Effectiveness of Matcha Tea Extract on Immune Functions of Cyclophosphamide induced- immunosuppression Rats

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Abstract

Matcha is a type of Japanese green tea (Camellia sinensis) of the Tencha type and rich source antioxidant compounds. The existing study was proposed research the effect of aqueous extract of matcha tea toward Cyclophosphamide (CYP) - induced immunosuppression in rats. The study lasted 8 weeks. Immunosuppression was induced by the injected with CYP 30 mg/Kg of b. wt in paraffin oil (1:1). The experimental groups consisted of 35 rats divided into five groups. The rats in the healthy-group (-ve) and untreated immunosuppress-group (+ve) were fed on a basal diet with the orally given of distilled water, while the other three treated immunosuppress-groups consisted were fed on the basal diet with the orally given of aqueous extract of matcha tea at dosses of 100, 200 and 300 mg/kg b wt, respectively. Results founded that Matcha tea has a higher content of phenolic acid, moderate contents of chlorogenic and caffeic acids while the quercetin was the lower content. Also, the obtained results revealed that immunosuppressed-groups treated with the three tested doses of Matcha had a significant increase in the levels of RBC, Hb, total protein, albumin, IgM and IgG and activity of GPx enzyme; and decreased ($P<0.05$) in the levels of PLT, WBC, MDA and the activities of liver enzymes, compared to untreated immunosuppressed-group (+ve). Finally, the current study concluded that Matcha tea has several phenolic compounds, antioxidant properties which are thought to improve the immune system and protect against oxidative damage.

Keywords: Immunosuppression; Matcha Tea; Liver Functions; Antioxidant Enzymes; Blood Pictures; Cyclophosphamide.

Introduction

Both plants and animals maintain immunity as a protective mechanism for disease tolerance through reproduction. It protects the host from infections caused by bacteria, viruses, fungi, and protozoa **(Soares *et al.*, 2017).** The cells of the immune system originate in the bone marrow and then go through the bloodstream and the lymphatic system, a specialized network of channels, to protect the peripheral tissues **(Charles *et al.*, 2001).** Two components of immunity are distinguished based on the reaction's particularity and rapidity. The innate and adaptive responses are the names given to these. Although physical, chemical, and microbiological barriers are all part of innate immunity, immune system components such neutrophils, monocytes, macrophages, supplements, cytokines, and acute phase proteins that offer immediate host protection are more frequently included defense (**Delves and Roitt, 2000a**).

Adaptive immunity, on the other hand, is the immune system of higher animals that uses T and B cells to maintain antigen-specific responses. The tailored response is precise but takes days or weeks to develop, whereas the innate reaction is quick but can often harm healthy tissues due to a lack of uniqueness. Additionally, the adaptive reaction includes memory, so subsequent display results in a more active and quick response, however this is not instantaneous. (**Delves and Roitt, 2000b**).

On the other hand, Immunosuppression is the decrease in the immune system's capacity to effectively react to external antigens, such as

tumor cell surface antigens. Killing immune activator cells or Immunosuppression can also result from inhibiting intracellular pathways necessary for antigen identification or additional components of the immunological reaction. **(Wieland *et al.*, 2014).**

A chemotherapeutic drug called cyclophosphamide (CP) is frequently used to treat a range of malignancies and illnesses, including multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus **(Raj and Gothandam, 2015).** CP treatment in clinical settings, however, may result in immunosuppression by inhibiting the growth of healthy immune cells. **(Tang and others, 2018).** Immunotherapy relies heavily on an efficient immune modulator, and natural plants have demonstrated promise immunomodulatory effects through a variety of sites. As a result, natural plants have been used extensively as a possible immunotherapy for illnesses involving the immune system **(Ibrahim *et al.*, 2019).**

Around the world, matcha, a powdered form of Japanese green tea (Camellia sinensis) of the Tencha kind **(Horie *et al.*, 2017),** is widely consumed, and its use is only growing **(Schröder *et al.*, 2019).** Because of its traditional production method, this variety of tea is a significant source of antioxidant chemicals **(Sharangi *et al.*, 2009 and Sano *et al.*, 2018).** Furthermore, Matcha is regarded as the highest-quality product and the most aromatic green tea **(Unno *et al.*, 2018).**

The present study was conducted to find out the potential effects of Matcha tea extract against cyclophosphamides-induced immunosuppressive in male rats. The foremost objective was accomplished by:

1- Estimation of serum complete blood count.

2- Assessment of liver functions.

3- Estimation of serum immunoglobulin level.

4- Determination of serum Malondialdehyde (MDA) level and activity of Glutathione Peroxidase (GPx) enzymes.

Materials and Methods:

Materials:

Matcha Tea: Matcha tea was purchased from herbalist shops in Cairo, Egypt and was identified at the Agricultural Research Center, Cairo, Egypt. **Rats:** Thirty-Five of adult male rate (Sprague Dawley Strain), weighing about 200±5g, were obtained from the Laboratory Animal Colony, Helwan, Egypt. **Diet:** Casein, cellulose, choline chloride, D-L methionine, vitamins and minerals, constituents were purchased from El-Gomhoriya Pharmaceutical Company, Cairo, Egypt. Starch, soybean oil, and sucrose were obtained from the local market.

Chemicals and Kits: Cyclophosphamide (CYP) was purchased from Baxter Oncology (Fermenta Biotech Limited Frankfurt, Germany). Kits were purchased from the Humatrol- Human Co-Germany for Pharmaceutical and Chemical, Germany.

Methods:

Preparation of Matcha Tea Extract: The extraction of the powdered matcha tea was conducted using the heat assisted method. The extraction was performed according to the procedures outlined in **(Sayuti et al., 2020).** Powdered samples (150 g) of matcha tea were extracted with deionized water (1000 ml) at 80 °C for 6 min. The extract then was filtered

with Whatman No. 1 filter paper (Cytiva, Little Chafont, United Kingdom). The filtrate was concentrated at 50 °C under medium pressure using a rotary evaporator.

Preparation of Basal Diet: The basal diet (AIN-93M) was consisted of protein (14%), corn oil (4%), minerals mixture (3.5%), vitamins mixture (1%), fiber (5%), sucrose (10%), choline chloride (0.25%) and the remainder were corn starch up to 100%. These constituents were thoroughly mixed and formulated according to (**Reeves *et al.*, 1993).**

Determination of Total Polyphenols, Caffeic Acid, Chlorogenic Acid, Phenolic Acid, Quercetin:

The total phenolic content was estimated using the Folin-Ciocalteu´s reagent according to the method of **Maurya and Singh (2010).**

The Induction of Immunocompromised: Rats were injected with one dose of Cyclophosphamide at 30 mg/ Kg/ BW in paraffin oil (1:1) according to **(Patwa et al., 2020),** random blood samples were collected from rats to determine IgM and IgG to ensure that there was immunocompromised in injected rats.

Experimental Design: All rats were housed at a room temperature of 25 ± 2 °C, relative humidity of 50–55% and 12 hr. light/12 hr. dark cycles in animal house of the Faculty of Home Economics, Cairo, Egypt for one week for acclimatization. After acclimatization period (one week), all animals were randomly divided into five equal groups (n=7 rats of each) as follows:

Group 1: Rats were represented as normal rats (negative control group), fed on the basal diet and given orally distilled water.

Group 2: Immunosuppressed Rats were represented as (positive control group), fed on the basal diet and given orally distilled water.

Group 3: Immunosuppressed Rats were fed on the basal diet and given orally matcha tea extract at the dose of 100 mg/kg of body weight.

Group 4: Immunosuppressed Rats were fed on the basal diet and given orally matcha tea extract at the dose of 200 mg/kg of body weight.

Group 5: Immunosuppressed Rats were fed on the basal diet and given orally matcha tea extract at the dose of 300 mg/kg of body weight.

Biological Evaluation: Feed intake (FI) was calculated every day. Body weight gain was calculated according to **Kratochvílova, (2002)** and feed efficiency ratio (FER) were determined at the end of the experimental period (8 weeks).

Rats were fasted then sacrificed at the end of the experimental period. Two Blood samples were collected from the portal vein, one of them was collected into a tube containing disodium salt of Ethylene Damien Tetra Acetic Acid (EDTA) as anticoagulant and was used for the assessment of the erythrocyte's indices. The other blood samples were collected into dry clean centrifuge tubes. Serum was separated by centrifuge at 3000 for 15 min and serum aliquots was stored at –20ºC until use for biochemical analysis.

according to the following equations.

BWG = Final Body Weight - Initial Body Weight

Body weight gain %= FBW-IBW/IBW X 100

FER = **(**Body weight gain (g/day)/feed intake (g/day)

Biochemical analysis:

Whole blood samples were used for the assay of the complete blood count test (CBC) Red blood cells count (RBC), platelets (PLT), hemoglobin concentration (Hb) and white blood cell (WBC) according to the methods of **Baker and Silverton, (1984).**

Serum was used to determine the liver markers alanine amino-transferase (ALT) and aspartate aminotransferase (AST) according to **(Murray, 1984)** and alkaline phosphate (ALP) according to **(Wenger et al., 1984).** Total protein and albumin were determined according to the method of **(Schultz,1984 and Jaffè,1886),** respectively**.** The mean value serum's immunoglobulins (IgM and IgG) were determined according to the method described by **Friedman and Young, (1997).** Glutathione Peroxidase (GPx) according to **(Aebi, 1984)** and malondialdehyde (MDA) **(Ohkawa et al., 1979).**

Statistical analysis: Data was evaluated statistically with computerized SPSS package program (SPSS 22.00 software for Windows) using one-way analysis of variance (ANOVA). Significant difference among means were estimated at $P<0.05$ **(Armitage and Berry, 1987).**

Results:

Table 1 presents the discovery of the total polyphenols, phenolic compounds content of caffeic acid, chlorogenic acid, phenolic acid and quercetin in matcha tea. Results proved that matcha tea possess total polyphenols (211.66±2.75mg GAE/100 g), a higher content of phenolic acid (349.33±2.70µg/g) followed by chlorogenic acid (54.53±2.65µg/g), caffeic acid (32.86±1.97µg/g) and quercetin (28.33±3.63µg/g), respectively.

Table (1): Total Polyphenols, Caffeic acid, Chlorogenic Acid, Phenolic acid and Quercetin Content in Matcha Tea.

Type	Amount
Total Polyphenols	211.66±2.75 (mg GAE/100 g)
Caffeic Acid	32.86 ± 1.97 (µg/g)
Chlorogenic Acid	54.53 ± 2.65 (µg/g)
Phenolic Acid	349.33 ± 2.70 (µg/g)
Quercetin	28.33 ± 3.63 (µg/g)

*Values are expressed as means ±SE.
*Gallic Acid Equivalent (GAE)

The Effect of Matcha Tea Extract on BW, FI and FER in Immunosuppressed-Rats:

The results of the effect of the oral administration of the matcha tea extract at the different three doses (100, 200 and 300 mg/kg of body weight) (BWG), (BWG %), (FI) and (FER) of immunosuppressed-Rats is recorded **Table (2)**. As shown, FBE, BWG, BWG %, FI and FER of the positive control group were significantly decreased ($P<0.05$) as compared to the negative control. While, BWG, BWG %, FI and FER were significantly increased in all treated groups with matcha tea extract in dose depended manner, compared to the positive control group.

Table (2): The Effect of Matcha Tea Extract on FBW, BWG, FI and FER in Immunosuppressed-Rats.

Parameter / Groups		IBW(g)	FBW(g)	BWG (g)	BWG (%)	FI (g/day)	FER
Negative Control		202.83±1.56^{a}	276.86±0.07^{a}	74.03±1.25 ^{a}	36.50±0.77^{a}	22.50±1.03^{a}	0.06±0.97^{a}
Positive Control		201.53±1.94^{a}	255.00±0.29^{c}	53.47±0.47^{c}	26.54±0.38^{c}	18.30±1.09^{e}	0.05±0.85^{b}
Treated rats with Matcha extract at a dose of:	**100 mg**	201.77±1.46^{a}	262.43±0.40^{b}	60.66±0.21^{b}	30.07±0.12^{b}	19.00±1.07^{c}	0.06±0.95^{a}
	200 mg	202.45±2.58^{a}	275.82±0.39^{a}	73.37±2.50^{a}	36.25±1.46^{a}	20.50±1.05^{b}	0.06±0.87^{a}
	300 mg	202.37±1.42^{a}	275.86±0.19^{a}	73.49±1.71^{a}	36.31±0.98^{a}	20.50±1.07^{b}	0.06±0.89^{a}

Values are expressed as means ±SE. Values at the same column with different letters are significant at $p< 0.05$. **IBW:** Initial body weight**; FBW:** Final body weight**; BWG:** Body weight gain**; BWG%:** Percent Change of body weight gain%; **FI:** Feed Intake; **FER**: Feed Efficiency Ratio.

Effect of Matcha Tea Extract on Hematological Parameters in Immunosuppressed-Rats:

The effect of giving matcha tea extract orally on the blood levels of (RBCs, PLT, Hb and WBC) is recorded in **Table (3)**. The obtained results illustrated that untreated immunosuppressed rats (positive group) had a significant ($p<0.05$) decrease in blood levels of RBCs, PLT, Hb and WBC, compared to the healthy rats (negative control group). In comparison to the untreated immunosuppressed-rats, treated immunosuppressed-groups by the oral administration of the three different levels of matcha tea have a significant ($p<0.05$) increase in serum levels of RBCs, PLT, Hb and WBC. There was also a notable difference in the serum levels of RBCs, PLT, Hb and WBC between treated immunosuppressed-groups with matcha tea, as the improvement rate increased with increasing extract dose taken.

Table (3): The Effect of Matcha Tea Extract on Hematological Parameters in Immunosuppressed-Rats.

Parameters / Groups		RBC ($x10^6$/ μL)	PLT ($x10^3$/ μL)	Hb (g/dL)	WBC ($x10^3/mm^3$)
Negative Control		50.41±2.53[a]	1160.73±5.96[a]	9.28±0.14[a]	11.33±0.27[a]
Positive Control		28.16±0.96[d]	468.48±2.02[e]	4.95±0.25[e]	4.81±0.11[e]
Treated rats with Matcha extract at a dose of:	**100 mg**	33.61±1.07[c]	557.43±1.96[d]	5.33±0.09[d]	6.95±0.06[d]
	200 mg	39.25±0.89[b]	655.97±1.38[c]	7.30±0.18[c]	7.88±0.32[c]
	300 mg	46.83±1.20[a]	736.66±3.46[b]	8.68±0.14[b]	9.12±0.15[b]

Values are expressed as means ±SE. Values at the same column with different letters are significant at $p<0.05$; **RBCs**: Red Blood Cells Count; **PLT**: platelet count; **Hb**: The Amount of Hemoglobin; **WBC**: white blood cell count.

The Effect of Matcha Tea Extract on Serum Levels of immunoglobulin (IgM and IgG) in Immunosuppressed-Rats:

Tabulated results in **Table (4)** exhibit the effect of matcha tea extract on the serum levels of immunoglobulin (IgM and IgG) of immunosuppressed-rats. The obtained results revealed that untreated immunosuppression-rats had a significant ($p < 0.05$) decrease in serum levels of IgM and IgG, compared to that of the normal rats. In contrast, oral administration with 100, 200 and 300 mg/kg b. wt of matcha tea extract for the treating of immunosuppression-rats produced a significant ($p<0.05$) increase in the serum IgM and IgG, in comparison to the positive rats. Also, the get better advancement in the serum levels of the immunoglobulin parameters was established in the treated group with the highest level (300 mg/kg b. wt) of Macha tea extract, followed by those treated with the 200 and 100mg/kg b. wt, compared to the positive rats. There are a significant ($p<0.05$) difference in serum immunoglobulin IgG and IgM between the three tested groups, it was observed that the higher dose of the extraction ,the most concentration level was seen.

Table (4): The Effect of Matcha Tea Extract on Serum Levels of IgM and IgG in Immunosuppressed-Rats.

Parameters / Groups		IgG (mg/dL)	IgM (mg/dL)
Negative Control		974.93 ± 4.59^{a}	78.78 ± 1.37^{a}
Positive Control		362.65 ± 5.46^{e}	33.88 ± 3.72^{e}
Treated rats with Matcha extract at a dose of:	**100 mg**	543.05 ± 2.77^{d}	42.11 ± 0.66^{d}
	200 mg	595.83 ± 4.45^{c}	59.21 ± 1.59^{c}
	300 mg	633.79 ± 3.77^{b}	64.65 ± 3.25^{b}

Values are expressed as means ±SE; Values at the same column with different letters are significant at
$p< 0.05$; **IgG**: Immune globulin G; **IgM**: Immune globulin M.

The Effect of Matcha Tea Extract on Liver Functions in Immunosuppressed-Rats:

The obtained results in **Table (5)** illustrate the effect of giving Macha tea extract orally on the serum activity of liver enzymes (AST, ALT, ALP) and serum concentrations of total protein (TP) and albumin (Alb)as a measurable of liver function in treated-rats with CP. Tabulated results created that CP resulted in liver injury as identified by the significant ($P<0.05$) increase in the activity of AST, ALT and ALP enzymes, and the decrease in serum levels of TP and Alb compared to normal control rats.

In comparison to the untreated immunosuppressed-rats, treating rats with immunosuppression by the oral administration of the three different doses of matcha tea extract caused a significant ($p<0.05$) lowering in the serum activity of AST, ALT and ALP enzymes, and increased the concentration of TP and Alb, compared to the positive control group. There was also a difference in the activity of liver enzymes, and concentrations of TP and Alb between treated groups with matcha tea extract, as the improvement rate increased with increasing taken dose.

Table (5): The Effect of Matcha Tea Extract on The Serum Activity of AST, ALT and ALP enzymes and serum Levels of TP and Alb in Immunosuppressed-Rats

Parameters / Groups		ALT (μ/L)	AST (μ/L)	ALP (μ/L)	TP (gm/dl)	Alb (gm/dl)
Negative Control		30.15±2.33^{e}	84.92±1.98^{e}	66.62±0.23^{e}	11.23±0.43^{a}	6.68±0.18^{a}
Positive Control		75.67±2.19^{a}	136.09±1.72^{a}	120.04±1.72^{a}	4.52 ± 0.25^{e}	2.69 ± 0.14^{e}
Treated rats with Matcha Extract at a dose of:	**100mg**	61.45±3.95^{b}	111.53±0.97^{b}	98.89±0.31^{b}	6.62 ± 0.23^{d}	3.96 ± 0.13^{d}
	200mg	54.18±2.12^{c}	101.72±2.29^{c}	83.32±0.24^{c}	8.32 ± 0.24^{c}	4.69 ± 0.33^{c}
	300mg	35.38±2.50^{d}	94.59±2.70^{d}	71.01±2.51^{d}	9.89 ± 0.31^{b}	5.84 ± 0.25^{b}

Values are expressed as means ±SE; Values at the same column with different letters are significant at $p< 0.05$; **AST**: Aspartate transaminase; **ALT**: Alanine transaminase; **ALP**: Alkaline phosphatase; **TP:** Total Protein; **Alb**: Albumin

Effect of Matcha Tea Extract on Serum Level of MDA and Activity of GPx in Immunosuppressed-Rats

Table (6) constitutes the effect of matcha tea extract on serum levels of MDA and the activity of GPx (antioxidant enzyme) in the treatment of rats from immunosuppression caused by CP. The present results proved that intraperitoneal injection of CP gave a significant ($p<0.05$) rise in serum MDA level, and a decline in GPx activity, in comparison to normal rats. However, intraperitoneal injection of CP, followed by the oral administration of the different three doses (100, 200 and 300 mg/kg b. wt) of match tea, significantly reduced serum levels of MDA and increased activity of GPx enzyme, compared to the positive control rats.

The results also showed that the better amelioration of the tested parameters was improved by increasing the dose of Macha tea extract taken.

Table (6): The Effect of Matcha Tea Extract on Serum Level of MDA and Activity of GPx in Immunosuppressed-Rats

Parameters / Groups		MDA (nmol/ml)	GPx (U/ml)
Negative Control		3.37 ± 0.17^{e}	67.82 ± 2.92^{a}
Positive Control		8.14 ± 0.35^{a}	36.50 ± 1.79^{e}
Treated rats with Matcha Extract at a dose of:	**100 mg**	6.89 ± 0.12^{b}	43.73 ± 1.86^{d}
	200 mg	5.73 ± 0.36^{c}	49.88 ± 1.43^{c}
	300 mg	4.85 ± 0.36^{d}	55.95 ± 1.45^{b}

Values are expressed as means ±SE;Values at the same column with different letters are significant at $p< 0.05$; **MDA**: Malondialdehyde; **GPx:** Glutathione Peroxidase.

Discussion:

The effective immune modulate is a key component for immunotherapy, and natural plants has been shown to have promising immunomodulatory effects via multiple actions. Therefore, natural plants may be widely used as a potential immunotherapy for immune-related diseases (**Ibrahim et al., 2019**). Matcha tea is an aromatic green tea powdered type of Japanese (Camellia sinensis) (**Horie et al., 2017**). The health benefits of matcha tea result from its higher content of the antioxidants compounds (**Komes et al., 2010**). Therefore, the existing study was proposed to research the effect of aqueous extract of matcha tea against cyclophosphamide - induced immunosuppression in rats.

The obtained results proved that matcha tea possesses polyphenols and was a higher content of phenolic acid followed by chlorogenic acid, caffic acid and quercetin, respectively. In the same line, detailed analysis finding by **Kolá˘cková et al., (2020)** found that matcha tea extract has the following maximum levels of phenolic acids, which differing in terms of various criteria, including origin: chlorogenic acid, caffeic acid and quercetin levels.

Regarding to explore the effect of intraperitoneal injection of cyclophosphamide to induce-immunosuppression in rats on their body weight, blood levels of RBC, PLT, Hb, and WBC, serum immunoglobulin level, liver functions and serum antioxidant enzyme activity, The obtained results revealed that immunosuppressed-rats (positive group) had a significant decrease in FBE, BWG, BWG %, FI and FER; blood levels of RBCs, PLT, Hb and WBC; serum levels of IgM, IgG, TP, Alb and activity of GPx enzyme, as well there is a significant increase in the activity of liver enzymes (AST, ALT and ALP) and serum MDA.

The current results were agreed with **Zhang et al., (2021)** who showed the weight gain of CP treated mice significantly inhibited ($p < 0.01$), as growth and development of mice were decreased, **Yang *et al.*, (2019)** observed a significant reduction of weight body/weight gain in mice after they exposed to CP at 80 mg/kg/d for 3 consecutive days via intraperitoneal injection. As well, **Hou and Jalali et al., (2007)** found that

5–10 mg/kg CP treatment for 28–30 days by oral gavages induced significant decreases in body weight and/or weight gain in rat. Also, **Kamiya et al., (2021)** revealed that cyclophosphamide significantly decreased food intake and body weight within 24 h after administration and the delayed phase.

Leukocyte, platelet and hemoglobin are important components of blood, which plays an important role in guaranteeing metabolism, functional regulation and balance of internal and external environment of animal body (**Zuo, 201**5). Leukocytes are immune cells and are "guardians" for animal/human health, which can kill bacteria, pathogens and prevent disease. The significant reduction of leukocytes indicated that the immune function of the body was impaired and weakened greatly. The result in this study was consistent with previous studies that the mice/rat exposed to CP will substantially reduce leukocytes levels in their body, and leukocytes were more sensitive to CP exposure as compared to other blood component (**Alrumaihi et al., 2019**). CP is a strong immunosuppressive agent that inhibits cell proliferation, and kills antigen-sensitive small lymphocytes nonspecifically, inhibiting macrophage responses/function and even cause the atrophy of immune organs (**Jordan and Waxman, 2016**) As well CP damage the antioxidant capacity of the tissue and show liver damage (**Mahmoud et al., 2017**) and can inhibit the growth and development of the body (**Hou et al., 2007**).

Additionally immune suppression and oxidative stress injury are most important toxicity induced by CP (**Khan, 2017**). As well, CP caused significant reduction in body weight ($p < 0.01$) and serum leukocyte, however SOD, MDA, ALT in liver significantly increased. The overall, the most sensitive parameters to CP toxicity may be associated with growth, immune system and the normal function of liver and kidney (**Zhang et al., 2021**).

Cyclophosphamide (CPA) is a cytotoxic alkylating agent that has been in clinical use for it is effective in the treatment of neoplastic diseases such as solid tumors and lymphomas as well as nonneoplastic diseases such as rheumatoid arthritis and systemic lupus erythematosus (**Lawson et al.,**

2008). However, the clinical use of CPA has been limited due to its ability to damage normal tissues which usually resulted in multiple organ toxicity (**Fraiser et al., 1991**). Hepatotoxicity is a major side effect of CPA as it is metabolized principally within the hepatocytes by hepatic microsomal cytochrome p450 mixed function oxidase system to produce its two active metabolite phosphoramide mustard and acrolein (**King and Perry, 2001**). Phosphoramide is associated with its immunosuppressive and antineoplastic effect, while acrolein is associated with its toxic effect (**Kern and Kehrer, 2002**). CP induced hepatic damage as indicated by significant elevation ($P < 0.05$) in serum AST and MDA, while the level of glutathione (GSH) peroxidase crashed (**Oyagbemi et al., 2016**). Studies have suggested that oxidative stress is associated with its hepatotoxic effect (**Zarei and Shivanandappa 2013**). CPA toxicity results from acrolein binding to cellular antioxidant nucleophiles such as glutathione (GSH) resulting in the depletion of the antioxidant defense system and it also initiates lipid peroxidation (LPO) (**Adams and Klaidman, 1993**).

In comparison to the untreated immunosuppressed-rats, the obtained results revealed that the orally administration of matcha tea extract at the three different levels (100, 200 and 300 mg/kg of body weight) significantly improved BWG, FI, FER, blood levels of RBCs, PLT, Hb and WBC, serum levels of IgM and IgG, TP and Alb and MDA, as well as activity of liver and GPx enzymes treated immunosuppressed-groups in dose dependent manner. These obvious enhancements in the tested parameters, may be due to the health benefits effect of matcha tea. The health benefits of matcha tea arise from the presence of natural antioxidants, such as polyphenols (**Kurleto et al., 2013**). Polyphenols are believed to be exceptionally powerful antioxidants, with effects comparable to those of vitamins, such as vitamins C and E, carotene and tocopherol (**Dufresne and Farnworth, 2001**). These results the same line with the results of **Zhou et al., (2021)** who investigated the consumption effect of matcha tea on obese mice model feed on a high fat diet for 6 weeks. They showed matcha tea caused a slight increase in weight, but

mice were still significantly heavier than control mice at all matcha tea concentrations.

In the same line, **Awad et al., (2024)** found that matcha enhanced the levels of IgA, IgM, IgG and activity of liver enzymes in obese rats fed on high fat diet. **Kolář cková et al., (2020)** reported that matcha have more than double the amount of vitamin C of other green teas, Vitamin C is a powerful exogenous antioxidant. Due to its properties, it reinforces the immune defense of the body. Quercetin, a flavonoid found in matcha tea as detected in the present study. These properties form the basis for potential benefits to overall health and disease resistance, including anti-carcinogenic, anti-inflammatory, antiviral, antioxidant, and psychostimulant activities, as well as the ability to inhibit lipid peroxidation, platelet aggregation and capillary permeability, and to stimulate mitochondrial biogenesis (**Aguirre et al., 2011**). Quercetin was reported as a long-lasting anti-inflammatory substance that possesses strong anti-inflammatory capacities (**Orsolic et al., 2004**). It possesses anti-inflammatory potential that can be expressed on different cell types, both in animal and human models (**Yang et al., 2015)**. Quercetin affects immunity and inflammation by acting mainly on leukocytes and targeting many intracellular signaling kinases and phosphatases, enzymes and membrane proteins are often crucial for a cellular specific function. However, the wide group of intracellular targets and the elevated number of natural compounds potentially effective as anti-inflammatory therapeutic agents, asks for further insights and evidence to comprehend the role of these substances in animal cell biology (**Chirumbolo, 2010**).

Our finding is similar with findings imply that matcha protects against hepatic damage by inhibiting the buildup of AGE in the kidneys, lowering hepatic glucose, triglyceride, and total cholesterol levels, and acting as an antioxidant **(El-Desouky et al., 2019).** Also, **Schrier et al., (2004)** demonstrated that the polyphenols in matcha tea, particularly epigallocatechin gallate (EGCG) is a type of plant-based compound called catechins, are known to exert hepatoprotective effects by attenuating oxidative stress. Additionally, **Ohishi et al., (2016)** investigated that

matcha also significantly improved obesity-related lipid accumulation and steatosis hepatitis, because. As well as **Zhou et al., (2021)** indicated that matcha green tea effectively decrease serum ALT and AST levels in obese C57BL/6 mice. These results may be attributed to that matcha green tea beverages have a high content of bioactive compounds, (-) epigallocatechin-3-gallate (EGCG), the main bioactive component regulating inflammatory processes attenuates the development of hepatitis, by suppressing gene and protein expression of inflammatory cytokines **(Shan et al., (2008) and Mahajan et al., (2008)**.

The bioactivity of phenolic acids is characterized by its protective antioxidant mechanisms capable of breaking chain reaction of oxidation which as a result protects the cells from the attack of free radicals. It also exhibits excellent anti-inflammatory, neuroprotective, anticancer, and hypoglycemic effects (**Albuquerque et al., 2021**). This study was consistent with our results that showed that matcha Study conducted by **Xu et al., (2016)** on mice subjected to high fat diet through 4 weeks, reveals that the administration of matcha tea resulted in promotes antioxidant benefits and further increase the level of glutathione peroxidase and superoxide dismutase because of containing caffeine that exhibits potent antioxidant activity, exerts various nutraceutical benefits. Moreover, it effectively reduces oxidative stress, inhibits and neutralizes reactive oxygen species (ROS) formation, improves the activity of antioxidant enzymes and glutathione levels (**Stefanello et al., 2019**). **Fujioka et al., (2016)** found that matcha tea have a protective effect against oxygen radicals was found to be significantly higher than the effect of normal tea leaves due to increased catechin levels. Additionally, matcha is one of the major sources of catechins, can be included in the normal diet. Catechin is a natural phenolic compound known for its strong antioxidant activity. Studies have shown that the catechins obtained from tea have high free radical scavenging activity, enhanced enzyme detoxification including catalase, glutathione reductase, and glutathione peroxidase optimizing redox homeostasis of the cell (**Sakurai et al., 2020**). As well, chlorogenic acid (CGA) family members are abundant dietary phenolic acid com

pounds in plants, CGA reduces malondialdehyde (MDA) levels, CGA suppresses inflammatory response by downregulation of F4/80+ and CD68+ macrophages in the liver and adipose tissues (**Ma et al., 2015**). CGA reduces glomerular hypertrophy and proliferation and mesangial cell expansions, decreases kidney malondialdehyde (MDA) levels in the kidney of a diabetic nephropathy rat model (**Bao et al., 2018**).

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