Original Research Article

Hepatoprotective effect of Honey and Green tea on Atrazine induced hepatotoxicity inAlbino Wistar rats

ABSTRACT

Aim: To evaluate the hepatoprotective effect of green tea and honey on atrazine induced hepatotoxicity 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: Please Thirty(30) albino Wistar rats weighing between 150-250g were randomly divided into six (6) groups of five rats each. Group I rats served as the negative control, group II rats served as the positive control as they were given oral administration of Atrazine (100mg/kg), Group III rats were given oral administration of atrazine (100mg/kg), and low dose green tea (50mg/kg), group IV received oral administration of atrazine (100mg/kg), and high dose green tea (200mg/kg), group V received oral administration of atrazine (100mg/kg) and 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 8 hours before they were anaesthetized using chloroform and blood samples were collected via cardiac puncture for the analysis of aspartate transferase (AST), Alanine transferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), Albumin, Total protein and 5 Nucleotides (5'NT). GraphPadPrism version 10.0 was used to analyze the Mean, standard deviation, ANOVA and Tukey's multiple comparison test, and $p<0.05$ was regarded to be statistically significant.
Results: The results showed a significant increase in AST ($p<0.0001$), ALT ($p<0.0001$), GGT($p<0.0001$), Albumin ($p<0.0001$), total protein ($p<0.05$) and 5'NT ($p<0.0001$) in group I I (positive control) compared to group I (negative control) and a significant decrease ($p<0.0001$) in the AST, ALT, GGT, 5'NT, total protein and Albumin Levels in group III, IV and V after green tea and honey administration. Grean tea exact a dose dependent decrease on the level of AST, ALT And GGT with group IV having a more pronounced reduction than group III. Group VI showed a significantly lower ($p<0.0001$) level of AST, ALT, GGT, albumin and total protein.
Conclusion: The findings of this study may suggest that honey and green tea possess the potential of ameliorating hepatotoxicity caused by Atrazine. Further related studies are however, recommended.

Keywords: Hepatoprotective effect, Honey, Green tea, Atrazine induced hepatotoxicity, Albino Wistar rats

1. INTRODUCTION

Atrazine is a widely used herbicide with known toxic effects. Prolonged exposure to this chemical can lead to oxidative stress, inflammation, and liver damage, posing significant health risks[1]. While synthetic drugs may offer some protection, they often come with side effects [2][3]. Therefore, there is a need to explore natural alternatives like honey and green tea. This study aims to address this problem by evaluating the potential of these natural substances to protect the liver from atrazine-induced damage.

Atrazine is primarily employed in agricultural settings to control broadleaf weeds in crops like corn and sugarcane, making it one of the most heavily used herbicides globally [4]. Despite its agricultural benefits, research has shown that atrazine can persist in the environment, leading to contamination of soil, water, and air. Prolonged exposure to atrazine has been linked to various toxic effects, including endocrine disruption, reproductive toxicity, andcarcinogenicity [5]. However, one of the most concerning

effects is its hepatotoxicity, as the liver is responsible for metabolizing and detoxifying atrazine. Studies have demonstrated that atrazine induces oxidative stress, inflammation, and liver damage in animal models, leading to elevated liver enzymes, lipid peroxidation, and histopathological changes [6].Given the growing concerns about atrazine-induced liver toxicity, there is an increasing interest in exploring natural substances that may offer protective effects against liver damage.

The liver is the largest internal organ in the human body, weighing approximately 1.5 kilograms in adults [7]. Itplays a crucial role in detoxification, metabolism, and the regulation of various biochemical processes that are essential for maintaining physiological balance in the body. It is in the right upper quadrant of the abdomen, beneath the diaphragm and above the stomach. The liver is divided into two primary lobes: the larger right lobe and the smaller left lobe. Each lobe is further subdivided into smaller functional units called liver lobules. The liver has a unique dual blood supply, receiving oxygenated blood from the hepatic artery and nutrient-rich blood from the portal vein. The hepatic artery provides approximately 25% of the liver's blood flow, supplying oxygen and essential nutrients. In contrast, the portal vein delivers about 75% of the blood flow, carrying nutrients absorbed from the gastrointestinal tract [7]. However, the liver is often the primary target for toxic injury due to its central role in filtering and processing harmful substances, including drugs, chemicals, and environmental toxins. One such environmental toxin is atrazine, a widely used herbicide that has garnered significant attention due to its potential harmful effects on both human and animal health [8].

Honey, a natural product made by honeybees, has long been recognized for its medicinal properties. Rich in phenolic compounds, flavonoids, and antioxidants, honey exhibits anti- inflammatory, antimicrobial, and antioxidant properties that make it a promising candidate for hepatoprotection [9]. Studies have indicated that honey can protect the liver from oxidative damage induced by toxins such as carbon tetrachloride and ethanol[10]. Its ability to scavenge free radicals and reduce oxidative stress suggests that it may also be effective in mitigating the harmful effects of atrazine on the liver [20-22].

Green tea (*Camellia sinensis*), which is widely consumed for its health benefits, is known for its rich content of polyphenols, particularly catechins, which have potent antioxidant and anti- inflammatory properties [11]. Epigallocatechin gallate (EGCG), the most abundant catechin in green tea, has been shown to protect against various forms of liver injury, including drug-induced liver damage and non-alcoholic fatty liver disease [12]. The hepatoprotective effects of green tea are attributed to its ability to reduce oxidative stress, inhibit pro-inflammatory cytokines, and enhance the activity of antioxidant enzymes in the liver [13]. Thus, the use of green tea as a natural hepatoprotective agent against atrazine-induced liver damage is an area of interest that warrants further investigation.

This study aims to investigate the hepatoprotective effects of honey and green tea on atrazine- exposed albino Wistar rats. By evaluating the potential benefits of these natural substances, this research could provide valuable insights into alternative therapies for managing liver damage caused by Atrazine.
The increasing use of herbicides like atrazine has raised concerns about the long-term health implications, particularly in relation to liver toxicity. Despite the known harmful effects of atrazine, there is limited research on the efficacy of natural substances like honey and green tea in counteracting these effects. This study is justified because it addresses the need for safe, natural, and affordable interventions to protect the liver from the toxic effects of atrazine. By focusing on honey and green tea, this research aims to fill a gap in the existing literature and provide scientific evidence on the use of these substances as potential hepatoprotective agents. The aim of this study therefore was to evaluate the hepatoprotective effect of green tea and honey on atrazine induced hepatotoxicity in albino Wistar rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

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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[17].

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.

2.3Experimental 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.

2.4Blood Collection and Preparation

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At the end of the 15th day of the experimental study, the animals in the respective groups were left to fast overnight and were then anaesthetized in a jar with chloroform, and blood samples were collected via cardiac puncture. Two mililitre (2ml) blood samples were collected aseptically into a plain bottle using 2ml sterile syringes. The blood was spun in a centrifuge for 5 minutes at 3000rpm. The serum was separated and transferred into another plain bottle for the analysis of AST, ALT, ALP, GGT, albumin, total protein and 5'NT

2.5Sample Analysis

2.5.1Determination of Gamma glutamyl Transferases (GGT) Method: UV-Assay according to Szasz

Principle: The substrate L-gamma-glutamyl-3-carboxy-4-nitroanilide, in the presence of glycylglycine is converted by gamma-glutamyl transferase in the sample to 5-amino-2- nitrobenzoate which can be measured at 405nm.

2.5.2Determination of Serum Alkaline phosphatase (ALP) Method of Estimation: Colorimetric Endpoint Method

Principle: Alkaline phosphatase hydrolyses the disodium phenylphosphatase to release phenol which reacts with 4-aminophenazone in the presence of alkaline potassium ferricyanide to give a red colored complex, which is measured at 520nm wavelength.

2.5.3Determination of Serum Aspartate Aminotransferase (AST) Level Method of Estimation: Enzymatic (Reitman-frankel) method

Principle: When AST is incubated at a temperature of 370C for 60 minutes in a pH of 7.5 buffered substrate containing aspartate and alpha-ketoglutarate, it catalyzes the reversible transfer of an amino group from aspartate to alpha-ketoglutarate, the Oxaloacetate reacts with 2,4- Dinitrophenylhydraize (DNPH) to form 2,4-Dinitrophenylhydrazone which in an alkaline medium gives a red-brown color. The absorbance of the color is read at 505nm wavelength.

2.5.4Determination of Serum Alanine Aminotransferase (ALT) Level Method of Estimation: Enzymatic (Reitman-frankel) method

Principle: ALT is incubated at 37oC for exactly 30 minutes in a pH of 7.4 buffered substrate containing alanine and alpha ketoglutarate. ALT catalyzes the transfer of the amino group from alanine to alpha-ketoglutarate forming pyruvate and glutamate. The pyruvate reacts with 2,4- Dinitrophenylhydrazine (DNPH) to form 2,4-Dinitrophenylhydrazone which in an alkaline medium gives a red-brown color. The absorbance of the color is read at 505nm wavelength.

2.5.5 Determination of Total Protein

Method of Estimation: Biuret Method

Principle: The Biuret reaction is based on the interaction of proteins with copper ions in an alkaline medium, forming a violet-colored complex. The intensity of the color is directly proportional to the concentration of proteins in the sample and is measured at a wavelength of 546 nm.

2.5.6 Determination of 5′ Nucleotidase (5′NT)

Method of Estimation: Spectrophotometric Method

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Principle: 5′-Nucleotidase catalyzes the hydrolysis of 5′-nucleotides to nucleosides and inorganic phosphate. The liberated phosphate reacts with ammonium molybdate to form a phosphomolybdate complex, which is then reduced to a blue-colored complex by ferrous sulfate. The absorbance of this blue complex is measured at 700 nm.

2.6Statistical Analysis

The data generated from the analysis was expressed as Mean ± standard deviation and analysed 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

Table1:ResultsofHepatocellular Enzymesforall ExperimentalGroups

Groups	AST (IU/L)	ALT (IU/L)
GroupI (NC)	70.36±0.850	43.54±0.456
GroupII (PC)	79.30±1.037	55.24±0.251
GroupIII	78.00±0.7906	47.54±0.456
GroupIV	7.48±0.5119	48.66±0.594
Group V	75.84±0.503	51.54±0.446
Group VI	72.40±0.418	39.74±4.030
F-value	184.4	28.85
P-value	<0.0001	<0.0001
Remark	S	S

Key: S=Significant NS- not Significant, Values are Significant at p<0.05 Aspartate transferase (AST), Alanine transferase (ALT).

Table2:ResultsofPost Hepatic Enzymesforall Experimental Groups

Groups	GGT (IU/L)	ALP (IU/L)	5'NT (IU/L)
GroupI (NC)	0.100±0.00	135.4±1.140	0.136±0.033
GroupII (PC)	0.5500±0.023	134.6±0.6519	0.170±0.010
GroupIII	0.100±0.00	133.7±0.4219	0.166±0.008
GroupIV	0.160±0.038	133.8±0.5841	0.164±0.008
Group V	0.558±0.0657	131.5±1.143	0.163±0.08
Group VI	0.310±0.1597	132.8±1.031	0.132±0.013
F-value	32.45	668.2	38.07
P-value	<0.0001	<0.42	<0.0001
Remark	S	NS	S

Key: S=Significant NS- not Significant, Values are Significant at p<0.05, alkaline phosphatase (ALP), gamma glutamyl transferase (GGT).

Table3:ResultsofAlbuminandTotalProtein forall Experimental Groups

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Groups	Albumin (g/dl)	TP(g/dl)
GroupI (NC)	4.768±0.01304	7.138±0.0134
GroupII(PC)	4.82±0.0109	7.294±0.0378
GroupIII(A)	4.728±0.0192	7.156±0.03772
GroupIV (B)	4.686±0.0114	7.226±0.0241
GroupV (C)	4.580±0.01581	7.172±0.01924
GroupVI (D)	4.676±0.04980	7.150±0.0790
F-value	61.77	10.49
P-value	<0.0001	<0.0001
Remark	S	S

S=Significant NS- not Significant, Values are Significant at p=0.05, *TP- Total protein*

Table 4: Tukey's Multiple ComparisonTest of Hepatocellular Enzymes Between Groups

Groups/Parameters	AST (IU/L)	ALT (IU/L)
Grp 1 vs Grp 2	<0.0001	0.340
Grp 1 vs Grp 3	<0.0001	<0.0001
Grp 1 vs Grp 4	<0.0001	<0.0001
Grp 1 vs Grp 5	<0.0001	<0.0001
Grp 1 vs Grp 6	<0.0001	<0.0001
Grp 2 vs Grp 3	0.100	0.310
Grp 2 vs Grp 4	<0.0001	<0.0001
Grp 2 vs Grp 5	<0.0001	<0.0001
Grp 2 vs Grp 6	<0.0001	<0.0001
Grp 3 vs Grp 4	<0.0001	0.345
Grp 3 vs Grp 5	<0.0001	<0.0001
Grp 3 vs Grp 6	<0.0001	<0.0001
Grp 4 vs Grp 5	<0.0001	0.732
Grp 4 vs Grp 6	<0.0001	<0.0001
Grp 5 vs Grp 6	<0.0001	<0.0001

Table 5: Tukey's Multiple ComparisonTest of Post Hepatic Enzymes Between Groups

Groups/Parameters	ALP (IU/L)	GGT IU/L)	5`NT IU/L)
Grp 1 vs Grp 2	0.563	0.982	0.0026
Grp 1 vs Grp 3	<0.0001	0.421	<0.0001
Grp 1 vs Grp 4	0.459	0.321	<0.0001
Grp 1 vs Grp 5	<0.0001	<0.0001	<0.0001
Grp 1 vs Grp 6	<0.0001	<0.0001	<0.0001
Grp 2 vs Grp 3	0.138	0.234	0.0138
Grp 2 vs Grp 4	<0.0001	0.178	<0.0001
Grp 2 vs Grp 5	0.231	<0.0001	<0.0001
Grp 2 vs Grp 6	<0.0001	<0.0001	0.2003
Grp 3 vs Grp 4	<0.0001	0.461	0.2893
Grp 3 vs Grp 5	<0.0001	<0.0001	0.0006
Grp 3 vs Grp 6	<0.244	<0.0001	0.231
Grp 4 vs Grp 5	<0.0001	0.732	<0.0001
Grp 4 vs Grp 6	0.543	<0.0001	0.442
Grp 5 vs Grp 6	<0.0001	<0.0001	<0.0001

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Table 6: Tukey's Multiple Comparison Test of Albumin and Total Protein Between Groups

Groups	Albumin (g/dl)	TP (g/dl)
Grp 1vs 2	<0.0001	0.314
Grp 1vs 3	0.057	0.782
Grp 1vs 4	<0.0001	<0.0001
Grp1 vs 5	<0.0001	0.233
Grp 1vs 6	<0.0001	0.183
Grp 2 vs 3	<0.0001	<0.0001
Grp 2 vs 4	<0.0001	0.431
Grp 2 vs 5	<0.0001	<0.0001
Grp 2 vs 6	<0.0001	0.0044
Grp 3 vs 4	0.398	<0.0001
Grp 3 vs 5	<0.0001	0.342
Grp 3 vs 6	<0.0001	0.291
Grp 4 vs 5	<0.0001	0.092
Grp 4 vs 6	0.056	0.083
Grp 5 vs 6	<0.0001	0.492

Atrazine is known to induce hepatotoxicity through oxidative stress and inflammatory pathways, which can lead to cellular damage and liver dysfunction. Both honey and green tea have been identified as natural substances with antioxidant and anti-inflammatory properties, which may counteract the harmful effects of atrazine. This study was aimed at investigating the potential hepatoprotective effects of honey and green tea in mitigating liver damage induced by atrazine exposure to albino wistar rats.

According to the results of this study group II (positive control) showed a significant increase in AST ($p<0.0001$), ALT ($p<0.0001$), and GGT ($p<0.0001$) levels compared to the group I (negative control), indicating hepatotoxicity caused by atrazine exposure. These findings align with the findings of (Song et al. (2015) which reported that atrazine can induce oxidative stress and hepatocellular damage, leading to increased liver enzyme release into the bloodstream. In contrast, treatment groups (III, IV, V and VI) displayed variable enzyme levels. Green tea and honey treatments reduced AST and ALT levels in group III, IV and V, with Group VI (honey post-atrazine exposure) showing the most pronounced improvement. This suggests honey's antioxidant properties may mitigate hepatotoxicity effectively, as supported by Singh et al. [14]reporting honey's ability to scavenge free radicals and reduce lipid peroxidation.

Green tea treatments of the dose (Groups III and IV) reduced AST and ALT compared to group II (positive control), although not to normal levels, suggesting a protective effect. Group IV (higher green tea dose) showed greater reductions than Group III, indicating a dose-dependent effect of green tea. These results are consistent with those of some studies which reported that green tea polyphenol's exact hepatoprotective effects by modulating antioxidant enzyme activity and reducing oxidative stress. However, GGT levels increased slightly in Group IV compared to Group III, possibly due to the stimulation of hepatic detoxification pathways, a phenomenon also observed in studies evaluating polyphenol-rich diets [15].It has been reported that green tea[18] andhoney [19] independently possess hepatoprotective effects and studies have shown it to reduce liver injury caused by alcohol, carbon tetrachloride, ischemic reperfusion, lead, viral hepatitis, phenobarbitol, microcystin, azathioprine, galactosamine, lipopolysaccharide, and cypermethrin.

GGT and 5′NT levels were significantly elevated ($p<0.0001$) in group II (positive control), consistent with hepatobiliary damage induced by atrazine. While green tea and honey treatments reduced these levels, complete normalization was not achieved in some groups. Interestingly, ALP levels did not vary significantly across groups, suggesting that atrazine-induced liver damage predominantly affects hepatocellular integrity (as reflected by AST and ALT) rather than cholestatic function. This finding

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aligns with the studies of Singh et al. [14], Zhou et al. [13],which reported no significant difference in ALP levels despite significant hepatocellular damage, likely due to differences in enzyme origin and activity.

The observed hepatoprotective effects of green tea and honey align with the study of Sun et al. [16]which research on the polyphenolic compounds in green tea and reported reduced liver enzyme levels in rats exposed to environmental toxins, emphasizing its antioxidative and anti-inflammatory properties. Honey has been shown to repair hepatic damage by enhancing glutathione production[14].

The results presented in Tables3 and 6show that the levels of albumin and total protein in group II (positive control) were significantly higher ($p<0.0001$) compared to group I (negative control) Treatment groups (group III, IV, V and VI) show variations, with a significant difference (<0.0001), indicating the treatments had a statistically significant effect. Notably, Group IV (higher green tea dose) and Group V (honey treatment) exhibited reduced albumin levels compared to the group II (positive control), suggesting potential restoration of protein metabolism. Similar trends were observed for total protein, with Group VI (honey post-atrazine exposure) showing a near-normalization of total protein, indicative of honey's hepatoprotective effect. These findings align with the study of Sun et al. [16]which reported that honey's antioxidant properties enhance protein synthesis by mitigating oxidative damage to hepatocytes.

In contrast, green tea treatments (Group III and IV) showed a dose-dependent reduction in albumin and TP compared to group II (positive control), though not fully normalized to negative control levels. This may be attributed to green tea polyphenols' modulation of protein metabolism by reducing inflammation and oxidative stress, as reported in studies on dietary polyphenols. The slight elevation of total protein in group II (positive control) might result from hepatotoxicity induced by atrazine. Overall, these findings suggest that honey and green tea offer hepatoprotection through distinct but complementary mechanisms, warranting further research into optimizing their doses and combined effects.

4. CONCLUSION

In conclusion, both green tea and honey demonstrated hepatoprotective effects against atrazine-induced hepatotoxicity, with honey showing superior efficacy, especially post-exposure. These effects likely stem from their antioxidant and anti-inflammatory properties, which mitigate oxidative stress and cellular damage caused by atrazine. The dose-dependent effects observed in green tea treatments further emphasize the importance of optimizing dosing for therapeutic benefits.

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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