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دور مستخلصات طحالب السبيرولينا والكلوريلا في تخفيف سمية الكبد، واختلال توازن المؤكسدات ومضادات الأكسدة، واضطرابات مستوى الدهون الناتجة عن العلاج بحمض الفالبرويك في الأرانب

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الخلاصة :

هدفت هذه الدراسة إلى تحديد قدرة مستخلصات السبيرولينا والكلوريلا على الحد من تلف الكبد، واختلال التوازن التأكسدي، واضطرابات مستوى الدهون في الدم الناجمة عن حمض الفالبرويك. أُجريت التجربة على 36 أنثى بالغة من الأرانب المحلية، قُسّمت إلى ست مجموعات، بواقع 6 أرانب في كل مجموعة. تلقت المجموعة الأولى محلول ملحي عادي، وكانت بمثابة المجموعة الضابطة. تلقت المجموعة الثانية حمض الفالبرويك بجرعة 400 ملغم/كغم. عُولجت المجموعة الثالثة بمستخلص السبيرولينا فقط بجرعة 500 ملغم/كغم. تلقت المجموعة الرابعة حمض الفالبرويك بجرعة 400 ملغم/كغم مع مستخلص سبيرولينا بجرعة 500 ملغم/كغم. عولجت المجموعة الخامسة بمستخلص كلوريلا فقط بجرعة 500 ملغم/كغم. تلقت المجموعة السادسة حمض الفالبرويك بجرعة 400 ملغم/كغم مع مستخلص كلوريلا بجرعة 500 ملغم/كغم. أُعطيت جميع العلاجات عن طريق الفم لمدة 21 يومًا. تم قياس إنزيمات الكبد في الدم، بما في ذلك ناقلة أمين الأسبارتات، وناقلة أمين الألانين، والفوسفاتاز القلوي. كما تم تقييم مؤشرات الدهون، بما في ذلك الكوليسترول الكلي، والدهون الثلاثية، والبروتين الدهني عالي الكثافة، والبروتين الدهني منخفض الكثافة، والبروتين الدهني منخفض الكثافة جدًا. تم تقييم مؤشرات الإجهاد التأكسدي، بما في ذلك مالونديالدهيد، وديسموتاز الفائق، والجلوتاثيون المختزل، والكاتالاز، باستخدام طرق المقايسة المناعية الإنزيمية المرتبطة. أظهرت النتائج زيادة ملحوظة في إنزيمات الكبد، والكوليسترول الكلي، لوحظ انخفاض ملحوظ في مستويات الدهون الثلاثية، والبروتين الدهني منخفض الكثافة، والبروتين الدهني منخفض الكثافة جدًا، والمالونديالدهيد، مع انخفاض ملحوظ في مستويات البروتين الدهني عالي الكثافة، وإنزيم ديسموتاز الفائق، والجلوتاثيون المختزل، وإنزيم الكاتالاز في مجموعة حمض الفالبرويك مقارنةً بالمجموعة الضابطة. كما أظهرت المجموعات المعالجة بسبيرولينا وكلوريلا تحسنًا ملحوظًا في جميع المؤشرات. في الختام، تُظهر مستخلصات سبيرولينا وكلوريلا تأثيرات وقائية ضد سمية حمض الفالبرويك على الكبد، والإجهاد التأكسدي، واضطرابات الدهون.

الكلمات المفتاحية: سبيرولينا، كلوريلا، حمض الفالبرويك، إنزيمات الكبد، الدهون، المالونديالدهيد، مضادات الأكسدة، الأرانب.

The role of *spirulina* and *chlorella* algae extracts in alleviating hepatotoxicity, oxidants-antioxidants status imbalances, and lipid profile Disorders resulting from valproic acid treatment in rabbits

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Abstract

This study aimed to determine the ability of *Spirulina* and *Chlorella* extracts to reduce liver damage,oxidative stress imbalance and lipid profile disturbances induced by valproic acid.The experiment was conducted on 36 adult female local rabbits divided into six groups,6 rabbits per group.The first group received normal saline solution and served as the control group. The second group received valproic acid at a dose of 400 mg/kg.The third group was treated with *Spirulina* extract only at a dose of 500 mg/kg. The fourth group received valproic acid at 400 mg/kg combined with *Spirulina* extract at 500 mg/kg.The fifth group was treated with *Chlorella* extract only at a dose of 500 mg/kg.The sixth group received valproic acid at 400 mg/kg combined with *Chlorella* extract at 500 mg/kg.All treatments were administered orally for 21 days.Serum liver enzymes including aspartate aminotransferase,alanine aminotransferase and alkaline phosphatase were measured.Lipid profile parameters including total cholesterol,triglycerides, high density lipoprotein,low density lipoprotein, and very low density lipoprotein were also evaluated.Oxidative stress markers including malondialdehyde,superoxide dismutase,reduced glutathione and catalase were assessed using enzyme linked immunosorbent assay methods.The results showed a significant increase in liver enzymes,total cholesterol, triglycerides,low density lipoprotein,very low density lipoprotein,and malondialdehyde levels,with a significant decrease in high density lipoprotein, superoxide dismutase, reduced glutathione, and catalase in the valproic acid group compared with the control group.*Spirulina* and *Chlorella* treated groups showed marked improvement in all parameters.In conclusion, *Spirulina* and *Chlorella* extracts exert protective effects against valproic acid induced hepatic toxicity,oxidative stress and lipid profile alterations.

Keywords: *Spirulina*, *Chlorella*, Valproic acid, liver enzymes, lipid profile, MDA, Antioxidants, rabbits.

Introduction

Valproic acid is classified as one of the essential drugs used in the treatment of epilepsy and psychiatric disorders. However, its long-term use is associated with severe adverse effects, especially in females, as many studies indicate its role in

causing a disruption in reproductive hormones, liver dysfunction, and its stimulation of oxidative stress, which leads to cell destruction and a disruption of the body s lipid profile (Nanau and Neuman, 2013) The study of microalgae has gained importance in recent years because they are considered natural materials and a source of biochemical compounds. (Chun *et al*.,2011). Microalgae biomass presents remarkable commercial interest in the food, nutraceutical, cosmetic and pharmaceutical fields due to its high value-added bioactive components (Khan *et al*., 2018). The discovery of high levels of natural antioxidants in microalgae has led the food industry to use microalgae-derived materials as a source of natural antioxidants instead of synthetic ones. (Azamanet *et al*., 2017).

Antioxidants are substances that reduce the effects of free radicals, which are compounds that damage living cells and may lead to their death. (Venableset *et a*l., 2008).

Spirulina is a free-floating filamentous microalgae with the helical properties of its filaments. It is officially called Arthrosporia powder and is related to the class of cyanobacteria with the ability to photosynthesize (Sapp, 2005) *Spirulina* is known for its wide-ranging biological and antioxidant activities (Miranda et al., 1998), so spirulina is considered a valuable source of antioxidants such as phycocyanin, carotenoids, and phenolic compounds (Ismaielet *et al*., 2016). These biologically active compounds in *Spirulina* reduce the risk of chronic diseases such as cardiovascular diseases, inflammatory diseases, type 2 diabetes, and cancer (Rodriguez *et al*., 2018).

Chlorella is a single-celled green algae. Therefore studies have shown that *Chlorella* cells contain a variety of nutrients and bioactive compounds that enhance human health and prevent some diseases (Rani *et al*., 2018 ; Ru *et al*., 2020) *Chlorella* supplements have been shown to have an effect antioxidant, antidiabetic, immunomodulatory, antihypertensive, and antihyperlipidemia effects (Fallah *et al*., 2018 ; Bito *et al*., 2020) . *Chlorella* contains secondary metabolites such as flavonoids and phenols that have antioxidant activity (Meshram *et al*., 2013 ; Adhoni *et al*., 2016). *Chlorella* is also high in fatty acids that can act as antioxidant compounds (Putri *et al*., 2021) .

y of Spirulina and Chlorella extracts This study aimed to determining the abilit
antioxidants status imbalances and adverse -to reduce liver damage, oxidants
.effects on the lipid profile resulting from valproic acid (VPA) treatment

Materials and Methods

Preparation of the aqueous extract of *spirulina* and *Chlorella:*

The cold aqueous extract of *spirulina* and *Chlorella* algae was prepared by taking 50 grams of dried algae powder and adding 500 ml of distilled water to it and placing the mixture in a magnetic stirrer for 24 hours, then filtering it using filter paper, then pouring the liquid after filtration into Petri dishes and leaving it to dry at laboratory temperature. Then the algae extract was collected in sterile opaque glass containers and placed in the refrigerator at a temperature of (4) ₀ C until use (Harborn, 1984) .

Depakine (valproic acid)

Depakine tablets were powdered, dissolved in distilled water, and administered orally to animals in the form of an emulsion.

Animals :

In this experiment, 36 adult female local rabbits were used and randomly divided into six groups as following: the first group received normal saline solution (0.9% sodium chloride, 5 ml/animal) as a control; the second group received valproate (400 mg/kg BW)(Elwakkad *et al.*, 2008), the third group was treated with *Spirulina* extract only (500 mg/kg ,BW) the fourth group received valproate (400 mg/kg BW) and *Spirulina* extract (500 mg/kg ,BW), the fifth group was treated with *Chlorella* extract only (500 mg/kg); and the sixth group received valproic acid (400 mg/kg) and *Chlorella* extract (500 mg/kg). All materials in this experiment were given orally via a gavage tube daily for 21 days.

Blood collectionand laboratory tests

After the experimental period, the animals were dissected and blood was collected using a fine needle (5 cc) via cardiac puncture. The collected blood samples were placed in a gel tube for blood collection and allowed to coagulate at room temperature. They were then separated by centrifugation for 15 minutes at 3000 rpm. The serum was then collected and stored in a plain tube at -20°C until use. Liver enzymes (aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP)) levels and lipid profile (cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol) were assessed for all groups using the Cobas integra 400 plus (Roche-Germany), oxidants-antioxidants system (lipid

peroxidation marker (MDA), Superoxide dismutase **(SOD),** reduced (GSH), and Catalase **(CAT)**) levels were assessed for all groups by ELISA technique (using kit, KIAZIST, Iran).

Statistical analysis:

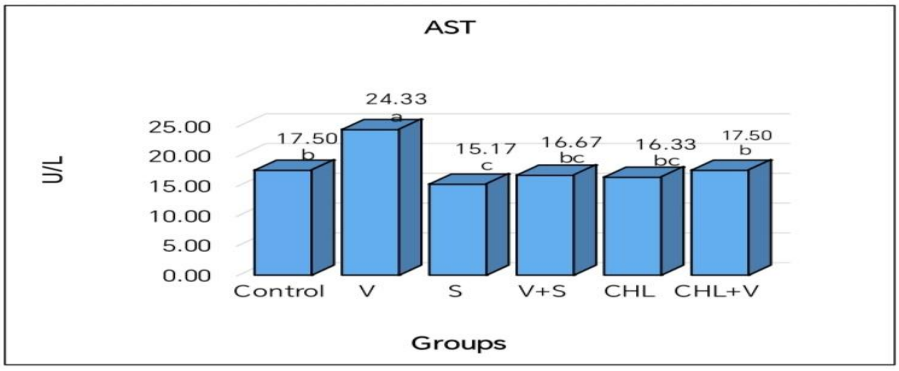
The results were represented as mean ± standard deviation (M±SD), and the experiments were analyzed using one-way analysis of variance (ANOVA) with SPSS and the LSD test, with a p-value <0.05.

Results

1: Liver enzymes

1- Aspartate aminotransferase (AST)

The findings indicated that AST levels were significantly elevated ($p < 0.05$) in the valproic acid–treated group relative to the control and all other experimental groups. In contrast, no statistically significant differences ($p < 0.05$) were observed among the valproic acid combined with *Spirulina* (500 mg/kg), *Chlorella* (500 mg/kg), and valproic acid combined with *Chlorella* (500 mg/kg) groups, nor when compared with the control group. Conversely, treatment with *Spirulina* (500 mg/kg), valproic acid combined with *Spirulina* (500 mg/kg), and *Chlorella* (500 mg/kg) resulted in significant variations when compared with the remaining groups (Figure 1).

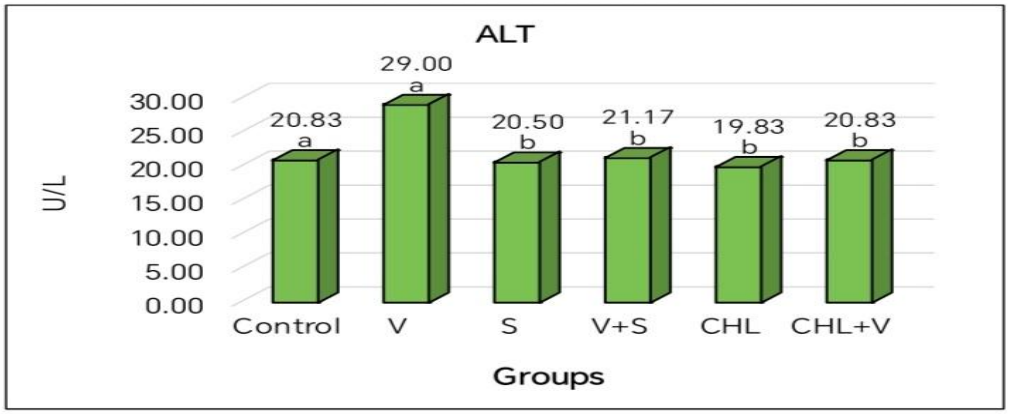


a-c Different letters indicate significant difference (P< 0.05)

Figure 1: Effect of *Spirulina* and *Chlorella* extracts on AST level in female rabbits treated with valproic acid .

2- Alanine aminotransferase (ALT)

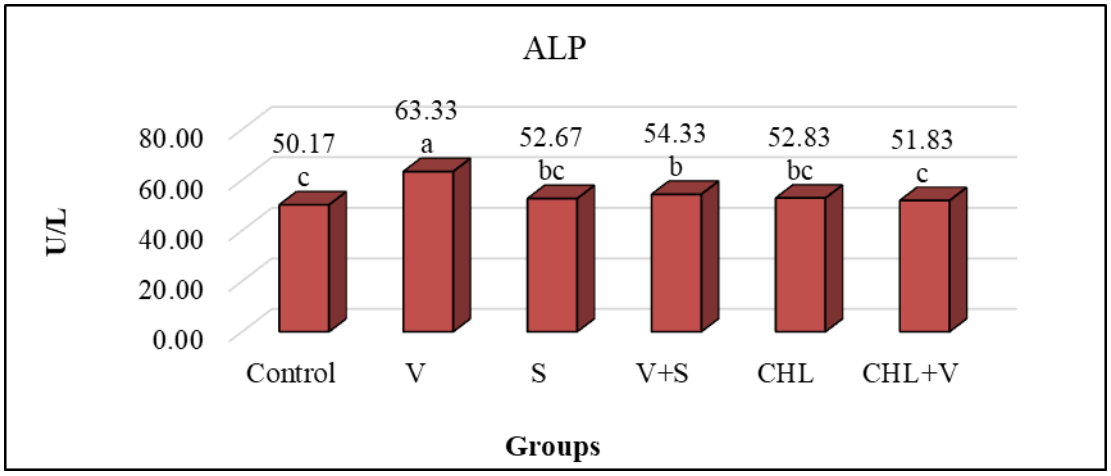
Figure 2 show a significant increase ($p<0.05$) in the level of ALT in the valproic acid group compared to the control group and the other groups. However, the *Spirulina* group (500 mg/kg), the valproic acid with *Spirulina* group (500 mg/kg), the *Chlorella* group (500 mg/kg), and the valproic acid with *Chlorella* group (500 mg/kg) show no significant difference ($p<0.05$) among themselves or compared to the control group **(figure 2).**



a-bDifferent letters indicate significant difference (P< 0.05

Figure 2: Effect of *Spirulina* and *Chlorella* extracts on ALT level in female rabbits treated with valproic acid .

3- **Alkaline phosphatase (ALP)**Analysis of the data revealed a statistically significant elevation ($p < 0.05$) in ALP activity in the valproic acid–treated group when compared with the control and all remaining experimental groups. Conversely, no significant differences ($p < 0.05$) were detected among the



Spirulina (500 mg/kg), *Chlorella* (500 mg/kg), and valproic acid combined with *Chlorella* (500 mg/kg) groups, nor relative to the control group. In contrast, administration of *Spirulina* (500 mg/kg), valproic acid combined with *Spirulina*

(500 mg/kg), and *Chlorella* (500 mg/kg) produced significant variations in ALP levels compared with the other groups **(Figure 3).**

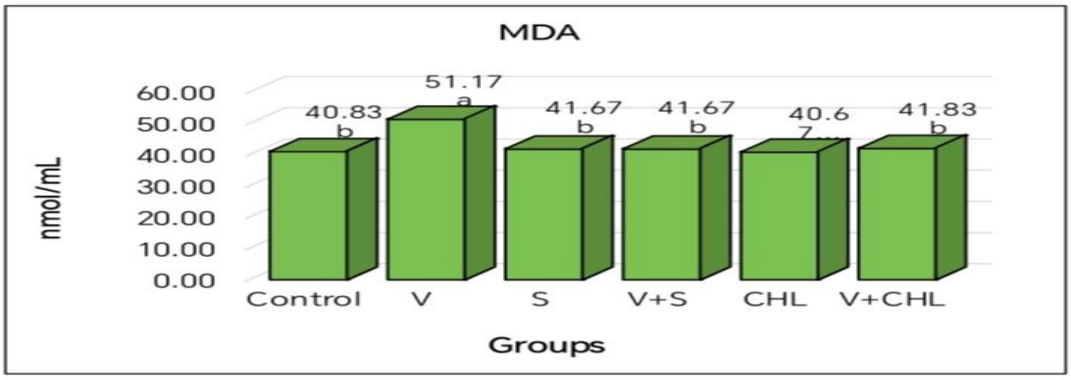
a–c Different letters indicate significant difference (P< 0.05)

Figure 3: Effect of *Spirulina* and *Chlorella* extracts on ALP level in female rabbits treated with valproic acid .

2- **oxidants-antioxidants system**

2.1 lipid peroxidation marker (MDA)

The results of this study showed a significant increase (p<0.05) in MDA levels in the valproic acid group compared to the control group and the other groups. The other groups show no significant difference (p<0.05) among themselves or compared to the control group **(figure 4) .**

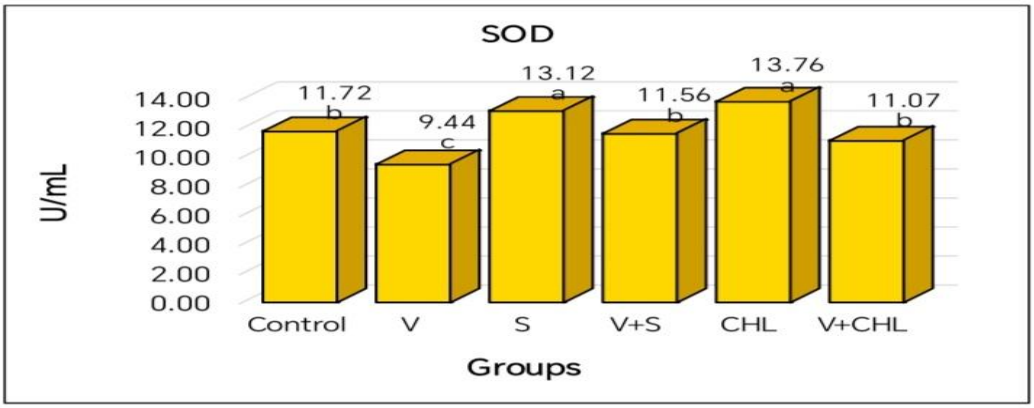


a–b Different letters indicate significant difference (P< 0.05)

Figure 4 : Effect of *Spirulina* and *Chlorella* extracts on MDA level in female rabbits treated with valproic acid .

2.2 Superoxide dismutase (SOD)

The results of this study showed a significant decrease (p<0.05) in SOD levels in the valproic acid group compared to the control group and other groups. However, the valproic acid with *Spirulina* (500 mg/kg) and valproic acid with *Chlorella* (500 mg/kg) groups show no significant difference (p<0.05) among themselves or compared to the control group. In contrast, the *Spirulina* (500 mg/kg) and *Chlorella* (500 mg/kg) groups showed a significant increase (p<0.05) compared to the control group and other groups **(figure 5)** .



a–c Different letters indicate significant difference (P< 0.05)

Figure 5 : Effect of *Spirulina* and *Chlorella* extracts on SOD level in female rabbits treated with valproic acid .

2.3 Reduced glutathione (GSH)

Assessment of GSH levels revealed a statistically significant decline ($p < 0.05$) in the valproic acid–treated group when compared with the control and all other experimental groups. In contrast, treatment with *Spirulina* (500 mg/kg) and valproic acid combined with *Chlorella* (500 mg/kg) resulted in significant variations ($p < 0.05$) relative to the control and the remaining groups (Figure 6). Conversely, no significant change in GSH levels was detected in the *Chlorella* (500 mg/kg) group when compared with the control group. Additionally, the valproic acid combined with *Spirulina* (500 mg/kg) group exhibited a statistically significant difference in comparison with both the control and other experimental groups. **(Figure 6).**

a–d Different letters indicate significant difference (P< 0.05)

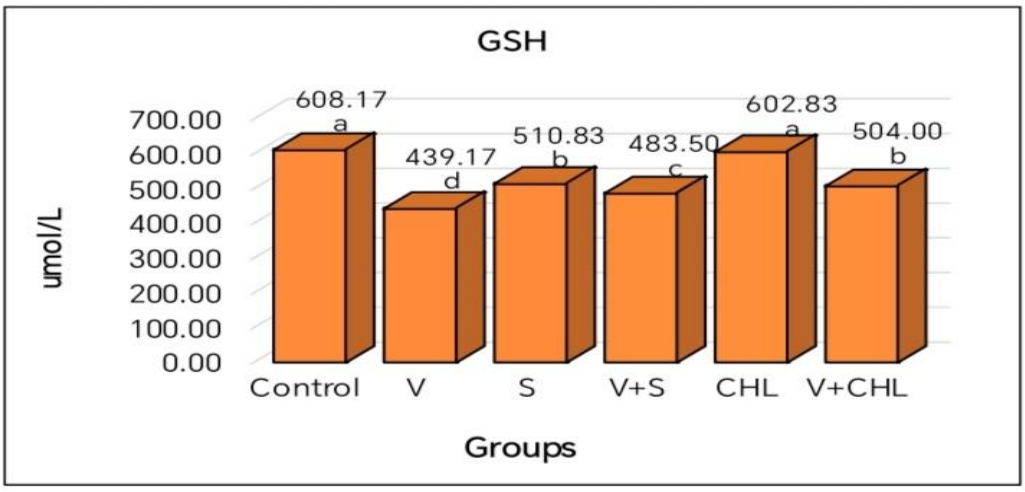
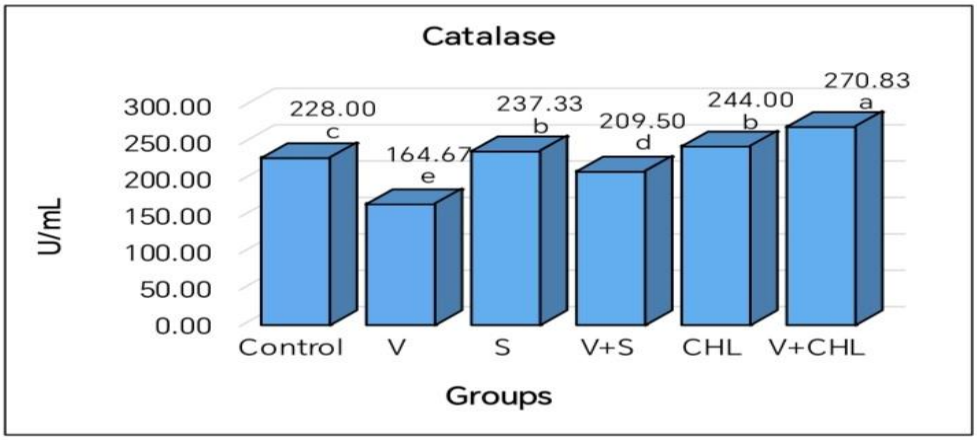


Figure 6 : Effect of *Spirulina* and *Chlorella* extracts on GSH level in female rabbits treated with valproic acid .

2.4 Catalase (CAT)

Evaluation of CAT activity demonstrated a statistically significant reduction ($p < 0.05$) in the valproic acid–treated group relative to the control and all other experimental groups. In contrast, administration of *Spirulina* (500 mg/kg) and *Chlorella* (500 mg/kg) resulted in a significant elevation ($p < 0.05$) in CAT levels when compared with the control group and the valproic acid combined with *Spirulina* (500 mg/kg) group. Notably, the most pronounced increase in CAT activity ($p < 0.05$) was recorded in the valproic acid combined with *Chlorella* (500 mg/kg) group in comparison with both the control and the remaining groups **(Figure 7).**



a–e Different letters indicate significant difference (P< 0.05)

Figure 7: Effect of *Spirulina* and *Chlorella* extracts on CAT level in female rabbits treated with valproic acid .

3- Lipid profile

1- Cholesterol

The results showed a significant increase ($p<0.05$) in cholesterol levels in the valproic acid group compared to the control group and the other groups. However, the *Spirulina* (500 mg/kg) and *Chlorella* (500 mg/kg) groups show no significant difference ($p<0.05$) among themselves or compared to the control group. The valproic acid with *Spirulina* (500 mg/kg) and valproic acid with *Chlorella* (500 mg/kg) groups showed a significant difference compared to the

other groups (figure 8).

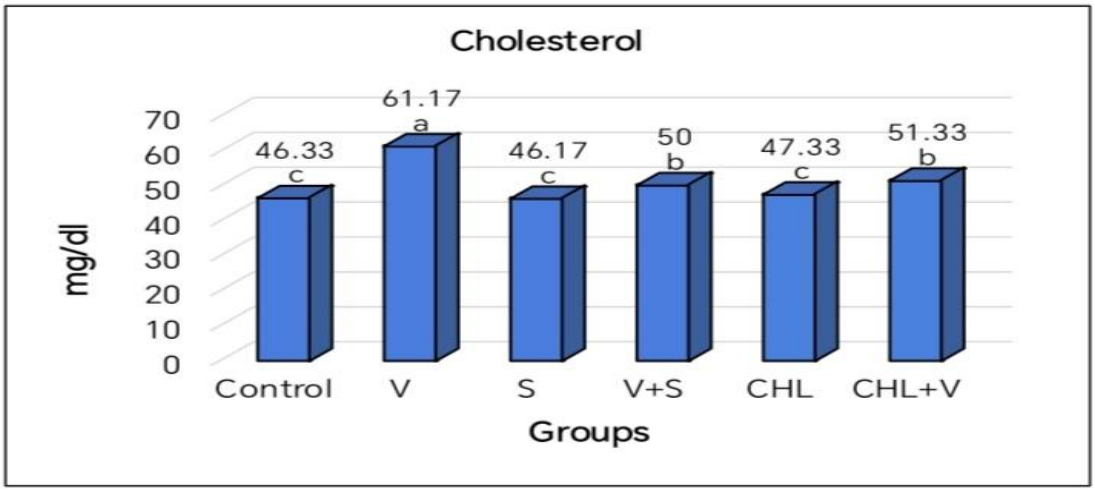


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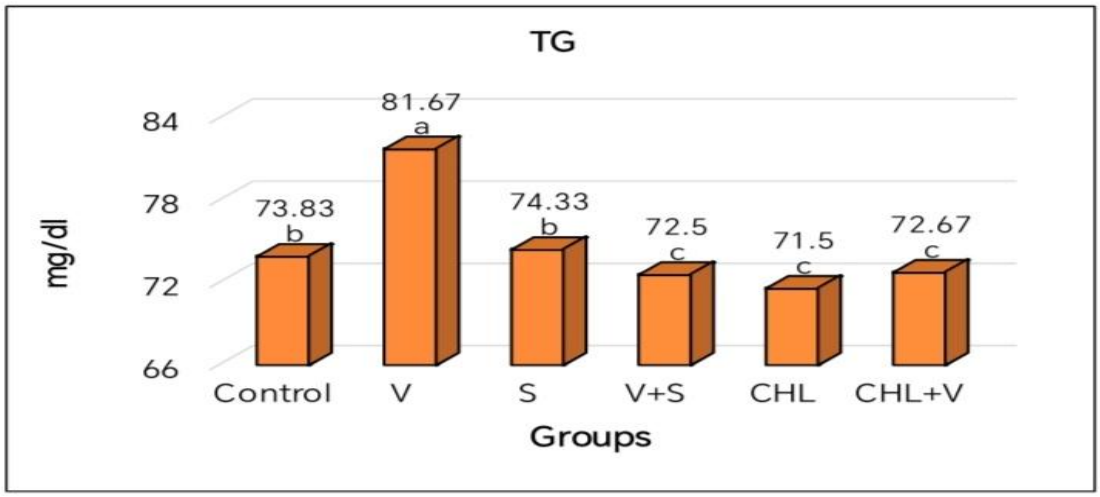
Figure 8 : The effect of *Spirulina* and *Chlorella* extracts on Cholesterol in female rabbits treated with valproic acid.

2- Triglyceride

Statistical analysis demonstrated a significant elevation ($p < 0.05$) in triglyceride concentrations in the valproic acid–treated group relative to the control and all other experimental groups. In contrast, the valproic acid combined with *Spirulina* (500 mg/kg), *Chlorella* (500 mg/kg), and valproic acid combined with *Chlorella* (500 mg/kg) groups exhibited statistically significant differences when compared with the remaining groups (Figure 9).



a-cDifferent letters indicate significant difference (P< 0.05)

Figure 9: The effect of *Spirulina* and *Chlorella* extracts on Triglyceride in female rabbits treated with valproic acid

3- High Density Lipoprotein. (HDL)

The results showed a significant decrease ($p<0.05$) in HDL level in the valproic acid group compared to the control group and the other groups. However, the *Spirulina* group (500 mg/kg), the valproic acid with *Spirulina* group (500 mg/kg), the *Chlorella* group (500 mg/kg), and the valproic acid with *Chlorella* group (500 mg/kg) show no significant difference ($p<0.05$) among themselves or compared to the control group (**figure 10) .**

a-bDifferent letters indicate significant difference (P< 0.05)

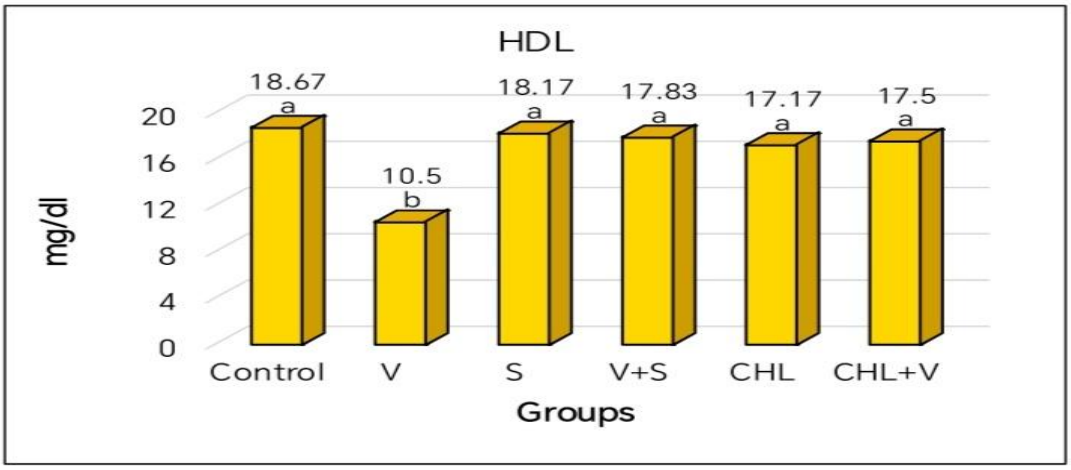
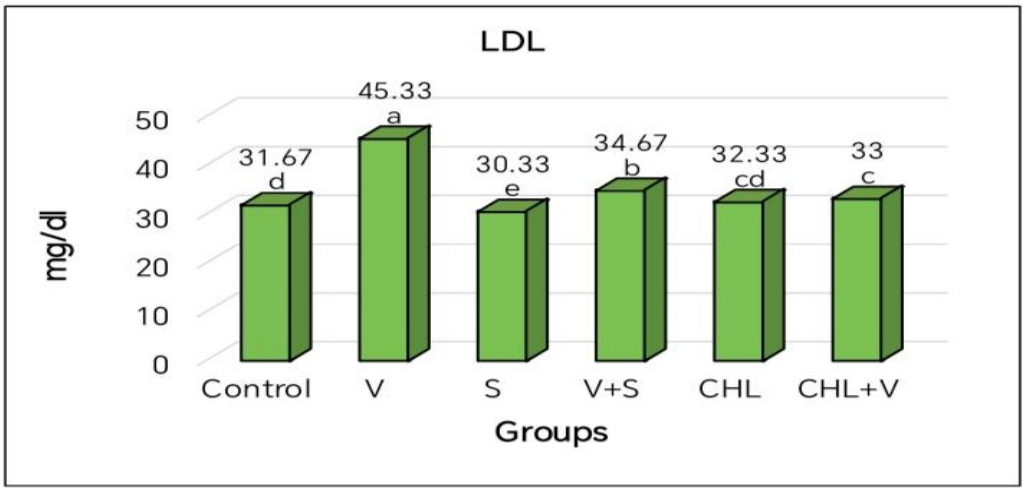


Figure 10 : The effect of *Spirulina* and *Chlorella* extracts on HDL in female rabbits treated with valproic acid.

4- Low Density. Lipoprotein (L. D. L)

The results showed a significant increase ($p<0.05$) in LDL level in the valproic acid group compared to the control group and the other groups. While the *Spirulina* (500 mg/kg) group and the valproic acid with *Spirulina* (500 mg/kg) group showed a significant difference between them and compared with the control group and the other groups. The *Chlorella* group (500 mg/kg) and the valproic acid group with *Chlorella* (500 mg/kg) also showed a significant difference compared to the control group and the other groups. **(figure 11).**



a-e Different letters indicate significant difference (P< 0.05)

Figure 11 : The effect of *Spirulina* and *Chlorella* extracts on LDL in female rabbits treated with valproic acid.

5- Very Low-Density Lipoprotein (VLDL)

VLDL(mg/dl) = serum TG/5

Discussion:

The results of the present study showed a significant increase in liver enzyme levels (ALT, ALP, and AST) in rabbits treated with valproic acid (VAP) compared with the control group, this elevation is considered an important biological indicator of structural and functional impairment of hepatocytes. These findings are consistent with the study of (Abdel-Dayem *et al.*,2014), which reported that VAP induced a marked increase in liver enzyme levels in rats, indicating its hepatotoxic effect. In addition, the current results agree with those reported by (Yang *et al.*,2019), who observed a significant elevation in liver enzymes associated with enhanced hepatic toxicity in treated mice.

The hepatotoxicity of valproic acid can be explained by its primary metabolism in the liver. Although hepatic L02 cells possess cytochrome P450 enzyme activity, only about 10% of VPA is metabolized through CYP450-mediated oxidation (Xu *et al.*, 2019). Its metabolic products lead to mitochondrial dysfunction and inhibition of oxidative processes, resulting in increased production of reactive oxygen species (ROS) and the development of oxidative stress. This oxidative

stress is considered a major factor responsible for hepatocyte damage and elevated liver enzyme levels.

In the present study, a significant decrease in antioxidant enzymes (SOD, CAT, and GSH) was observed in the VAP-treated group, which is consistent with previous reports (Ibrahim *et al*., 2017; Hamza *et al*., 2025). This reduction was accompanied by a marked increase in malondialdehyde (MDA) levels, as reported in several studies (Tūrkylmaz *et al*., 2023; Asghar *et al*., 2025; Chang *et al*., 2006).

Furthermore, the results demonstrated a significant increase in blood lipid levels, including total cholesterol, triglycerides, and low-density lipoprotein (LDL), along with a significant decrease in high-density lipoprotein (HDL) levels in rabbits treated with VAP compared with the control group, these findings are consistent with the study of (Owoade *et al*.,2022) conducted on rats, which showed that valproic acid disrupts lipid metabolism in various tissues, leading to disturbances in the regulation of major blood lipid concentrations.

The present results also agree with the study of (Bari ,2018) conducted on rabbits, which reported that valproic acid treatment induces lipid oxidation and causes a sharp increase in lipid levels. In addition, (Xu *et al*.,2019) demonstrated that valproic acid aggravates lipid disorders by significantly increasing lipid concentrations and promoting the accumulation of long-chain free fatty acids within hepatocytes in rats.

Exposure to valproic acid has been reported to induce hepatotoxic effects primarily through the enhancement of oxidative stress, which arises from excessive generation of reactive oxygen species alongside depletion of key antioxidant defenses. This includes reductions in critical antioxidant components such as reduced glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD), ultimately leading to impaired hepatic redox homeostasis. Elevated oxidative stress is closely linked to increased lipid peroxidation, as evidenced by higher levels of malondialdehyde (MDA), and concomitant depletion of glutathione reserves, thereby promoting hepatocellular injury and aggravating liver dysfunction. Furthermore, oxidative imbalance interferes with lipid metabolic pathways, as valproic acid alters the activity of enzymes and the expression of genes involved in fatty acid metabolism. These disruptions result in elevated serum triglycerides, total cholesterol, and low-density lipoprotein (LDL) levels, accompanied by a reduction in high-density lipoprotein (HDL),

collectively contributing to a dysregulated lipid profile and increased hepatic injury markers, including ALT, AST, and ALP (Asghar et al., 2025; Kadam et al., 2025; Grünig et al., 2020).

Conversely, the results showed a significant reduction in liver enzyme levels (ALT, AST, and ALP) in both the *Spirulina* and *Spirulina* + VAP groups compared with the VAP group, with enzyme levels returning close to normal values. These findings are consistent with previous studies on rats (Mohamed *et al*., 2021; Rani and Chandravadana, 2008), which demonstrated the protective role of *Spirulina* against liver damage caused by free radicals generated from reactive oxygen species, through preservation of hepatic cell membrane integrity and reduction of oxidative stress induced by VAP.

Moreover, treatment with Spirulina alone or in combination with VPA resulted in a marked enhancement of antioxidant defenses, as evidenced by increased levels of SOD, CAT, and GSH, along with a significant reduction in malondialdehyde concentrations when compared with the VPA-treated group. These observations are consistent with earlier reports demonstrating the protective role of *Spirulina* supplementation against VPA-induced oxidative stress (Indrawati, 2023; Hossain et al., 2025). The antioxidative capacity of Spirulina is largely attributed to its rich content of bioactive compounds, including phycocyanin, carotenoids, and various phenolic constituents, which collectively contribute to the mitigation of oxidative damage (Rodriguez et al., 2018).

Moreover, the study results showed a significant decrease in total cholesterol, triglycerides, and LDL levels, along with a significant increase in HDL levels in the *Spirulina* and *Spirulina* + VAP groups compared with the VAP group, as illustrated in the Figure. These findings are consistent with previous studies (Elmeleh *et al*., 2023; Mohamed *et al*., 2025).

In line with these findings, a significant reduction was observe in liver enzyme levels (ALT, AST, and ALP) in both the *Chlorella* and *Chlorella* + VAP groups compared with the VAP group, with values approaching normal levels. These findings are in agreement with previous studies (Khadrawy *et al*., 2023; Eissa *et al*., 2021), which confirmed the protective role of Chlorella against VAP-induced oxidative stress and hepatotoxicity.

In addition, a significant increase in antioxidant levels (SOD, CAT, and GSH) and a significant decrease in malondialdehyde levels were observed in the *Chlorella* and *Chlorella* + VAP groups compared with the VAP group. This protective effect is attributed to the presence of various bioactive antioxidant compounds in *Chlorella*, including carotenoids, phenolic compounds, flavonoids, as well as essential vitamins and minerals (Bito *et al.*, 2020; Almutairi *et al*., 2025).

Finally, the study results showed a significant improvement in the lipid profile of rabbits treated with *Chlorella*, characterized by reduced total cholesterol, triglycerides, and LDL levels, and increased HDL levels compared with the VAP group. These findings are consistent with previous studies (Moradi *et al*., 2021; Bargchi *et al.*, 2023), which demonstrated the ability of *Chlorella* to ameliorate VAP-induced lipid disturbances by enhancing HDL levels and inhibiting LDL oxidation, thereby improving blood lipid status and reducing lipid peroxidation.

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