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Identification of xylene and toluene in commercial matcha tea and their associated hepatorenal toxicity in wistar rats

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Abstract

Matcha tea (*Camellia sinensis*), a fine-powder green tea, has become popular worldwide for its claimed antioxidant and weight loss effects. Nevertheless, safety and authenticity of commercial matcha products, especially those from local sources, remain poorly controlled. This study was aimed to investigate potential hepatotoxic and nephrotoxic effects of a locally marketed matcha in an animal model. Forty-eight male Wistar rats were randomly divided into 4 groups (n= 12): a control group and three matcha extract treatment groups of low, moderate, and high doses (10, 20, and 30 mg matcha/kg/day), by gavage for two months. Biochemical markers, including urea, creatinine, pro-inflammatory cytokines (interleukin-6 and tumor necrosis factor-alpha), hepatic enzymes (alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase), were determined. The matcha extracts were subjected to the analysis of their chemical compositions by gas chromatography-mass spectrometry (GC-MS). Liver and kidney histopathological examination was carried out using routine staining procedures. GC-MS analysis revealed the presence of toxic contaminants, including xylene and toluene, which are not naturally occurring constituents of authentic matcha. Biochemical results revealed dose-dependent increases in proinflammatory cytokines, hepatic enzymes, creatinine and urea were significantly higher ($P < 0.01$) in the treatment groups when compared to the control group. Histopathology demonstrated dose-related hepatic and renal degenerative and inflammatory changes. Overall, the local matcha product exhibited clear hepatotoxic, nephrotoxic, and pro-inflammatory effects, likely associated with chemical contamination or poor product quality. These results emphasize the importance of strict quality control and thorough safety assessment in commercialized matcha products for human health

Keywords: Matcha tea, hepatorenal toxicity, inflammatory cytokines, xylene contamination

1. Introduction

Matcha is a powdered form of Japanese green tea (*Camellia sinensis*) derived from the Tencha varietal(Horie et al., 2017). This popular drink has gained worldwide popularity due to its unique cultivation process, which increases its content of polyphenols, catechins, and other antioxidant constituents (Sano *et al.*, 2018, Schröder *et al.*, 2019). Matcha consumption has increased sharply in recent years, not only as a traditional beverage but also as an ingredient in functional foods, snacks, chocolates, and desserts in Japan and internationally (Kochman *et al.*, 2020),(Kurauchi *et al.*, 2019). Originally used in ceremonial practices, matcha is now widely incorporated into commercial products due to its perceived health benefits. Matcha, a ground powder grown and processed green tea leaves, has been the subject of intensive research as a traditional beverage for health benefits studied in obesity and metabolic disorders. Obesity is defined by an abnormal fat accumulation that may harm health and life(Alsaffar *et al.*, 2024). It's a problem which affects millions of people worldwide and haunts many different chronic diseases like high blood pressure and diabetes. Obesity refers to increased body fat, which can lead to health problems, functional impairment, reduced life expectancy, and increased mortality. (de Abreu *et al.*, 2022). To reduce health problems due to unhealthy eating, many people today look for healthier food items in supermarkets(Topolska *et al.*, 2021). Many herbal medicines are developed in the markets to solve the problem of obesity, which are the new generation products and green weight reduction with fast results. Though the trend of matcha consumption has been increasing in recent years, scientific information about its physiological effects on major internal organs has not yet been well established. Since the function of the liver and kidney involves controlling metabolism, detoxification, and waste elimination, organs usually become compromised by obesity, and dietary intervention would be necessary to know possible effects that matcha might have on these organs to assess its safety as well as efficacy.

In view of the expanding global market and increasing consumer demand, it is essential to assess the chemical composition and biological effects of matcha products, particularly those produced locally, where quality control may be insufficient. Recent studies have emphasized the need for comprehensive analysis of matcha composition, bioactivity, and potential contaminants using advanced analytical techniques such as GC-MS (Devkota et al., 2021). Therefore, the aim of this study was to evaluate the hepatic and renal effects of a locally marketed matcha tea product through biochemical, inflammatory, and histopathological assessments in Wistar rats. Additionally, GC-MS analysis was conducted to identify the chemical profile of the product and detect possible contaminants that may contribute to toxicity. This approach provides greater insight into the potential health risks associated with unregulated commercial matcha and highlights the importance of safety evaluation and regulatory oversight

2. Materials and Methods

2.1. Plant material

In this study, matcha tea (*Camellia sinensis*) (Matcha Premium Japanese, Matcham, Turkey) figure (1) samples were obtained from local pharmacies in Baghdad, Iraq, for chemical composition analysis and toxicological evaluation. The preparations of aqueous extracts were as follows: 0.5 g of matcha powder was combined with 50 mL of distilled water and processed in an ultrasonic bath at 80°C for 30 minutes. Following cooling, the mixtures were centrifuged, filtered, and then frozen and freeze-dried for analysis. Analysis of the volatile constituents in the extracts was performed by GC-MS. The method allowed identification of major volatile

and semi-volatile components to assess product authenticity and potential contaminants (Luo et al., 2022).

Figure 1. Matcha Premium Japanese

2.2. Animals and Experimental Design

The study was carried out on forty-eight male Wistar rats, weighing between 170 and 200 g, with an age of 8 weeks. Rats were obtained from Tikrit city and randomized to four groups: Control, G1, G2, and G3. The study was carried out in accordance the with ethical requirements of the University of Baghdad. Rats were maintained in a conventional condition of standard ventilation cages (12 rats/cage). The study was conducted at Al-Nahrain University, Biotechnology Research Center. Matcha powder was dissolved in distilled water, and rats were orally fed for three dosages (10, 20, and 30 mg/kg/day).

2.3. Sample collection and preparation

At the end of the experimental period (two months), all animals were sacrificed. Blood and tissue samples were taken, blood in gel tubes was centrifuged at 3000 rpm for 15 mins to obtain necessary serum without red blood cells. The serum was then collected and placed in a 2ml Eppendorf tube, which was maintained sterile and stored at -20 °C until used (Obakiro *et al.*, 2021). At the end of the dosing period, rats were sacrificed to excise vital organs (liver and kidney) for histopathological examination in 10% formalin.

2.4. Hematological analysis

The hematological parameters including WBC, HGB, RBC, neutrophils, basophils, eosinophils monocytes and lymphocytes of the samples were measured by an automatic blood analyzer (Mindray BC-5000)(Marref *et al.*, 2024).

2.5. Biochemical analysis

Liver function (Aspartate aminotransferase (GOT), Alanine aminotransferase (GPT), and Alkaline phosphatase (ALP)) and Kidney function (creatinine and urea) concentrations in serum were measured using the commercial kits (Fuji, Japan, dry chemical slide technology) (Mulyati *et al.*, 2019).

2.6. Histopathological examination of the kidney and liver

Autopsy samples were extracted from the kidneys and liver of rats belonging to various groups and immersed in a 10% formal saline solution for twenty-four hours. The washing procedure included the use of tap water, followed by the use of alcohol (namely methyl, ethyl, and absolute ethyl) in subsequent dilutions for dehydration purposes. The specimens were subjected to xylene clearing and subsequently embedded in paraffin wax to form tissue blocks. The blocks were prepared for sectioning at a thickness of 4 microns with a rotating LEITZ microtome. The tissue sections that were acquired were placed onto glass slides, treated to remove paraffin, and then stained using the hematoxylin and eosin stain (Aljaghmani *et al.*, 2024) (K Abbas *et al.*, 2020) for inspection under the light microscope.

2.7. Enzyme-Linked Immunosorbent Assays (ELISA)

The serum concentrations of TNF-α and IL-6 were quantified using an enzyme-linked immunosorbent assay (ELISA) kit (Reed Biotech, China) according to the manufacturer's guidelines (Mohammed and Yenzeel, 2024).

2.8. Statistical analysis

A Statistical Program for Social Sciences (SPSS version 2019) was used to determine the effects of different groups on the study variables. Least significant difference test was employed to compare means significantly in this study (George and Mallery, 2024).

3. Results

Bioactive compounds of the matcha sample

The Key chemicals detected with GC-MS are given in Figure 2and Table 1. Analysis of the aqueous extract revealed eleven major compounds, including fatty acids, esters, aromatic hydrocarbons, and other small organic molecules. Ethane 1,1-diethoxy-, which accounted for approximately 81.12% of the total ion chromatogram area, was the most abundant component. It is also referred to as an acetal (diethyl acetal). It is the main constituent of the GC-MS profile, yet it has no pharmacological significance and could not possibly be an extraction artifact. It also shows toluene and xylene. Prolonged or high-level exposure to toluene can cause neurotoxicity, hepatotoxicity, and reproductive toxicity. Xylene is also associated to respiratory irritation as well as central nervous system depression and organ damage from central nervous system depression, especially in the kidneys and liver. But tea constituents raise serious toxicological concerns, especially those destined for human consumption(Saeedi *et al.*, 2024). These materials that are not normally found in genuine high-quality matcha, when compared to authentic matcha. These chemicals, which are absent from the validated reference samples, indicate potential adulteration. Such disparity in chemical com-

position would suggest either low quality raw material or of production standard and subsequently affect product quality. Furthermore, there are major questions about this material's suitability for human ingestion given the presence of potentially dangerous chemicals.

Table 1. Chemical analysis of aqueous extract of Matcha

Peak#	RT	area%	Compound	CAS	Qual
1	5.459	1.79	1-Pentadecene	013360-61-7	35
2	5.487	1.52	Acetic acid, propyl ester	000109-60-4	50
3	5.665	81.12	Ethane, 1,1-diethoxy-	000105-57-7	74
4	6.356	1.02	Isobutyl acetate	000110-19-0	78
5	6.448	2.92	Toluene	000108-88-3	91
6	7.162	0.56	Acetic acid, butyl ester	000123-86-4	78
7	8.076	0.6	Oxirane, 2-ethyl-3-propyl-	056052-95-0/94-9	64
8	8.316	1.42	Crotonic acid	003724-65-0	86
9	8.671	0.89	3-Nonanone	000925-78-0	47
10	8.939	1.85	Xylene	001330-20-7	95
11	54.005	6.31	n-Hexadecanoic acid	000057-10-3	99

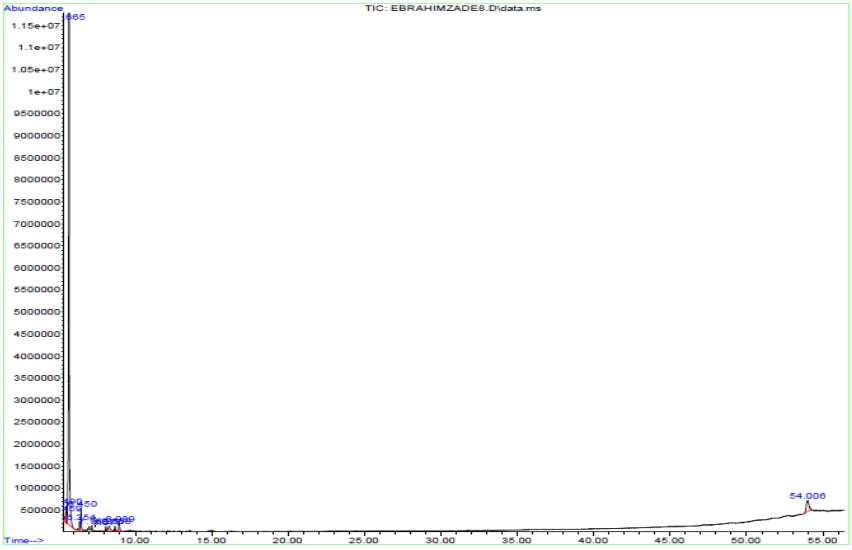

Figure 2. GC-MS analysis of the aqueous extract of matcha tea

Complete Blood Count with Differential Analysis

Statistical analysis revealed significant differences in hematological parameters between the experimental groups compared with the control group. The red blood cell (RBC) count in the G1 group was significantly lower ($7.21 \pm 0.46 \times 10^6$/µl, $P \leq 0.01$) compared with the control group ($10.85 \pm 0.18 \times 10^6$/µl), while the RBC counts in the G2 ($10.28 \pm 0.89 \times 10^6$/µl) and G3 ($8.94 \pm 0.17 \times 10^6$/µl) groups were intermediate. Hemoglobin concentration (HGB) was significantly lower in the G1 group (11.12 ± 0.83 g/dL, $P \leq 0.01$), while no significant differences were observed among the control, G2, and G3 groups. The platelet count (PLT) in the G1 group ($565.16 \pm 10.61 \times 10^3$/µL) was significantly higher than that in the control group

(788.00 ± 226.22 × 10^3/µL, P ≤ 0.05), while there was no significant difference in the mean platelet count between the G2 and G3 groups (Table 2).

Table 2. Comparison between different groups in CBC parameters

Group	Means ±SE		
	RBC (10*6/µL)	HGB (g/dL)	PLT (10*3/µL)
Control	10.85 ±0.18 a	14.62 ±0.18 a	565.16 ±10.61 b
G1	7.21 ±0.46 c	11.12 ±0.83 b	788.00 ±226.22 a
G2	10.28 ±0.89 ab	14.01 ±1.06 a	737.75 ±88.75 ab
G3	8.94 ±0.17 b	15.69 ±0.29 a	649.41 ±56.79 ab
L.S.D.	1.477 **	1974 **	255.97 *
P-value	0.0001	0.0002	0.0498
Means having with the different letters in same column differed significantly. * (P≤0.05), ** (P≤0.01).			

There was no significant change in total WBC counts between the control, G1, and G2 groups. Only G3 showed a significant decrease (11.21 ± 0.58 ×10^3/µL; P = 0.0476). The differential count indicated that the decrease in monocytes, eosinophils, and basophils in treated groups was statistically significant when compared to the control group (P ≤ 0.01). The percentage of lymphocytes was significantly less in G3 (7.96 ± 0.38%) as compared to control (9.55 ± 0.18%; P = 0.0484). Results infer that higher levels of treatment may exert suppressive effects on some leukocyte populations, while lower doses have minimal effects (Table 3).

Table 3. Comparison between different groups in WBC and their differentials

Group	Means ±SE					
	WBC (10*3/µL)	Neutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)	Basophil (%)
Control	14.54 ±0.24 a	2.86 ±0.29	9.55 ±0.18 ab	2.99 ±0.17 a	0.082 ±0.014 a	0.00 ±0.00 b
G1	14.59 ±1.38 a	1.84 ±0.21	10.74 ±1.05 a	1.99 ±0.26 b	0.016 ±0.004 b	0.009 ±0.003 a
G2	14.73 ±1.64 a	2.28 ±0.39	11.07 ±1.22 a	0.882 ±0.37 a	0.016 ±0.001 b	0.003 ±0.001 b
G3	11.21 ±0.58 b	2.18 ±0.25	7.96 ±0.38 b	0.749 ±0.22 a	0.003 ±0.01 b	0.00 ±0.00 b
L.S.D.	2.195 *	0.943 NS	2.376 *	0.767 **	0.016 **	0.0057 **
P-value	0.0476	0.1224	0.0484	0.0001	0.0001	0.0064
Means with the different letters in same column differed significantly. * (P≤0.05), ** (P≤0.01).						

Renal and Hepatic Parameters in albino rats

Blood urea concentrations were markedly elevated in groups G1 and G2 compared to the control group, with values of 43.19 ± 2.12 mg/dL and 42.81 ± 5.48 mg/dL, respectively (P ≤ 0.05). Moreover, serum creatinine levels showed a significant increase in the treated groups compared with those of the control group (P ≤ 0.01). The rise was more pronounced among G3 group (0.759± 0.03 mg/dl) which may suggest a potential cumulative or dose-related nephrotoxic effect table (4).

Table 4. Effect of different concentrations of matcha tea on kidney functions

Group	Means ±SE	
	Blood urea (mg/dl)	S. Creatinine (mg/dl)
Control	32.10 ±0.52 b	0.489 ±0.02 c
G1	43.19 ±2.12 a	0.615 ±0.02 b
G2	42.81 ±5.48 a	0.691 ±0.02 a
G3	35.14 ±2.55 ab	0.759 ±0.03 a
L.S.D.	9.164 *	0.070 **
P-value	0.0411	0.0001
Means with the different letters in the same column differed significantly. * (P≤0.05), ** (P≤0.01).		

Table 5 shows a remarkable and dose dependent increase ($P \leq 0.01$) in the activity of liver enzymes: GOT (AST), GPT (ALT), and ALP when compared with that of control rats. GOT levels increased from 49.25 ± 3.69 IU/L (control) to 116.91 ± 0.89 IU/L in G3, an increase of more than two times the control value, specifically. Likewise, GPT levels steadily increased to 49.83 ± 1.29 IU/L in G3 when compared to the control (27.41 ± 2.37 IU/L). ALP also showed a similar pattern, increasing significantly in the treated groups particularly G3 and reached its peak at this group (135.33 ± 0.97 IU/L vs 90.67 ± 2.15 IU/L in control).

Table 5. Comparison between different groups in liver enzymes

Group	Means ±SE		
	GOT (IU/L)	GPT (IU/L)	ALP (IU/L)
Control	49.25 ±3.69 d	27.41 ±2.37 d	90.67 ±2.15 d
G1	75.41 ±4.33 c	35.58 ±0.89 c	111.83 ±2.59 c
G2	96.91 ±0.99 b	42.08 ±1.13 b	125.17 ±3.14 b
G3	116.91 ±0.89 a	49.83 ±1.29 a	135.33 ±0.97 a
L.S.D.	8.332 **	4.375 **	6.24 **
P-value	0.0001	0.0001	0.0001
Means with the different letters in the same column differed significantly. ** (P≤0.01).			

Serum level of IL-6 and TNF-α

To evaluate the inflammatory effect of matcha, IL-6 and TNF-α levels in the serum was determined in three experimental groups (G1, G2 and G3) and also in control group on this study .The results presented in Table 6 indicated that the IL-6 level among groups was statistically significantly different ($P = 0.0001$, L.S.D. = 28.398, $P \leq 0.01$), which means that the treatment had a diverse effect on systemic immune response. IL-6 levels varied from 40.07 to 180.84 ng/mL, with the lowest value of the mean obtained in the control group (82.73 ± 12.55 ng/mL).Without any experimental intervention, this relatively low level is indicative for a physiological inflammatory situation. In contrast, the levels of IL-6 in all treated groups were higher, indicating that the matcha treatment elicited an inflammatory response. IL-6 levels of Group 1 (144.71±61.47 ng/mL) were significantly higher than Intergroup control, reflecting a mild to moderate inflammatory reaction. In this group, the range of results (112.13 – 226.92 ng/mL) suggests inter-individual variability in response. Another higher IL-6 level in G2 187.26 ± 4.61 ng/mL with less variation (range: from 140.62 to 261.35 ng/mL) had shown the more consistent and increasing inflammatory response. There was no statistical difference between G2 and G1 or G3 as evidenced by common superscript letter "a-b". This refers to a moderate inflammatory response, which is influenced by high exposure or treatment. However, with a range of 180.00 to 276.35 ng/mL, Group G3 had the highest mean IL-6 level (213.47 ± 8.77 ng/mL). This group differs considerably from G1 and the control group, but

not significantly from G2, as indicated by the superscript "a." These results suggest that, in comparison to the other groups, G3 produced the strongest pro-inflammatory response, possibly as a result of larger dosage, toxicity, or more forceful action.

Table 6. Comparison between different groups in IL-6

Group	Mean ±SD of IL-6 (ng/ml)	Range (Min. – Max.)
G1	164.57 ±11.88 b	112.13 – 226.92
G2	187.26 ±4.61 ab	140.62 – 261.35
G3	213.47 ±8.77 a	180.00 – 276.35
Control	82.73 ±12.55 c	40.07 –180.84
L.S.D.	28.398 **	--
P-value	0.0001	--
Means with the different letters in the column differed significantly. ** (P≤0.01).		

Serum TNF-α analysis revealed that there were significant differences between the groups (P = 0.0001). Group 2 recorded the highest mean level of TNF-α (138.95 ± 15.14 ng/mL), it was significantly higher than that of any other group, indicating acute pro-inflammatory response. Also, group 3 showed remarkably higher (106.48 ± 5.02 ng/mL), though it was less than that of Group2 but rather more than group 1 and control table (7).

Table 7. Comparison between different groups in TNF-α

Group	Mean ±SD of TNF-α (ng/ml)	Range (Min. – Max.)
G1	65.25 ±9.22 c	24.23 –113.46
G2	138.95 ±15.14 a	84.14 –261.35
G3	106.48 ±5.02 b	88.75 –138.18
Control	41.37 ±6.27 c	20.04 –90.42
L.S.D.	27.753 **	--
P-value	0.0001	--
Means having the different letters in the column differed significantly. ** (P≤0.01).		

Histopathological Findings of the Liver

Liver histology appeared normal on control sections; there was no evidence of pathological lesions. The structural organization of central vein and surrounding hepatocytes in hepatic parenchyma was within normal limit. Group G1 (matcha 10 mg/kg/day) showed multifocal inflammatory reactions, primarily affecting the portal triad and surrounding parenchyma, with approximately 25% of tissue affected, accompanied by mild congestion, without degeneration, necrosis, tissue destruction, or hemorrhage. Group G2 (matcha 20 mg/kg/day) exhibited a more severe inflammatory reaction, with approximately 55% of the tissue affected, characterized by congestion and focal degeneration, but without severe necrosis, tissue destruction, or hemorrhage. Group G3 (matcha 30 mg/kg/day) showed significant multifocal inflammation, with over 75% of tissue affected. This was accompanied by severe congestion and degenerative changes, but without severe necrosis, destruction, or hemorrhage (Figure 3).

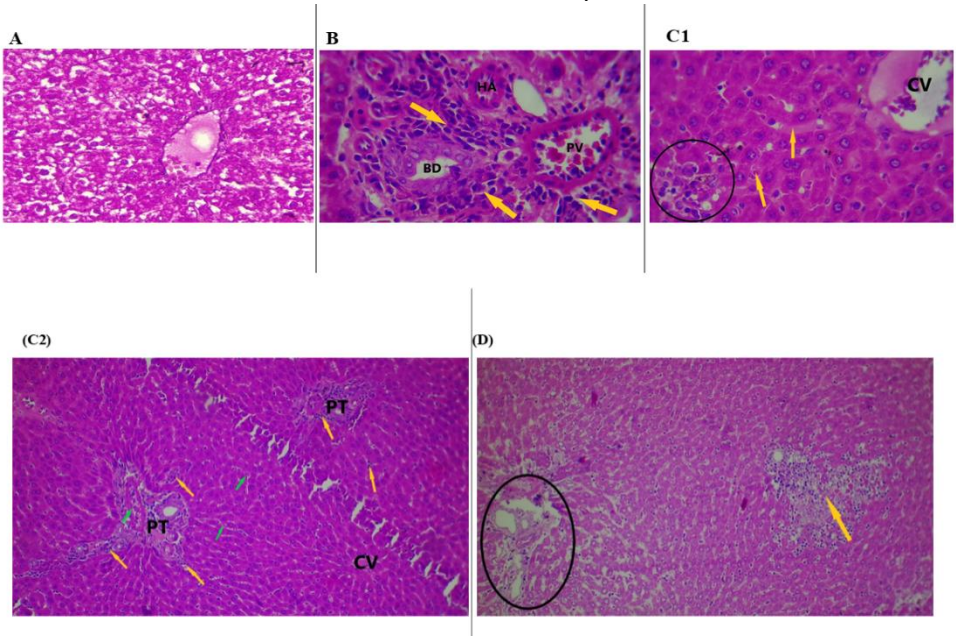

Figure 3. control group (H&E, 40x) (A). Stained liver sections showed well-preserved architecture, normal arrangement of hepatic cords, portal triad including portal vein (PV), bile duct (BD), and hepatic arteriole (HA). Liver tissue of the G1 group (10 mg/kg/day), 40x (B). A small number of multifocal mononuclear inflammatory cells infiltrated the portal vein (PT) channel (yellow arrows). No degenerative lesions or necrosis were observed. In the G2 group (20mg/kg/day) (H&E, 40x) (C1) Section from a stained liver shows central vein (CV) lined by endothelial cells, with mild congestion, few multifocal mononuclear inflammatory cells infiltration through parenchymal hepatocytes (Black circle), some dilated sinusoids noticed (yellow arrow). No damage or necrosis was detected. The stained liver sections of the same group (C2) showed well-preserved structure, slight dislocation of the hepatic cords, multifocal mononuclear inflammatory cells infiltrating into the liver parenchyma through the portal vein (PT) (yellow arrows), slight congestion of the portal vein and sinusoids (green arrows), and central vein (CV), and no degenerative changes in hepatocytes were observed. Liver-stained sections show separation of hepatic cords and multifocal infiltration of mononuclear inflammatory cells through the portal triad (black circles) and into the liver parenchyma. Liver tissue exhibits degenerative necrosis (yellow arrows). Liver tissue in the G3 group (30mg/kg/day) (H&E, 10x) (D) Liver-stained sections show separation of hepatic cords and multifocal infiltration of mononuclear inflammatory cells through the portal triad (black circles) and into the liver parenchyma. Liver tissue exhibits degenerative necrosis (yellow arrows).

Histopathological Findings of the Kidney

Kidney sections from the control group showed normal architecture, with intact glomeruli and tubules, indicating no nephrotoxic effects under the experimental conditions used. In group G1 (matcha 10 mg/kg/day), multifocal inflammation and degenerative alterations of the cortex medulla zone were observed. This was accompanied by tubular degeneration signs, such as dark eosinophilic cytoplasm, thickening of the basement membrane and nuclear condensation. Infiltration of mononuclear inflammatory cells involved both proximal and distal tubules, and some medullary tubules had focally necrotic deposits. In G2 (matcha

20 mg/kg/day), a multi-focal inflammatory reaction involved about 50% of tissue sections with perivascular edema, over imposed interstitial congestion, hemorrhage and degenerative changes, although severe central necrosis were not present. The kidney tissue of the G3 (matcha 30 mg/kg/day) group revealed diffuse inflammation, severe interstitial congestion and hemorrhage throughout the section with no degenerative changes or necrosis being observed (Figure 4).

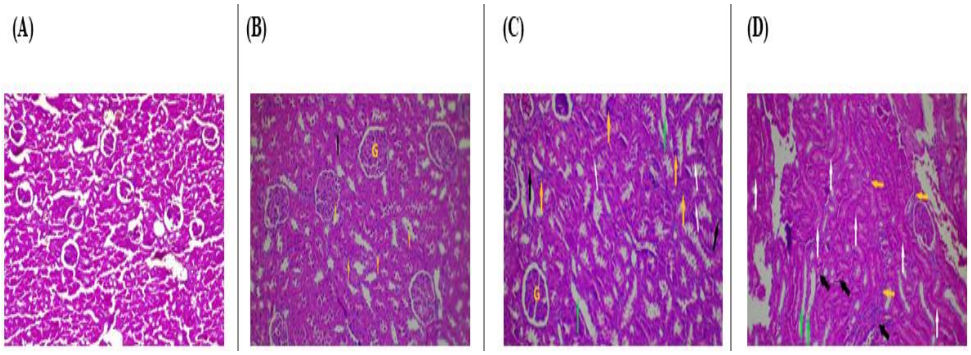

Figure 4. The H&E-stained kidney section at 10X magnification of the control group (A) reveals that glomeruli and tubules in the cortex retained their normal histological structure, and no histopathological alterations were seen.G1 group (B) shows a multifocal inflammatory process (yellow arrows) involving the tissue section associated with mild interstitial congestion (black arrows)and hemorrhage, no degenerative changes, and no necrosis detected.G2 group (C)Section from stained kidney shows renal tissue with preserved architecture, some proximal and distal tubules show signs of degeneration (dark pinkish cytoplasm and condensed nuclei) (White arrows), mononuclear inflammatory cells infiltration (yellow arrows) involving the proximal and distal tubules, some of the tubules are dilated (green arrows), multifocal interstitial congestion and hemorrhage (black arrow).G3 group (D)Section from stained kidney shows degenerative renal tissue at the cortico-medullary junction, tubules shows signs of degeneration(dark pinkish cytoplasm, thick basement membrane and condensed nuclei) (White arrows), mononuclear inflammatory cells infiltration (yellow arrows) involving the proximal and distal tubules, some of the tubules are dilated (green arrows), multifocal interstitial congestion and hemorrhage (black arrow).

4. Discussion

Matcha, an ultra-fine green tea powder, has attracted significant study interest as a desirable beverage and nutritional supplement due to its potential efficacy in reducing obesity and associated metabolic disorders (Wang *et al.*, 2022). Its rising popularity has also increased the marketing of low-quality or counterfeit products, especially in regions with limited regulatory oversight. In this study, we investigated the toxic effect of Commercial matcha tea, which the GC-MS analysis showed the presence of both Toluene and xylene, which, interestingly were either absent or minimally reported in other matcha-related studies *(Kika et al., 2024, Luo et al.*, 2022). These chemicals have, especially after chronic exposure, been found to cause Central Nervous System (CNS), liver and kidney injury (Bolden *et al.*, 2015). Xylene exposure is harmful to the nervous system, respiratory system, liver and kidneys and also the reproductive system(Chaudhary, 2022). The results of this investigation raise concerns about the safety and quality of the tested product, which is marketed as a substance that resembles matcha. In contrast to actual matcha (*Camellia sinensis*), which has a well-established anti-inflammatory and antioxidant profile.

In treated groups, the matcha sample under investigation showed histological evidence of tissue injury and inflammation. Microscopic analysis revealed a multifocal inflammatory process, accompanied by congestion and focal degenerative alterations, although widespread tissue necrosis, destruction, or hemorrhage was not observed in the liver and kidney sections especially at the dose 20mg/kg/day and 30mg/kg/day, when compares with GI and control group. These findings indicate a pro-inflammatory and potentially toxic reaction.

These results suggest that the tested material may lack the protective bioactive compounds: catechins, theanine, and polyphenols that are typical of authentic matcha. Although previous studies have reported the general safety or low toxicity of matcha (Xu et al., 2016),other animal toxicity studies indicated dose-dependent hepatotoxicity of catechins (Hunjadi *et al.*, 2021). Research conducted on tea leaves revealed that increased consumption of matcha tea can induce acute cytotoxicity in hepatic cells (Takabayashi *et al.*, 2004). A study discovered that excessive intake of green tea may induce oxidative DNA damage in the pancreas and liver of hamsters (Takabayashi *et al.*, 2004). A study indicated that a high dosage (5% of the diet for 13 weeks) of green tea extract induced thyroid enlargement (goitre) in healthy rats (Chandra and De, 2010). Research suggests the ability of tea plants to collect excessive quantities of aluminium may lead to nephrological disorders, as aluminium can be retained in the bodies of individuals with renal failure(Sueoka *et al.*, 2001).

These inconsistencies may be related to differences in test design, dose, duration of exposure, and extraction method, or the overall quality and purity of the tested product. Thus, while the agent's teste has been shown to be non-toxic in previous studies, we suggest that they should be re-evaluated under different experimental conditions and with stricter quality-control measures.

The hepatotoxicity found in this experiment is most likely due to the xylene and toluene contaminants. Xylene and toluene are established organic solvent hepatotoxins. Xylene exposure has documented evidence of structural liver damage, elevation of serum transaminases in experimental models, and disruption of bile acid transport. Toluene is known to produce oxidative stress and inflammatory response as well as mitochondrial respiration inhibition within hepatocytes(Nsonwu-Anyanwu *et al.*, 2021). Mechanistically, these solvents cause hepatotoxicity via increasing reactive oxygen species (ROS) generation, reducing glutathione level, inducing lipid peroxidation in hepatocytes, impairing mitochondrial membrane potential and upregulating pro-inflammatory cytokines such as TNF-α and IL-6 (Sui *et al.*, 2022).

Alterations in hematological parameters seen in all treated groups, imply possible haematotoxicity induced by this compound. Reduction in the number of red blood cells and in hemoglobin concentration, which was statistically significant in Group 1, indicates clearly anemia, possibly due to ineffective erythropoiesis or increased haemolysis. The decrease was particularly significant in the actively treated group 1, suggesting that hematotoxicity is not dose-dependent but may be related to other parameters such as bioavailability, absorption, and metabolism of the compound. Stable HGB levels in G2 and G3 amidst the decrement of RBC counts could be due to such compensatory mechanisms, probably by the maintenance of hemoglobin synthesis or increased erythrocyte turnover, which would more likely sustain for quite a time. Moderately declining RBCs with increasing HGB observed in group 3 may be due to hemoconcentration or increasing erythrocyte size and hemoglobin content (Turner *et al.*, 2018). An experiment using New Zealand White (NZW) rabbits found that the red blood cell (RBC), hemoglobin concentration, and ferritin significantly increased in the matcha tea group(Mohamed *et al.*, 2025). The results are in contrast to a previous study using rats, which reported no significant effects of green tea on the complete blood count. These results also indicate that the bone marrow function is preserved and hematopotoic

formation might not be affected by the intake of green tea(Essex *et al.*, 2019).In a different study on the matcha tea group, there was no statistically significant difference in complete blood count and different throughout the 12 weeks consumption of matcha tea ($p>0.05$)(El-Elimat *et al.*, 2022). This result is in sharp contrast to the present findings. In order to clarify the effects of matcha tea on iron metabolism, more precise examinations should be performed in a follow-up study, including serum ferritin, total iron-binding capacity, and transferrin to confirm iron deficiency anemia.

Platelets participate in thrombus formation and hemostasis regulation, so their functional evaluation remains important for the clinical management of thrombocytopenic patients(Boknäs *et al.*, 2019). In this study, the G1 group had significantly lower platelet counts than the G2 and G3 groups shown along with significant declines in RBC count and hemoglobin level. Such a result accords with earlier reports that severe anemia can be present as a thrombo-cytopenia(Jundi *et al.*, 2025). Notably (El-Elimat *et al.*, 2022) , found no significant hematological differences between the matcha tea-treated and control groups under normal conditions, indicating that the observed effects may be context-dependent. The paradoxical occurrence of anemia exclusively in the lowest-dose group suggests a biphasic, dose-dependent response. Low or sub-therapeutic doses of bioactive compounds may induce oxidative stress, resulting in bone marrow suppression and reduced erythropoiesis. Conversely, higher doses appear to activate endogenous antioxidant defense mechanisms, including upregulation of glutathione peroxidase and catalase, thereby mitigating hematological toxicity (Jodynis-Liebert and Kujawska, 2020). Decreased red blood cell counts and hemoglobin levels may reflect compromised erythrocyte production or excessive loss from hemopoietic tissue (because the liver is one of the major sites of erythropoiesis) with secondary damage to circulating erythrocytes. These indicate that RBC and platelet counts were sensitive hematological biomarkers in rats exposed to matcha with xylene and toluene. The action of xylene in causing anemia relates to several metabolic abnormalities such as augmented inflammatory signaling, disturbed hepatocellular integrity, defective erythropoiesis and perhaps malnutrition(Baldari *et al.*, 2021).

Our findings contradict the results of some previous studies, such as (Ogar et al., 2023) found that there is no statistically significant difference in packed cell volume, hemoglobin, red blood cells, white blood cells, neutrophils, lymphocytes, monocytes, and platelets between the treatment group and the control group, and (Agarwal *et al.*, 2004) failed to observe any significant changes in hematologic profiles in rats following the administration of green tea catechins This means to say that *Camellia sinensis* extract does not bear any significant effect on hematologic profiles in Wistar rats. A previous study reported that administration of green tea extract as a dietary supplement for 14 days enhanced leukocyte activity and increased total plasma antioxidant status, suggesting a potential role in the prevention of inflammatory diseases (Lowe *et al.*, 2015). Related studies have reported a decrease in WBC, Hb, and RBC count among people exposed to chemical contaminants (xylene and toluene). A significantly reduced amount of white blood cells may suggest immunosuppression *(Wang et al.*, 2016).

Certain medical disorders that are linked to pathologically significant amounts of haematological indices have been linked to xylene and toulune exposure.(Goldstein and Smith, 2021; Spatari *et al.*, 2021). (Adebola *et al.*, 2022) report that Benzene administration resulted in a significant reduction ($p < 0.05$) in white blood cell (WBC) count, leading to leukopenia. This decrease in WBCs has been linked to metabolic processes linked to the development and spread of leukemia and indicates an immune system that is compromised. It's also crucial to consider that matcha tea has been shown to contain trace amounts of volatile chemical compounds, including xylene and toluene, which were probably added during processing and cultivation. These substances are known to adversely impact hematopoietic activity, namely

leukocyte dynamics, and they resemble benzene in both structure and toxicity. Consequently, even though matcha is well known for its bioactive ingredients and health-promoting benefits, the possibility that it contains aromatic hydrocarbons highlights the significance of strict quality control and careful evaluation of its overall safety profile. Furthermore, additional research should be carried out to determine the mechanism of lymphocyte elevation after matcha tea consumption in a larger clinical study. Drug use encompasses the non-medical use of psychoactive substances, including legal drugs such as benzene. These harsh chemicals are commonly used in various industrial processes(Adebola *et al.*, 2022),Toluene and xylene are primarily hepatotoxicants, and the liver experiences oxidative stress as a result of their metabolism (Sayed *et al.*, 2023).

In addition, serum cytokine concentrations were also measured to assess damage caused by matcha tea on liver and kidney tissues. Cytokine analysis showed that matcha tea induced a dose-dependent pro-inflammatory response, with particularly significant increases in IL-6 and TNF-α concentrations in the G3 and G2 groups. Furthermore, serum leptin concentrations were significantly increased in all treatment groups, regardless of dose, suggesting a consistent metabolic response related to adipocyte activity. In disagreement with anti-inflammatory effects, matcha supplementation may dramatically reduce the release of inflammatory cytokines (including TNF-α and IL-6) brought on by saturated fatty acids via the hypothalamic ARC's JAK2/STAT3 signaling pathway (Zhou *et al.*, 2020). As stated in (Kany *et al.*, 2019), Proliferative inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF), are associated with a variety of inflammatory diseases. In multiple animal studies, this inflammatory response was reduced in several animal studies following matcha extract as it prevented the release of major inflammatory cytokines into blood (Devkota *et al.*, 2021) (Ramez *et al.*, 2021). Previous studies have also reported that matcha treatment can inhibit the release of important inflammatory cytokines(Ohishi *et al.*, 2016) ; (Khateeb and Taha, 2024).(Hamed *et al.*, 2025) found that it is possible that matcha extract can protect liver tissues from inflammation-induced harm by blocking TNFα.While generally considered beneficial, matcha can, in some cases, lead to hepatotoxicity (liver damage). This is more likely to occur with high doses of green tea extract, particularly when consumed in supplement form, rather than as a beverage (Diabetes *et al.*, 2012).The observed elevation in blood urea and creatinine concentrations and serum ALT, AST, and ALP levels in groups G1 and G2, relative to the control group, suggests a disruption in renal and hepatic function that may be attributable to early nephrotoxic effects of the administered treatment. These findings may be since the kidneys are impaired by reduced uric acid excretion with subsequent accumulation of uric acid in the blood stream which was already reported (AlAni and Al-Lami, 2024). Several studies have shown that matcha green tea can effectively inhibit lipid accumulation,improve dyslipidemia, reduce hyperglycemia, and reduce ALT and AST levels in obese C57BL/6 mice (Zhou *et al.*, 2021). Additionally, (El-Kholie et al., 2022) demonstrated that Green tea helps restore urea, creatinine, and uric acid levels and improves liver function. Drinks rich in green tea are highly recommended for preventing kidney damage, especially for those who are exposed to drug poisoning, environmental toxins, and pollutants, such as those working in laboratories, chemical plants, and factories. Herein, we suggest that the locally sourced matcha product obtained from a pharmacy may not be authentic and may appear to exert toxic effects, as evidenced by its adverse impact on renal and hepatic biomarkers. The findings raise concerns about the purity, composition and safety of the formula and highlight the potential risks of consuming substandard or counterfeit matcha products.

5. Conclusion

The present study demonstrates that the indigenous matcha tea product tested has dose-related hepatotoxic and nephrotoxic effects in Wistar rats. The toxicants detected using the GC-MS are xylene and toluene, which infers that these contaminants are probably responsible

for the observed biochemical derangement with increase in liver enzymes, renal markers and pro-inflammatory cytokines (IL-6 and TNF-alpha) of treated group rats. Mediated through histopathological findings, degenerative and inflammatory alterations are evident in the liver and kidney tissues especially at higher doses. Further studies are recommended to explore the underlying molecular mechanisms and confirm its clinical applicability.

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Conflict of interests

The authors declare that they have no conflicts of interest that could have influenced the conduct, analysis, or presentation of the research.

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