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Research Article

EVALUATION OF THE ANTIOXIDATIVE EFFECTS OF *HIBISCUS SABDARIFFA* L. EXTRACT ON *SACCHAROMYCES CEREVISIAE* WITH MOLECULAR BIOLOGICAL AND BIOCHEMICAL BIOMARKERS

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ABSTRACT. It has been determined by studies that the *Hibiscus sabdariffa* L. Plant has many biological activities such as antimicrobial, antifungal, antiviral, anti-inflammatory, antioxidant, anticarcinogenic, anticholesterol, antidiabetic and antihypertensive. This experiment tested whether Hibiscus sabdariffa L. plant extract could protect *S. cerevisiae* from oxidative damage caused by carbon tetrachloride (CCl_4). In this study, four groups were formed. Groups: (i) Control Group: Group planted only with yeast; (ii) *Hibiscus* Group: *Hibiscus*-provided group (10%); (iii) CCl_4 Group: CCl_4 given group (15 millimeters); (iv) *Hibiscus* +CCl_4, granted group: *Hibiscus* given group (10%). *S. cerevisiae* cultures were grown at 30 degrees Celsius for one, three, five, and twenty-four hours. A spectrophotometer was used to measure the time of cell development, lipid peroxidation, malondialdehyde (MDA) analysis, catalase activity determination (CAT), and glutathione (GSH) levels. The total protein changes in *S. cerevisiae* cultures at 1, 3, 5, and 24 hours were identified using SDS-PAGE electrophoresis and calculated using the Bradford method. Therefore, the purpose of this experiment was to determine the therapeutic action of plant extract from the polyphenol-rich species *Hibiscus sabdariffa* L. and to uncover the plant's potential for use as a medication to treat a variety of ailments. Our study is unusual since there hasn't been enough research done on *Hibiscus sabdariffa* L. plant extract in the literature that is currently available in our nation. It has special significance, particularly when it comes to examining the role and promoting mechanism of plant extract *Hibiscus sabdariffa* L. in protein synthesis, which is referred to as the genesis of life. Because of its potent bioactive qualities, *Hibiscus sabdariffa* L. plant extract is believed to assist in eradicating a wide range of health issues when utilized on a daily basis. Issues that exist in society, it will favorably contribute to the improvement of the nation's medical expenses.

Keywords: *Hibiscus sabdariffa L., oxidative stress, Saccharomyces cerevisiae, SDS-PAGE.*

INTRODUCTION

Medicinal plants play a significant role in a variety of businesses today, including food additives, cosmetics, herbal compounds, and dyes, in addition to modern medicine. Epidemiological research conducted recently has demonstrated the critical role that a plant diet high in polyphenols plays in maintaining human health. Rich in polyphenolic chemicals, *Hibiscus sabdariffa* L. is a plant that is well-known for both its potential medicinal benefits and its favorable bioactivity. *Hibiscus sabdariffa* L., also referred to as Rosella or Roselle is a species that is commonly used as a Halloween flower and is a

member of the Malvaceae family of plants. *Hibiscus sabdariffa* L., which is distributed in tropical and subtropical regions, has an eye-catching flower. It is a long-lived plant and is a woody-based subshrub form that can grow up to 2-2.5 meters tall. The leaves of this plant are arranged alternately on straight, cylindrical red stems [1].

The main components of the *Hibiscus sabdariffa* L. plant are polysaccharides, organic acids, flavonoids, and anthocyanins. It is known that dried calyx extracts also contain chemical components such as organic acids (citric acid, ascorbic acid, hibisc acid, oxalic acid, tartaric acid), phytosterols, polyphenols, anthocyanins, and other water-soluble antioxidants (Fig. 1). Thanks to the organic acids in its structure, it eliminates free radicals together with bioactive components. This plant is frequently used in traditional medicine as it is rich in phytochemicals such as polyphenols, especially anthocyanins, polysaccharides, and organic acids [2, 3]. Nutritionally, it is known to provide strong antioxidant protection due to its high amount of vitamin C [4].

Fig. 1. *Dried and powdered image of Hibiscus sabdariffa L. plant [1].*

The model organism *Saccharomyces cerevisiae* (*S. cerevisiae*) is an effective tool for researching basic eukaryotic cell biology concepts. 16 chromosomes make up *S. cerevisiae*. There are roughly 13,117,000 nucleotide pairs in the entire genome, of which 78,520 are found in the mitochondrial DNA. Approximately 50 times more protein-coding genes exist than there are genes in the human genome. It is believed to be roughly 23% similar to the human genome because of these genomic properties [5].

S. cerevisiae was selected as our model organism because its traits are comparable to those of the human genome. According to this study, a xenobiotic that causes a variety of cytotoxicities in both people and animals is carbon tetrachloride (CCl_4), which will be used as a source of oxidative stress in *S. cerevisiae* (Fig. 2). Four chlorine atoms arranged in a configuration with a center carbon atom connected by a single covalent connection make up CCl_4. It produces free radicals, which harm cells [6].

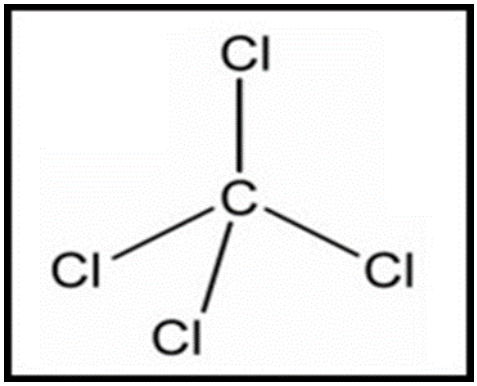

Fig. 2. *Organic structure of carbon tetrachloride (CCl_4) [7].*

In addition to playing a role in the etiology of many diseases, free radicals cause oxidative stress by causing changes in the structure of lipids, proteins, and nucleic acids. Oxidative stress that occurs as a result of these changes creates irreversible changes in the structures of lipid membranes and proteins, causing tissue damage. It causes the formation and pathogenesis of many diseases, especially liver, lung, kidney, neurodegenerative and cancer [8].

Many medicinal plants with antioxidant properties provide protection against pathological conditions by reducing the damage caused by free radicals. Active components such as polyphenols, flavonoids, vitamins, carotenoids and tannins contained in these medicinal plants create an antioxidant defense mechanism by clearing free radicals formed in the organism [9]. *Hibiscus sabdariffa* L., one of the important therapeutic plants, is believed to reduce oxidative stress caused by CCl_4 and therefore eliminate free radicals.

MATERIALS AND METHODS

Experimental Groups

This work examined the potential therapeutic benefit of *Hibiscus sabdariffa* L. plant extract against *S. cerevisiae* damage caused by carbon tetrachloride (CCl_4). In the study, four groups were created. The following are the groups:

I. Control Group: This group only had yeast planted;
II. Hibiscus Group: This group received 10% of the plant;
III. CCl_4 Group: This group received 15 millimolars of the compound;
IV. Hibiscus + CCl_4 Group: This group received 10% of the plant plus 15 millimolar of CCl_4

Application of Hibiscus sabdariffa L. Plant Extract and Carbon Tetrachloride (CCl_4) to the Culture

Hibiscus sabdariffa L. was commercially supplied from the covered bazaar in Elazığ city center. Growth medium of *S. cerevisiae*: For the growth and propagation of yeasts, YEPD (for 250 ml; 7.5 g yeast extract, 7.5 g tryptone, and 7.5 g glucose) was produced. Next, 50 ml of the 250 ml media that had been prepared was added to each of the five

conical flasks. It was taken out and allowed to cool after an hour at 121 degrees Celsius in the autoclave. Yeast was seeded 800 µl each flask near the burner flame. After 20 minutes of sitting in the oven, a blank measurement was made. Ten grams of *Hibiscus sabdariffa* L. plant material were weighed and steeped for fifteen to twenty minutes in one hundred milliliters of boiling distilled water in order to prepare ten percent of the plant extract. It was then prepared for sowing in culture by filtering it through sterile cheesecloth. Furthermore, in the experimental investigation, 15 millimolar CCl_4 was introduced into every flask bearing the CCl_4 label and allowed to mature at 30 degrees Celsius [10].

Analysis of Malondialdehyde (MDA)

The fundamental idea behind MDA, the most significant marker of lipid peroxidation, is that when heated in an acidic environment, it combines with thiobarbuturic acid to produce a pink chromogen. The amount of MDA present in the sample is directly correlated with the intensity of the pink color. To create a 10% homogenate, 1.15% KCl solution was added to the culture samples. The homogenate was then homogenized on ice at 15000 rpm for one to two minutes. The MDA analysis was conducted using the generated homogenates. Following the addition of 2 mmol/L 1,1',3,3' tetraethoxypropane, 20% acetic acid, 0.8% 2-thiobarbuturic acid (TBA), and 8.1% sodium dodecyl sulfate (SDS), vortexing was performed. After that, the mixture was incubated and cooled for 45 minutes at 95°C in a boiling water bath. It was vortexed with the addition of 2 ml of n-butanol. The tubes were centrifuged at 5000 rpm for 10 minutes after being held in boiling water (95°C) for an hour. The spectrophotometer was calibrated to measure zero absorbance using a blank wavelength of 532 nm. Erdemli [11] evaluated the absorbance of the pink supernatants at 532 nm and recorded the results in nmol/ml.

Analysis of Glutathione (GSH)

Distilled water was added to form a 10% homogenate taken from the cultures and homogenized at 12000 rpm for 1-2 minutes on ice. The tissue homogenates were centrifuged for 20 minutes at 5000 rpm and +4 degrees. Supernatant samples were employed in GSH analysis. TCA solution was added to the resultant supernatant, mixed, and centrifuged one more at 3000 rpm, +4 degrees, for 20 minutes, to ensure the proteins precipitated. 10% trichlor acetic acid, 1% trisodium citrate, 0.4% 5,5'-Dithiobis 2-nitrobenzoic acid, and 0.3 molar disodium hydrogen phosphate reagents were combined in test tubes, and the mixtures were thoroughly mixed using a vortex. After the samples were left at room temperature for five minutes, a color appeared. This allowed the spectrophotometer to read the absorbance values at 410 nm, and the results were recorded as µmol/ml [11].

Measurement of Catalase (CAT) Activity

250 µl of the cultures' supernatant was taken out, treated with 100 mM 250 µl Tris-HCl and 1 M 500 µl KI (potassium iodide), and let to rest at room temperature in the dark for ninety minutes in order to measure the amount of H_2O_2. After 90 minutes, 200 µl of the samples were removed, and measurements were made at a wavelength of 390 nm using a spectrophotometer against a blank. The results to read the absorbance change of H_2O_2 for 1 minute were recorded as U/ml catalase activity [12].

Bradford's Measurements of Total Protein Density

Using a spectrophotometer set to 595 nm (OD_{595}), the total protein density was calculated using the Bradford method. BSA (bowin serum albumin) protein standards were produced utilizing BSA protein at various concentrations. Therefore, it was determined how much protein overall each *S. cerevisiae* group had in relation to this standard value [10, 13].

Protein Isolation for SDS-PAGE

A milliliter of the culture sample was extracted and centrifuged for five minutes at 13,000 rpm. Subsequently, 500 µl of Tris-EDTA Acetic Acid (pH: 7.5) was added to the pellet to dissolve it after the pellet portion was removed. The cells were subjected to two 10-second blasts of a power 2 sonicator, five minutes of cold treatment, and ten minutes of centrifugation at 13.000 rpm. To prepare for electrophoresis, pellet segments were collected and combined with an equal volume of sample staining solution (Sample Amplification Buffer) for SDS-PAGE investigations [14].

SDS-PAGE (Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis) Analysis

Protein isolation was performed from *Saccharomyces* cultures prior to electrophoresis. The isolated proteins were prepared for loading into protein electrophoresis after being treated with SDS-PAGE sample application buffer. For this purpose, firstly the separation gel was prepared (12%), then the loading gel was prepared (10%) to load the protein samples and then Protein samples from *S. cerevisiae* cells were boiled for five minutes and then added an equal volume of SDS-PAGE SAB (Sample Amplification Buffer) dye before being placed into the wells. One buffer tank was used for the electrophoresis procedure. Then, until the blue band of the bromophenol blue dye, which indicates the movement of the proteins in the gel, reached the end of the gel, a 30 mA current was applied. The gel was electrophoresed and then allowed to stand at room temperature for 30 to 60 minutes before Coommasie blue staining was applied. Subsequently, the protein bands in the gel were washed until they were visible using a dye removal solution, and gel images were captured to examine the variations in protein bands between the groups [15, 16].

The Examination of Statistical Data

One Way ANOVA tests were used to ascertain group differences in the statistical analyses of the study, which were conducted using the SPSS 22 package program. The mean ± standard deviation (Mean ± Sd) represents the data for the study groups.

RESULTS AND DISCUSSION

Hibiscus sabdariffa L., belonging to the Malvaceae family, is frequently used in traditional medicine because it is rich in phytochemicals such as polyphenols, especially anthocyanins, polysaccharides, and organic acids [3]. Research on the phytochemical properties of *Hibiscus sabdariffa* L. has shown that it has various health benefits and can be used as a powerful material for the therapeutic treatment of various diseases. Therefore, it has tremendous potential in modern therapeutic uses in recent years [17].

The phytochemistry and therapeutic potential of the *Hibiscus sabdariffa* L. plant were studied by Riaz and Chopra [3]. They claimed that the active ingredients in *Hibiscus sabdariffa* L. have the ability to counteract the effects of toxicity. According to Ojulari et al. [17] the bioactive chemicals in *Hibiscus sabdariffa* L. plant significantly reduce body weight, block fat accumulation, and suppress adipogenesis. *Hibiscus sabdariffa* L., a plant rich in phenolic compounds, has been shown by Guardiola and March [18] to offer protection against oxidative stress, lipid profile, hypertension, and atherosclerosis. They discovered that it greatly lowers LDL oxidation due to the anthocyanins it contains. They also revealed that certain microRNAs modulate gene expression by regulating adipogenic signaling pathways and transcription factors, as well as inhibiting adipogenesis. As a result of the experimental analysis of this study, it was determined that *Hibiscus sabdariffa* plant extract is a powerful antioxidant in the therapeutic treatment of diseases.

According to the experimental results, it is observed in Fig. 3 that there is a significant difference between the groups that developed in different time periods ($p<0.05$). It was observed that *Hibiscus sabdariffa* plant extract added to the culture medium increased cell growth against CCl_4-induced damage in *S. cerevisiae*.

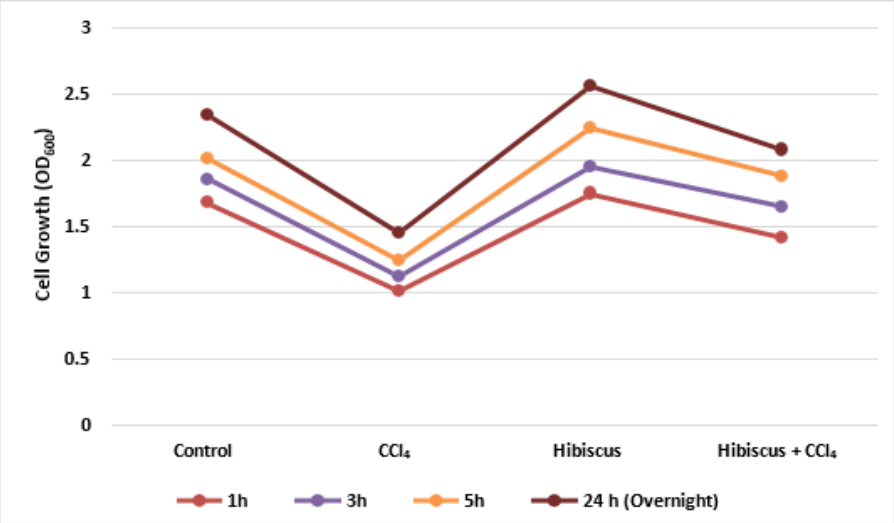

Fig. 3. *S. cerevisiae cell growth at 1, 3, 5, and 24 hours after treatment with Hibiscus sabdariffa L.*

Mezni et al. [19] found that *Hibiscus sabdariffa* L. plant calyx extract protected against cypermethrin-induced oxidative stress in mice. Mahmoudi et al. [20] determined that plant combinations of *Chrysanthemum morifolium, Hypericum perforatum, Humulus lupulus,* and *Hibiscus sabdariffa* provide effective protection against COVID-19. They found that these combination plants have high affinity as well as antioxidant, vasoprotective, anticarcinogenic and antiviral properties.

Beyaz [16] found that *Melissa officinalis* and *Lavandula angustifolia* increased total protein, GSH, CAT activity and reduced MDA level against chromium-induced damage in *S. cerevisiae.* Thus, it was revealed that *Melissa officinalis* and *Lavandula angustifolia* provide effective protection against oxidative damage thanks to their strong antioxidant and anti-inflammatory properties. Rambe et al. [21] revealed that *Hibiscus sabdariffa* L.

plant extract, which has various properties such as anti-inflammatory, antioxidant and antimicrobial, has very strong effects on wound healing in rats.

The results of the total protein pellet and supernatant, as shown in Fig. 4 and Fig. 5, indicate that *Hibiscus sabdariffa* plant extract boosted *S. cerevisiae* protein synthesis. Furthermore, compared to the CCl_4 group, the *Hibiscus* (10%) + CCl_4 (15 mM) group had a greater total protein level.

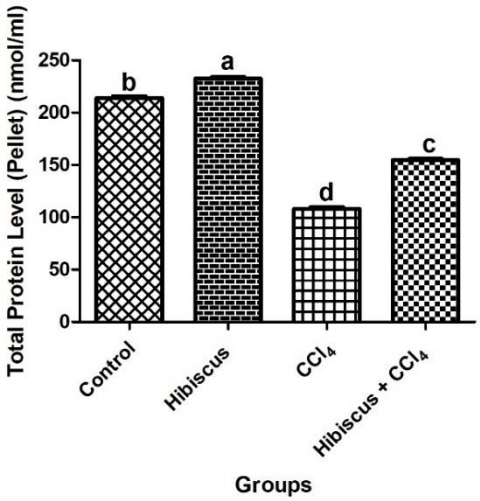

Fig. 4. *Total protein pellet level*

Saral [22] studied the protective effects of apitherapeutic bee products (propolis, honey, pollen, and royal jelly) against liver damage caused by carbon tetrachloride (CCl_4) and how they can help avoid liver damage. According to him, the groups who applied CCl_4 had higher MDA levels and lower CAT activity than the groups that used bee products. Yang et al. [23] discovered that by lessening renal epithelial-mesenchymal transition, plant polyphenols from *Hibiscus sabdariffa* L. alleviated diabetic nephropathy.

Beyaz [24] studied how an extract from the clove plant (*Syzygium aromaticum* L.) protected *S. cerevisiae* from oxidative stress caused by CCl_4. The researchers concluded that the clove plant has a potent anti-oxidative stress medicinal action. According to him, adding CCl_4 to the groups resulted in a large increase in MDA levels, while adding clove to the groups caused a considerable increase in total protein levels.

The study's MDA values, as presented in Figure 6, showed that the CCl_4 group had the highest MDA level, whereas the *Hibiscus* (10%) + CCl_4 (15 mM) group had the lowest MDA level. The GSH levels shown in Figure 7 were shown to have significantly increased in the *Hibiscus* (10%) + CCl_4 (15 mM) group, whereas they had reduced in the CCl_4 group.

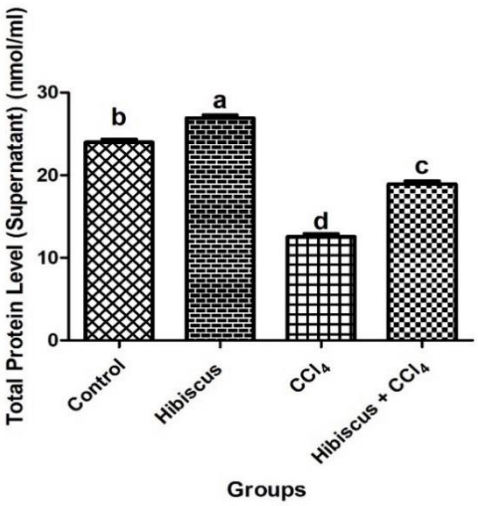

Fig. 5. *Total protein supernatant level*

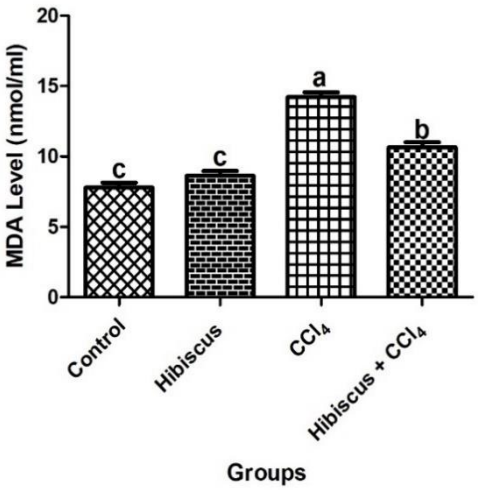

Fig. 6. *MDA level*

Gökçe [25] states that in order to examine their impact on antioxidant activity against CCl4-induced oxidative stress, MDA and SOD measured GSH levels and CAT activities in *S. cerevisiae*. The groups under investigation showed significantly higher GSH and CAT activity levels and lower MDA and SOD levels, according to the CCl_4. Significant reductions in oxidative damage were observed in the development of cells in *S. cerevisiae* cultures treated with peanut extract. The effect of green tea and tart tea (*Hibiscus sabdariffa* L.) supplementation on oxidative stress and muscle injuries in athletes has been studied by Hadi et al. [26]. Those given green tea and acidic tea (*Hibiscus sabdariffa* L.) have been shown to have lower levels of MDA, a bio-enhancer of oxidative stress.

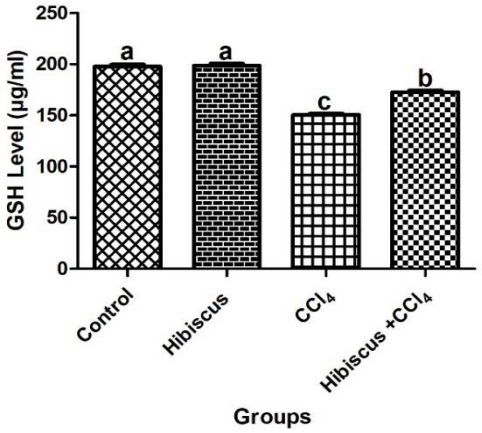

Fig. 7. *GSH level*

Ellis vd. [27], who studied the effects of *Hibiscus sabdariffa* L. on blood pressure and cardiometabolic markers, concluded that its regular consumption reduces the risk of cardiovascular disease. Chiu vd. [28] studied the clinical effects of the extract of *Hibiscus sabdariffa* L. on antioxidant activity, blood pressure, and skin condition. A significant decrease in blood pressure was observed in groups treated with 200 ml *Hibiscus* per 39 healthy adult individuals for 6 months. After 6 months of *Hibiscus* therapy, serum phenolic content, antioxidant capacity (TEAC), glutathione, superoxide dismutase (SOD), glucose-6-phosphate dehydrogenase (G-6-PDH), and glutathione peroxidase (GSH-Px) levels increased significantly. They also noted improvements in skin redness and moisture ratio in patients treated with *Hibiscus*. Chang et al. [29] found that *Hibiscus sabdariffa* L. plant extract improved fatty liver in humans by preventing obesity and fat accumulation. Asgary et al. [30], studied the activity of *Hibiscus sabdariffa* L. on oxidative stress and insulin resistance in adult patients with metabolic syndrome. *Hibiscus* therapy has been shown to regulate systolic and diastolic blood pressure in adult patients. They also found that it causes significant decreases in triglycerides and MDA levels.

In this study, when examining the definition of CAT activity given in Fig. 8, CAT activity was the lowest in the CCl_4 group and significantly decreased in the *Hibiscus* (10%) + CCl_4- (15 mM) group.

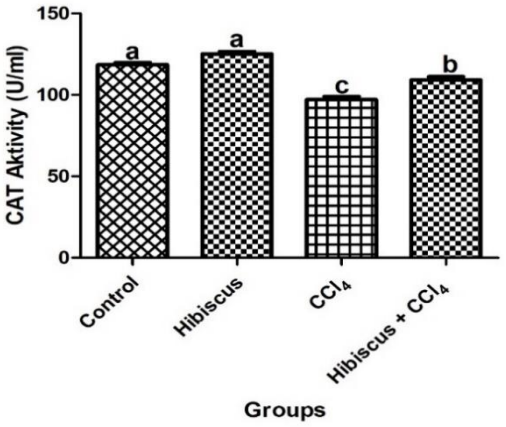

Fig. 8. *CAT activity*

The protein density rose more quickly in the groups provided *Hibiscus* (%) and *Hibiscus* (10%) + CCl_4 (15 mM) than in the group given CCl_4, as shown by an examination of the pellets and supernatant gel images acquired with SDS-PAGE analysis in Fig. 9. in groups that were given CCl_4, there was a decrease in protein density due to cellular damage caused by the generation of free radicals. These findings indicate that by lessening the oxidative damage brought on by CCl_4, the plant extract of *Hibiscus sabdariffa* L. has a beneficial effect on the growth of *S. cerevisiae.*

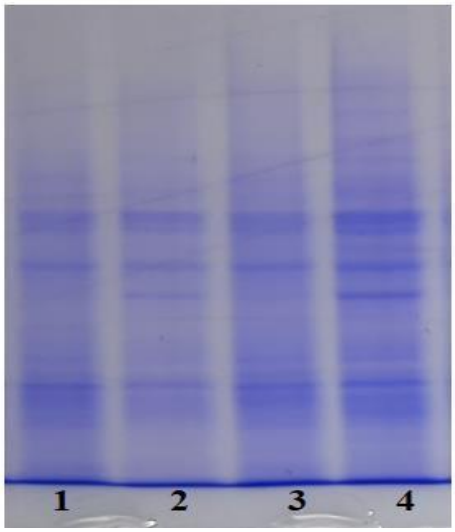

Fig. 9. *Bands; SDS-PAGE protein (pellets) image; 1: Control; 2: CCl_4; 3: Hibiscus + CCl_4; 4: Hibiscus*

CONCLUSION

Hibiscus sabdariffa L., which contains rich bioactive compounds such as flavonoids, phenolic acids, anthocyanins, and polyphenols, is known to have many biological activities such as anti-cancer, anti-inflammatory, and antioxidant. Thanks to these properties, *Hibiscus sabdariffa* L. neutralizes free radicals and helps reduce the damage to cells caused by oxidative stress.

In this study, the MDA levels were significantly reduced due to increased oxidative damage within the cell in groups that were added to CCl_4 and significantly increased levels of CAT and GSH from antioxidant bio-enhancers in groups treated with Hibiscus plant extract. In line with these findings, *Hibiscus sabdariffa* L. consumption has shown that it can strengthen the antioxidant defense system and limit the damage caused by free radicals.

Conflict of Interest. The authors declared that there is no conflict of interest.

Authorship Contributions. Concept: A.A., S.B., O.G., Design: A.A., S.B., O.G., Data Collection or Processing: S.B., O.G., M.A.N., Analysis or Interpretation: S.B., O.G., M.A.N., Literature Search: S.B., O.G., M.A.N., Writing: A.A., S.B., O.G., M.A.N.

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