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Assessment of the Effect of Propolis Extract on Enzymatic Antioxidants and Lipidperoxidation

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Abstract: ***The current study aimed to evaluate the effect of propolis (aquatic, alcoholic) and vitamin E on the state of experimentally induced oxidative stress status in the male white rats. The animals were distributed into five groups: The first group (negative control) was given only water and food until the end of the study period. The second group (positive control) was dosed with H_2O_2 through drinking water at a concentration of 0.5% for 21 days. The third group was dosed with H_2O_2 at a concentration of 0.5% and treated with aqueous extract of propolis at a concentration of 4% for 21 days. The fourth group was dosed with H_2O_2 with drinking water at a concentration of 0.5% and treated with an alcoholic extract of propolis at a concentration of 1% for 21 days. The fifth group was dosed with $H_2O_2$with drinking water at a concentration of 0.5%. It was treated with vitamin E at a concentration of 50 mg/kg for 21 days. The results showed that the treatment with the water and alcoholic extract of propolis and vitamin E has led to a significant increase ($p \leq 0.05$) in the concentrations of the antioxidants enzyme (superoxide dismutase, catalase, glutathione peroxidase) and a moral decrease in the Malmondialdehyde compared to the negative control.***

Keywords: Propolis, Antioxidant, Oxidative Stress, Lipidperoxidation, Hydrogenperoxide.

1. INTRODUCTION

Humanity has known the use of herbs and natural products in medication from eternity and this situation is still ongoing until the present time, and with the scientific progress that occurred after the industrial revolution and the manufacture of chemical alternatives to the active substances of plants and medicinal herbs less dependence on these plants in treatment for a limited period ended with the emergence of the negative side effects of the medicines Chemical [1]. Propolis is a natural product with a gummy consistency and a complex

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composition of a mixture of beewax, resin materials, pollen, and essential oils collected by honey bees from tree buds and beard, and produces after its treatment and mixing with enzymes [2], It contains among its compounents many secondary metabolites such as Flavonoids, Phenolic Compounds, alkaloids, and Saponins, which stimulates its various effects, especially its work as a strong antioxidant oxidation [3]. Oxidation processes in live cells occur naturally with the sharing of oxygen (O_2) for carbon -containing molecules for the purpose of power generation to run vital activities, and at the same time the formation of secondary oxidants is caused by free radicals (FRS) [4], The accumulation of these oxidizing compounds, including free radicals (FRs), inside the body in high concentrations that exceed the ability of antioxidants (AO) to remove them and their interaction with various biological compounds leads to damage within the body and the occurrence of oxidative stress (OS) [5] Which works to destroy biological molecules in the cell [6] This makes it a major pathogenic factor and the cause of many Diagnosis conditions, such as coronary heart disease (CHD) [7], Ocular diseases [8] .Diabetes,Cancer, Rheumatoid arthritis, Parkinson's diseases, Alzheimer's diseas ... etc [9]. Common oxidized molecules include oxygen are known as reactive oxygen Species (ROS), and they are either in the form of unstable, short -age and interactive chemical structures [10] or it can be in the form of non-free radical compounds such as Hydrogen Peroxide (H_2O_2), which works as an important medium for operations that regulate oxidation and reduction or may be caused by oxidative damage according to the place and time of its collection [11] It participates in oxidative damage directly through its work as a partial oxidant or indirectly through its work in generating free radicals [12]. Free radicals are eliminated and effects using antioxidants that are prescribed as molecules that prevent or delay the oxidation of biomus by working on removing free radicals or by adjusting the cellular activities responsible for their production [5] Some of them are manufactured in the form of drugs and medicines, and some of them are found naturally [13] As in medicinal plants, vegetables, spices, fruits and microorganisms [14] and honey bee product known as propolis [15].

2. RELATED WORKS

[16] referred in their studies to the role of propolis in protecting the lung tissue from the oxidative damage caused by radiation by reducing oxidation factors and increasing the production of enzymatic antioxidants, in addition to the study of [17] which indicated the role of extracts of propolis in causing a significant increase in In the levels of antioxidants such as glutathione (GSH), superoxide dismutase (SOD), and glutathione peroxidase (GPx).

3. METHODOLOGY

Animals Used in the Study

Fivety-five of the males of the Sprague Dawley white rats were used with weights ranging between (160-200)g, which were equipped from the Faculty of Veterinary Medicine at Tikrit University and placed in plastic cages whose dimensions (46x28x13) cm and under the appropriate conditions of the appropriate temperature (25) C° and a light period divided into

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12 hours of light and 12 hours of darkness in addition to the availability of good ventilation for a period of 21 days.

Collection of Samples Propolis and Preparing the Extracts
Propolis samples were collected using propolis traps directly from previously classified *Apis melifera* honey bee hives. After collecting them, the samples were stored in plastic containers in the freezer and below at temperature (-20) C° to make it solid and able to be ground using an electric grinder to turn it into a powder for the purpose of increasing its surface area exposed to the solvent when preparing extracts,

Preparing the Alcholic Extract
The extract attended according to the method described by [18] to melt 30 g of propolis powder with 100 ml of absolute ethyl alcohol with a concentration of 99 % for 7 days at room temperature with the mixing, and the solution nominated the solution using Watman no: 1 and then the disposal took place From alcohol by exposing the Rotary Evaporatuore at a temperature (40) C° for 35 minutes to obtain the raw extract.

Preparing the Aqueous Extracts
The water extract was prepared according to the method described by [19] by dissolving 20 g of powder in 200 ml of distilled water inside a 250ml volumetric spin The medical and then using the filter paper for the purpose of obtaining the candidate and drying it with the electric oven at a temperature of 40 C° to obtain the raw extract in powder.

Collection of Blood Samples
The animals were fasted for 12 hours by cutting off their water and food, and were anesthetized using chloroform, after that they were then dissected, and blood samples were drawn directly from the heart (3 ml) for each animal and placed in test tubes. The tubes were transferred to the incubator for 30 minutes at a temperature of (37) C° and separate the serum from the rest of the blood components using a centrifuge at a speed of 3000 rpm for 15 minutes. Micropipettes were used to withdraw the serum and keep it in the freezer at a temperature of (-20°C) until the required tests are performed.

Antioxidant Tests
Determination of Superoxide Dismutase (SOD) Concentration in Serum
The activity of the superoxide dismutase enzyme was estimated using the modified photochemical nitroblue tetrazolum (NBT) method [20] it is measured according to the following equation [21]:

$$\text{SOD activity} = \frac{A0-A1}{A0} \div 50\% \times \frac{\text{System Volum}}{\text{Simple volum}} \times \text{Dilution factor}$$

Determination of Catalase (CAT) concentration in serum
The method described by [22] was used to measure the concentration of catalase enzyme in the serum.

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Determination of Glutathione Peroxidase (Gpx) Concentration in Serum
The enzyme was estimated according to the method of [23].

Determination of Lipidperoxidation Concentration in Serum
The thiobarbituric acid (TBA) reaction method modified used by researchers [24] was used to measure MDA.

Statistical Analysis
Statistical results were analyzed by using the SPSS statistical program and extracted morale through the Anova-One Way test and then the moral differences were identified according to the Duncans Multiple Ranges test (P≤0.05) [25].

4. RESULTS AND DISCUSSIONS

The Effect of the Studied Parameters on the Concentration of Super Oxide Dismutase (SOD) in Blood Serum
Figure (1) indicates a significant decrease (p≤ 0.05) in the Super Oxide dismutase in the group transaction with hydrogen peroxide H_2O_2 (2.68 ± 0.41) µmol /l when compared to the negative control group (5.35 ± 0.06) µmol /L,While In the groups treated with water extract, alcoholic extract and vitamin E, respectively (3.45 ± 0.24) (4.25 ± 0.10) 3.31 ± 0.09)) µmol /L when compared to the group of hydrogen peroxide H_2O_2 (2.68 ± 0.41) µmol /L.

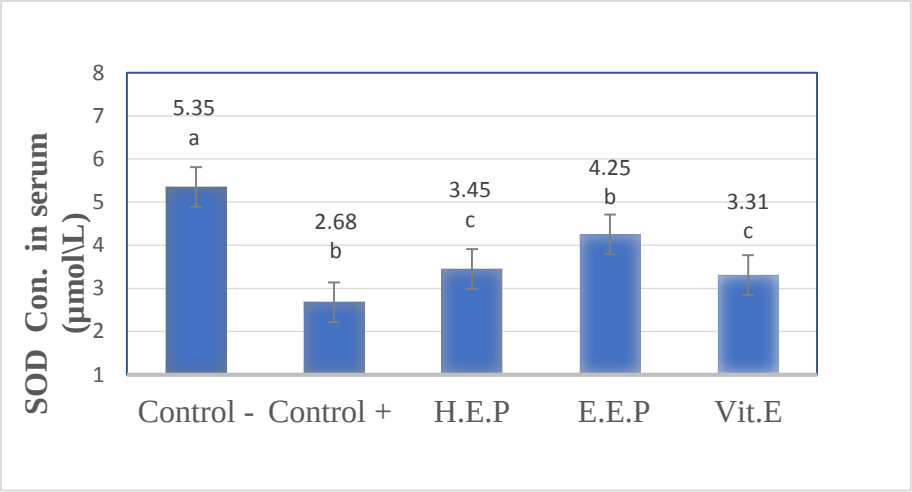

Figure 1 Effect of aqueous extract propolis (HEP), alcoholic extract propolis (E.EP), and vitamin E on the concentration of super oxide dismutase (SOD).

The Effect of the Studied Parameters on the Concentration of Glutathione Peroxidase (Gpx) in Blood Serum.
The results of the current study shown in Figure (2) show the presence of a moral decrease (P≤ 0.05) in the concentration of glutathione peroxides in the group translated by H_2O_2 (10.682 ± 0.169) U/ L when it is compared to the negative control group (13.260 ± 0.668) U/ L ,While In the groups treated with water extract, alcoholic extract and vitamin E,

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respectively (11.970 ± 0.227) ,(12.962 ± 0.259) ,(11.067 ± 0.157) U/L when it is compared to the positive control group (10.682 ± 0.169) U/L.

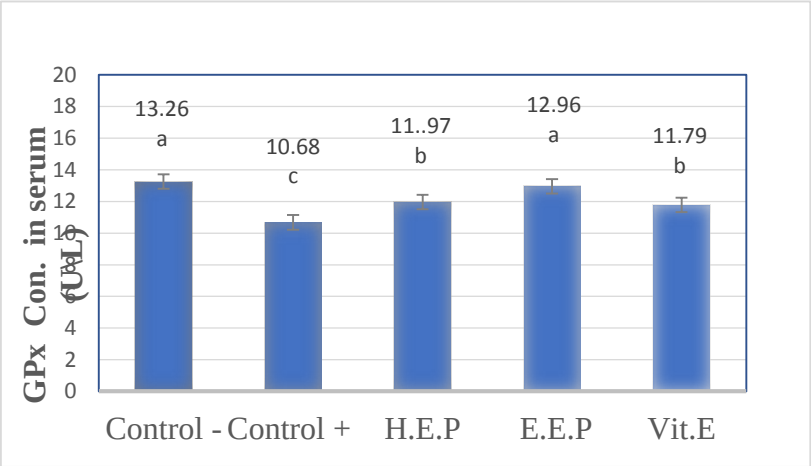

Figure 2 Effect of aqueous extract propolis (HEP), alcoholic extract propolis (E.EP), and vitamin E on the concentration of glutathione peroxidase (GPx).

The effect of the studied parameters on the concentration of catalase (CAT) in blood serum. Figure (3) indicates a significant decrease (p≤ 0.05)) in the concentration of Catalase in the group of positive control (G2) (14.802 ± 0.372) U/ mg units when compared to the negative control group (20.790 ± 0.786) U/ mg and a height Moral in groups treated with water extract, alcoholic extract and vitamin E, respectively (17.640 ± 0.541) (20.335 ± 0.366) (16.305 ± 0.470) U/mg when it is compared to the positive control group.

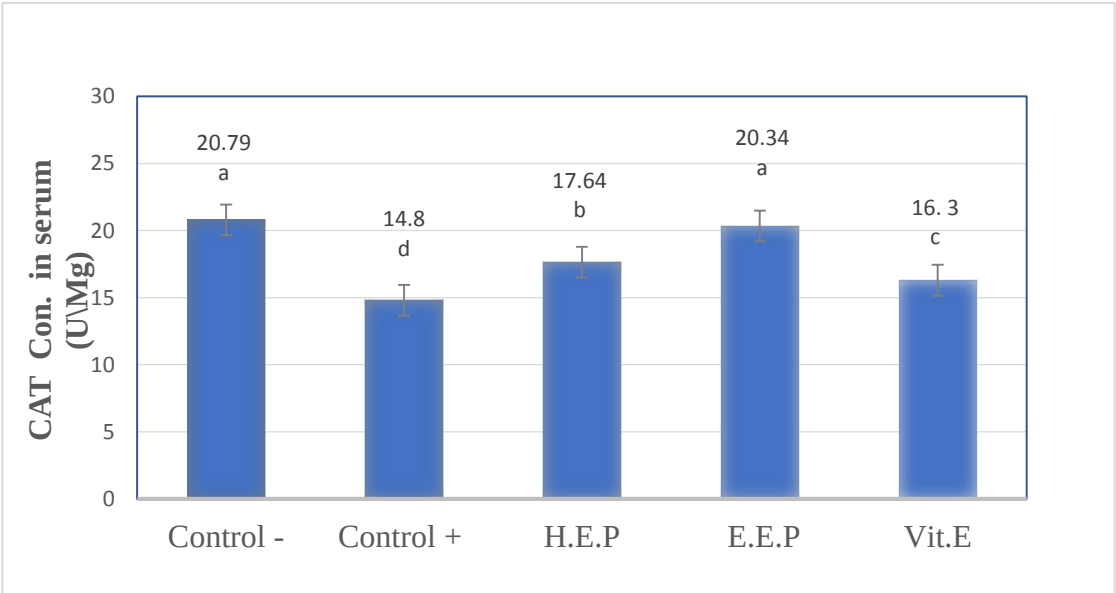

Figure: 3 Effect of aqueous extract propolis (HEP), alcoholic extract propolis (E.EP), and vitamin E on the concentration of catalase (CAT).

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The Effect of the Studied Parameters on the Concentration of Lipid Peroxidation in Blood Serum (MDA).

The results of the current study shown in Figure (4) appear a significant increase ($p \leq 0.05$) in the MDA concentration of the group translated with H_2O_2 (58.853 ± 1.592) µmol/ L when compared to the control group of negative control (28.935 ± 0.904) µmol/L as shown The presence of a moral height ($p \leq 0.05$) in the group treatment of water extract and treatment with alcoholic extract and treatment with vitamin E in succession (31.917 ± 3.145) (31.04 ± 1.597) (36.117 ± 4.429) µmol /L when it is compared to the possitive control group (58.853 ± 1.592) µmol / L.

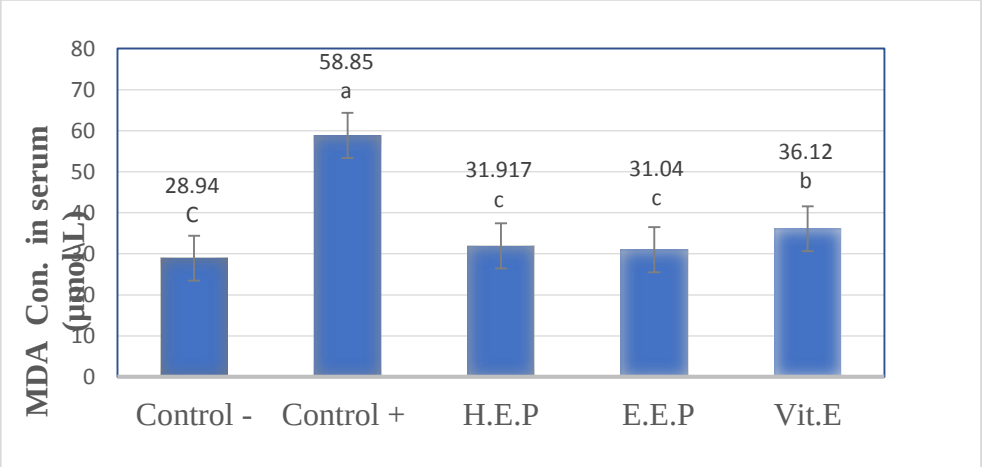

Figure 4 Effect of aqueous extract propolis (HEP), alcoholic extract propolis (E.EP) and vitamin E on the concentration of lipid peroxidation.

Effect of Hydrogen Peroxide on Enzymatic Antioxidants and Malondialdehyde

The results of the present study shown in Figures (1,2,3,4) showed a significant decrease ($p \leq 0.05$) in enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) a significant increase in the concentration of malondialdehyde (MDA) in the possitive control when compared with the negative group. These results were consistent with [26] as well as with the study of [27] , which indicated a significant decrease in some antioxidants as superoxide dismutase (SOD) and a significant increase in the concentration of malondialdehyde (MDA) as a result of increased oxidative stress, which causes increased free radical formation and depletion of antioxidants. The decrease in the activity of (SOD) in the blood serum in the group dosed with H_2O_2 may be due to the exposure of the molecules of this enzyme to oxidative damage due to the Hydroxyl radical derived from H_2O_2, which directly contributes to the fragmentation of this enzyme and its loss of its activity [28]. In oxidation and reduction reactions that increase in this group due to the presence of H_2O_2 hydrogen peroxide in drinking water and causes increased oxidative stress and works to deplete the GSH in its reduced form of blood and tissues [29]. This is done by inhibiting the pentose phosphate shunt pathway producing NADPH that is essential for the effectiveness of the enzyme glutathione reductase (GRx) in reductase GSH from its oxidized form GSSH [30], at the same time and at the same course it works to take off the

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enzyme of the glutathione peroxidase (GPx) that contributes to converting hydrogen peroxide into water in the body [31]. The reason for the moral rise of the malondialdehyde is the important vital indicator for evaluating the lipid peroxidation [32] ,because of the imbalance in the oxidant-antioxidant balance in pathological conditions in favor of free radicals, this encourages them to attack and oxidize fatty acids (PUFAs) located within the phospholipid layers of cell membranes [33] . Producing lipid hydroperoxides, the most important of which is MDA, which is characterized by its high toxicity to cells and its ability to inhibit antioxidant enzymes [34]. [35] pointed out in their study the role of oxidative stress and free radicals in the development of various diseases for both humans and animals, as hydrogen peroxide was used in the laboratory to cause many diseases associated with oxidative stress in laboratory animals, at a concentration of 0.5% along with drinking water in the development of diabetes [36], atherosclerosis lesions in rats [37] and ovarian diseases in mice [38].

Effect of Aqueous and Alcoholic Extracts of Propolis on Antioxidants and Malondialdehyde

Forms (1,2,3,4) show a significant significance of ($p \leq 0.05$) in the activity of antioxidants in the tiles with the water extract and the alcohol for the propolis E.E.P when compared to the positive group and this result is compatible with [39], [40] as well as the study of [17] that referred to the role of alcoholic extracts of propolis in the events of a significant increase in the levels of antioxidants such as the SOD and the GPX. The study of [41] and [42] also agreed with the results of our study, which indicated the role of aqueous extract in preventing the damage and toxic effects of oxidative stress by increasing the capacity of antioxidants such as catalase and superoxide dismutase. The moral increase that occurred after the dose with alcoholic and water extract is due to the chemical composition of propolis, which is characterized by its containing many biologically active chemical compounds (more than 850 compounds) Phenolic and caffeic acid phenethyl ester (CAPE) and cumaric acid ... [43] Which indirectly contributes to the reduction of oxidative stress by inhibiting enzymes that participate in the formation of ROS reactive species such as mono-oxigenase and NADPH-oxidase enzymes or directly by capturing reactive species to maintain antioxidant concentrations and this is what flavonoids do [44] In particular because of its ability to donate electrons from the hydroxyl group present in its structure to unstable ROS reactive species [45]. CAPE is also one of the compounds that are mainly available in propolis in addition to its presence in natural materials such as plants and fruits [46] It is characterized by its protective role in cells from oxidative stress damage [47][16] have pointed to its role in protecting lung tissue from radiation-induced oxidative damage by reducing oxidative factors and increasing antioxidant production by increasing the expression of antioxidant enzymes, inhibiting the expression of inflammatory cytokines, and modifying the pathways of the nuclear factor Nrf2 [48] has a pivotal role in regulating the gene expression of antioxidant structures [49] and in particular the expression of the enzyme heme oxygenase 1 (HO1), which activates cellular defense mechanisms against oxidative stress including superoxide dismutase (SOD), catalase (CAT) and glotathione-s-transferase (GST) [50]. As well as its role in producing a powerful antioxidant known as bilibrubin by breaking down heme into non-toxic by-products such as carbon monoxide (CO), free Fe and Biliverdin, which is reduced to bilirubin [51][52] is a source of antioxidant in certain physiological

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concentrations due to its unique mechanisms of antioxidant action by inhibiting the enzyme NADPH-oxidase producing superoxide radical O• [53]. The low MDA concentration in groups treated with propolis extracts is mainly due to the increase in antioxidant enzymes and the inhibition of enzymes that make up the types of interactive oxygen by some biologically active compounds in propolis such as flavonids [44], Many studies have pointed to the role of propolis supplements in influencing lipid metabolism and oxidative stress by reducing the products of lipid oxidation such as theobarbituric acid reactive substances (TBARS) and increasing antioxidant levels [54] In a pilot study on male rats exposed to cigarette smoke, propolis extract was found to reduce MDA levels and improve sperm quality [55] and in other study the application of propolis gel was shown to reduce MDA levels in the blood and increase the number of bone cells, which are important for bone health [56]. The results shown in figures ((1,2,3,4)) also showed that the antioxidant activity of propolis was higher in alcoholic extract compared to aqueous extract, because its alcohol-extracted phenolic content is ten times higher compared to water extract [57] due to the fact that the majority of its active compounds are poorly soluble in water [58].

Effect of Vetamin E on Antioxidants and Malondialdehyde

The results of the current study confirmed that vitamin E was able to improve the levels of enzymatic antioxidants and reduce the MDA concentrations and thus protect cells from the dangers of lipidperxidation. These results were compatible with the results of the study of both [59][60][61], which indicated the role of vitamin E in increasing the concentration of enzymatic antioxidants and reducing MDA. The significant increase in the concentration of enzymatic antioxidants and the decrease in the concentration of MDA in the group treated with 50 mg/kg vitamin E are mainly due to its antioxidant properties [62] by suppressing the production of free radicals resulting from lipid peroxidation, especially Brussels radical ROO• which oxidizes unsaturated fatty acids (PUFAs) in cell membranes [63] through its removal of these radicals and the formation of alpha-tocopheroxyl radicals - α as an intermediate [64], which are reduced directly to tocopherol-α as a result of its interaction with vitamin C, which regenerates its antioxidant properties and contributes to maintaining its levels in tissues within the normal range [65]. The work of vitamin E as an antioxidant is through several important mechanisms, including its absorption of free radicals and its conversion to harmless vehicles, and its interaction with the oxidizing fat, which protects the cells from the harmful effects of the oxidation process [64], as well as its contribution to preventing the occurrence of a kind of programmed death known Ferroptosis through its effect on the Lipoxygenase -15 it was found that there is a final future for vitamin E called the α -Tocopherol Hydroquinone it works as an effective inhibitor of this enzyme [66].

5. CONCLUSIONS

We conclude from the results of the current study that the propolis has an important role in preventing the harmful effects of oxidative stress, through its ability to increase the concentrations of enzymatic antioxidants and reduce lipid peroxidation. The results of the study also showed that the activity of propolis extracts as antioxidants differ in the different

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solvents used in the process of extraction, as the alcoholic extract showed a stronger effect compared to the water extract.

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