*Original Research Article*

Assessment of Protective and Therapeutic Effects of Green Tea and Honey on Atrazine-Induced Nephrotoxicity in Albino Wistar Rats

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ABSTRACT

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| **Aim:** To assess the effect of green tea and honey on atrazine induced nephrotoxicity in albino Wistar rats.  **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) rats both male and female 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 provided with free access to standard feed and water. Group I rats served as the negative control, group II rats served as the positive control as they were given oral administration of 0.23ml Atrazine orally for 15 days, rats in group III were given 0.23ml of 100mg/kg of atrazine and 0.2ml of 50mg/kg (low dose) of green tea orally once daily for 15 days, rats in group IV were given 0.23ml of 100mg/kg of atrazine and 0.9ml of 200mg/kg (high dose) of green tea orally once daily for 15 days, rats in group V were given 0.23ml of 100mg/kg of atrazine, 0.5ml of 100mg/kg of green tea and 10% honey solution at 0.7ml (high dose) of honey orally once daily for 15 day, and rats in group VI were given 0.23ml of 100mg/kg of atrazine after 15 days of administering 10% honey solution at 0.7ml (high dose) of honey orally once daily. The rats were then anaesthesized, using chloroform and 2ml blood samples were collected each through cardiac puncture after a fasting period of 24 hours for the analysis of Sodium, Potassium, Chloride, and Bicarbonate all using enzymatic method, Urea using enzymatic method and Creatinine using spectrophotometric method. GraphPad Prism Version 10.0 was used for the data analysis with statistical tools such as mean (x), standard deviation (SD), Analysis of Variance (ANOVA) and Tukey’s multiple comparison test and p<0.05 was regarded to be statistically significant.  **Results:** Kindly The results showed a significant difference inUrea (4.02±0.64mmol/l, 6.94±0.41mmol/l, 6.08±0.33mmol/l, 5.66±0.35mmol/l, 4.96±0.35mmol/l, 4.90±0.31mmol/l and P=0.0001) and Creatinine (51.00±9.05umol/l, 106.60±27.82umol/l, 108.40±17.47umol/l, 95.20±13.81umol/l, 85.40±8.32umol/l, 98.20±6.83umol/l and P=0.0001), Sodium level of groups I, II, III, IV, V and VI was 140.8 ± 3.89 mmol/L, 163.0 ± 3.39mmol/L, 153.8 ± 2.38mmol/L, 143.0 ± 6.78mmol/L, 142.2 ± 5.31mmol/L, and 137.6 ± 5.32mmol/L and p<0.0001, Potassium level of groups I, II, III, IV, V and VI was 4.30 ± 0.31mmol/L, 6.16 ± 0.39mmol/L, 5.96 ± 0.19mmol/L, 4.68 ± 0.30mmol/L, 4.70 ± 0.33mmol/L, and 4.32 ± 0.38mmol/L, p<0.0001, Chloride level of groups I, II, III, IV, V and VI was 86.00 ± 12.33mmol/L, 92.00 ± 4.12mmol/L, 85.20 ± 3.96mmol/L, 80.60 ± 4.03mmol/L, 83.80 ± 3.96mmol/L, and 76.40 ± 3.57mmol/L, p=0.0138, and bicarbonate level of groups I, II, III, IV, V and VI was 23.40 ± 1.81mmol/L, 27.60 ± 1.14mmol/L, 25.20 ± 1.48mmol/L, 24.00 ± 1.58mmol/L, 22.40 ± 1.14mmol/L, and 23.80 ± 1.78mmol/L, p=0.0003, in the treatment groups compared to the positive control group (group II).  **Conclusion:** These results may suggest that green tea and honey possess the potential of ameliorating nephrotoxicity caused by Atrazine. Further related studies on the mechanisms of nephrotoxicity are, however, recommended. |

*Keywords: Protective, Therapeutic Effects, Green Tea, Honey, Atrazine-Induced Nephrotoxicity, Albino Wistar Rats*

1. INTRODUCTION

A growing number of people who are directly or indirectly exposed to atrazine through environmental contamination may be at risk for health problems because of this practice in agriculture. Chronic renal problems, which are expensive and difficult to treat, can result from hazardous exposure-induced kidney damage. Green tea and honey are widely accessible and have been used traditionally for a variety of health benefits. They are renowned for their antioxidants and nephro-protective qualities. Nevertheless, there is little scientific data to support their effectiveness in stopping or lessening atrazine-induced nephrotoxicity. The purpose of this study is to provide scientific insights into the possible application of these natural chemicals as protective agents against kidney damage, potentially resulting in a more affordable, safer, and accessible treatment option for toxin-induced nephrotoxicity. The herbicide atrazine, which is frequently used in agricultural operations, is known to be harmful to several organs, including the kidneys [1]. Renal inflammation, oxidative stress, and nephrotoxicity have all been related to extended atrazine exposure [2].

The use of herbal plants as natural medicines is growing in popularity today. It could potentially be because many herbs' primary ingredients contain antioxidant properties that either prevent Reactive Oxygen Species (ROS) from forming or scavenge those that have already been produced. Furthermore, by upregulating vitagenes, which shield cells from a variety of electrophiles and oxidants, herb components can reduce the generation of reactive oxygen species (ROS). Ferritin, glutathione reductase, haemeoxygenase-1 (HO-1), and repair enzymes like the 26S proteasome are a few examples of these cytoprotective proteins [3]. Organic components such as green tea and honey are gaining a lot of attention increasingly for their nephro-protective properties, especially when the kidneys are exposed to toxins or dangerous chemicals. Atrazine, a commonly used herbicide with a reputation for causing major health concerns, including kidney damage, is one such toxin [1].

The kidney is an organ with multiple biological functions, the most significant of which is maintaining the homeostatic balance of bodily fluids by filtering and secreting minerals, urea, uric acid, and other metabolites from the blood and excreting nitrogenous waste in the form of urine together with water [4][5]. Because of its high blood flow and ability for highly specialized cells to collect and concentrate hazardous compounds, it is frequently the target of toxic xenobiotics [6]. Nephrotoxicity occurs when endogenous or external toxins cause the kidneys to fail, impairing the kidneys' ability to excrete and detoxify waste products [7]. It is one of the most prevalent kidney issues that arise from drug or toxin exposure [8]. According to Azab et al. [9], nephrotoxicity is defined as kidney illness or dysfunction resulting from exposure to pharmaceuticals, industrial or environmental toxins, or both in an absolute or aberrant manner. There are several acknowledged elements that make the kidney vulnerable to toxic damage from traditional medicines [10]. This contains high metabolic activity, high blood flow rate, high pH of the urine, high endothelial surface area, active tubular cell absorption, and medullary interstitial concentration. According to Rivastava et al. [11], the toxins can cause renal ischemia, haemoglobinuria, myoglobinuria, or direct abuse of the tubules.

Green tea, a known herbal plant which is manufactured from the leaves of the Camellia sinensis plant. It is rich in antioxidant polyphenolic flavonoids, which are found in water. Additionally, according to Bitu Pinto et al. [12], it has anti-mutagenic, anti-carcinogenic, and anti-apoptotic properties. It has very high vitamin C content, which has significant positive impacts on human health. More than 3000 phytochemical substances have been found in green tea (GT), with polyphenols making up roughly one-third of these components [13].

One of these natural products, bee honey (BH), which is made by honeybees (Apis millifera) from nectar, has gained more attention lately. A Sumerian tablet and an Egyptian papyrus both attest to its long history of usage as a traditional medicine. It has a variety of advantages, including antibacterial, anti-inflammatory, hepatoprotective, antioxidant, and anti-hypertensive actions. Furthermore, some investigations have demonstrated the potential anticancer effects of raw honey on a variety of tumor cell lines both in vivo and *in vitro.* We hypothesize that green tea and honey taken together may have a synergistic effect on kidney protection against atrazine-induced damage. Therefore, the aim of this study was to assess the effect of green tea and honey on atrazine induced nephrotoxicity in albino Wistar rats.

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.

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 served as 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**

At the end of the 15 days of experimental study, the rats in the respective groups were allowed to fast overnight and were then anaesthetized in a jar containing cotton wool soaked with chloroform, and blood samples were collected via cardiac puncture. Two mililitre(2ml) of blood samples was collected aseptically into plain bottles using 2ml sterile syringes. The blood was then taken to the laboratory where it was spun in a centrifuge for 5 minutes at 3000rpm. The serum was separated and transferred into another plain bottle for the analysis of kidney function (Urea, Creatinine, Sodium, Potassium, Chloride, and Bicarbonate).

**2.5 Sample Analysis**

**2.5.1 Determination of Urea Concentration**

**Method:** Enzymatic method [14].

**Principle:** Urea is hydrolyzed in the presence of the enzyme urease to give ammonia which

**2.5.2 Determination of Creatinine Concentration**

**Method:** Jaffe’s Method [15]

**Principle:** Creatinine reacts with picric acid in an alkaline medium to produce yellow-red colour which is proportional to the amount in the sample.

**2.5.3 Determination of Potassium**

**Method:** Enymatic Method [16]

**Principle:** The enzymatic reaction for K+ assay is based on conversion of phosphoenolpyruvate into pyruvate by K+ dependent pyruvate kinase. In presence of reduced nicotinamide adenine dinucleotide (NADH), the pyruvate generated is converted to lactate in a reaction catalyzed by lactate dehydrogenase. The K+ concentration present in the sample is proportional to reduction of the optical density at 630 nm as a function of NADH oxidation to nicotinamide adenine oxidized dinucleotide.

**2.5.4 Determination of Sodium**

Method: Enzymatic Method [16]

**Principle:** The method relies on the activation of a reaction between sodium-dependent β-D-galactosidase and its substrate, O-nitrophenyl-D-galactopyranose (ONPG), which is converted into O-nitrophenyl and galactose in the presence of Na+ in the sample. The rate of product formation, measured at 578 nm, is directly proportional to the amount of sodium present in the sample.

**2.5.5 Determination of Chloride**

**Method:** Enzymatic Method [16]

**Principle:** The chloride ion displaces thiocyanate from non-ionized mercuric thiocyanate to form mercuric chlorite and thiocyanate ions. The released thiocynates ions reacts with ferric ions to form a colour complex that absorbs light at 480nm. The intensity of the colour produced is directly proportional to the chloride concentration.

**2.6.6 Determination of Bicarbonate**

Method: Enzymatic Method [16]

**Principle:** Bicarbonate reacts with Phosphoenolpyruvate (PEP) in the presence of Phosphoenolpyruvate Carboxylase, forming Oxaloacetate. This Oxaloacetate is then reduced to Malate-by-Malate Dehydrogenase, utilizing NADH, which is oxidized to NAD+. The decrease in absorbance at 340nm correlates with bicarbonate concentration.

**2.6 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 for Control and Treatment Groups (Urea and Creatinine)**

|  |  |  |
| --- | --- | --- |
|  | **Urea (mmol/L)** | **Creatinine (umol/L)** |
| Group I (NC) | 4.02 ± 0.64 | 51.00 ± 9.05 |
| Group ii (PC) | 6.94 ± 0.41 | 106.60 ± 27.82 |
| Group iii (Atrazine and low dose green tea) | 6.08 ± 0.33 | 108.40 ± 17.47 |
| Group iv (Atrazine and high dose green tea) | 5.66 ± 0.35 | 95.20 ± 13.81 |
| Group v (Atrazine and Green Tea and high dose Honey) | 4.96 ± 0.35 | 85.40 ± 8.32 |
| Group vi (Atrazine after 15 days of high dose honey) | 4.90 ± 0.31 | 98.20 ± 6.83 |
| F-value | 30.03 | 9.184 |
| *P*-value | 0.0001 | 0.0001 |
| Remark | S | S |

***Key:*** *S = significant. Values with different superscripts are significantly different (p<0.05), NC= Negative Control, PC= Positive Control.*

**Table 2: Descriptive and Inferential Results for Control and Treatment Groups (Serum Electrolytes)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Groups/Parameters** | **Sodium**  **(mmol/L)** | **Potassium (mmol/L)** | **Chloride (mmol/L)** | **Bicarbonate (mmol/L)** |
| Group I (NC) | 140.8 ± 3.89 | 4.30 ± 0.31 | 86.00 ± 12.33 | 23.40 ± 1.81 |
| Group II (PC) | 163.0 ± 3.39 | 6.16 ± 0.39 | 92.00 ± 4.12 | 27.60 ± 1.14 |
| Group III (Atrazine and low dose Green Tea) | 153.8 ± 2.38 | 5.96 ± 0.19 | 85.20 ± 3.96 | 25.20 ± 1.48 |
| Group iv (Atrazine and high dose Green Tea) | 143.0 ± 6.78 | 4.68 ± 0.30 | 80.60 ± 4.03 | 24.00 ± 1.58 |
| Group v (Atrazine and Green Tea and high dose Honey) | 142.2 ± 5.31 | 4.70 ± 0.33 | 83.80 ± 3.96 | 22.40 ± 1.14 |
| Group vi (Atrazine after 15 days of high dose Honey) | 137.6 ± 5.32 | 4.32 ± 0.38 | 76.40 ± 3.57 | 23.80 ± 1.78 |
| F-value | 20.80 | 22.34 | 3.629 | 7.130 |
| P-value | 0.0001 | 0.0001 | 0.0138 | 0.0003 |
| Remark | S | S | S | S |

***Key:*** *NS = not significant, S = significant. Values with different superscripts are significantly different (p<0.05), NC= Negative Control, PC= Positive Control.*

**Table 3: Tukey's Multiple Comparison of Urea and Creatinine Between Groups**

|  |  |  |
| --- | --- | --- |
| Groups | Urea(mmol/L) | Creatinine(umol/L) |
| Grp 1vs 2 | -2.920 | -55.60 |
| Grp 1vs 3 | -2.060 | -57.40 |
| Grp 1vs 4 | -1.640 | -44.20 |
| Grp1 vs 5 | -0.9400 | -34.40 |
| Grp 1vs 6 | -0.8800 | -47.20 |
| Grp 2 vs 3 | 0.8600 | -1.800 |
| Grp 2 vs 4 | 1.280 | 11.40 |
| Grp 2 vs 5 | 1.980 | 21.20 |
| Grp 2 vs 6 | 2.040 | 8.400 |
| Grp 3 vs 4 | 0.4200 | 13.20 |
| Grp 3 vs 5 | 1.120 | 23.00 |
| Grp 3 vs 6 | 1.180 | 10.20 |
| Grp 4 vs 5 | 0.7000 | 9.800 |
| Grp 4 vs 6 | 0.7600 | -3.000 |
| Grp 5 vs 6 | 0.06000 | -12.80 |

**Table 4: Tukey's Multiple Comparison of Serum Electrolytes Between Groups**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Groups | Sodiu(mmol/L) | Potassium  (mmol/L) | Chloride  (mmol/L) | Bicarbonate  (mmol/L) |
| Grp 1vs 2 | 22.20 | 1.860 | 6.000 | 4.200 |
| Grp 1vs 3 | 13.00 | 0.8600 | 0.8000 | 1.800 |
| Grp 1vs 4 | 2.200 | 0.3800 | 5.400 | 0.6000 |
| Grp1 vs 5 | 1.400 | 0.4000 | 2.200 | 1.000 |
| Grp 1vs 6 | 3.200 | 0.02000 | 9.600 | 0.4000 |
| Grp 2 vs 3 | 9.200 | 1.000 | 6.800 | 2.400 |
| Grp 2 vs 4 | 20.00 | 1.480 | 11.40 | 3.600 |
| Grp 2 vs 5 | 20.80 | 1.460 | 8.200 | 5.200 |
| Grp 2 vs 6 | 25.40 | 1.840 | 15.60 | 3.800 |
| Grp 3 vs 4 | 10.80 | 0.4800 | 4.600 | 1.200 |
| Grp 3 vs 5 | 11.60 | 0.4600 | 1.400 | 2.800 |
| Grp 3 vs 6 | 16.20 | 0.8400 | 8.800 | 1.400 |
| Grp 4 vs 5 | 0.8000 | 0.02000 | 3.200 | 1.600 |
| Grp 4 vs 6 | 5.400 | 0.3600 | 4.200 | 0.2000 |
| Grp 5 vs 6 | 4.600 | 0.3800 | 7.400 | 1.400 |

Atrazine is known to induce nephrotoxicity through oxidative stress and inflammatory pathways, which can lead to cellular damage and kidney dysfunction according to Abarikwu et al. [17]; Kassim et al. [18]. Both green tea and honey have been identified as natural substances with antioxidants and anti-inflammatory properties, which may counteract the harmful effects of atrazine on the kidneys [1]. This study was aimed at investigating the potential protective effects of green tea and honey in mitigating kidney damage induced by atrazine exposure to albino Wistar rats.

The findings of this sudy are consistent with previous studies, which have shown that atrazine can cause kidney damage and alter the levels of kidney function parameters including urea and creatinine [1].

The results also show that the administration of green tea had a protective effect against atrazine-induced nephrotoxicity. The rats in Group III, which received atrazine and low-dose green tea, had significantly lower mean levels of urea and creatinine, likewise serum electrolytes including Na, K, Cl, and HCO3 compared to Group II. Similarly, the rats in Group IV, which received atrazine and high-dose green tea, had even lower mean levels of these enzymes. These findings are consistent with previous studies, which suggest that green tea has antioxidants and anti-inflammatory properties that can act against kidney damage [19].

The administration of honey also had an effect against atrazine-induced nephrotoxicity. The rats in Group V, which received atrazine, green tea, and high-dose honey, had significantly lower mean levels of urea and creatinine, likewise that of serum electrolytes including Na, K, Cl, and HCO3 compared to Group II. These findings are consistent with previous studies, which reported that honey possesses antioxidants and anti-inflammatory properties that can act against kidney damage or dysfunction, according to Kassim et al. [18]. The results also show that the administration of honey before atrazine exposure had a protective effect against nephrotoxicity. The rats in Group VI, which received high-dose honey before atrazine exposure, had significantly lower mean levels of urea and creatinine, likewise that of serum electrolytes including Na, K, Cl, and HCO3 compared to Group II. These findings suggest that honey may have a prophylactic effect against atrazine-induced nephrotoxicity.

The possible reasons for the increase in urea and creatinine levels across the groups may be due to the toxic effects of atrazine on the kidney. Atrazine has been shown to induce oxidative stress and inflammation in the kidney, leading to increased levels of urea and creatinine in subjects [1]. The decrease in urea and creatinine levels in groups that received green tea and honey both separately and in collaboration may be due to the antioxidant and anti-inflammatory properties of these compounds, which can protect against kidney damage leading to kidney dysfunction.

The possible reasons for the increase in serum electrolyte levels across the groups may be due to the toxic effects of atrazine on the kidney. Atrazine has been shown to induce oxidative stress and inflammation in the kidney, leading to an increased levels of serum electrolytes [1]. The decrease in serum electrolyte levels across groups that received green tea and honey both separately and in collaboration may be due to the antioxidant and anti-inflammatory properties of these compounds, which can protect against kidney damage.

4. Conclusion

In conclusion, the results of this study showed that atrazine induces nephrotoxicity in albino Wistar rats, as evidenced by increased levels of kidney function parameters such as urea and creatinine and serum electrolytes such as Na, K, Cl and HCO3. The administration of green tea and honey both separately and in collaboration had a protective effect against atrazine-induced nephrotoxicity, as evidenced by decreased levels of kidney function parameters such urea and creatinine and serum electrolytes such as Na, K, Cl, and HCO3. These findings suggest that green tea and honey may be useful in preventing or treating kidney damage induced by atrazine exposure.

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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