td>
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Data are expressed as mean±standard error mean, n=8. *Significant difference at □0.05. AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase.

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<th>Table 3. Toxicological effect of Solignum® on serum electrolytes in rats.</th>
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<tr>
<td>K+ (mEq/L)</td>
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<td>Na+ (mEq/L)</td>
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<td>Urea (mEq/L)</td>
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<td>Creatinine (μmol/L)</td>
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and interstitial hemorrhage in the kidney. This observation was similar to the findings of Chargui et al., who reported the histopathological alterations in the liver and kidney of female rats exposed to low doses of deltamethrin - a synthetic pyrethroid insecticide. The histopathological pattern in this study is comparable with the report of Grewal et al., who reported that cypermethrin administration at 14.5 mg/kg for 30 consecutive days produced congestion, marked degenerative changes of hepatocytes and kidneys. Our finding in this present study is also in agreement with the report of Saleh who reported that liver of treated rats with diflubenzuron, cypermethrin and fenitrothion showed different phases of degenerative changes in the form of cloudy swelling, hydropic degeneration, chromatolysis, pyknosis, fatty degeneration, necrosis and karyorrhexis. Hence, the observed effects of Solignum® are associated, in part at least, to the presence of permethrin. In the same manner, data obtained by Farrag and Shalaby showed that 1/10 LD₅₀ of lufenuron in the kidney sections of newly hatched chick (Gallus domesticus) treated with permethrin (100 and 200 ppm). These histopathological changes in the liver and kidney may be explained by the fact that Solignum® induced oxidative stress or inflammation which may account for the degenerative changes in various organs such as liver and kidney. Data from the current study suggest that the active ingredient of Solignum®, permethrin may have caused adverse effects on biochemical parameters and subsequent damage to various organs such as liver and kidney through the generation of reactive oxygen species. The underlying mechanism may be attributed to 3-phenoxy benzoic acid, the major metabolite of several pyrethroid insecticides. However, further studies are needed to explore the mechanism underlying the role of this metabolite to induce oxidative stress as the contributing effects of the commercial formulation (solvents, dispersive agents etc.) cannot be ruled out in the present study.

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