***Original Research Article***

# Cardioprotective effects of Green Tea and Honey on Albino Wistar Rats exposed to Atrazine


## ABSTRACT

**Aim:** To evaluate thecardioprotective effect of green tea and honey on the blood levels of creatinine kinase and troponin of albino Wistar rats exposed to atrazine.
**Study design:** Experimental study
**Place and Duration of Study:**Department of Clinical Chemistry and Department of Anatomy, Rivers State University, Port Harcourt, between August 2024 and December 2024.
**Methodology:**Thirty (30) male rats weighing between 150-250g were randomly divided into six (6) groups of five rats each. Prior to the study, the rats were allowed to acclimatize for 14 days and were allowed standard feed and water*ad libitum*. Group I rats served as the negative control, group II rats served as the positive control as they were given oral administration of Atrazine (2.3mg/ml), Group III oral administration of atrazine (2.3mg/ml), low dose green tea (0.23ml), group IV received oral administration of atrazine (2.3mg/ml), high dose green tea (1ml), group V received oral administration of atrazine (2.3mg/ml), honey (1ml) once daily, and group VI received oral administration of atrazine after 15 days of oral administration of honey. After the end of the study the rats were allowed to fast for 24 hours before they were anaesthetized, using chloroform and blood samples were collected via cardiac puncture for the analysis of creatine kinase-muscle/brain (CK-MB), creatine kinase-Brain/Brain (CK-BB), creatine kinase-Muscle/Muscle (CK-MM), Troponin-I and Troponin-II. GraphPad Prism version 10.0 was used to analyze the mean,standard deviation, ANOVA and Tukey multiple comparison test, and $p<0.05$ was regarded to be statistically significant
**Results:**The results showed a significant difference in CK-MB ($p<0.0001$), CK-MM ($p<0.0001$), CK-BB ($p<0.0001$), Trop-I ($p<0.0001$) and Trop-T ($p<0.0001$) in the treatment groups (group III, IV, V &VI) compared to the positive control group (group II). The multiple comparison test also showed a significant difference ($p<0.05$) between the mean value of CK-MB, CK-BB, CK-MM, Troponin-I and Troponin-T across the various in-between groups comparisons.
**Conclusion:**The findings of this study suggest that honey and green tea possesses the potential of ameliorating cardiotoxicity caused by Atrazine. Further related studies are, however, recommended.



*Keywords:CardioprotectiveGreen Tea, Honey, Creatinine Kinase, Troponin, Albino Wistar Rats, Atrazine*


## 1. INTRODUCTION

Atrazine's widespread use in agriculture has raised concerns about its toxic effects on human health and the environment. Atrazine exposure has been linked to cardiovascular diseases, which are a leading cause of morbidity and mortality worldwide. Creatine kinase and Troponin are biomarkers used to diagnose cardiac damage [1], and elevated levels of these biomarkers indicate cardiac damage. Limited research exists on the effects of natural products like honey and herbal tea on creatine kinase and Troponin levels in atrazine-

exposed animals. This study will investigate the effects of honey and green tea on creatine kinase and Troponin levels in albino Wistar rats exposed to atrazine, providing insights into potential alternative therapies for cardiovascular diseases caused by atrazine exposure.

Atrazine (ATR), a widely utilized herbicide, is employed extensively to control broadleaf and grassy weeds on various crops, including corn, sorghum, and sugarcane. With an estimated annual application of approximately 73-78 million pounds, ATR stands as one of the most frequently applied pesticides in the United States. Due to its high mobility and persistence in water, ATR is commonly detected in streams, rivers, and groundwater across many countries. In certain areas, such as Iowa, ATR concentrations in surface waters have been reported to reach levels of up to 300μgL−1[2].

Cardiac damage is the leading cause of human morbidity and mortality. Exposure to atrazine (ATR) can lead to severe cardiac damage, which may result in CVD [3][1].ATR has been one of the most widely used herbicides in recent decades. Due to its widespread presence and continued use in most countries, there are increasing concerns about the potential adverse health effects of ATR. ATR and/or its metabolites (such as atrazine-desethyl-desisopropyl; DACT) have been detected in heart tissues, and ATR contributes to cardiovascular disorders during intoxication [4].

Creatine kinase and Troponin are biomarkers used to diagnose cardiac damage. Elevated levels of these biomarkers indicate cardiac damage. Honey and herbal tea have been used for centuries for their medicinal properties, including antioxidant and anti-inflammatory effects. Studies have shown that honey and herbal tea can protect against cardiac damage caused by various toxins. The antioxidant properties of honey and herbal tea can help reduce oxidative stress, which is a major contributor to cardiac damage. Additionally, the anti-inflammatory properties of honey and herbal tea can help reduce inflammation, which is also a major contributor to cardiac damage [5].

In recent years, the prevention of cardiovascular disease (CVD) has been associated with the consumption of fresh food items and plants rich in natural antioxidants, which have been shown to be more effective and safer than synthetic products[6]. Honey, a natural liquid produced by honeybees, has recently received significant attention due to its therapeutic potential in CVD, which is attributed to its diverse composition of at least 181 different substances [7]. The therapeutic value of honey is partly due to its antioxidant properties [8], which are attributed to its various constituents, including phenolics, flavonoids, ascorbic acid, proteins [9], certain enzymes (glucose oxidase, catalase) [10], α-tocopherol, and beta-carotene, a precursor of vitamin A that may reduce the risk of certain fatal diseases, such as cancer, CVD, and stroke [8].

Green tea leaves are rich in polyphenols, comprising approximately 30% of their dry weight, with flavonoids being the primary constituent. The main flavonoid class in tea is flavanols, which encompass catechin, epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) [11]. Although the total polyphenol content is similar across various tea types, the specific components differ due to factors like polyphenol oxidation during processing. In green tea, catechins make up 80-90% and flavanols 10% of the total flavonoids, whereas in black tea, theaflavins account for 50-60% and catechins only 20-30% [12]. Recently, there has been growing interest in the potential cardiovascular benefits of tea consumption, with some observational studies suggesting a positive association, while others have found no correlation. However, mechanistic studies have demonstrated that tea and its polyphenols have beneficial effects on systemic risk factors and direct impacts on the vasculature and platelets, which may contribute to reduced cardiovascular risk [12].

This study is Important because it investigates the potential effects of honey and herbal tea on creatine kinase and Troponin levels in albino Wistar rats exposed to atrazine. The findings of this study will provide valuable insights into the toxic effects of atrazine on cardiovascular health and the potential health benefits of natural products like honey and herbal tea. This research will also contribute to the development of alternative therapeutic approaches for cardiovascular diseases caused by atrazine exposure and inform environmental and public health policies on the safe use of atrazine in agriculture.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Animals

Thirty (30) randomly selected albino rats that weighed 150- 200g were used for the study. The animals were obtained from the Department of Anatomy, College of Medical Sciences, Rivers State University. They were transported in well-ventilated wired cages to the Animal House in the Department of Anatomy, Rivers State University, Port Harcourt. The rats were maintained in a 12- hour light/ dark cycle and were allowed solid poultry chow as feed and water*ad libitum* andwere allowed to acclimatize for 2 weeks.

### 2.2 Determination and Preparation of Solution

#### 2.2.1 Atrazine

The Atrazine was administered at 100mg/kg of rat. Each rat was weighed and 100mg of Atrazine was administered to a 1kg rat[18].

For a rat that weighed 230g, 23mg of Atrazine was administered 1000g >1kg
230g > 230/1000 = 0.23kg
Determining the dose 1kg. > 100mg
0.23kg > 0.23×100= 23mg
A stock solution of 100ml was prepared in the chemistry laboratory at Rivers State University, Port Harcourt. To create 23mg/ml solution; 2.3grams of atrazine was dissolved in 100ml of distilled water.

#### 2.2.2 Green Tea

Dose per rat: low dose = 50mg/kg × 0.23kg = 11.5mg High dose = 200mg/kg × 0.23kg = 46mg
Volume: low volume = 11.5mg ÷ 50mg/ml = 0.23ml High volume = 46mg ÷ 50mg/ml = 0.92ml.
Green tea in the form of green tea bags were purchased from the supermarket in Port Harcourt. Green tea is usually prepared as an extract (aqueous) from commercially available green tea leaves or bags. A known green tea bag weighing 2g per 100ml of distilled water. Distilled water was boiled and poured over the tea bags. The tea is allowed to steep for 10-20 minutes to extract the active component(s), the tea is filtered to remove the tea bags, leaving a clear green tea extract.

#### 2.2.3 Honey Solution

The standard concentration of honey per body weight used for 1g/kg was 1g/ml. For the dilution process to take place, the standard concentration was first determined. Dilution was carried out with distilled water to achieve the desired result. In the dilution of the honey, 10% honey solution was prepared by dissolving 10g of honey in 90ml of distilled water to make a total amount of 100ml of a 10% solution.

### 2.3 Experimental Design

After acclimatization, the rats were assigned into six (6) groups of five (5) rats each, and the study lasted for 15 days.

**Group 1**: Rats in this group were given only food and water for 15 days. They served as negative control for the study.
**Group 2**: Rats in this group were given 23mg atrazine for 15 days to induce toxicity. They servedas positive control for the study.
**Group 3:** Rats in this group were given 23mg atrazine and low dose (11.5mg) of green tea for 15 days.
**Group 4**: Rats in this group were given 23mg of atrazine and high dose (46mg) of green tea for 15 days.
**Group 5**: Rats in this group were given 23mg of atrazine and 11.5mg of green tea and high dose of honey for 15 days.
**Group 6**: Rats in this group were given 23mg of atrazine after 15 days of administering high dose of honey.

## 2.4 Blood Collection and Preparation

### 2.4.1 Analysis of CK-MB, CK-MM, CK-BB, Trop-I and Trop-II

**Method:** Enzyme-linked Immunosorbent Assay (ELISA)[13]

**Principle:**The Seamaty analyzer uses dry chemistry technology to detect biochemical markers in serum. The pre-filled reagent discs contain immobilized reagents that react with specific analytes (CK isoforms and troponins) in serum samples. Changes in color intensity during the reaction are optically analyzed, and the results are digitally displayed in concentration units.

## 2.5 Statistical Analysis

The data generated from the analysis was expressed as Mean ± standard deviation and analyzed using the GraphPad prism version 10.0. Comparison of the mean and standard deviation values were made for the various parameters for the various groups using the one-way ANOVA and Tukey's tests. Results were considered statistically significant at 95% confidence interval ($p<0.05$).

## 3. RESULTS AND DISCUSSION

**Table 1: Descriptive and Inferential Results of CK-BB, CK-MM and CK-MB for Control and Treatment Groups**

| Groups | CK-MB (µ/L) | CK-MM(µ/L) | Ck-BB (µ/L) |
|---|---|---|---|
| Group I (NC) | 5.80±0.84 | 135.0±5.00 | 2.60±0.55 |
| Group II(PC) | 10.0±0.707 | 162.0±8.37 | 4.80±0.84 |
| Group III (ATR 2.3g/ml, green tea 0.23ml) | 13.60±0.0.54 | 217.0±6.71 | 6.80±1.09 |
| Group IV (ATR 2.3g/ml, green tea 1ml) | 19.0±1.0 | 268.0±5.701 | 8.00±0.707 |
| Group V (ATR 2.3g/ml, honey 0.5) | 11.6±0.55 | 209.0±8.44 | 4.5±1.08 |
| Group VI (High dose honey and Atrazine) | 9.60±0.8944 | 145.0±7.07 | 3.60±0.55 |
| F-value | 164.9 | 309.0 | 26.41 |
| P-value | <0.0001 | <0.0001 | <0.0001 |

| | | | |
|---|---|---|---|
| Remark | S | S | S |

*S- Significant NS- Not Significant NC= negative control, PC = positive control, Ck-MB = creatine kinase-muscle /Brain, Ck-BB = creatine kinase-Brain /Brain, Ck-MM = creatine kinase-muscle, Value is significant at p<0.05)*

**Table 2: Descriptive and Inferential Results of Troponin-I and Troponin-T for Control and Treatment Groups**

| Groups | Trop-I (ng/mL) | Trop-T (ng/mL) |
|---|---|---|
| Group I (NC) | 0.026±0.0055 | 0.010±0.00 |
| Group II (PC) | 0.034±0.0054 | 0.020±0.00 |
| Group III (Atrazine and low dose green tea) | 0.042±0.0084 | 0.036±0.00 |
| Group IV (Atrazine and high dose green tea) | 0.054±0.0055 | 0.042±0.00 |
| Group V (Atrazine, green tea and honey) | 0.034±0.0054 | 0.024±0.00 |
| Group VI (high dose honey and Atrazine) | 0.030±0.0071 | 0.016±0.00 |
| F-value | 12.53 | 40.44 |
| P-value | <0.0001 | <0.0001 |
| Remark | S | S |

S- Significant NS- Not Significant, NC= negative control, PC = positive control, Value is significant at p<0.05)

**Table3: Tukey's Multiple Comparison Table for CK-BB, CK-MM and CK-MB for Control and Treatment Groups**

| Groups | CK-MB (µ/L) | CK-MM(µ/L) | Ck-BB (µ/L) |
|---|---|---|---|
| Grp 1vs 2 | <0.0001 | <0.0001 | 0.0056 |
| Grp 1vs 3 | <0.0001 | <0.0001 | <0.0001 |
| Grp 1vs 4 | <0.0001 | <0.0001 | <0.0001 |
| Grp1 vs 5 | <0.0001 | <0.0001 | 0.0057 |
| Grp 1vs 6 | <0.0001 | 0.183 | 0.4843 |
| Grp 2 vs 3 | <0.0001 | <0.0001 | 0.0138 |
| Grp 2 vs 4 | <0.0001 | <0.0001 | <0.0001 |
| Grp 2 vs 5 | 0.0341 | <0.0001 | 0.9885 |
| Grp 2 vs 6 | 0.9616 | 0.0044 | 0.2893 |
| Grp 3 vs 4 | <0.0001 | <0.0001 | 0.2893 |
| Grp 3 vs 5 | <0.0001 | 0.3983 | 0.0006 |
| Grp 3 vs 6 | <0.0001 | <0.0001 | <0.0001 |
| Grp 4 vs 5 | <0.0001 | <0.0001 | <0.0001 |
| Grp 4 vs 6 | <0.0001 | <0.0001 | <0.0001 |
| Grp 5 vs 6 | 0.0051 | <0.0001 | 0.4423 |

*Value is significant at p<0.05*

**Table4: Tukey's Multiple Comparison Table for Troponin-I and Troponin-T for Control and Treatment Groups**

| Groups | Trop-I (ng/mL) | Trop-T (ng/mL) |
|---|---|---|
| Grp 1vs 2 | 0.3713 | 0.0129 |

| | | |
|---|---|---|
| Grp 1vs 3 | 0.0062 | <0.0001 |
| Grp 1vs 4 | <0.0001 | <0.0001 |
| Grp1 vs 5 | 0.3713 | 0.0004 |
| Grp 1vs 6 | 0.9134 | 0.02674 |
| Grp 2 vs 3 | 0.3713 | <0.0001 |
| Grp 2 vs 4 | 0.0005 | <0.0001 |
| Grp 2 vs 5 | >0.999 | 0.6813 |
| Grp 2 vs 6 | 0.9134 | 0.6813 |
| Grp 3 vs 4 | 0.0608 | 0.2674 |
| Grp 3 vs 5 | 0.3713 | 0.0022 |
| Grp 3 vs 6 | 0.0608 | <0.0001 |
| Grp 4 vs 5 | 0.0005 | <0.0001 |
| Grp 4 vs 6 | <0.0001 | <0.0001 |
| Grp 5 vs 6 | 0.9134 | 0.0670 |

*Value is significant at p<0.05*

The widespread use of atrazine, a commonly used herbicide, has raised concerns about its potential toxic effects on human health and the environment. This study was aimed at investigating the effects of green tea and honey on the levels of creatine kinase and troponine in atrazine-induced toxicity in albino Wistar rats.

According to the results of this study presented in table 1 showed that Atrazine exposure irrespective of the dose,caused a significant increase ($p<0.05$) on the level of CK-MB, CK-BB and CK-MM in group II (positive) when compared to the values for group 1rats. The increase in the level of CK-MB, CK-BB and CK-MM in the treatment groups may be accounted for by the ability of atrazine to induce oxidative stress and inflammation which may have resulted in the increase in the assayed parameters. These findings are consistent with studies of Singh et al. [14] and Sharma et al. [15], which reported increased creatine kinase levels following exposure to atrazine.

The results also revealed that the administration of green tea failed to significantly reduce the levels of the assayed markers, but on combination with honey, there was a significantly reduced ($p<0.05$) levels of CK and Trop in atrazine-exposed rats.Group IV, which received high doses of green tea, did not show any significant decrease in the levels of CK-MB, CK-MM, and CK-BB compared to Group II (PC). Group V, which received green tea and honey, had significantly lower levels of CK-MB, CK-MM, and CK-BB compared to Group II (PC). The finding of a reduction in the assayed markers after the administration of green tea and honeysuggests that green tea and honey may have worked synergistically to cause a protective effect against atrazine-induced muscle damage.The results of these studies agree with the findings of Khan et al. [16], Erejuwa et al. [17] on which they reported a significant effect of honey on the level of cardiac markers of albino rats exposed to toxic substances like atrazine. The protective effect of honey and green tea against atrazine-induced cardiotoxicity may be accounted for by the presence of their phytochemical parameters such as polyphenols, flavonoids, terpenoids and alkaloids (Khan et al.[16].

The results revealed that pre-treatment with honey before atrazine exposure significantly reduced the levels of CK and Trop. Group VI, which received high doses of honey before atrazine exposure, had significantly lower levels of CK-MB, CK-MM, and CK-BB compared to Group II (PC). These findings suggest that honey may have pre-emptive protective effects against atrazine-induced muscle damage. Previous studies have reported that honey has an antioxidant and anti-inflammatory properties, which may contribute to its protective effects [17].

The mean levels of Trop-I and Trop-T were significantly higher ($p<0.05$) in Group II (PC) compared to Group I (NC), indicating that Atrazine may induce oxidative stress and inflammation leading to the increase in the levels of Troponin-I and Troponin-T. The results also showed thatthe administration of green tea and honey significantly reduced the levels of Trop-I and Trop-T in atrazine-exposed rats. These findings suggest that green tea and honey may have protective effects against atrazine-induced toxicity. The protective effect of honey and green tea may be accounted for by the presence of physiochemical parameters such as polyphenols, flavonoids, terpenoids and alkaloids. The result of this study aligns with the findings of Khan et al. [16], Erejuwa et al.[17] which reported that the green tea and honey possess some antioxidants and anti-inflammatory properties which can act against cardiotoxicity induced by toxic substances such as Atrazine.

The findings of this study are consistent with other studies which have reported an increased CK and Trop levels following exposure to atrazine [14][15]. The study's findings also suggest that green tea and honey may have protective effects against atrazine-induced cardiotoxicity. However, further studies are needed to fully understand the mechanisms of action of green tea and honey in protecting against atrazine-induced toxicity.

## 4. CONCLUSION

In conclusion, the findings of this study suggest that exposure to atrazine significantly increases the levels of CK and Trop in albino Wistar rats, indicating cardiotoxicity. The study's findings also suggest that the administration of green tea and honey may have protective effects against atrazine-induced cardiotoxicity.

## ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable.

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