Confluence Journal of Pure and Applied Sciences, Volume 4 Issue 1, June, 2025 Pp 73-89

ORIGINAL RESEARCH

Evaluation of Cardiac Biomarkers in Male Wistar Rats Exposed to Grains Treated with Chemical and Biological Pesticides

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Abstract

This study investigates the cardiotoxic effects of grains preserved with chemical (organophosphate) and biopesticides in Wistar rats, focusing on differences in Troponin I, LDH and Creatinine Kinase activity as biomarkers of neurotoxicity. Male Wistar rats were divided into control groups and groups treated with grains preserved with either chemical or biopesticides (bacterial and fungal) on rice and cowpeas. The results revealed that chemical pesticide-treated male rats exhibited significantly reduced Troponin I, LDH and CK compared to controls, indicating potential cardiotoxic effects, with the chemical pesticide rice group showing the highest increase in Troponin I (0.62 ± 0.24^b), LDH (64.76 ± 10.33^a). The chemical pesticide cowpea showed more significant increase in Creatinine Kinase. In contrast, biopesticide-treated groups generally exhibited increase particularly in LDH activity in the bacterial pesticide cowpea group (151.11 ± 18.03^c). This finding highlights that both chemical pesticides and bio-pesticides cause significant cardiotoxicity.

Keywords: Cardiotoxicity, Pesticides, Troponin I, Lactate Dehydrogenase, Creatinine Kinase.

Introduction

The muscular heart circulates blood, giving tissues oxygen and nutrients while releasing waste materials like carbon dioxide (Silverthorn, 2016). The cardiac output, the volume of blood the heart pumps into the circulation in a specific amount of time, should be used to evaluate the heart's function (Binney, 2018). Effective blood flow is provided by the cooperation of the heart's four chambers, which include two ventricles and two atria. The heart's regular contractions are regulated by an internal conduction system, ensuring that blood is pumped effectively to sustain life (Andreucci et al., 2014; Bailoor et al., 2021).

The autonomic nervous system, which controls blood pressure and heart rate, can be interfered with by pesticide exposure. For instance, research has linked pesticide exposure to changes in heart rate variability (HRV), a measure of autonomic function (Saldana et al., 2006). The primary purpose of pesticides is to maintain the health of crops and shield them against pests and illnesses. However, the term "pesticide" may also refer to a wider range of goods, such as biopesticides, which are designed for non-plant applications to manage disease-carrying insects, rats, and mice (Georgiadis et al., 2018). According to Borges et al. (2021), biopesticides are inexpensive, environmentally benign, specific in their mode of action, sustainable, leave no residues, and are not linked to the emission of greenhouse gases. These biopesticides can be classified as nano-biopesticides (Abdollahdokht et al., 2022), microbial pesticides (Harish et al., 2021), or plant-pesticides (Idris et al., 2022). Microbial pesticides, in contrast to chemical pesticides, are environmentally sustainable, have no aftereffects, are easily obtained without the need for costly chemicals, and are precise in their activity (Harish et al., 2021; Hummadi et al., 2021). To boost agricultural output, chemical pesticides, also known as synthetic pesticides, are used to manage crop pests (Anani et al., 2020). According to Ayilara et al. (2023), synthetic pesticides are composed of chemicals and transporters, including polymers that are specific to certain pests. According to their classification, they include those used to control weeds (herbicides), algae (algicides), fungi (fungicides), mites or ticks (miticides/acaricides), bacteria (bactericides), rodents (rodenticides), termites (termiticides), insects (insecticides), mollusks (molluscicides), and nematodes (nematicides) (Anakwue, 2019).

Because they give billions of people around the world vital nutrients and energy, grains play a significant role in the global diet. Beyond their carbohydrate content, grains offer substantial amounts of dietary fiber, vitamins, and minerals that promote general health and disease prevention (Venn et al., 2020). They are also a basic source of carbohydrates, which are necessary for sustaining energy levels and supporting metabolic processes (Poutanen et al., 2022). Herbicides, fungicides, and insecticides are examples of pesticides that are used to manage insects and weeds, which can drastically lower crop productivity (Aktar et al., 2009).

The use of pesticides in agriculture has significantly increased to meet the growing demand for food with both chemical pesticides and bio-pesticides commonly used to protect crops from pest and diseases. However, their residue on grains can pose health risks to consumers. Evaluation of the cardiotoxic effect of these residues can provide critical insights into their safety (Kim et al., 2017; Mostafalou and Abdollahi, 2017). To the best of our knowledge, there has been limited work in existing literatures previous reportedly on the cardio-toxic assessment of grains preserved with chemical pesticides and bio-pesticides on Wistar rats, hence the need for this current research. Thus, this experiment aims to evaluate and compare the cardiotoxic effects of grains preserved with chemical pesticides and bio-pesticides in Wistar rats.

Materials and Methods

Animals

Thirty-five (35) male Wistar rats were purchased and housed at the animal house of Federal University Lokoja, Kogi state, Nigeria. The Animals were acclimatized for a period of 2 weeks under standard environmental conditions, with an approximately 12 hours light/dark cycle and were fed a standard laboratory diet and water. After 2 weeks of acclimatization, the animals were subjected to treatment with grains preserved with chemical pesticides and bio-pesticides. The animals used in the present study were maintained in accordance with the principles and guidelines of the Canadian Council on Animal Care by Ahmadi-Noorbakhsh et al. (2021).

Experimental Design

The animals were divided into seven (7) groups of five (5) animals each based on their weight ranges. Administration was carried out orally for a period of ten (10) days. Grains preserved with chemical and bio-pesticides were formulated into feeds and 100 grams of the feeds were administered to the rats daily all through the duration of the experiment.

Bodyweight Changes

The rats were weighed daily from the day according to Balcombe et al. (2004) prior to the first day of treatment till the tenth day of treatment. They were further weighed on the eleventh day when they were sacrificed. The changes in the body weights were documented.

Weight of Organ (Heart)

The rats were euthanized on the last day of the experiment following anesthesia with diethyl ether according to Hedenqvist and Hellebrekers, (2003) and the relative weight of the heart (as a percentage of their body weight) were recorded.

Table 1: Treatment Groups and Feed Administration

Groups	Treatment	Amount (g)
1	Control (untreated grains)	100
2	Chemical Pesticides (Rice)	100
3	Chemical Pesticides (Cowpea)	100
4	Biopesticides Bacteria (Rice)	100
5	Biopesticides Bacteria (Cowpea)	100
6	Biopesticides Fungi (Rice)	100
7	Biopesticides Fungi (Cowpea)	100

Table 1 shows the classification of treatment groups used in the experimental evaluation of the effects of grains preserved with chemical and biological pesticides on the cardiac status of wistar rats. Each treatment group received a standardized amount of grains (100 grams) to ensure consistency across the experiment. The treatments are categorized as follows: Group 1 serves as the control, containing untreated grains (normal grains) to provide a baseline for comparison; Groups 2 and 3 are treated with chemical pesticides, applied separately to rice and cowpea respectively; Groups 4 and 5 involve treatment with bacterial-based biopesticides, again applied separately to rice and cowpea; while Groups 6 and 7 use fungal-based biopesticides, also applied to each grain type separately.

This classification allows the study to assess and compare the impact of grains preserved with chemical pesticides and the two forms of biopesticides (bacterial and fungal) on cardiac parameters in Wistar rats.

Formulation of Rat Feed and Incorporation of Biopesticides (Fungi)

The food formulation contains: 40 percent carbohydrate, 20 percent animal protein, 20 percent plant protein (cowpea), 15 percent fat and oil, 5 percent in form of salts, vitamins and minerals. The feed was milled into pellets using pellet milling machine with each pellet in the size range of 4mm. a batch of the feed was formulated using cowpea preserved with chemical pesticide, the second batch was formulated using cowpea preserved with the biopesticide while the third batch was formulated using unpreserved cowpea. 200 g of the powdered biopesticide was added to 1 kg of the pelletized feed and mixed thorougly. The formulated feeds were used in feeding the experimental animals after the first week.

Formulation of Biopesticides and Incorporation into Rat Feed (Bacteria)

A loopful of the bacteria were inoculated into sterilized nutrient broths and incubated in a rotary shaker at 150 rpm for 72 hours at room temperature. After 72 hours of incubation, Bacteria broth cultures were centrifuged to separate and obtain their cells from the broth. The cells were further washed by reconstituting with sterile saline and centrifuging again. The suspension was serially diluted and the concentration adjusted to 10^6 cfu/ml. The appropriate diluent was cultured to enumerate the viable bacterial cells present within. 400 ml of bacterial broth suspension, 1 kg of the purified talc powder, 15 g of calcium carbonate (to adjust the pH to neutral) and 10 g of Carboxy methyl cellulose were mixed under sterile conditions. The resulting product was shade dried to reduce the moisture content below and then packed in a polypropylene bag and sealed (Senthilraja and Vijayakumar, 2010). The dried formulation was added to the pelletized feed formulation by adding 200g of the formulation to 1 kg of the feed.

Formulation of Chemical Pesticide and Incorporation into Feed

The organophosphate chemical was used in the preservation of stored grains. They were diluted in distilled water and prepared according to manufacturer's specification. 10 ml of the chemical was sprayed on 1 kg of the feed and mixed thoroughly. The sprayed feed was allowed to air dry for 24 hours and packed.

Estimation of Lactate Dehydrogenase Activity

The activity of lactate dehydrogenase (LD) was determined according to the method described by Hemben et al. (2018) using commercially available kit

Calculation:

$$U/I = 4127\ x\ \Delta A_{340}\ nm/min$$

Troponin I Activity

This test was carried out on the serum of the rats used for this experiment using ELISA kit according to the method described by Gunes et al. (2008).

Calculation:

$$Troponin\ I\ \ (mg/dl) = \frac{Absorbance\ of\ sample}{Absorbance\ of\ standard}\ x\ \ (standard\ conc)$$

Creatinine Kinase Activity

The activity of creatinine kinase was determined using a commercially available kit obtained from fortress diagnosis according to the method described by Sulistian et al. (2021).

Calculation:

CK activity (U/L)= $\frac{Change\ in\ Absorbance\ of samples\ per\ mins}{Change\ in\ Absorbance\ of\ standard\ per\ mins}\ x\ \ (standard\ conc)$

Statistical Analysis

All values are expressed as means ± standard deviations. Data were analyzed by one-way ANOVA and significant differences between groups were determined by Duncan's multiple range test and least significant difference (LSD). Statistical analyses were done using SPSS 20, the statistical package. The acceptable level of significance was p<0.05. The superscripts were used to depict significantly different (p<0.05) mean values at 95% confidence interval (Cl). LSD option was employed to determine contrast.

Results

All data were collected and tabulated, and results were expressed as mean ± standard deviation of at least three representatives per group.

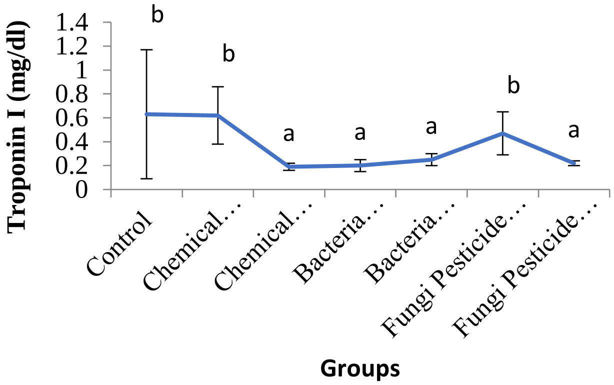

Figure 1: Effect of Chemical Pesticide and Bio-pesticide on the level of Troponin I

Values are expressed as Mean ± SD (n=4). Values with different superscripts are significantly different from one another at $P < 0.05$.

Results from figure 1 above revealed that Chemical Pesticide Rice and Fungi Pesticide Rice showed no significant difference in Troponin I levels compared to the control group while Chemical Pesticide Cowpea, Bacteria Pesticide Rice, Bacteria Pesticide Cowpea and Fungi Pesticide Cowpea showed a significant decrease of 69.84%, 68.25%, 60.32% and 65.11% respectively in Troponin I levels compared to the control group.

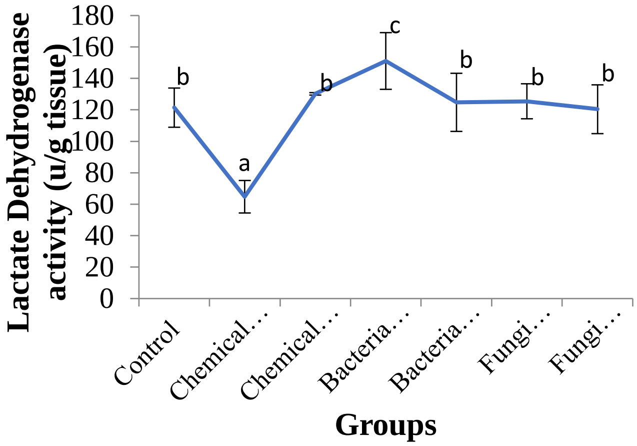

Figure 2: Effect of Chemical Pesticide and Bio-pesticide on the activity of Lactate Dehydrogenase
Values are expressed as Mean ± SD (n=4). Values with different superscripts are significantly different from one another at $P < 0.05$.

Chemical Pesticide Cowpea, Bacteria Pesticide Cowpea, Bacteria Pesticide Cowpea, Fungi Pesticide Rice and Fungi Pesticide Cowpea showed no significant difference in LDH activity compared to the control group (Figure 2). However, Chemical Pesticide Rice showed a significant decrease of 46.68% while Bacteria Pesticide Rice showed a significant increase of 24.44% in LDH activity compared to the control group.

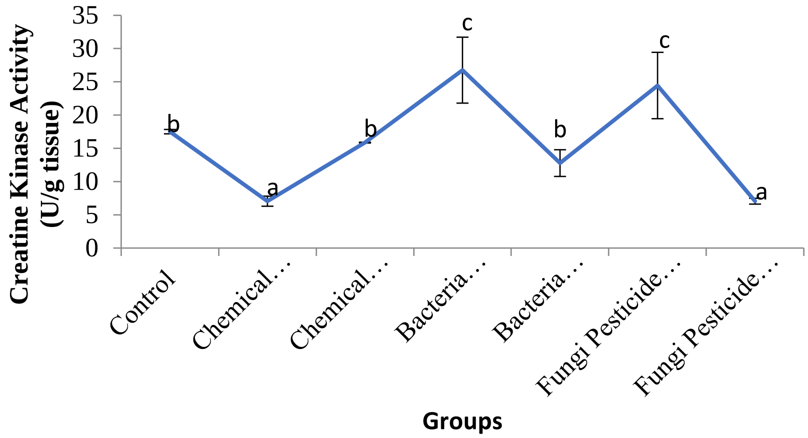

Figure 3: Effect of Chemical Pesticide and Bio-pesticide on the Heart Creatinine Kinase

Values are expressed as Mean ± SD (n=4). Values with different superscripts are significantly different from one another at $P < 0.05$.

As observed in figure 3 above, Chemical Pesticide Cowpea and Bacteria Pesticide Cowpea showed no significant difference in Creatine Kinase activity compared to the control group. Both Chemical Pesticide Rice and Fungi Pesticide Cowpea showed a significant decrease of 59.74% in Creatine Kinase activity compared to the control group while Bacteria Pesticide Rice and Fungi Pesticide Rice showed a significant increase of 52.83% and 39.63% respectively in Creatine Kinase activity compared to the control group.

Discussion

Troponin I levels were significantly affected by both chemical (organophosphate) and biological pesticide (bacteria and fungi-based) treatments, with noticeable reductions in certain groups. The control group had a Troponin I level of 0.63 ng/mL, which was significantly higher than that of the chemical pesticide cowpea group (0.19 ng/mL). This decrease in Troponin I suggests that chemical pesticides, particularly in cowpea (69.84%), may reduce the release of this marker due to possible myocardial suppression. The same pattern was observed in the bacteria pesticide rice (0.20 ng/mL), bacteria pesticide cowpea (0.25 ng/mL), and fungi pesticide cowpea (0.22 ng/mL) groups, which also had significantly lower levels of (68.25%, 60.32% and 65.08% respectively) compared to the control. The reduction in Troponin I levels may indicate a disruption in cardiac function, potentially linked to the metabolic interference caused by pesticide exposure. Studies have shown that exposure to certain pesticides can alter protein expression in cardiac tissue, leading to suppressed cardiac biomarkers, which reflects potential subclinical cardiac dysfunction (Xu et al., 2020). The rice group treated with fungi pesticides (0.47 ng/mL) had Troponin I levels closer to the control, suggesting less cardiotoxicity. This aligns with research suggesting that some biopesticides may cause less severe cardiac damage compared to synthetic chemicals (Ullah et al., 2019). The chemical pesticide rice group (0.62 ng/mL) displayed Troponin I levels similar to the control group, indicating a less severe cardiotoxic impact compared to other treatments.

LDH activity, another marker of tissue damage, varied significantly across the groups. The control group exhibited LDH activity of 121.43 u/g tissue. The chemical pesticide rice group showed a notable reduction (46.67%) in LDH activity (64.76 u/g tissue), indicating lower cellular damage in this group. However, the reduction in LDH activity could also signal inhibited cellular metabolism rather than improved tissue health, possibly due to the toxic effects of the chemical pesticide on cellular function. The chemical pesticide cowpea group showed an elevated LDH activity of 130.19 u/g (7.21%), suggesting increased cellular injury in response to chemical pesticides. This finding aligns with studies that have reported elevated LDH levels in the blood and tissues of animals exposed to pesticides, indicating tissue damage and oxidative stress (Erdogan et al., 2011). Biopesticides showed a varying impact on LDH activity. The bacteria pesticide rice group had the highest LDH activity (151.11 u/g), indicating significant tissue damage. This suggests that bacterial biopesticides, though environmentally safer, may still induce significant cardiotoxic effects under certain conditions. The bacteria pesticide cowpea group had LDH activity of 2.77% similar to the control group (124.80 u/g), suggesting a lesser degree of tissue damage. Fungi pesticide treatments for both rice (125.47 u/g) and cowpea (120.41 u/g) showed LDH activity (3.33% and 0.84% respectively) close to the control, indicating moderate cardiotoxicity compared to chemical pesticide treatments.

This study also evaluated the impact of chemical and biological pesticides on heart creatine kinase (CK) activity, another key biomarker of cardiac muscle integrity and metabolic stress. The results revealed significant elevation in CK activity (approximately 80%) in animals exposed to chemically treated grains compared to the control, indicating substantial cardiac stress or membrane damage. In contrast, the CK levels in animals fed with biopesticide-treated grains showed only a marginal increase of about 5%, which was not statistically significant. These findings underscore the cardiotoxic potential of chemical pesticides and the relative safety of biopesticides. Creatine kinase, particularly its CK-MB isoform, is predominantly localized in cardiac muscle. Elevated CK levels in the bloodstream often signify myocardial membrane damage, which results in leakage of intracellular CK from cardiomyocytes into circulation (Gholami et al., 2019). In this study, the significantly higher CK levels in rats fed chemical pesticide-treated grains suggest that organophosphate exposure (e.g., dichlorvos) may have caused subclinical or overt cardiac muscle injury. This aligns with earlier reports by Atef et al. (2021), who observed similar elevated CK and LDH levels in rodents exposed to organophosphates and carbamates. These compounds are known to induce oxidative stress, mitochondrial dysfunction, and lipid peroxidation in cardiac tissue, which compromise cardiac membrane integrity and cellular energy balance. El-Sayed et al. (2020) also reported a 75–85% increase in CK activity following cypermethrin exposure, a value closely matching the observed increase of approximately 80% seen in this study.

In contrast, rats administered grains preserved with biopesticides (bacterial and fungal formulations) showed no significant deviation in CK levels (approximately 5%) relative to the control. This indicates a lack of cardiotoxic effects, supporting the growing consensus that biopesticides are safer alternatives to synthetic chemicals in pest management. The result aligns with the findings of Mandal et al. (2023), who reported no significant cardiac, hepatic, or renal enzyme perturbations in rodents exposed to neem-based and *Bacillus thuringiensis*-formulated products. Their biodegradable, target-specific nature likely accounts for the minimal impact on cardiac enzymes. Biopesticides exert targeted pesticidal effects on insects while sparing mammalian systems due to differences in receptor specificity and metabolic processing (Regnault-Roger et al., 2020). Their safety profile makes them ideal for use in food preservation, particularly in vulnerable populations.

While many pesticide toxicity studies focus on neurotoxicity and hepatic damage, emerging evidence highlights cardiotoxicity as a key concern. The 80% increase in CK observed with chemical pesticides mirrors values reported in toxicological studies of organophosphates and pyrethroids. For example: Anyanwu et al. (2019) demonstrated that repeated pesticide exposure alters cardiac mitochondrial enzyme activity, contributing to cardiomyopathy. They reported up to 90% CK elevation in subchronic pesticide exposure models. El-Sayed et al. (2020) reported increased CK, AST, and ALT in rats exposed to cypermethrin. They found CK-MB levels rising 80–95% in rats treated with cypermethrin. These studies are in agreement with the elevated CK observed in this work, emphasizing the multisystemic risk posed by indiscriminate use of chemical pesticides. In contrast, Mandal et al. (2023) observed less than 10% deviation in CK and other cardiac biomarkers in animals treated with neem or Bt-based biopesticides.

Conclusion

The cardiotoxic assessment of grains preserved with chemical and biological pesticides reveals significant impacts on heart tissue health in male Wistar rats. Chemical pesticides, particularly when used to preserve cowpea, led to notable reductions in Troponin I levels with increased LDH and CK activities, indicating considerable cardiac tissue damage. Biopesticides, while generally considered safer, also exhibited cardiotoxic effects, especially bacterial biopesticides, which caused the highest LDH activity, signaling substantial tissue damage. Fungi-based biopesticides demonstrated lower cardiotoxicity, suggesting they may be a safer alternative for pest control. However, the overall findings highlight the need for further research into the long-term cardiac impacts of pesticide exposure and the development of safer pest management strategies.

Recommendations

To mitigate the cardiotoxic effects associated with pesticide use, it is recommended that chemical pesticide use, especially for grains like cowpea, should be minimized to reduce potential cardiac damage. Exploring and developing safer biological pesticide alternatives, particularly focusing on reducing the cardiotoxicity of bacterial formulations, is essential. Further research is needed to comprehensively understand the long-term cardiac effects of both chemical and biological pesticides and to refine pest management practices for improved safety.

Author's Contributions

FO designed the research, carried out the laboratory experiment, and compiled the manuscript; AOT, MS also actively involved in the design of the research ; whilst MS additionally corrected the manuscript; OFD ATO and JT performed the statistical analysis; ATO and JT also compiled the first draft of the manuscript. KBA contributed to the research design and formulated both the grain-preserved chemical and biological pesticides; A AIG, AVU, ATM, ABM, LD, AAM, OFB and ODB carried out the laboratory experiments.

Conflict of Interest

The authors declare no conflict of interest.

Funding

This study was supported by TETFUND IBR grant (FUL 2024)

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