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# Effects *Amaranthus cruentus* Contaminated with Pesticides on Liver and Renal Function of Male Wistar Rats

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## Abstract

Today, a number of pathologies are emerging in countries with high levels of agricultural activity. Burkina Faso, for example, is one of the countries in the sub-region with the highest market-growing activity. This study investigates the effects of pesticide-contaminated *Amaranthus cruentus* on liver and kidney function in male Wistar rats. *Amaranthus cruentus* samples were collected from market gardens and aqueous extracts were prepared. After determination of pesticide residues in the extract, the rats were force-fed the extract at various doses for 90 days. Weight gain, hematological parameters, biochemical parameters (liver and kidney function) and liver and kidney histopathology were evaluated. Analysis of pesticide residues revealed the presence of various pesticides, many of which exceeded maximum residue limits (MRLs). Results showed significant increases in liver enzymes (ASAT, ALAT, PAL) and alterations in renal histopathology, suggesting adverse effects due to pesticide contamination. Long-term consumption of *Amaranthus cruentus* leaves would be harmful to the health of the population if the use of pesticides in market gardening were not regulated.

## Keywords

Histopathology, Male Rat, Plant Protection Products, Biochemical Parameters

## 1. Introduction

Burkina Faso, like many other African countries, has experienced strong demo-

graphic growth in recent years. This is correlated with an increase in food requirements. At the same time, the use of pesticides is steadily increasing in these countries. The use of these pesticides implies health risks linked to the consumption of these market garden products. *Amaranthus cruentus*, a plant rich in fiber, is widely grown and consumed in Burkina Faso. It is used in many traditional dishes for its digestibility and high fiber content [1]. In addition, some market gardeners cultivate on sites sometimes subject to several sanitary constraints [2]. In recent years, toxicologists have been alerted to the considerable existence of adverse effects following exposure to plant protection products [3]. The different physicochemical and toxicological properties of pesticides can lead to major risks for human health following exposure [4]. Products known to be highly toxic and banned in developed countries (DDT, etc.) are still sold and used in developing countries [5]. Furthermore, several studies in Burkina Faso have highlighted the existence of poor phytosanitary practices on market garden sites [6] [7]. More than 20% of phytosanitary products are carcinogenic, and most of them contain molecules identified as endocrine disruptors (EDs). Pathologies associated with pesticides include Parkinson's disease, Alzheimer's disease, lateral sclerosis and cognitive disorders [3]. The widespread use of pesticides has clearly led to recurrent poisoning in certain regions and provinces. Moreover, the Burkina Faso Ministry of Health in 2019, reported that around thirty (30) people died as a result of food contaminated with pesticide residues in the Centre-Ouest and Sud-Ouest regions [8]. The aim of the present study was to assess the impact of plant protection products on the biochemical and renal profile of male rats.

## 2. Materials and Methods

### 2.1. Plant Material

The survey enabled us to identify *Amaranthus cruentus*, one of the vegetables most commonly used in traditional dishes. The species was identified at the Botany Laboratory of the Joseph KI-ZERBO University in Ouagadougou, where an authenticated specimen was left. Amaranth was harvested in the fields of Loumbila in November 2022 from several market gardeners. The *Amaranthus cruentus* was washed with water and then dried in the shade. After drying, it was ground and used for aqueous extraction.

### 2.2. Animal Materials

Twenty-one (21)-day-old male rat Wistar were used for the experiment. They were raised in polycarbonate cages and subjected to the same standard laboratory animal husbandry conditions. These conditions included a photoperiod of 12 hours of light and 12 hours of darkness, an ambient temperature of 22˚C ± 3˚C and a relative humidity of 50% ± 10%. They were reared at the Animal Physiology Laboratory of Joseph KI-ZERBO University and had free access to drinking water and pellets from the Livestock Feed Manufacturing Workshop (LFMW) containing an average of 29% protein.

Validation of the experimental protocols was carried out in compliance with the European Convention for the Protection of Vertebrate Animals used for laboratory experiments. The opinion of the institutional animal ethics committee of the Joseph KI-ZERBO University was authorized according to the approval code: CE-UJKZ/2023-15.

### 2.3. Aqueous Extraction of *Amaranthus cruentus*

After harvesting the *amaranthus creuentus*, it was dried in the shade in a natural circulation dryer, at an average temperature of 37˚C in the chamber. After drying, it was ground using various fine sieves for further use. The ground material was used for the aqueous extraction of *Amaranthus cruentus*.

*Amaranthus cruentus* plant powder (3 × 100 g) was added to 750 mL of distilled water. Then the mixture was homogenized using a glass rod. It was boiled on a hot plate for one hour with a glass rod. The decoctate was filtered after cooling by pressing on a fine-mesh nylon cloth. The filtrates obtained were centrifuged at 2000 rpm for 10 min. The supernatants obtained were collected and transferred to freezer jars for lyophilization. The total mass of dry extract obtained and the extraction yield were determined after freeze-drying.

### 2.4. Analysis of Pesticide Residues in *Amaranthus cruentus* Aqueous Extracts

Pesticide residues were quantified using the QuEChERS method described by Borowiak [9] in both *Amaranthus cruentus* powder and aqueous extract. In each *Amaranthus cruentus* sample, 5 g were weighed and then introduced into 50 mL centrifuge tubes. After centrifuging the sample, a 6-mL aliquot was taken and transferred to a 15-mL tube containing the purification reagent. The purification reagent consists of 300 mg PSA (Protein S A), 400 mg activated carbon (C18) and 900 mg magnesium sulfate ($MgSO_4$). The whole is vortexed for one minute, then centrifuged at 3000 rpm for 5 minutes. Finally, tubes containing *Amaranthus cruentus* powder or extract and extraction reagents were centrifuged at 3000 rpm for 5 minutes. A 6 ml aliquot from each tube was removed and transferred to 15 ml vials containing the purification reagent. Tubes were vortexed for 1 minute, then centrifuged at 3000 rpm for 5 minutes. Supernatants of 2 ml were collected and transferred to vials for chromatographic analysis.

### 2.5. Subchronic Toxicity of *Amaranthus cruentus* Extract on Biochemical and Hematological Parameters in Male Wistar Rats

The subchronic toxicity test was performed according to OECD guideline 408 2018 [10]. Twenty (20) male rats of 21-day-old were divided into four groups of five rat each: control, 1, 2 and 3. They were gavaged with distilled water and aqueous *Amaranthus cruentus* extracts corresponding to doses of 50, 100 and 200 mg/kg, respectively. Each rat was weighed at the start of the test and every week until the end of treatment. They were anesthetized with ketamine (50 mg/ml) and

xylazine (20 mg/ml) (1/0.7 v/v), then blood was collected by cardiac puncture for biochemical and hematological parameters. Liver and kidneys were fixed in 10% formalin for histological sections.

### 2.6. Determination of Biochemical and Hematological Parameters

Blood collected from rats by cardiac puncture was placed in dry tubes and centrifuged at 3000 rpm for 15 min. Serum was collected for determination of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), total bilirubin (BT), alkaline phosphatase (PAL), creatinine and complete blood count (NFS). The assay was performed automatically using a Cobas 6000-type device.

### 2.7. Statistical Analysis

The data collected were analyzed using Excel software version 2016. Results were expressed as mean ± standard deviation, compared by ANOVA (Analysis of Variance) and separated by Tukey's test at the 5% threshold using Graph pad 10.2.0 software.

## 3. Results

### 3.1. Extraction of Pesticide Residues from Amaranth Powder and Aqueous Extract

Pesticide residue analysis identified a number of active ingredients in amaranth powder and aqueous extract. This study revealed a multitude of active ingredients belonging to different families and classified as dangerous for consumers. These included aldrin, lindane, HCB (hexachlorobenzene) and OP'DDT (Organochlorine dichlorodiphenyltrichloroethane) all of which were predominantly organochlorines followed by organophosphates (Diazinon, Dimethoate, Mevinphos). Pyrethroids (Alpha cypermethrin and tretrametrin), carbamates (carbofuran and methomyl) and triazines (atrazine and simazine) were identified in the minority (Table 1). The percentage of pesticide residues was higher in the extract than in the amaranth powder (Table 1). Most of the residues were well above the maximum residue limit.

### 3.2. Effect of Amaranth Aqueous Extract on Weight Gain in Rats

Aqueous *Amaranthus cruentus* extract produced significant increases at the second and eighth week, respectively at doses of 50 and 100 mg/kg ($p < 0.05$). At the sixth and tenth week, a significant increase ($p < 0.05$) was observed at the dose of 100 mg/kg compared with the control group (Figure 1).

### 3.3. Effect of *Amaranthus cruentus* Aqueous Extract on Haematological Parameters

Aqueous extracts of amaranth had no significant influence ($p > 0.05$) on the hematological parameters of treated group compared with the control group (Figure 2).

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Table 1. Identification of pesticide residues in *Amaranthus cruentus* powder and extract.

| | Chemical familles of pesticides | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Organochlorines | | | | | | Pyréthroids | | Carbamates | | Organophosphates | | | Triazines | |
| Active ingrédients | Aldrin | HCB) | βHCB | Heptachlor | Lindane | OP'DDT | Alpha cyperméthrin | Tétrametrine | Carbofuran | Méthomyl | Diazinon | Dimethoate | Mevinphos | Atrazine | Simazine |
| Active ingredient concentration in powder (mg/kg) | X | 0.52 | X | 0.28 | 0.2 | X | X | X | X | X | 9.36 | X | 1.68 | 2.6 | 2.88 |
| Active ingredient concentration in extract (mg/kg) | 0.16 | 1.1 | 2.64 | 0.74 | 1.44 | 0.06 | 0.14 | 0.26 | 34.5 | 0.12 | 0.26 | 0.38 | 0.64 | 8.8 | 9.76 |
| Maximum residue limit (mg/kg) | 0.05 | 0.2 | 0.2 | 0.1 | 0.5 | 0.01 | 0.05 | 0.05 | 0.1 | 0.01 | 0.25 | 0.02 | 0.5 | 0.5 | 0.02 |

HCB: Hexachlorobenzen, *β*HCB: Betahexachlorobenzen, X = Not present.

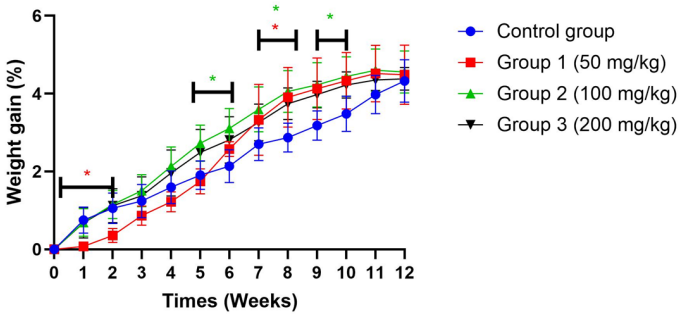


**Figure 1.** Variation in rat body weight gain with different doses of amaranth extract.

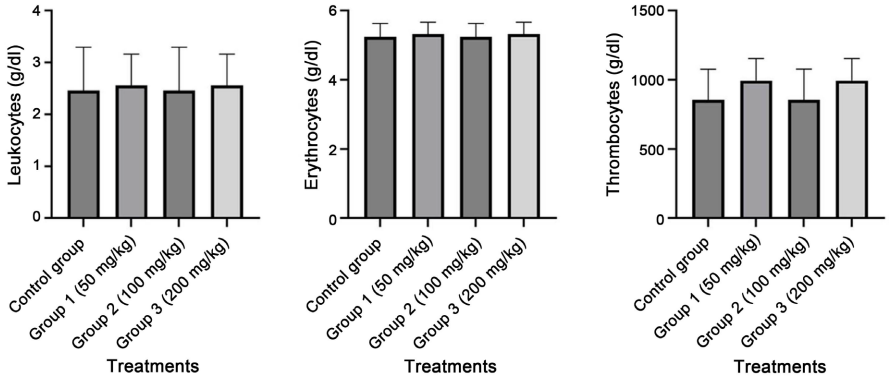


**Figure 2.** Variation in hematological parameters of rats at different doses of amaranth extracts.

### 3.4. Effect of Aqueous *Amaranthus cruentus* Extracts on Biochemical Parameters

Serum levels of ASAT, ALAT and PAL showed moderately to highly significant increases compared with the control group at the different doses of 50, 100 and 200 mg/kg ($p < 0.01$ and $p < 0.001$) for ASAT, at 100 and 200 mg/kg ($p < 0.05$ and $p < 0.001$) for ALAT and at 200 mg/kg ($p < 0.01$) for PAL (Table 2). Creatinine and total bilirubin were not significantly increased ($p > 0.05$) compared with the control group (Table 2).

**Table 2.** Serum levels of ASAT, ALAT, total bilirubin, PAL and creatinine in male rats treated with different doses of aqueous extracts.

| Biochemical parameters | Aqueous extract of *Amaranthus cruentus* | | | |
|---|---|---|---|---|
| | Control (D W) | 50 mg/kg | 100 mg/kg | 200 mg/kg |
| ASAT (UI/L) | 107.65 ± 0.7858 | 121.57 ± 1.411** | 125.6 ± 1.587** | 131.37 ± 3.79*** |
| ALAT(UI/L) | 53.07 ± 1.41 | 51.33 ± 0.50 | 58.25 ± 0.49* | 66.7 ± 1.58*** |
| PAL(UI/L) | 171 ± 24.25 | 132.33 ± 2.333 | 183.33 ± 3.84 | 264.67 ± 15.38** |
| BT (mg/L) | 0.082 ± 0.01 | 0.0753 ± 0.00 | 0.069 ± 0.00 | 0.074 ± 0.00 |
| Créatinine (mg/L) | 0.55 ± 0.01 | 0.43 ± 0.02 | 0.53 ± 0.048 | 0.57 ± 0.05 |

*yes ($p < 0.05$); **yes ($p < 0.01$); ***yes ($p < 0.001$); Distilled water: DW.

### 3.5. Effect of *Amaranthus cruentus* Extract on Liver Histopathology in Male Rats

Histopathological findings in the livers of rats treated with aqueous *Amaranthus cruentus* extracts showed dilation of the centrilobular vein and a large number of sinusoids at 200 mg/kg compared with the control group (Figure 3(A), Figure 3(B)).

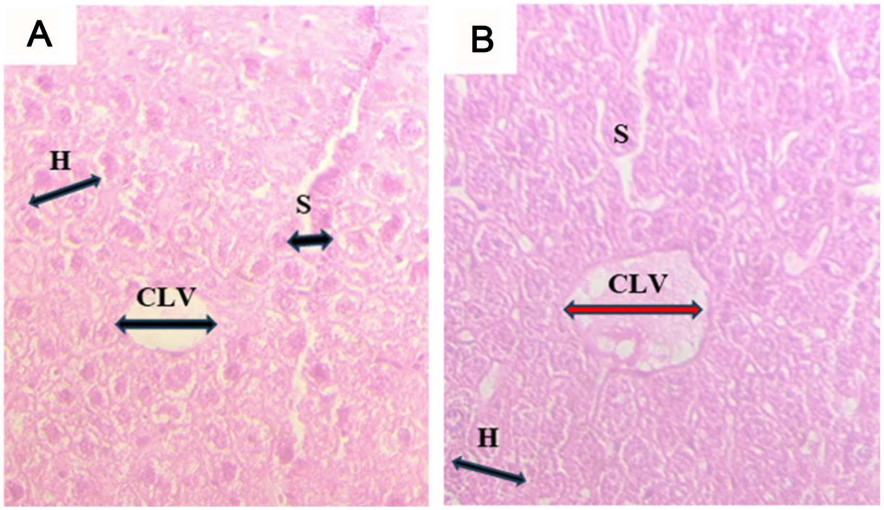


Figure 3. Histological sections of liver (Gr ×400) from male rats treated with aqueous amaranth extracts. A: Control group, B: Group at 200 mg/kg, H: Hepatocytes, S: Sinusoid, CLV: Centro-lobular vein.

### 3.6. Effect of *Amaranthus cruentus* Extract on Histopathology of Male Rat Kidney

Histological sections of the kidneys show tubular dilatation (G), leading to enlargement of the glomerular chamber in group treated with 200 mg/kg compared with the control group (Figure 4(A), Figure 4(B)).

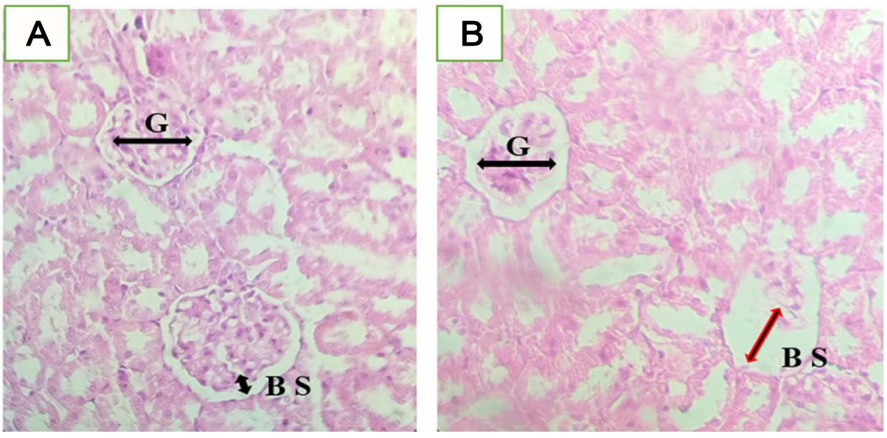


Figure 4. Histological sections of kidney (Gr ×400) from male rats treated with aqueous amaranth extracts. A: Control group, B: Group at 200 mg/kg G: gromerule, B S: Bowman Space.

## 4. Discussion

The detection of active ingredients in both powders and extracts could appear to be the result of non-compliance with good practices at the sites. Residue levels were higher in extracts than in powders. This is because the residue particles were concentrated during extraction. This means that extracts cause higher levels of intoxication than powders. As a result, regular or prolonged consumption puts consumers at greater risk. Residues were predominantly those of organochlorines, even though these are banned for use. According to the literature, the half-life of organochlorines ranged from 28 days to 12.8 years [11]. It is classified as a very persistent organic pollutant (POP) in the environment. This persistence would lead to intoxication of the environment and of market crops such as vegetables.

Active ingredients such as lindane and aldrin, tetrametrin, carbofuran, dimethoate and atrazine as well as other materials found in the extracts were well above the maximum residue limit. They belong to families of pesticides, some of which are classified as endocrine disruptors. In the case of our study, amaranth leaves store a large quantity of pesticide residues. A predominance of active ingredients from the organochlorine family was also found in the *Amaranthus cruentus* extract. Organochlorines have been declared endocrine disruptors. They could be one of the causes of today's metabolic diseases [12]. These results are similar to those of Rouamba [13], who showed that market garden produce was the most contaminated.

Nephrotoxicity refers to all functional or structural renal alterations caused by chemical agents. It mainly concerns the renal tubule and sometimes the glomerulus. Toxic wastes are filtered by the kidneys, which play a major role in the body's homeostasis. Histopathology of the kidney shows alterations in Bowman's capsule adhering to the glomerulus. These alterations affect glomerular filtration. These results concur with those of Fétoui [14]. The probable renal dysfunction suggested by the increase in biological parameters is confirmed by histological examination of the kidneys. It shows structural alteration of the zone of Bowman's capsules adhering to the glomeruli at the expense of the glomerular chamber spaces, which are greatly reduced in the proximal tubule. Vacuolization and hypertrophy of the epithelial cells are also noted, at the expense of the renal interstitium and vessels, which are compressed, thus impairing renal filtration and tubular secretion functions. Similar results were obtained by Zuhair [15] in rats treated with organophosphate insecticides.

For Kalender [16], transaminase disruption is an indicator of liver damage. The results of extract administration showed a significant increase in ASAT and ALAT levels at different doses. These would be due to hepatic lesions in the liver caused by the chemical substances found in the extracts. These substances were well above the maximum residue limits and could cause damage to the liver and other organs responsible for transaminase production. This is because ASAT is found in a variety of tissues, 80% of them in the liver. Our results are comparable to studies by several researchers who have investigated the effect of carbamates and

other pesticides on serum transaminase levels [17]-[19].

## 5. Conclusion

The use of pesticides in market gardening could have adverse effects on the population consuming them. Our study highlighted the existence of several active substances present in amaranth extracts. The transaminases and histopathology of certain organs such as the liver and kidneys were affected by the effect of pesticide residues in these extracts. It also revealed that all the active substances found exceeded the maximum acceptable residue limits. Some of these substances are classified as endocrine disruptors. The increase in certain transaminases (ASAT and ALAT) was influenced by the presence of the active substances in the extracts. We can conclude that the administration of amaranth extracts would affect biochemical parameters and kidney histopathology. These observations suggest a limited use of these toxic pesticides could improve men's quality of life.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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