# Effect of Matcha Tea on Fatty Liver
# in Experimental Rats


## Abstract

Fatty liver disease is characterized by a progressive pattern of intrahepatic fat accumulation that can lead to hepatocellular lipotoxicity. This study aims to show the effect of matcha tea aqueous extract on fatty liver in male rats. Forty adult male Wistar albino rats, weighing (١٥٠±١٠g) were randomly divided into four groups (١٠ rats each) as follows: **Group (١):** Comprised of normal rats as negative control group (-). **Group (٢):** Comprised of rats that received oral administration of matcha tea aqueous extract (٣٠٠ mg/kg/day). **Group (٣):** Comprised of rats injected with Oxytetracycline (١٢٠ mg/kg body weight of rat for three consecutive days) as a control positive group (+). **Group (٤):** Comprise of Oxytetracycline-induced fatty liver rats that were treated with oral matcha tea aqueous extract (٣٠٠ mg/kg/day) for six weeks. At the end of the experiment, rats were sacrificed and serum was collected for biochemical analyses. Results revealed that the administration of Oxytetracycline in the positive control group revealed a significant reduction in body weight gain. Also, matcha tea aqueous extract showed a significant reduction of total cholesterol, triglycerides, LDL-c, VLDL-c, glucose and liver functions (ALT, AST, ALP, GGT, LDH and Bilirubin) besides a rise in HDL-c, proteins, albumin and globulin compared to the positive control group. In conclusion, matcha tea exhibits a high content of antioxidants that contribute to fat reduction in fatty liver rats. So, this study recommends that the consumption of matcha tea may be beneficial for individuals who suffer from fatty liver disease.



## Keywords:

Matcha tea, fatty liver, lipid profile, liver functions, rats.

## Introduction

The liver is a critical organ in the human body that is responsible for an array of functions that help support metabolism, immunity, digestion, detoxification, and vitamin storage among other functions. It comprises around ٢٪ of an adult's body weight. The liver is a unique organ due to its dual blood supply from the portal vein (approximately ٧٥٪) and the hepatic artery (approximately ٢٥٪) **Devarbhavi *et al.*, (٢٠٢٣).** Several human diseases particularly, obesity, insulin resistance, and type ٢ diabetes were shown to be linked with the accumulation of triglyceride within the cytoplasm of hepatocytes which is collectively known as fatty liver, or steatosis **Ratziu *et al.*, (٢٠١٠)**. Where, fatty liver disease is called the silent epidemic mortality for young adults **El-Abbassy *et al.*, (٢٠٢٤)**. Where, the prevalence of fatty liver was ٣٨,٥٪ in obese Egyptian children and adolescents, and the prevalence of fatty liver in general was ٦٢,١٩٪ for males and ٥٣,٩٦٪ for females **Halaby *et al.*, (٢٠١٥).**

Recently, the term "metabolic-associated fatty liver disease" was used to rename fatty liver disease, which is a hepatic manifestation of metabolic syndrome of liver steatosis, with fat build-up occurring in over ٥٪ of hepatocytes. Non-alcoholic fatty liver disease (NAFLD) can be separated into two types of diseases: Non-alcoholic fatty liver (NAFL) with earlier stages of hepatic steatosis and worsened stage of non-alcoholic steatohepatitis (NASH). Both categories are among the most prevalent causes of liver disease globally and are increasingly becoming common causes of cirrhosis. Fatty liver disease and its more severe form, steatohepatitis (NASH), can promote the development of cirrhosis, hepatocellular carcinoma, cardiovascular disease, and type ٢ diabetes. Although lifestyle changes are critical to success, early implementation of pharmacological treatments for obesity and type ٢ diabetes are essential to treat NASH and avoid disease progression **Duggan *et al.*, (٢٠٢٢); Méndez-Sánchez *et al.*, (٢٠٢٢) and Genua & Cusi, (٢٠٢٤).** On the other hand, with the growing burden of liver dysfunction, using natural plant products is increasing because of their sturdy antioxidant contents, negligible side effects, and economic features **Elsadek and Habib, (٢٠١٨).**

Tea is one of the most popular liquids consumed by humans **Pastoriza *et al.*, (٢٠١٧)**. Due to its distinctive taste and aroma and numerous health-promoting benefits, as well as sociocultural factors, it is one of the world's favorite beverages **Luo *et al.*, (٢٠٢٢).** Tea is first used as a beverage in China and has expanded throughout the world **Hinojosa *et al.*, (٢٠٢١) and Perez *et al.*, (٢٠٢١).** According to the processing degree of leaves, teas can be classified into green tea, white tea, yellow tea, dark tea, and so on **Kochman *et al.*, (٢٠٢١).** From time immemorial, it has been in use as a health product, stimulant, and medicine for the prevention of various diseases. Antioxidant, bacteriostatic, anti-cancer, anti-obesity, anti-diabetic, anti-cardiovascular, anti-infectious, anti-neurodegenerative effects, and regulation of lipid metabolism are few outstanding health benefits of tea **Suzuki *et al.*, (٢٠١٦) and Hayakawa *et al.*, (٢٠١٨).**

So, matcha tea (*Camellia sinensis*), which in the botanical classification belongs to the tea family (*Theaceae*), the genus (*Camellia*) is a special variant of Japanese green tea grown and cultivated in a unique traditional way **Kochman *et al.*, (٢٠٢١)**. The term matcha translates to "powdered green tea" in Japanese **Bonanno *et al.*, (٢٠٢٠)**. It is a rich source of various bioactive antioxidant compounds accounting for its multiple health benefits **Wu *et al.*, (٢٠٢٢)** However, the rich antioxidant profile of matcha is mainly attributed to its unique cultivation method. During the growth period, the tea leaves are kept protected from direct sun rays by covering them with bamboo mats. Thus, this specific step enhances the synthesis and accumulation of various potent compounds like amino acids, chlorophyll, caffeine, theanine, and catechins **Bhandari *et al.*, (٢٠٢٣)**. Matcha tea is sold as a powder for direct consumption. The tea powder is increasingly used in the confectionery sector, as a food coloring and in capsule form **Sokary *et al.*, (٢٠٢٣)**. The total contents of phenols, flavonoids, flavanols and antioxidants were higher in matcha tea. Catechins account for ٩٠٪ of the polyphenolic content in matcha and are responsible for the majority of the biological activity offered by green tea. Whereas, polyphenols are considered excellent antioxidants comparable to those of vitamin E, tocopherol, carotene, and vitamin C. Matcha plays an important role as an antioxidant, anticarcinogen and anti-hypercholesterolemia. Studies confirm that matcha is one of the condensed sources of rutin and epigallocatechin-٣-gallate (EGCG) that helps in support the immune system, cardiovascular health, reduce inflammation, etc. **Devi and Bhasin, (٢٠٢٣)**. So, this study aims to investigate the effect of matcha tea on fatty liver in experimental rats.

# Materials and methods

## Materials

### Plant materials

Matcha tea (*Camellia sinensis*) was obtained from a local supplier (Abd El-Rahman Harraz, Bab El-Khalk zone, Cairo, Egypt).

### Chemicals

- Oxytetracycline (OTC) was purchased from Sigma Aldrich **(St. Louis, MO, USA).**
- Kits were used to determine total cholesterol (T.C), triglycerides (T.G), high-density lipoprotein-cholesterol (HDL-c), low-density lipoprotein-cholesterol (LDL-c), very low-density lipoprotein-cholesterol (VLDL-c), glucose, alanine aminotransferases (ALT), aspartate aminotransferases (AST),

alkaline phosphatase (ALP), gamma-glutamyl transaminase (GGT), lactate dehydrogenase (LDH), Bilirubin, proteins, albumin and globulin were obtained from Sigma Aldrich, Egypt.

## Methods

## Preparation of matcha tea aqueous extract

Matcha aqueous extract of dry powder was carried out according to the modified method of **Tan *et al.*, (٢٠٢١)**; ١٠ g of the matcha powder was added to ٥٠٠ ml of distilled water for ٧ days at ٢٥ °C in the dark medium at a stable position. Finally, the solution was filtered using a sterile syringe filter to obtain matcha extract. All samples were stored in sterile polypropylene containers until use at ٢٥ °C.

## Chemical methods

## Determination of the gross chemical composition of matcha tea powder

Moisture, ash, protein, crude fat, and crude fiber were determined according to the method outlined by **A.O.A.C., (٢٠١٠).**

## Carbohydrate content

The total carbohydrate content of the studied matcha tea powder sample was calculated by difference ١٠٠ - (moisture + ash + protein + fat + fiber) according to the method described in **A.O.A.C., (٢٠١٠).**

The caloric value was calculated according to the methods of **Seleet, (٢٠١٠).**

**Total calories** = (Fat ×9) + (Protein ×4) + (Total carbohydrates ×4).

## Antioxidant of matcha aqueous extract

## Estimation of total extract yield

The combined filtrates were transferred to a quick fit round bottom flask with known weight (W١ in grams), freeze-dried and weighted again (W٢ in grams) and finally the yield was calculated from the following formula: Extract yield (% crude herb) = (W٢ −W١)/W٣

Where,

W١ is the weight of clear and dry quick fit flask in grams,

W٢ is the weight of the flask after lypholization in grams

W٣ is the weight of the crude powdered herb in grams used in the extraction process **Ashry *et al.*, (٢٠٢١).**

## Determination of total phenolic content (TPC)

The content of total phenolic compounds in the extract was estimated spectrophotometrically by the modified Folin–Ciocalteu colorimetric method by **Jayaprakasha *et al.*, (٢٠٠٣).**

## Determination of matcha aqueous extract radical scavenging activity (RSA)

The capacity of antioxidants in the extract to quench the Diphenyl pair Picryl Hydrazyl (DPPH) radical was determined using the method of **Nogala-Kalucka *et al.*, (٢٠٠٥).**

## Determination of matcha aqueous extract of phenols content

HPLC analysis was carried out using an Agilent ١٢٦٠ series and was determined using the method of **kujala *et al.*, (٢٠٠٠).**

## Experimental design

Forty adult male Wistar albino rats (*Rattus norvegicus*), weighing (١٥٠±١٠g) were obtained from the Animal Colony, Assuit University, Egypt; the rats were kept in suitable plastic cages and maintained free access to food and water for a week before starting the experiment for acclimatization rats fed on a basal diet. They received human care in compliance with the standard institution's criteria for the care and use of experimental rats according to the ethical committee of the Faculty of Science, Al-Azhar University, Assuit, Egypt **(AZHAR ١٥/٢٠٢٣)**. After the rats were acclimatized to experimental room conditions, they were divided randomly into four groups (١٠ rats each). The groups of rats were divided as follows: **Group (١):** Comprised of normal rats as negative control group (-). **Group (٢):** Comprised of rats that received oral administration of matcha tea aqueous extract (٣٠٠ mg/kg/day). **Group (٣):** Comprised of rats injected with Oxytetracycline (١٢٠ mg/kg body weight of rat for three consecutive days) as a control positive group (+). **Group (٤):** Comprise of Oxytetracycline-induced fatty liver rats that were treated with oral matcha tea aqueous extract (٣٠٠ mg/kg/day) for six weeks.

## Body weight changes

At the beginning and the end of the experimental study, each rat was weighted; and the changes in body weight (body gain) were calculated from the following formula:

$$\textbf{Body weight gain (\%)} = \frac{\text{(Final body weight} - \text{Initial body weight)}}{\text{Initial body weight}} \times 100$$

## Biochemical determination

- Serum Total Cholesterol (T.C), Triglycerides (T.G), High-Density Lipoprotein-cholesterol (HDL-c), Low-Density Lipoprotein-cholesterol (LDL-c), Very Low-Density Lipoprotein-cholesterol (VLDL-c) and Serum glucose were determined according to **Artiss and Zak, (١٩٩٧); Cole *et al.*, (١٩٩٧); Lopes-Virella *et al.*, (١٩٧٧); Wieland and Seidel, (١٩٨٣); Lee & Nieman, (١٩٩٦) and Young, (٢٠٠١[a]);** respectively. Serum Alanine Aminotransferases (ALT), Aspartate Aminotransferases (AST), Alkaline Phosphatase (ALP), Gamma-Glutamyl Transaminase (GGT) and Lactate Dehydrogenase (LDH) were determined according to **Schumann and Klauke, (٢٠٠٣); Moss and Henderson, (١٩٩٩); IFCC, (١٩٨٣); Trinder, (١٩٦٩) and Tietz *et al.*, (١٩٨٣)**; respectively. Serum total bilirubin (T. Bilirubin); direct bilirubin (D. Bilirubin) and indirect bilirubin were determined according to **Doumas *et al.*, (١٩٧٣); Sharma and Chary, (٢٠٠٤) and Young, (٢٠٠١$^{b}$)**; respectively. Serum proteins and albumin were determined according to the method described by **Henry, (١٩٦٤).** Serum globulin was determined according to the method described by **Dumas, (١٩٧١).** The A/G ratio for each sample was obtained by dividing the albumin level by the globulin level.

## Statistical Analysis

The obtained data were statistically analyzed by SPSS computer software. Expressed as mean ± SD. Effect of different treatments analyzed by one-way (ANOVA) followed by Duncan's multiple range tests. Differences were considered significant at $p \leq$ ٠,٠٥ according to **Snedecor and Cochran, (١٩٨٦).**

## Results and discussion

### ١- Gross chemical composition and caloric value of matcha tea on a dry weight basis

Data given in **Table (١)** showed that matcha tea powder contained moisture (١,٥٢٪), ash (٨,٢٦٪) and protein (١٩,٠٩) in addition to crude fiber and crude fat (٣٩,١٥٪ and ٨,٠٠٪); respectively. Whereas, total carbohydrates and caloric value (٢٣.٩٨٪ and ٢٤٤,٢٨ k.cal./١٠٠g); respectively, our results are similar to those obtained by **Abd El Zahir and Ghaffar, (٢٠٢٣)** they noted that fiber, ash and total carbohydrates content were (٣٩,٥٠٪, ٨,٦٠٪ and ٢٤,٣٪); respectively in matcha tea powder. Also, **Kika *et al.*, (٢٠٢٤)** reported that protein and fat content were (١٧,٢٨٧٪ and ٧,٢٨٥٪); respectively. Given their unique farming and harvesting processes, the concentrations of bioactive compounds are higher in matcha tea than in other types of green tea. Matcha tea leaves are protected from sunlight before harvesting.

**Table (١): Gross chemical composition and caloric value of matcha tea powder on dry weight basis (g/١٠٠g)**

| Sample | Moisture % | Ash % | Protein % | Crude fiber % | Crude fat % | Total carbohydrates % | Caloric value (k.cal./١٠٠g) |
|---|---|---|---|---|---|---|---|
| Matcha tea powder | ١,٥٢ | ٨,٢٦ | ١٩,٠٩ | ٣٩,١٥ | ٨.٠٠ | ٢٣,٩٨ | ٢٤٤,٢٨ |

- Mean of three replicates - Carbohydrates were calculated by difference.

## ٢- Antioxidants of matcha tea aqueous extract

The yields (٦،٢ %), total phenolic content (TPC) (٢،١ mg/g), and radical scavenging activity (RSA) (٦٩,٨٪) of matcha tea aqueous extract. These results agree with **(Pannucci *et al.*, ٢٠٢٤)** they said that the aqueous extracts from matcha powders were prepared in an ultrasonic bath at ٦٠°C and ٨٠°C. All the extract's levels of antioxidant compounds and antioxidant potential were significantly high. Where labels indicate significant statistical differences (*P*< ٠،٠٥) in the total phenolic content (TPC) of matcha extracts. The radical scavenging activity (RSA) of matcha extracts was recorded (٤٢،٤٪ ± ٢،٩ and ٤٢،٥٪ ± ١،٢); respectively. And, these results disagree with **Sayuti *et al.*, (٢٠٢١)** they showed that the highest polyphenol and antioxidant content yield in matcha green tea aqueous extract was reached at a temperature of ٨٠ °C, an extraction time of ٢٠ min. and a liquid-to-solid ratio of ١٠٠ ml/g. The experimental values for total phenolics under the optimum extraction conditions were ٣١٧،٦٢±٣،٤٥ mg GAE/g and ٢٩،٢١±٠،٣٨ mg RE/g for the total flavonoids. The antioxidant activity (AA) was evaluated using ٢،٢ diphenyl-١-picrylhydrazyl (DPPH) and ٢،٢-azinobis (٣-ethylbenzothiazoline-٦-sulfonic acid) (ABTS), which showed radical scavenging activities at ٨٨،٢٨±٠،١٤٪ and ٩٠،٠٢±٠،١٤٪; respectively. Where, **Lee *et al.*, (٢٠١٣)** they reported that this can be explained by the long extraction periods and high temperatures, which will result in degradations and epimerization of catechins and lower the matcha green tea extract antioxidant activity (AA). And, as the extraction temperature decreased and the liquid-to-solid ratio increased, the (DPPH) increased. Taken together, these optimized conditions are promising to improve the total phenolic content (TPC) and antioxidant activity (AA) in matcha green tea extract. These differences may be attributable to the different growing conditions.

**Table (٢): Matcha tea aqueous extract yield (%), total phenolic content (TPC) and radical scavenging activity (RSA)**

| Parameter / Extract | Yield (%) | TPC (mg / g) | RSA (%) |
|---|---|---|---|
| **Matcha tea aqueous extract** | **٦.٢** | **٢,١** | **٦٩,٨** |

## ٣- Phenolic constituents of matcha tea aqueous extract

As shown in **Table (٣)** ١٢ phenolic compounds were identified in matcha tea aqueous extract using HPLC analysis. The compounds identified were found to include high contents of gallic acid, chlorogenic acid and rutin. While, the lowest values were Rosmarinic acid, Coumaric acid and Vanillin. These results are in agreement with **Wang *et al.*, (٢٠١٩) and Abd El Zahir & Ghaffar, (٢٠٢٣)** they reported that the data indicate using high-performance liquid chromatography, the active phenolic components of the generated matcha powder were characterized. Where matcha powder contains a high concentration of phenolic chemicals. Chlorogenic acid, gallic acid and caffeic acid were isolated from matcha the highest quantities of phenolic compounds reported for chlorogenic acid and gallic acid were ٤٧٣٠ g/g and ٤٢١g/g; respectively followed by caffeic acid. **Unno *et al.*, (٢٠١٨)** confirmed that the presence of gallic acid and caffeic acid in matcha, these acids have a greater antioxidant capacity than others. And, matcha brewed at ٩٠ °C for ١٠ minutes had the highest rutin content.

**Table (٣): Phenolic constituents of matcha tea aqueous extract using HPLC analysis**

| Parameters | Conc. (μg/g) |
|---|---|
| Gallic acid | ٢٠٣٢,٦٨ |
| Chlorogenic acid | ٤٢٥٦,٨٣ |
| Catechin | ٩٢,٤٨ |
| Methyl gallate | *N.D |
| Coffeic acid | ٤٠٢,٣٠ |
| Syringic acid | *N.D |
| Rutin | ٧٢٩,٢٥ |
| Ellagic acid | *N.D |
| Coumaric acid | ٤٠,١٠ |
| Vanillin | ٤٧,٣٨ |
| Ferulic acid | ١٢١,٦٤ |
| Naringenin | ٨٠,٤٢ |
| Rosmarinic acid | ٣٥,١٦ |
| Daidzein | ٩٨,٥٥ |
| Quercetin | ٤٤٣,٩٢ |

* N.D. Not detected

## ٤- Body weight gain

The effect of Oxytetracycline (OTC) and matcha tea aqueous extract on the rat's body weight gain(%) is shown in **Table (٤)**, These results showed that rats treated with (OTC) had a significant decrease in body weight (%); while rats treated with matcha tea aqueous extract alone showed a significant at (*p*≤٠,٠٥) changes in body weight when compared with the rats of the control group. In addition, when rats treated with (OTC) companied with matcha tea aqueous extract dose showed a significant increase in body weight when compared with the rats group treated with (OTC) alone, reflecting the protective potential of extract. These results agreement with **Abdel-Samad *et al.*, (٢٠٢١)** they indicate that oxytetracycline treatment decreased body weight, immunological parameters and increase oxidonitrositive parameters. And, these results disagree with **Xu *et al.*, (٢٠١٦)** they showed that intake of both water-soluble and water-insoluble parts of matcha was more helpful in controlling weight that was elevated by a high-fat diet (HFD). **Zhou *et al.*, (٢٠٢١)** established that dietary supplementation with matcha could effectively inhibit weight gain and fat accumulation against HFD-induced obese mouse models. Green tea and its catechins, particularly EGCG, have been shown to lower body weight, adipose tissue, and blood lipid levels. The mechanism of action of EGCG includes a decrease in energy intake, an increase in energy expenditure, as well as changes in the activities of fat, liver, muscle and intestinal cells **Singla *et al.*, (٢٠١٠) and Henao-Mejia *et al.*, (٢٠١٢).**

**Table (٤): Effect of matcha tea aqueous extract on body weight gain in experimental rats (%)**

| Groups \ Parameter | Body weight gain (%) |
|---|---|
| **Group (١): Control (-ve)** | **٤١,٦±٠,٤٣D** |
| **Group (٢): Control (-ve) + Matcha tea aqueous extract (٣٠٠ mg/kg/day)** | **٤٤,٦±٠,٥٥D** |
| **Group (٣): Control (+ve) oxytetracycline (١٢٠ mg/Kg)** | **١١,٢± ٠,٧٦A** |
| **Group (٤): Control (+ve) + Matcha tea aqueous extract (٣٠٠ mg/kg/day)** | **٣٧,١±٠,٣٨B** |

The same column means that different superscript letters are significantly different at (*p* ≤ ٠,٠٥).

## ٥- Effect of matcha tea aqueous extract on lipids profile and serum glucose

Data in **Table (٥)** revealed that positive control rats had a significant (*p*≤٠,٠٥) increases in serum levels of T.C, T.G, LDL-c VLDL-c and a significant decrease in HDL-c when compared to the negative control group. Rats that were administrated with matcha tea aqueous extract orally treated (٣٠٠ mg/kg/day) for six weeks had significant (*p*≤٠,٠٥) reduction in the elevated serum T.C, T.G, LDL-c and VLDL-c levels and an increase in serum HDL-c when compared with positive control group. These results agree with **Helal *et al.*, (٢٠١١)** they reported that Oxytetracycline injection to rats resulted in high significant increase in serum T.C, T.G and LDL-c, while HDL-c was significantly decreased. It was also induced acute pathological changes in the liver including narrowed blood sinusoidal lumina due to the enlarged, fat-laden hepatocytes together with necrosis. This finding correlates with the marked increase in serum T.C, T.G and LDL-c. The increase of these parameters in the blood is in correlation with the fatty degeneration of the liver. On the other hand, the present results were in agreement with **Noor *et al.*, (٢٠٢٤)** they found that matcha tea had the lowest cholesterol and triglyceride, LDL-c and VLDL-c levels, as well as the greatest levels of HDL-c, with significant differences. This might be explained by the fact that matcha tea contained higher fiber which could lower the levels of T.C and T.G in the blood, and also might be effective in controlling body weight, improving lipid levels and increasing the antioxidant status of mice and human beings **Sokary *et al.*, (٢٠٢٣).** Moreover, **Table (٥)** shows the effect of matcha tea aqueous extract on the glucose levels of fatty liver rats. The data suggested that the positive control group had a higher glucose level than the negative control group. Compared to the positive control group, serum glucose levels decreased significantly ($p \leq$ ٠,٠٥) in rats that were administrated with matcha tea aqueous extract. These findings are consistent with those reported by **Abd El Zahir and Ghaffar, (٢٠٢٣)**, they found that matcha tea can significantly lower blood glucose levels. Where, matcha has beneficial effects by suppressing blood glucose accumulation and promoting lipid metabolism and antioxidant activities. In addition, the majority of the dietary fiber was water-insoluble. Matcha tea was found to have a positive effect on hyperglycemia, dyslipidemia, and oxidative stress. Furthermore, in rat adipocytes, matcha green tea has been shown to increase basal and insulin-stimulated glucose absorption. The major matcha tea catechin, (-)-Epigallocatechin Gallate (EGCG), has been shown to inhibit Sodium-dependent Glucose Transporter (SGLT١) intestinal glucose uptake **Xu *et al.*, (٢٠١٦)**.

**Table (٥): Effect of matcha tea aqueous extract of serum (T.C), (T.G), (HDL-c), (LDL-c), (VLDL-c) and glucose in experimental rats**

| Parameters / Groups | T.C (mg/dl) | T.G (mg/dl) | HDL-c (mg/dl) | LDL-c (mg/dl) | VLDL-c (mg/dl) | Glucose (mg/dl) |
|---|---|---|---|---|---|---|
| **Group (١): Control (-ve)** | ١٣٩,٧ ± ٦,٢$^{A}$ | ١٥٦,٥ ± ٣,٨$^{D}$ | ٤٣,٧ ± ٠,٢$^{C}$ | ٦٤,٧ ± ٦,٩$^{A}$ | ٣١,٣ ± ٠,٧$^{D}$ | ٨٦,٨ ± ٧,٨$^{A}$ |
| **Group (٢): Control (-ve) + Matcha tea aqueous extract (٣٠٠ mg/kg/day)** | ١٣٢,٢ ± ٣,٦$^{A}$ | ١٤٦,٢ ± ٩,١$^{D}$ | ٤٣,٤ ± ٠,٥$^{C}$ | ٦١,٥ ± ٢,٢$^{A}$ | ٢٩,٢ ± ١,٨$^{CD}$ | ٨٥,١ ± ٦,١$^{A}$ |
| **Group (٣): Control (+ve) oxytetracycline (١٢٠ mg/Kg)** | ٢٧٧.٠ ±١١,٠$^{B}$ | ٣١٢,٦ ±١٨,٠$^{A}$ | ٣٨,٠ ± ٠,٢$^{A}$ | ١٧٦,٣ ±١٢,٩$^{B}$ | ٦٢,٥ ± ٣,٦$^{A}$ | ١٢٩,٤ ± ٩,٨$^{B}$ |
| **Group (٤): Control (+ve) + Matcha tea aqueous extract (٣٠٠ mg/kg/day)** | ١٣٢,٠ ±٧,٣$^{C}$ | ١٦٧,٤ ± ٢,٧$^{B}$ | ٤٥,٢ ± ٠,٢$^{B}$ | ٤٥,٧ ± ٨,٨$^{CD}$ | ٣٣,٥ ± ٠,٥$^{B}$ | ٩٧,٩ ± ٤,٩$^{C}$ |

The same column means that different superscript letters are significantly different at ($p \leq$ ٠,٠٥).

## ٦- Effect of matcha tea aqueous extract on serum liver functions

Results in **Table (٦)** showed that serum levels of ALT, AST, ALP, GGT, LDH and Bilirubin significantly increased ($p \leq$ ٠,٠٥) in the positive control group when compared to the negative control group. Rats that were administrated with matcha tea aqueous extract orally treated (٣٠٠ mg/kg/day) for six weeks had a significant reduction (*p*≤ ٠,٠٥) in serum levels of ALT, AST, ALP, GGT, LDH and Bilirubin. Where, treated rats with Oxytetracycline for three consecutive days caused fatty liver, necrosis and inflammation. These histological changes were associated with a significant increase in activities of serum ALT, AST, ALP, GGT and LDH. This is in agreement with **Asha *et al.* (٢٠٠٧)**, they reported that this significant increase may be due to a rise in free radicals decrease in the antioxidant enzyme levels. Moreover, **Zhou *et al.*, (٢٠٢١)** confirmed that the administration with an aqueous extract of matcha tea effectively decreased serum AST, ALT and ALP activities, as found in the present study, these results agree with **Hunjadi *et al.*, (٢٠٢١)** they obtained that the test group of white rabbits obtained a standard diet supplemented with matcha green tea led to reduce of GGT and LDH compared with the control group. Also, **Karmakar *et al.*, (٢٠١١)** showed that treatment of all ٣٢ teas significantly reduced the elevated bilirubin level induced by alcohol in fatty liver rats. This is due to matcha tea supplementation effectively preventing excessive visceral and hepatic lipid accumulation, elevated blood glucose, dyslipidemia, abnormal liver function, and steatosis hepatitis **Li *et al.*, (٢٠٢٠)**. The hepatoprotective activities of matcha tea are attributed to its catechins that scavenge ROS/RNS in liver samples, Matcha tea treatment decreased the activity of lipid droplet-associated proteins and increased the activity of cytidine dehydrogenase **Hasanein *et al.*, (٢٠١٢).**

## Table (٦): Effect of matcha tea aqueous extract on liver functions in experimental rats

| Parameters / Groups | ALT (U/L) | AST (U/L) | ALP (U/L) | GGT (U/L) | LDH (U/L) | Bilirubin | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Total Bilirubin (mg/dL) | Direct Bilirubin (mg/dl) | Indirect Bilirubin (mg/dl) |
| Group (١): Control (-ve) | ٧٧,٨ ± ٣,١$^{A}$ | ١٠٧,٠ ± ٤,٧$^{D}$ | ٢٧٢,٢ ± ٢٦.٧$^{C}$ | ٦,٠٠ ± ٠,٤$^{C}$ | ٢٠٧٥ ± ٥٩,٤ | ٠,١٣ ± ٠.٠٠٤$^{A}$ | ٠,٠٣ ± ٠,٠٠٨$^{D}$ | ٠,٠٨ ± ٠,٠٢$^{D}$ |
| Group (٢): Control (-ve) + Matcha tea aqueous extract (٣٠٠ mg/kg/day) | ٧٥,٥ ± ٣,٢$^{A}$ | ١٠٥,٤ ± ٣,٨$^{CD}$ | ٢٦٨,٢ ± ١٣,٦$^{C}$ | ٥,٨ ± ٠,٣$^{CD}$ | ١٩٣٣± ٨٤,٩$^{D}$ | ٠,١٣± ٠,٠٢$^{A}$ | ٠,٠٣ ± ٠,٠٠٤$^{D}$ | ٠,٠٧ ± ٠,٠١$^{D}$ |
| Group (٣): Control (+ve) oxytetracycline (١٢٠ mg/Kg) | ١٢٤,٤ ± ٣,٤$^{B}$ | ٢١٦,٢ ± ١٠,٤$^{A}$ | ٣٣٧,٦ ± ١٤,٧$^{A}$ | ١٢,٤ ± ١,٨$^{A}$ | ٢٧٧٦ ± ٢٦٧,٦$^{A}$ | ٠,٨٠ ± ٠.٠٧$^{B}$ | ٠,١٦ ± ٠,٠١٥$^{A}$ | ٠,٦٤ ± ٠,٠٦$^{A}$ |
| Group (٤): Control (+ve) + Matcha tea aqueous extract (٣٠٠ mg/kg/day) | ٨٠,٢ ± ٣,٥$^{C}$ | ١١٨,٠٨ ± ٣,٩$^{B}$ | ٢٤٣,٢ ± ١٥.٣$^{BC}$ | ٧,٠ ± ٠,٧$^{B}$ | ١٨٠٤ ± ٨٧,٣$^{B}$ | ٠,١٨± ٠,٠٣$^{CD}$ | ٠,٠٢± ٠,٠٠٦$^{B}$ | ٠,٠٧ ± ٠,٠٢$^{B}$ |

The same column means that different superscript letters are significantly different at (*p* ≤ ٠,٠٥).

## ٧- Effect of matcha tea aqueous extract on protein status in experimental rats

The obtained data in **Table (٧)** showed that serum levels of total protein, albumin and globulin significantly decreased (*p*≤٠,٠٥), while the A/G ratio was non-significant in the positive control group when compared to the negative control group. Rats that were administrated with matcha tea aqueous extract orally treated (٣٠٠ mg/kg/day) for six weeks had a significant increase (*p*≤٠,٠٥) in serum levels of total protein, albumin and globulin, while the A/G ratio had non- a significant. Our results are in keeping with those of **Helal *et al.*, (٢٠١١)** they obtained that this decrease in the positive control group could be due to hepatic dysfunction and decreased protein synthesis. Also, it may be related to the damage of vital biological processes or changes in the permeability of the liver, kidney and other tissue cells leading to leakage of protein via the kidney. On the other hand, **Ramez *et al.*, (٢٠٢١) and Aljaghmani *et al.*, (٢٠٢٤)** they mentioned that the study uncovered notable disparities in the levels of total protein, albumin, and globulin among the groups. Matcha supplementation helped maintain higher levels of these proteins, indicating better preservation of liver function. Where, it contains a high amount of polyphenols, specifically epigallocatechin gallate (EGCG). The EGCG in matcha has antioxidant and anti-inflammatory effects likely contribute to the protective effects.

**Table (٧): Effect of matcha tea aqueous extract on serum total protein, albumin and globulin in experimental rats**

| Groups \ Parameters | Total protein (g/dl) | Albumin (g/dl) | Globulin (g/dl) | A/G ratio |
|---|---|---|---|---|
| Group (١): Control (-ve) | ٨,٣ ± ٠,٢$^{A}$ | ٤,٣ ± ٠,٠٢$^{D}$ | ٤,٠ ± ٠,٢٢$^{A}$ | ١,٠ ± ٠,٠٧$^{A}$ |
| Group (٢): Control (-ve) + Matcha tea aqueous extract (٣٠٠ mg/kg/day) | ٨,٥ ± ٠,١$^{A}$ | ٤.٣ ± ٠,١٠$^{D}$ | ٤,١ ± ٠,١$^{A}$ | ١,٠ ± ٠,٠٢$^{A}$ |
| Group (٣): Control (+ve) oxytetracycline (١٢٠ mg/Kg) | ٦,٠ ± ٠,٠٩$^{B}$ | ٣,٣ ± ٠,٠٨$^{A}$ | ٢,٧ ± ٠,٠٧$^{B}$ | ١,٢ ± ٠,٠٤$^{B}$ |
| Group (٤): Control (+ve) + Matcha tea aqueous extract (٣٠٠ mg/kg/day) | ٨,٢ ± ٠,٠٩$^{C}$ | ٤,٥ ± ٠,١$^{B}$ | ٣,٧ ± ٠,١$^{C}$ | ١,٢ ± ٠,٠٢$^{C}$ |

The same column means that different superscript letters are significantly different at (*p* ≤ ٠,٠٥).

## Conclusion

In conclusion, the present findings illustrate that matcha tea aqueous extract consumption led to an improvement in the biochemical changes resulting from fatty liver. These therapeutic effects of matcha tea aqueous extract can be attributed to their antioxidant properties due to their containing phenolic compounds.

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# تأثير شاي الماتشا على الكبد الدهني في فئران التجارب


**المستخلص العربي**

يتصف مرض الكبد الدهني بنمط تدريجي من تراكم الدهون داخل الكبد والذي يمكن أن يؤدي إلى سمية دهنية للخلايا الكبدية. تهدف هذه الدراسة إلى إظهار تأثير المستخلص المائي لشاي الماتشا على الكبد الدهني لدى ذكور الفئران. تم تقسيم أربعين من ذكور فئران ويستار البيضاء البالغة، بوزن (١٥٠ ± ١٠ جم) عشوائياً إلى أربع مجموعات (١٠ فئران لكل منها) على النحو التالي: **المجموعة (١)**: تتألف من فئران سليمة كمجموعة ضابطة سالبة، **المجموعة (٢)**: تتألف من الفئران التي تم إعطائها المستخلص المائي لشاي الماتشا عن طريق الفم (٣٠٠ مجم / كجم / يوم)، **المجموعة (٣)**: تتألف من فئران تم حقنها بأوكسي تتراسيكلين (١٢٠ مجم / كجم من وزن جسم الفئران لمدة ثلاثة أيام متتالية) كمجموعة ضابطة موجبة، **المجموعة (٤)**: تتألف من فئران الكبد الدهني الناجم عن أوكسي تتراسيكلين والتي عولجت بالمستخلص المائي لشاي الماتشا عن طريق الفم (٣٠٠ مجم / كجم / يوم) لمدة ستة أسابيع. في نهاية التجربة، تم ذبح الفئران وجمع السيرم للتحليل الكيميائي. أظهرت النتائج أن إعطاء الأوكسي تتراسيكلين للمجموعة الضابطة الموجبة أدى إلى إنخفاض كبير في زيادة وزن الجسم. كما أظهر المستخلص المائي لشاي الماتشا إنخفاضاً معنوياً في الكوليسترول الكلي والدهون الثلاثية والبروتين الدهني منخفض الكثافة و البروتين الدهني منخفض الكثافة جداً والجلوكوز وإنزيمات الكبد (ALT ، AST، ALP، GGT، LDH والبيليروبين) وإرتفاعاً في HDL والبروتينات والألبومين والجلوبيولين مقارنة بالمجموعة الضابطة الموجبة. وفي الختام، أظهر شاي الماتشا إرتفاع محتواه من مضادات الأكسدة التي ساهمت في خفض الدهون للفئران المصابة بالكبد الدهني. لذلك، توصي الدراسة بأن إستهلاك شاي الماتشا قد يكون مفيداً للأفراد الذين يعانون من مرض الكبد الدهني.



**الكلمات المفتاحية:**

شاي الماتشا، الكبد الدهني، دهون الدم، وظائف الكبد، الفئران.