



# Biochemical and Nutritional Studies on the Effect of Red Pitaya, Matcha Green Tea and Spirulina Algae on Obesity and Immunity Using Male Albino Rats

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**ABSTRACT:**

This work investigated the effect of red pitaya, matcha tea, Spirulina algae, and a mixture on enhancing immunity and reducing obesity in male albino rats. Sixty adult male rats were divided into two main groups; the first was control negative (C-; n=6) fed on a basal diet (BD). The second main group was split into nine groups (n=6 for each group), and the other main group (54 rats) was fed with diet-induced obesity for 8 weeks then classified into nine groups; group (2) fed on diet-induced obesity as positive control; groups (3,4,5,6,7,8,9 and 10) fed on diet-induced obesity containing plus 2.5 & 5% pitaya powder, 2.5 & 5% Spirulina algae powder, 2.5 & 5% matcha tea powder and 2.5 & 5% Mix of theme by equal parts, respectively. At the end of the experiment, after a week, collected blood serum samples were analyzed for liver enzymes, serum glucose, lipid profile, and immunoglobulins. The data collected displayed that HFD caused significant increases in BWG, FI, FER, TC, TG, LDLc, VLDLc, liver enzymes, serum glucose, and immunoglobulins. At the same time, consumption of red pitaya, matcha tea, and Spirulina algae and the mixture showed a significantly reduced serum HDLc. Conclusion: It can be recommended that the consumption of red pitaya, spirulina algae, matcha green tea and their combination in diets have obesity-reducing effects, which plays a safer role in treating obesity and preventing its complications.

**Keywords:** Dragon fruit, *Arthrospira platensis*, *Camellia sinensis*, weight loss, lipids

## 1. INTRODUCTION

Obesity is a global epidemic that poses major health risks and is regarded as a social problem. Morbidity indicators have shown a steady increase due to obesity. It

is the most common health issue affecting people of all ages and causes several of complications, including type 2 diabetes, chronic heart disease, and stroke [1]. The body's natural defense mechanism

against a wide range of illnesses and conditions is referred to as immunity. Among vertebrates, the immune system is incredibly complex and advanced, with the ability to produce an infinite number of cells and molecules to combat a wide range of infections and harmful substances [2].

One popular variety of pitaya fruit is red pitaya (*Hylocereus polyrhizus*, red pulp with pink peel), sometimes referred to as dragon fruit. Total polyphenols, betacyanins, and amino acids have all been found to be more concentrated in pitaya seeds and peels than in pulp; on the other hand, anthocyanins, or cyanidin 3-glucoside, delphinidin 3-glucoside, and pelargonidin 3-glucoside, were only found in pulp extracts. Red pitaya fruits contain bioactive substances, particularly betacyanin, which has been shown to enhance lipid profiles, control glycaemic response, reduce the risk of diabetes, and modify the immune system [3]. Spirulina is a type of prokaryotic, multicellular, filamentous blue-green algae (BGA). Another name for it is a cyanobacterium. It belongs to the group of algae called blue-green algae. When compared to other food sources, the amount of total fat and cholesterol found in these algae is significantly lower, even though they contain a high concentration of nutrients like proteins, vitamins, minerals, and fatty acids—in particular, the essential omega-3 and omega-6 fatty acids. Furthermore, Spirulina contains a high concentration of bioactive substances like

polysaccharides, phenols, and phycocyanin pigment, all of which are involved in various biological processes [4]. Because of its numerous biological activities and high concentration of natural nutrients, Spirulina is beneficial for nutrition and plays a significant role in enhancing immunity [5]. Spirulina may help in weight control by inhibiting the migration of macrophages into visceral fat, stopping the buildup of fat in the liver, lowering the body's levels of oxidative stress, and enhancing satiety and insulin sensitivity [6].

Japanese matcha is a kind of traditional-grown powdered green tea. Plants that are shaded during their growth phase produce more biologically active compounds, such as theanine, caffeine, chlorophyll, and different kinds of catechins. Matcha is the most condensed form of the four main catechins found in green tea: (–)-epicatechin (EC), (–)-epicatechin-3-gallate (ECG), (–)-epigallocatechin (EGC), and (–)-epigallocatechin-3-gallate (EGCG). The latter is the most active and abundant. Matcha provides many remarkable benefits, including anti-inflammatory, antioxidant, antidiabetic, blood pressure lowering, neuroprotection, anticancer, cell repair, and energizing effects. These benefits are primarily attributable to the presence of numerous bioactive substances in matcha [7].

Therefore, this study was carried out to evaluate the possible effects of red

pitaya, Spirulina algae, matcha green tea and their mix of all against obesity in male rats

## 2. MATERIALS AND METHODS

### 2.1. MATERIALS

#### 2.1.1 Red Pitaya, and Spirulina Algae

Red pitaya, and spirulina algae obtained from Ministry of Agriculture farms, Giza, Egypt. While matcha green tea purchased from hypermarkets, Cairo, Egypt.

#### 2.1.2 Experimental Animals

Sixty adult male rats weighing approximately  $150 \pm 10$  g were used. The animals were bought from the Ministry of Health's Vaccine and Immunity Organization at Helwan University in Egypt.

#### 2.1.3 Casein

Casein as main source of protein obtained from Morgan Company, Cairo, Egypt.

#### 2.1.4 Vitamin mixture and salt mixture

Vitamin mixture and salt mixture were purchased from El-Gomhoriya Company, Cairo, Egypt.

#### 2.1.5 Chemical kits.

Al-Gomhoria Company for Trading in Chemical, Drug, and Medical Equipment, Cairo, Egypt, supplied the chemical kits that were used to determine the lipid fractions, renal biomarkers, liver activity, and glucose.

## 2.2 METHODS:

### 2.2.1 Obesity induction:

Feeding on a high-fat diet (20% sheep fat) in addition to the basal diet, and using

them as positive control group, caused obesity in normally healthy male albino rats for four weeks according to [8].

### 2.2.2 Preparation of plants:

Every component was ground into a fine powder using an electric grinder and stored in dark, cork-sealed glass bottles in a cool, dry place until needed [9].

### 2.2.3 Experimental and animal models design:

In this study, sixty adult male rats weighing approximately  $150 \pm 10$  g were used. The animals were bought from the Ministry of Health's Vaccine and Immunity Organization at Helwan University in Egypt. The experiment was carried out at the Experimental Animal Research Unit, Faculty of Medicine, Ain Shams University, Cairo, Egypt. Rats were maintained under normal, safe conditions in wire cages at  $25^\circ\text{C}$ . Each rat was split up into two main groups. All rats were fed on basal diet for one-week before starting the experiment for acclimatization. After one week period, the rats were divided into two main groups, the first group (Group 1 contain 6 rats) still fed on basal diet and the other main group (54 rats) was feed with diet-induced obesity for four weeks and then classified into nine equal groups as follow: group (2), fed on diet-induced obesity as a positive control; groups (3,4,5,6,7,8,9and 10) fed on diet-induced obesity containing plus 2.5 &5% pitaya powder, 2.5 &5%, Spirulina alage powder , 2.5 &5% matcha tea powder and 2.5 & 5% mix of them by equal parts,

respectively. Herbs were added to diet by replacing fiber of diet. All rats were weighted each week to calculate the body weight gain (RWG).

Following the experiment, body weight gain (BWG) and blood serum samples were preserved for additional biochemical analyses.

#### 2.2.4 Biochemical blood analysis:

At the end of the experiment, the rats were first scarified under ether anesthesia, and blood samples were taken via the abdominal aorta following a 12-hour fast. Samples of blood were placed in sterile, dry centrifuge tubes, allowed to clot at room temperature, and then centrifuged for ten minutes at 3000 rpm to extract the serum. In order to prepare the serum for biochemical analysis, it was carefully divided, placed in sanitized cuvette tubes, and frozen at -20 °C described by [10].

The following techniques were used to measure the serum levels of total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL-c) by using the methods of [11], [12] and [13] respectively and also, the measurements of low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) were made using the equations explained by [14] as follows:

$$\text{VLDL-c (mg/dl)} = \text{Triglycerides}/5$$

$$\text{LDL-c (mg/dl)} = \text{Total cholesterol} - \text{HDL-c} - \text{VLDL-c.}$$

Serum glucose is determined according to [15]. The serum levels of alkaline phosphatase (ALP) and aspartate aminotransferase (AST) and alanine

aminotransferase (ALT) were measured using the techniques described in [16] and [17]. Immunoglobulin's (IgG, IgM and IgA) levels were determined by single radial immunodiffusion [18].

#### 2.2.5 Histopathological examination :

Small specimens from kidneys were collected from all experimental groups, fixed in 10% neutral buffered formalin, dehydrated in ascending concentration of ethanol (70, 80 and 90%) cleared in xylene and embedded in paraffin. Sections of (4-6)  $\mu\text{m}$  thickness were prepared and stained with Hematoxylin and Eosin according to [19].

#### 2.2.6 Statistical Analysis:

Using a computerized Costat program, a one-way ANOVA was used to statistically analyze the data. The findings are shown as mean  $\pm$  SD. Treatment differences that were statistically significant at ( $P \leq 0.05$ ) [20].

### 3. RESULTS AND DISCUSSION

Data presented in table (1) demonstrated the mean value of body weight gain (BWG), food intake (FI) and feed efficiency ratio (FER) of obese rats fed on different diets by feeding on red pitaya, Spirulina algae, matcha tea and combination (Mix) at levels of 2.5 and 5% in diets.

It is clear to notice that due to obesity BWG was raised in control positive group. As mainly obesity increased the metabolism of food leading to increase of rats weight. Meanwhile, Feeding on suggested plants reduced the BWG of

rats, which was a target of present work. The increase of pitaya in diet raised the weight loss. Combination of all tested diet raised the weight loss. In then common best group was that of the mix 5% indicating maximum weight loss.

It was found that due to obesity consumed feed increased, which may be expected, since more cells are to be fed by the body. Meanwhile due to feeding on diet containing tested plants, FI markedly reduced. In this connection FI was highest for control (-ve) group and lowest for mix 5% diet.

It is obviously by obesity FER increased. This may be expected since FI was increased and BWG raised indirection possible increase of appetite. Meanwhile when feeding on diets containing the targeted tested plants, FER decreased, in the following descending order: Mix followed by pitaya, Spirulina algae and matcha tea. This indicated that best

group was that of Mix 5% diet, followed by pitaya 5%, Spirulina algae 5% and matcha tea 5%, possibly due to synergistic action occurs for those tested plants. According to [21] a high-fat diet containing 0.05% Matcha (MMD) decreased body weight gain because of its high fiber content, which was more beneficial for weight control. [22], who investigated that red dragon fruit flesh (*Hylocereus costariensis*) reduced body weight gain in obese rats fed on high fat diet. It was demonstrated that an extract from dragon fruit flesh exhibited hypolipidemic and anti-obesity biological effects. Consuming extract from dragon fruit flesh could increase the concentration of fat and cholesterol in feces by binding fat and cholesterol from the feed as well as cholesterol from the liver, which was excreted into the intestine and subsequently into the feces..

**Table (1): Nutritional indicators of negative control and obese rats treated by consumption with red pitaya, Spirulina algae, matcha tea and their mixture**

| Groups              | Parameters | BWG (g/d)      | FI (g/d/rat) | FER        |
|---------------------|------------|----------------|--------------|------------|
| G1: Control -ve     |            | 1.4±0.0b       | 15.6±0.13e   | 0.1±0.0b   |
| G2: Control +ve     |            | 1.679±0.0011a  | 17.0±0.25a   | 0.1±0.0a   |
| G 3: Pitaya 2.5%    |            | -0.723±0.0025g | 15.4±0.20f   | -0.05±0.0g |
| G 4: Pitaya 5%      |            | -0.857±0.0003h | 15.1±0.10g   | -0.06±0.0h |
| G 5: Spirulina 2.5% |            | -0.536±0.0043d | 15.8±0.00d   | -0.03±0.0d |
| G 6: Spirulina 5%   |            | -0.679±0.0005f | 15.0±0.0gh   | -0.05±0.0f |
| G7: Matcha 2.5%     |            | -0.429±0.0015c | 16.6±0.02b   | -0.03±0.0c |
| G8: Matcha 5%       |            | -0.571±0.0002e | 16.3±0.08c   | -0.04±0.0e |
| G9: Mix 2.5%        |            | -1.000±0.0002i | 14.8±0.01h   | -0.07±0.0i |
| G10: Mix 5%         |            | -1.107±0.0008j | 14.9±0.0gh   | -0.07±0.0j |
| LSD                 |            | 0.003          | 0.200        | 0.000      |

Significant differences ( $P \leq 0.05$ ) exist between the values in each column containing different letters. \*BWG: Body weight gain, FI: food intake and FER: Feed efficiency ratio.

Also, [23] reported that supplementing with *Spirulina* significantly promotes weight loss, particularly in obese people. Additionally, [24] found that in obese anemic rats, dried green algae (*Spirulina platensis*) decreased (FI), (BWG%), and (FER).

The results of table (2), revealed the levels of liver enzymes (ALT, AST, ALP) of obese rats as affected by feeding of obese rats on pitaya, *Spirulina* algae, matcha tea and combination (Mix) at levels of 2.5 and 5% in diets. Results of this table indicated the activity of liver enzymes increased significantly due to obesity. Nevertheless by feeding on pitaya, *Spirulina* algae, matcha tea and combination (Mix) of all plant diets the liver activity reduced significantly in obese rats provided that at the level combination (5%) the decrease was pronounced. Moreover activity of different groups could be managed descending in variable groups as the following: Mix, pitaya, *Spirulina* and matcha. Accordingly best group may that of Mix 5%, indicating the synergistic action. Suggested plant diets proved to be excellent means to cope with obesity side effects, mainly the liver disorder. Supplementing with matcha tea effectively prevented abnormal liver function, high blood glucose, dyslipidemia, steatosis hepatitis, and excessive visceral and hepatic lipid accumulation. [25] discovered that matcha tea reduced serum AST and ALP in rats fed a high fat diet (HFD). Matcha treatment increased the activity of cytidine dehydrogenase and decreased

the activity of lipid droplet-associated proteins, according to RNA sequencing analyses of differentially expressed genes in liver samples. According to [26], matcha tea at doses of 2, 4, and 6% decreased the AST and ALT in obese rats. Also, [27], who show that *Spirulina* administered orally at a dose of 800 mg/kg b.wt/day reduced serum ALT, AST, and ALP activities because *Spirulina* has a potent antioxidant activity and activates an enzyme system that scavenges free radicals. Because fresh red pitaya peel has higher levels of phenolic compounds and betanins, the most prevalent type of betacyanin, and shows superior antioxidant properties compared to fruit flesh. [28] observed that by regulating oxidative stress, hepatic TLR4–MyD88 pathway, and lipid metabolism, fresh red pitaya peel supplements can lower inflammation and hepatic steatosis. In accordance with [29], the liver enzymes (AST, ALT, and ALP) of diabetic rats were lowered by pitaya flesh juice at doses of 5 and 7.5 mg/kg BW and pitaya peel powder at doses of 2.5 and 5%.

The results displayed in table (3) show the serum glucose of obese rats as affected by feeding on 2.5 and 5% of pitaya, *Spirulina* algae, matcha and Mix diets. It is obvious that due to obesity serum glucose raised significantly from 104.5 to 191.5 mg/dl. Biological reference internals for serum glucose: Normal 70-64 mg/dl, Pre diabetic 141-200 mg/dl, Diabetic > 200 mg/dl. So control (+) being (191.5 mg/dl) means diabetes. Diabetes mellitus is one of the side effects



of obesity. When feeding on diets containing pitaya, Spirulina algae, matcha tea and combination (Mix) at levels of 2.5 and 5% serum glucose reduced. Best group was that of the mix 5% showing rats of normal glucose level (70-140 mg/dl). [30], who demonstrated that the pitaya fruit's dietary fiber serves to lessen the rate at which food is broken down in the intestines, which lowers the production of

blood glucose. When used as a non-pharmacological therapy, red pitaya fruit has been demonstrated by [30] to be effective in lowering blood glucose levels in patients with type 2 diabetes mellitus (T2DM). This study has demonstrated that red pitaya fruit can help lower blood glucose levels in individuals with type 2 diabetes mellitus (T2DM).

**Table (2): Some liver function parameters of negative control and different experimental obesity rats group at the end of the study**

| Groups              | Parameters | Liver enzymes (U/L) |             |           |
|---------------------|------------|---------------------|-------------|-----------|
|                     |            | ALT                 | AST         | ALP       |
| G1: Control -ve     |            | 56.6±0.02d          | 59±1.11g    | 174±1.19h |
| G2: Control +ve     |            | 78±1.16a            | 190.5±2.25a | 306±1.35a |
| G 3: Pitaya 2.5%    |            | 55.5±0.003e         | 83±0.99d    | 201±0.8h  |
| G 4: Pitaya 5%      |            | 51.5±0.08g          | 76±0.9e     | 179±0.02g |
| G 5: Spirulina 2.5% |            | 56.5±0.11d          | 89.5±1.34d  | 245±2.6d  |
| G 6: Spirulina 5%   |            | 53±0.4f             | 77.5±0.99e  | 210±1.23e |
| G7: Matcha 2.5%     |            | 63±0.55b            | 90.5±2.16c  | 248±0.25c |
| G8: Matcha 5%       |            | 61.5±0.06c          | 102.5±0.8b  | 250±0.8b  |
| G9:Mix 2.5%         |            | 53.5±0.88f          | 64±0.38f    | 169±0.25i |
| G10: Mix 5%         |            | 40.5±0.02h          | 62.5±0.3f   | 160±0.04j |
| LSD                 |            | 0.87                | 2.18        | 1.93      |

Significant differences ( $P \leq 0.05$ ) exist between the values in each column containing different letters. \*AST: Aspartate aminotransferase, ALT: Alanine transaminase and ALP: Alkaline phosphatase.

In the study conducted by [31], administering methanolic extract of *S. platensis* at doses of 10 and 15 mg/kg BW produced an anti-hyperglycemic effect by reducing the raised blood sugar level. Potential mechanisms include improved insulin secretion from  $\beta$ -cell islets or improved blood glucose transportation to peripheral tissue in diabetic rats treated with Spirulina [32]. [33], who discovered that Spirulina decreased serum glucose at levels of 2.5, 5, and 7% in rats that were

hyperglycemic due to streptozotocin (STZ)-induced renal impairment. The findings of [25], matcha tea at doses of 2, 4, and 6% lowered the blood glucose levels of obese rats. Matcha's EGCG may have the ability to improve insulin sensitivity and inhibit gluconeogenesis as well as the gastrointestinal tract's absorption of lipids and glucose [34]. The results table (4) indicated that due to obesity TC and TG increased while HDL declined. Therefore obesity was damaging

for the lipids profile. Meanwhile feeding on diets containing pitaya, Spirulina algae, matcha and Mix of all reversed such changes. Best group seems to be diet of mix 5%, where serum recorded lowest TC and TG, and highest HDL.

**Table (3): Fasting levels of serum glucose of negative control and obesity rats treated by red pitaya, Spirulina algae, matcha tea and their mixture at the end of the study**

| Groups               | Mean $\pm$ SD     |
|----------------------|-------------------|
| G 1 : Control (- Ve) | 104.5 $\pm$ 1.11h |
| G 2: Control (+Ve)   | 191.5 $\pm$ 0.99a |
| G 3: Pitaya 2.5%     | 106.5 $\pm$ 0.08g |
| G 4: Pitaya 5%       | 100.5 $\pm$ 1.02i |
| G 5: Spirulina 2.5%  | 137 $\pm$ 0.55c   |
| G 6: Spirulina 5%    | 121.5 $\pm$ 0.03e |
| G7: Matcha 2.5%      | 139.5 $\pm$ 0.36b |
| G8: Matcha 5%        | 125 $\pm$ 0.8d    |
| G9: Mix 2.5%         | 110 $\pm$ 0.33f   |
| G10: Mix 5%          | 98.5 $\pm$ 0.93j  |
| LSD                  | 1.33              |

Significant differences ( $P \leq 0.05$ ) exist between the values in each column containing different letters.

It could be observed that VLDL followed the levels of TG, being increased by obesity induction, and decreased when obese rats fed on pitaya, Spirulina, matcha and the MIX diets. LDL was also highest for C (+) group and decreased when feeding on pitaya, Spirulina, matcha and the Mix diets. Best group seems to be that of the MIX 5% diet.

Red pitaya ingestion was shown to lower TC, TG, and LDL levels while raising HDL levels in type 2 diabetics, according to Song et al., [35].

According to [36] adding pitaya peel powder to diet might help avoiding hyperlipidemia because to its composition's benefits: increased levels of antioxidants, phenol, and tocotrienol (vitamin E) in particular reduce plasma total cholesterol and LDL-cholesterol concentrations as well as liver cholesterol levels. 69.30% total dietary fiber, or 56.50% insoluble food fiber and 14.82% soluble food fiber, is found in high concentration in the peel. This fiber can enhance insulin sensitivity, promote satiety, and help reduce energy intake by trapping cholesterol and bile acids in the small intestine. [37] and [38] discovered that Spirulina treatment significantly improved the lipid profile. According to the current study's findings, taking a Spirulina supplement was linked to a lower blood fat concentration [39], [40] and [41].

[42], reported that consuming green tea reduces TC and LDL cholesterol in people who are both normal weight and overweight/obese, but not HDL cholesterol or triglycerides. Nevertheless, more carefully planned trials with a wider range of participants and a longer time frame are required. Green tea catechins have been demonstrated to dramatically lower plasma triglyceride, total cholesterol (TC), and low-density lipoprotein (LDL) cholesterol levels in both in vitro and animal studies [43].



**Table (4): Fasting levels of some serum lipid patterns of negative control and obesity rats treated by red pitaya, Spirulina algae, matcha tea and their mixture at the end of experimental period**

| Parameters         | Lipids Profile (mg/dl) |           |            |            |            |
|--------------------|------------------------|-----------|------------|------------|------------|
|                    | TC                     | TG        | HDLc       | VLDLc      | LDLc       |
| G1:Control –ve     | 115.5±0.44g            | 105±0.11h | 90.1±0.02a | 21±0.09e   | 4.4±0.03g  |
| G2:Control +ve     | 198±0.02a              | 165±0.09a | 57±0.03h   | 33±0.21a   | 88±0.08a   |
| G 3: Pitaya 2.5%   | 119±0.07f              | 108±0.97e | 88±0.07c   | 21.6±0.08e | 9.4±0.06f  |
| G 4: Pitaya 5%     | 111±0.02h              | 105±0.18f | 89±0.08b   | 21±0.10e   | 1.0±0.005h |
| G 5: Spirulina2.5% | 125±0.5d               | 115±0.16c | 70.4±0.05e | 23±0.09c   | 31.6±0.08d |
| G 6: Spirulina 5%  | 120±0.56e              | 111±0.05d | 70.8±0.41d | 22.2±0.02d | 27±0.07e   |
| G7:Matcha 2.5%     | 136±0.41b              | 120±0.19b | 60±0.08g   | 24±0.31b   | 52±0.02b   |
| G8:Matcha 5%       | 133±0.17c              | 115±0.14c | 65.5±0.03f | 23±0.07c   | 44.5±0.03c |
| G9 :Mix 2.5%       | 110.4±0.45i            | 106±0.06g | 89±0.09b   | 21.2±0.19e | 0.2±0.001i |
| G10: Mix 5%        | 110.2±0.96i            | 100±0.02h | 90±0.01a   | 20±0.026f  | 0.2±0.001i |
| LSD                | 0.78                   | 0.56      | 0.24       | 0.57       | 0.13       |

Significant differences ( $P \leq 0.05$ ) exist between the values in each column containing different letters. \*TC: Total cholesterol, TG: Triglycerides, HDLc: High density lipoprotein, LDLc: Low density lipoprotein and VLDLc: Very low density lipoprotein

Data presented in table (5) show the levels of IgG, IgM & IgA ( $\mu\text{g/ml}$ ) of obese rats as affected by feeding on pitaya, Spirulina algae, matcha and mixture of all at 2.5 & 5% level.

It is evident that by obesity IgG was raised. it is known that 75% of antibodies in blood belongs to IgG. This increase indicates the risk of diseases and inflammation. Accordingly the feeding of obese rats, on pitaya, Spirulina, matcha and specially the MIX 5% diet significantly lowered the level of IgG compared to C (+) group.

It is clear that due to obesity rats may be at risk of inflammation, while feed obese rats on diets containing pitaya, Spirulina, matcha and the MIX diet showed the lowest. This indicated that the mentioned diet raised the immunity of obese rats.

It could be noticed that by obesity the IgA increased meaning the obese rats are at risk of microbe's infection, inflammations

and possibly Covid-19. This indicate the loss in immunity, but the reverse was recorded when feeding obese rats on pitaya, Spirulina, matcha and particular the MIX 5% diet. This reflection showed that obese rats restored much of their immunity.

Particularly, the oligosaccharides found in dragon fruit (pitaya) have prebiotic effects on gut health. Beneficial microorganisms ferment prebiotics to produce short-chain fatty acids (SCFA), which lower the pH of the colon. It has been demonstrated that products of the fructan type inhibit the growth of dangerous microorganisms [44] and also influence the immune system, resulting in T cells' reduced ability to proliferate in response to nonspecific mitogens and viruses. [45], T cell formation is accompanied by a decrease in lymphokines, an increase in serum IgG and IgA, but not serum IgM; a decrease in the titer of antibodies to particular

foreign antigens, an increase in autoantibodies, and a decline in the cell-mediated immune response with age [46]. [47], suggested that immune changes in IgG and IgM caused by aflatoxin B1 could

be reversed by supplementation of *Spirulina platensis* extract in mice.[48], indicated that green tea leaves powder reduced IgG and IgM in broiler chickens.

**Table (5): Some immunity Parameters of negative control and obese rats treated by consumption with red pitaya, *Spirulina* algae, matcha tea and their mixtur**

| Groups              | Parameters | Immunity Parameters ( $\mu\text{g/ml}$ ) |                 |                 |
|---------------------|------------|--|-----------------|-----------------|
|                     |            | IgG                                      | IgM             | IgA             |
| G1: Control -ve     |            | 245 $\pm$ 0.41d                          | 25 $\pm$ 0.11i  | 271 $\pm$ 0.2c  |
| G2: Control +ve     |            | 810 $\pm$ 0.52a                          | 246 $\pm$ 0.16a | 450 $\pm$ 0.21a |
| G 3: Pitaya 2.5%    |            | 272 $\pm$ 0.47cd                         | 125 $\pm$ 0.22e | 221 $\pm$ 0.19g |
| G 4: Pitaya 5%      |            | 270 $\pm$ 0.41cd                         | 112 $\pm$ 0.14f | 187 $\pm$ 0.23h |
| G 5: Spirulina 2.5% |            | 288 $\pm$ 0.5c                           | 135 $\pm$ 0.9b  | 250 $\pm$ 0.28f |
| G 6: Spirulina 5%   |            | 275 $\pm$ 0.49cd                         | 130 $\pm$ 0.25d | 264 $\pm$ 0.3e  |
| G7: Matcha 2.5%     |            | 305 $\pm$ 0.58b                          | 134 $\pm$ 0.12c | 280 $\pm$ 0.17b |
| G8: Matcha 5%       |            | 280 $\pm$ 0.4c                           | 130 $\pm$ 0.19d | 270 $\pm$ 0.25d |
| G9 :Mix 2.5%        |            | 300 $\pm$ 0.76b                          | 110 $\pm$ 0.23g | 180 $\pm$ 0.32i |
| G10: Mix 5%         |            | 250 $\pm$ 0.43d                          | 92 $\pm$ 0.13h  | 170 $\pm$ 0.18j |
| LSD                 |            | 8.50                                     | 0.57            | 0.40            |

Significant differences ( $P \leq 0.05$ ) exist between the values in each column containing different letters. \*IgG: Immunoglobulin G, IgA: Immunoglobulin A and IgM: Immunoglobulin M.

## Histopathological Results:

### A- Histopathological Examination of Liver:

Light microscopic examination of liver sections of rats from group 1 (control -) showed the normal histological architecture of hepatic lobule (Photos 1). In contrast, liver of rats from group 2 (control+) showed hepatocytes degenerating ballooning, the portal triad fibroplasia, and the creation of newly formed bile ductules (Photo 2). On the other hand, liver of rats from group 3 (Pitaya 2.5%) showed cystic bile duct dilatation and portal triad fibroplasia (Photo 3). Meanwhile, liver of rats from group 4 (Pitaya 5%) described slight

fibroplasia in the portal triad and mild hydropic degeneration of the hepatocytes (Photo 4). However, the liver of rats from group 5 (Spirulina 2.5%) had only a slight proliferation of Kupffer cells (Photo 5). Furthermore, hepatic tissue of rats from group 6 (spirulina 5%) revealed slight proliferation of Kupffer cells and slight congestion of central vein (Photo 6). Moreover, liver of rats from group 7 (matcha 2.5%) showed slight activation of Kupffer cells (Photo 7). Furthermore, liver of rats from group 8 (matcha 5%) exhibited slight hydropic degeneration of some hepatocytes (Photo 8). Meanwhile, liver sections from group 9 (MIX 2.5%) showed slight hydropic degeneration of

certain hepatocytes and slight activation of Kupffer cells (Photo 9). On the other hand, some sections from group 10 (MIX 5%) revealed minimal fibroplasia in the

portal triad, focal hepatocellular necrosis linked to inflammatory cell infiltration, and minimal Kupffer cell activation (Photo 1).

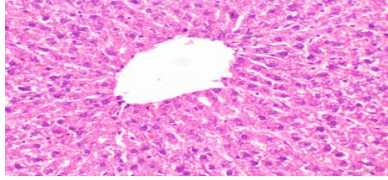


Photo (1): Photomicrograph of liver of rat from group 1 (control -) displaying the hepatic lobule's typical histological architecture (H & E X 400).

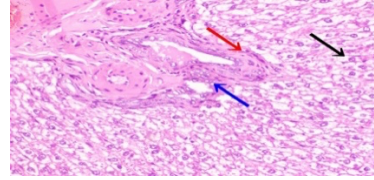


Photo (2): Photomicrograph of liver of rat from group 2 (control +) showing fibroplasia in the portal triad (red arrow), formation of newly formed bile ductules (blue arrow), and ballooning degeneration of hepatocytes (black arrow) (H & E X 400).

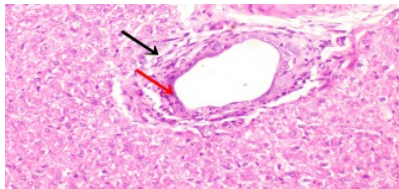


Photo (3): Photomicrograph of liver of rat from group 3 (Pitaya 2.5%) demonstrating cystic bile duct dilatation (red arrow) and fibroplasia in the portal triad (black arrow). (H & E X 400).

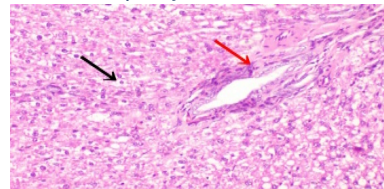


Photo (4): Photomicrograph of liver of rat from group 4 (Pitaya 5%) showing mild fibroplasia in the portal triad (red arrow) and mild hydropic degeneration of the hepatocytes (black arrow). (H & E X 400).

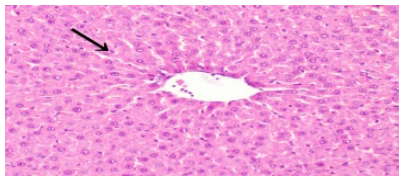


Photo (5): Photomicrograph of liver of rat from group 5 (Spirulina 2.5%) showing slight proliferation of Kupffer cells (arrow) (H & E X 400).

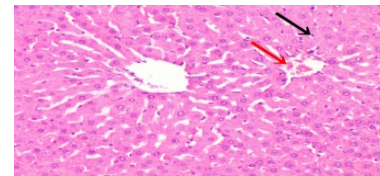


Photo (6): Photomicrograph of liver of rat from group 6 (spirulina 5%) showing slight proliferation of Kupffer cells (black arrow) and slight congestion of central vein (red arrow) (H & E X 400).

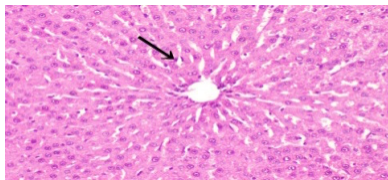


Photo (7): Photomicrograph of liver of rat from group 7 (matcha 2.5%) showing slight activation of Kupffer cells (black arrow) (H & E X 400).

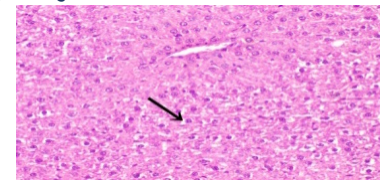


Photo (8): Photomicrograph of liver of rat from group 8 (matcha 5%) showing slight hydropic degeneration of some hepatocytes (black arrow) (H & E X 400).

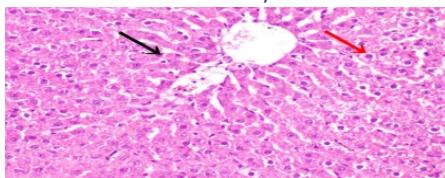


Photo (9): Photomicrograph of liver of rat from group 9 (MIX 2.5%) showing slight activation of Kupffer cells (black arrow) and slight hydropic degeneration of some hepatocytes (red arrow) (H & E X 400).

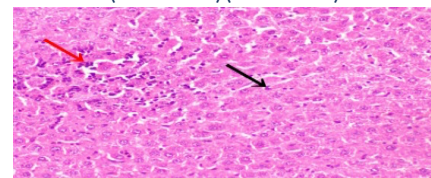


Photo (10): Photomicrograph of liver of rat from group 10 (MIX 5%) showing slight activation of Kupffer cells (black arrow) and focal hepatocellular necrosis associated with inflammatory cells infiltration (red arrow) (H & E X 400).



## B- Histopathological examination of kidneys:

Light microscopic examination of kidneys of rats from group 1 (control -) revealed the normal histological structure of renal parenchyma (Photo 11). On contrary, kidneys of rats from group 2 (control +) showed proteinaceous cast in the lumen of renal tubules, congestion of renal blood vessel, periglomerular and inflammatory cells infiltration (Photo 12). Meanwhile, kidneys of rats from group 3 (Pitaya 2.5%) exhibited no histopathological alterations (Photo 13). Furthermore, kidneys of rats from group 4 (Pitaya 5%) described no histopathological alterations (Photo 14) except congestion of renal blood vessels

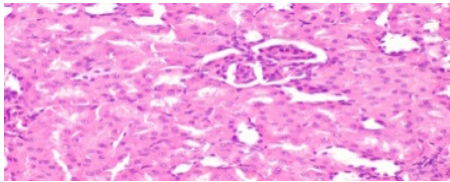


Photo (11): Photomicrograph of kidney of rat from group 1 (control -) showing the normal histological structure of renal parenchyma (H & E X 400).

in some sections (Photo 15). Otherwise, renal tissues of rats from group 5 (spirulina 2.5%) showed vacuolization of epithelial lining some renal tubules (Photo 16). On the other hand, kidneys of rats from group 6 (spirulina 5%) and some sections from group 7 (matcha 2.5%) revealed no histopathological alterations (Photo 17&18), whereas, other sections from group 7 (matcha 2.5%) showed proteinaceous cast in the lumen of some renal tubules (Photo 19). Moreover, kidneys of rats from groups 8 (matcha 5%), 9 (MIX 2.5%) & 10 (MIX 5%) revealed no histopathological alterations (Photo 20&21).

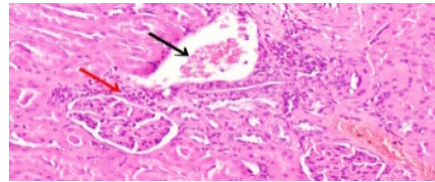


Photo (12): Photomicrograph of kidney of rat from group 2 (control+) showing congestion of renal blood vessel (black arrow) and periglomerular inflammatory cells infiltration (red arrow) (H & E X 400).

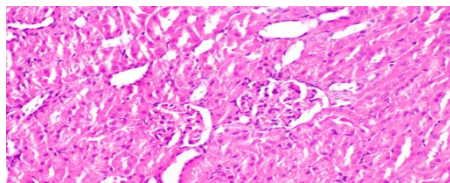


Photo (13): Photomicrograph of kidney of rat from group 3 (Pitaya 2.5%) showing no histopathological alterations (H & E X 400).

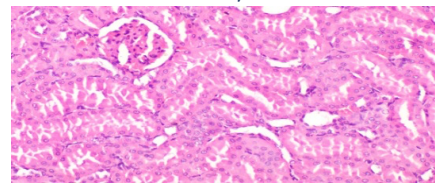


Photo (14): Photomicrograph of kidney of rat from group 4 (Pitaya 5%) showing no histopathological alterations (H & E X 400).

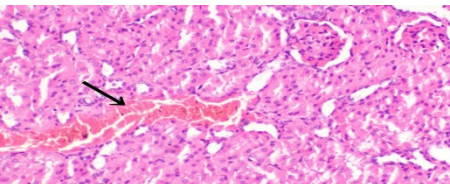


Photo (15): Photomicrograph of kidney of rat from group 4 (Pitaya 5%) showing congestion of renal blood vessel (H & E X 400).

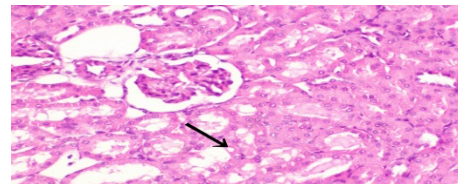


Photo (16): Photomicrograph of kidney of rat from group 5 (spirulina 2.5%) showing vacuolization of epithelial lining some renal tubules (black arrow) (H & E X 400).

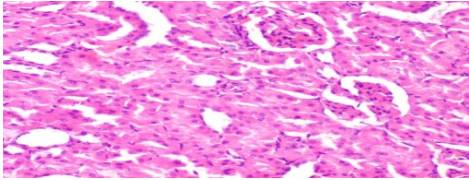


Photo (17): Photomicrograph of kidney of rat from group 6 (spirulina 5%) showing no histopathological alterations (H & E X 400).

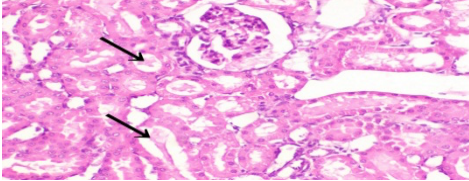


Photo (19): Photomicrograph of kidney of rat from group 7 (matcha 2.5%) showing proteinaceous cast in the lumen of some renal tubules (black arrow) (H & E X 400).

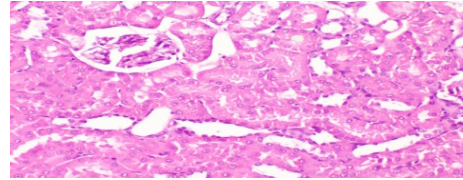


Photo (18): Photomicrograph of kidney of rat from group 7 (matcha 2.5%) showing no histopathological alterations (H & E X 400).

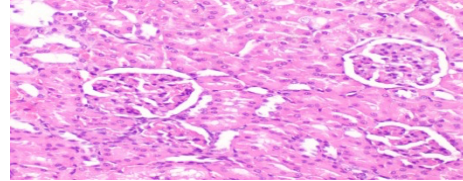


Photo (20): Photomicrograph of kidney of rat from group 8 (matcha 5%) showing no histopathological alterations (H & E X 400).

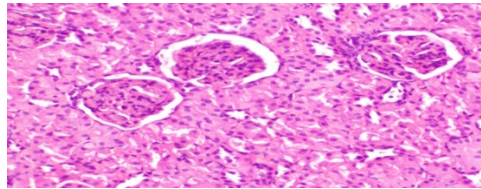


Photo (21): Photomicrograph of kidney of rat from group 9 and 10 (MIX 2.5 and 5%) showing no histopathological alterations (H & E X 400).

#### 4. CONCLUSION

Based on the findings, the 5% blend of pitaya, Spirulina, and matcha tea demonstrated the most favorable results. This combination notably enhanced the levels of TG, TC, LDL, VLDL, IgA, IgM, IgG, liver enzymes, and serum glucose in obese rats. Consequently, a 5% mixture of pitaya, Spirulina, and matcha tea holds potential for bolstering immunity and mitigating obesity.

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## دراسات بيوكيميائية وتغذوية حول تأثير البتايا الوردي وشاي الماتشا الأخضر وطحلب السبيرولينا علي السمنة والمناعة باستخدام ذكور الفئران البيضاء

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|---|--|
| <b>الملخص العربي:</b>   | <b>نوع المقالة</b>   |
| الهدف من هذه الدراسة هو تقدير تأثير البتايا الحمراء وشاي الماتشا وطحلب السبيرولينا وخليطهم على تعزيز المناعة وتقليل السمنة لدى ذكور الفئران البيضاء. تم تقسيم ستين من الفئران الذكور البالغين إلى مجموعتين رئيسيتين؛ الأولى هي المجموعة الضابطة السالبة التي تحتوي على ستة فئران تم تغذيتها على نظام غذائي أساسي وتم تغذية بقية الفئران وعددهم أربعة وخمسون فأر على نظام غذائي عالي الدهون لمدة أربع أسابيع. وبعد إصابتهم بالسمنة تم تقسيم المجموعة الثانية إلى تسع مجموعات؛ المجموعة الثانية: الضابطة الموجبة، المجموعة الثالثة والرابعة: التي تتغذى على نظام غذائي يحتوي على البتايا الوردي بنسبة (5&2.5%)، المجموعة الخامسة والسادسة: التي تتغذى على نظام غذائي يحتوي على طحلب السبيرولينا بنسبة (5&2.5%)، المجموعة السابعة والثامنة: التي تتغذى على نظام غذائي يحتوي على شاي الماتشا بنسبة (5 و 2.5%)، و المجموعة التاسعة والعاشر: التي تتغذى على نظام غذائي يحتوي على خليطهم بنسبة متساوية (5 و 2.5%) على التوالي. واثناء التجربة تم تقدير كل من الوزن المكتسب والمتناول من الطعام وفي نهاية التجربة، تم تحليل عينات سيرم الدم لقياس انزيمات الكبد، ونسبة الجلوكوز، ومستوى الدهون، والجلوبيولينات المناعية. أظهرت النتائج أن النظام الغذائي العالي الدهون تسبب في الزيادة في الوزن والمتناول من الطعام والكوليستيرول الكلي والدهون الثلاثية والليبوبروتينات منخفضة الكثافة، إنزيمات الكبد، والجلوبيولينات المناعية، بينما انخفض مستوي الليبوبروتين مرتفع الكثافة. بينما أظهرت النتائج عن استهلاك البتايا الحمراء وشاي ماتشا وطحالب سبيرولينا وخليطها تحسن معنوي في التحليل المختلفة. الخلاصة: يمكن التوصية بأن استهلاك البتايا الحمراء وطحلب سبيرولينا وشاي الماتشا الأخضر. ومزيجها في الوجبات الغذائية لها تأثيرات مخفضة للسمنة وتقوية المناعة. | بحوث اصلية   |
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|   | <b>الاستشهاد الي:</b>  |
|   | Awad et al., 2024,   |
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