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Effect of Honey Consumed of COVID-19 Patients over Time and Changes in Concentrations Levels of Antioxidants and Oxidative Stress in the Patients over Time and Compare with Control Group.

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Abstract

This study is concerned with COVID-19pandemic and subjects were divided into three main groups honey consumed of COVID-19 patients(n = 90),without honey consumed of COVID-19 patients(n = 30) and control group(n = 30). The determine patients in the city of Najaf during the period between July 2020 to august 2020.Patients were identified using a PCR technique, after which a 5 milliliter was withdrawnon the first day to identify(VitaminA**,**VitaminC,VitaminE,GSH,GPx,SOD,CAT,MDA) and then repeat the process of determining the variables every seven days.In addition to giving COVID-19patients a kilogram of honey divided into different quantities and different times and compere with second group (without honey consumed of COVID-19patients)and examined by PCR technique.These variables measured the concentration by using assay (ELISA) technique and spectrophotometer instrument. All above variables there was a significant difference and correlation in patients ($p<0.05$).Also results showed changes in concentrations levels variablesof honey consumed COVID-19 a significant difference between the mean value in patients ($p<0.05$)but little changesin concentrations variableswithout honey consumed of COVID-19 patients a significant difference between the mean value in patients ($p<0.05$).The results showed the effect of honey consumed of COVID-19 patients over timeon patients three times a day and the patients are examined byPCR test showed Negative which more than 14 days.

KeywordsCOVID-19**,**antioxidants**,**oxidativestress**,**honeyandantioxidant enzymes.

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Introduction

The COVID-19 is a pandemic the infected over 74 million people, died morethan 1,600,000 million people in the word inthe December 2020[1].

The COVID-19 which find from China in 2019, is caused by thesevere acute respiratorysyndrome anda positive-sense of single-stranded RNA virus3–5and taxonomically is the member of the Betacoronavirus genus[2].COVID-19 patients have the fever and cough[3]gastrointestinal tract manifestations such as the diarrhea,vomiting and abdominal pain [4].Also showed the increased morbidity inthe COVID-19 infections other compound factors aredyspnea, lymphopenia and higher plasma levels ofthe cytokines (IL2, IL7 and IL10),MIP1A, IP10, MCP1,GSCFand interferon alpha (TNF-α) [5].

Antioxidants are the damage of the cellscaused by the free radical's reactions.The activityof the radical molecules by antioxidants is scavenging power by stopping chain reactions,peroxide decomposition, metal-chelating and inductionof antioxidant enzymes[6].Administration of higher than recommended daily doses of nutrients such as vitamins A,C, E, Zinc and omega-3 fatty acidshaveeffect on potentially reducing COVID-19 viral load and length of hospitalization[7].Oxidative stress is the instrumentalin the etiology of numerous human diseases.Oxidative stress can arisethrough the increased production of reactive oxygen species (ROS) because of a deficiency of antioxidantdefenses and this may further worsen respiratorydiseases(all COVID-19), especially when the level free radicalsare high,oxidative stress is the defined as an imbalance between toxic reactive oxygen species (ROS) and antioxidants infavor of oxidants, leading to the disruption of redox signaling and the irreversible oxidativedamage to lipids, deoxyribonucleic acid (DNA) or proteins[8].Oxidative damages arethe included in the development of different the pathologies including cancer, cardiovascularand lung diseases[9].

Honey as a folk medicine isreferred in the utmostancient written archives[10].Demarcation of its usesin the current professional medicine as a potential therapy isentirely underutilized,there is an affinity for someresearchers that usage of thehoney as a natural product supplement is well intentionedfor reflection as a therapy or adjuvant antioxidant therapyin current medicine[11,44].Honey also showed such as anti-inflammatory activity that is inhibits the expression of thesepro-inflammatory cytokines,the role of the honey has attributed to itsantioxidant properties[12].The antioxidantactivity of the honey is positively the correlated to its phenolic compoundscontent[13].The antimicrobial activities of the honey have been well studied against many fungi and bacteria, also its antiviral activities still need an extensive exploration so that it can be used asprevention and treatment of viral infections[14,45].

Methodology

The subjects were divided into three main groups, honey consumed overtimeon theCOVID-19patients(n = 90),without honey consumed COVID-19 patients (n = 30)and control group(n = 30). The determine the patients in the city of Najaf during the period between July 2020 to august 2020.Patients were identified using a PCR technique, after which a 5 milliliter was withdrawn on the first day to identify(VitaminA,VitaminC,VitaminE,GSH,GPx,SOD,CAT,MDA) and then repeat the process of determining the variables every seven days.In addition to giving COVID-19patients a kilogram of honey divided into different quantities and different times and then repeat the process of determining using a PCR technique to patients and compere with the other group, without honey consumed COVID-19 patients.

Procedure of Vitamin A

In the Serum the concentrations of Vitamin A were determined by using ELISA assays. ELISA kits for testing Vitamin A were obtained from lifespan Biosciences co (M-B-SMBS729269).

Procedure of Vitamin C

In the Serum the concentrations of Vitamin Cwere determined by using ELISA assays. ELISA kits for testing Vitamin C were obtained from lifespan Biosciences co (LS-F-25060).

Procedure of Vitamin E

In the Serum the concentrations of Vitamin E were determined by using ELISA assays. ELISA kits for testing Vitamin E were obtained from lifespan Biosciences co (LS-F-4970).

Procedure of GSH

Serum GSH level was determined by using a procedure utilizing Elaman's reagent (DTNB) [15].When takes three tubes simple, plank and standard, the first tubes sample put 100µL of serum and 800 µL DDW and 100 µL TCA , second tubes the blanks put 900 µL DDW and 100 µL TCA and the third tubes standard put 100 µL from standard and 800 µL from DDW and 100 µL from TCA. Tubes were mixed in vortex mixture for 10 minutes and centrifuged for 15 minutes and readat absorbance 412nm.

Procedure of GPx

In the Serum activity of GPx were determined by using Colorimetricmouthed were obtained from lifespan Biosciences co. The GPx number is (ab102530).

Procedure of SOD

SOD activity in serum was determined using (NBT) when takes three tubes simple, plank and standard, the first tubes sample put 3ml of Reacting mixture , 0.04ml Sodium cyanide , 0 .15ml Serum and 0.52 Working buffer solution, for the blank tubes put 3ml of Reacting mixture, 0.04ml Sodium cyanide and 0.67 Working buffer solution, for the standard tubes put3ml of Reacting mixture , 0.04ml Sodium cyanide and 0.67 Working buffer solution, All tubes were mixed, and read at absorbance 560 nm[16].

Procedure of serum Catalase

Dilution of 50 µl of serum by 5 ml of phosphate buffer solution (50 mM), the pH was adjusted to 7.0 immediately prior to assay. Two tubes were prepared as followed, the

als of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 8961 - 8974
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first tubes sample put 2ml of Diluting serum and 1ml H_2O_2, for second tubes is the blank tubes put 2ml of Diluting serum and 1mlPhosphatebuffersolution, pH 7.0.initial absorbance was measured after 15 seconds (t1) and the final absorbance was measured after 30 seconds (t2) and read at 240 nm[17].

Procedure of MDA

Serum MDAwas determined usingBurtis method,when takes two tubes simple and plank, the first tubes simply put 1ml of TCA 17.5%, 1ml TBA,1mlTCA 70 % and 150µlserum and the second tubes plank put 1ml of TCA 17.5%, 1ml TBAand 1mlTCA 70 % and read at absorbance 532 nm.

Statistical analysis

The statistical analysis was done by using SPSS (statistical package for social sciences) version 17 in which mean, stander error,t-test and ANOVA test were used for data comparison. Correlation between of all of the studied variables were evaluated using person's correlation significant and a p value of <0.05 was considered to be statistically significant.

Results

The purpose of this study was to evaluate the level of some variables (Vitamin A**,**Vitamin C, Vitamin E,GSH,GPx,SOD,CAT,MDA)associated withCOVID-19patients and control group. These results measured concentration of these variables by using assay (ELISA) technique and spectrophotometer instrument and this study showed effect of honey consumed overtimeon the COVID-19 patients and compere with COVID-19 patients without honey consumedand examined byPCRtechnique.The concentrations of these variables in serum of patients and control are shown in table(1).The lower mean value of Vitamin A was shown in patients (24.5± 2.1µg /dL) compared with control group (26.0 ± 0.9µg /dL).In the value of Vitamin C in patients waslower (0.31± 0.41mg/dL**)**than control group was (0.42 ± 0.30mg/dL).In the value of Vitamin E in patients was lower (0.61± 0.03 mg/dL**)**than control group was (0.85 ± 0.40mg/dL).For GSH value lower in patients (1.9± 0.37µmol/l) compared with control group (2.5 ± 0.22 µmol/l).ForGPx value lower in patients (30.5± 2.1 U/g) compared with control group (36.5 ± 2.6 U/g).Regarding to SOD the mean value in was recorded aslower (1.71 ± 0.37U/ml) than control group (2.82 ± 0.36 U/ml). On the other hand, the mean value of CAT in patients higher (111.5± 2.8MU/l) than control group (107.0 ± 4.1MU/l).Also mean value of MDA in patients higher (4.7± 0.48 mmol/l) than control group (3.38 ± 0.19 mmol/l).All above variables there was a significant difference between the mean value inpatients and control group ($p<0.05$).

Table 1. Concentrations levels of antioxidants andoxidative stressmarkers of COVID-19 patients and compare with control groups.

Parameters	**Variables**	**COVID-19 patients**	**Controlgroups (*n* = 30)**	***p* value**

		(*n* = 120)		
Antioxidant vitamins	**Vitamin A (µg /dL)**	**24.5 ±2.1**	**26.0±0.9**	**<0.05**
	VitaminC (mg/dL)	**0.31±0.41**	**0.42±0.30**	**<0.05**
	VitaminE (mg/dL)	**0.61±0.03**	**0.85±0.04**	**<0.05**
Antioxidant enzymes	**GSH (µmol/l)**	**1.9±0.37**	**2.5±0.22**	**<0.05**
	GPx(U/g)	**30.5±2.1**	**36.5±2.6**	**<0.05**
	SOD (U/ml)	**1.71±0.37**	**2.82±0.36**	**<0.05**
	Catalase (MU/l)	**111.5±2.8**	**107.0±4.1**	**<0.05**
Oxidative stress markers	**MDA(mmol/l)**	**4.7±0.48**	**3.38±0.19**	**<0.05**

The correlation value between eachvariable(Vitamin A**,** Vitamin C,Vitamin E,GSH,GPx,SOD,CAT,MDA)in serum of COVID-19 patientsshowed intable(2),theresultrevealed that correlation between all variables andsignificant difference (p<0.05).

Table2.Thecorrelation coefficient test of antioxidants andoxidative stress between eachpatientin COVID-19.

Parameters	**Correlation (r)**	***p* value**
Vitamin A (µg /dL)	**−0.403**	**<0.05**
VitaminC (mg/dL)	**−0.303**	**<0.05**
VitaminE (mg/dL)	**−0.284**	**<0.05**
GSH(µmol/l)	**0.042**	**<0.05**
GPx(U/g)	**−0.091**	**<0.05**
SOD(U/ml)	**−0.141**	**<0.05**
Catalase(MU/l)	**−0.162**	**<0.05**
MDA(mmol/l)	**0.177**	**<0.05**

The results showed changes in concentrations levels of antioxidants and oxidative stress of honey consumed of COVID-19 patientsover time are shown in table (3).The mean value of Vitamin A it increases over time except after 7days decrease was shown (24.5± 2.1, 24.1±1.9, 24.8±1.7, 25.2±1.5, 25.6±1.3µg /dL).In the value of Vitamin C it increases over time except after 7days decreasewas shown (0.31±0.41, 0.29±0.40, 0.32±0.39,0.34±0.37,0.37±0.37 mg/dL).In the value of Vitamin E it increases over time except after 7days decrease was shown (0.61±0.03,0.55±0.02, 0.69±0.03, 0.73±0.04, 0.81±0.04 mg/dL).For GSH value it increases over timeexcept after 7days decrease was shown(1.9±0.37,1.8±0.34,2.0±0.29,2.1±0.27,2.3±0.26 µmol/l).For GPx value it increases over time except after 7days decrease was shown (30.5±2.1, 29.2±1.9, 31.8±2.3, 32.7±2.4, 34.1±2.5 U/g).Regarding to SOD the mean value it increases over time except after 7days decrease was recorded (1.71±0.37,1.58±0,33., 1.93±0.36, 2.31±0.39, 2.64±0.36 U/ml). On the other hand mean value of CAT it decreases over time except after 7days increase was recorded (111.5±2.8,112.4±1,9, 110.2±2.1,109.1±3.5, 107.8±4.3 MU/l).Also mean value of MDA it decreases over time except after 7days increase was recorded (4.7±0.48, 5.1±0,39, 4.2±0.42, 3.9±0.23, 3.3±0.28 mmol /l). All above variables there was a significant difference between the mean value in patients ($p<0.05$).

Table3.The changesin concentrations levels of antioxidants and oxidative stress of honey consumed of COVID-19 patients over time(n = 90).

Parameters	Variables	COVID-19 patients (n= 90) First day	COVID-19 patients (n = 90) After 7 day	COVID-19 patients (n = 90) After14 day	COVID-19 patients (n = 90) After 21 day	COVID-19 patients (n = 90) After 28 day	*p*value
Antioxidant vitamins	Vitamin A (µg /dL)	24.5 ±2.1	24.1 ±1.9	24.8 ±1.7	25.2 ±1.5	25.6 ±1.3	<0.05
	VitaminC (mg/dL)	0.31±0.41	0.29±0.40	0.32±0.39	0.34±0.37	0.37±0.37	<0.05
	VitaminE (mg/dL)	0.61±0.03	0.55±0.02	0.69±0.03	0.73±0.04	0.81±0.04	<0.05
Antioxidant enzymes	GSH (µmol/l)	1.9±0.37	1.8±0.34	2.0±0.29	2.1±0.27	2.3±0.26	<0.05
	GPx (U/g)	30.5±2.1	29.2±1.9	31.8±2.3	32.7±2.4	34.1±2.5	<0.05
	SOD (U/ml)	1.71±0.37	1.58±0.33	1.93±0.36	2.31±0.39	2.64±0.36	<0.05
	Catalase (MU/l)	111.5 ±2.8	112.4 ±1.9	110.2 ±2.1	109.1 ±3.5	107.8 ±4.3	<0.05
Oxidative stress markers	MDA (mmol/l)	4.7±0.48	5.1±0.39	4.2±0.42	3.9±0.23	3.3±0.28	<0.05

The results showed little changes in concentrations levels of antioxidants and oxidative stress without honey consumed of COVID-19 patientsare shown in table(4).The mean value of Vitamin A little change over time was shown (24.5 ± 2.1,

ls of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 8961 - 8974
ived 25 April 2021; Accepted 08 May 2021.

24.1±1.9, 23.6±1.8, 23.9±1.7, 24.0±1.8 µg /dL).In the value of Vitamin C no changeover timewas shown (0.31±0.41,0.28±0.40, 0.29±0.38, 0.30±0.38, 0.30±0.37 mg/dL).In the value of Vitamin E little changeover timewas shown(0.61±0.03,0.57±0.02,0.58±0.03,0.59±0.03,0.58±0.04 mg/dL).For GSH value no changeover timewas shown (1.9±0.37, 1.8±0.34, 1.9±0.33, 1.9±0.33, 2.0±0.32 µmol/l).For GPx value no changeover time was shown (30.1±2.0, 29.9±1.9, 30.0±2.0, 30.5±2.1,29.9±1.9U/g).Regarding to SOD the mean value little changeover time was recorded (1.71±0.37, 1.70 ±0,36, 1.69±0.35, 1.67±0.36, 1.7±0.35 U/ml). On the other hand mean value of CAT little changeover time was recorded (111.5±2.8,111.2±1,8, 111.1±1.9,111.4±1.9, 111.5±1.9 MU/l). Also mean value of MDA little changeover time was recorded (4.7±0.48, 4.8±0,47, 4.8±0.46, 5.0±0.44, 4.9±0.49 mmol /l). ThevariablesVitamin A,Vitamin E,SOD,CAT and MDA there was asignificant difference between the mean value in patients (p<0.05), but inVitamin C, GSH, GPx and there was no significant difference between the mean value in patients (p>0.05).

Table4. The changesin concentrations levels of antioxidants and oxidative stress of the group without honey consumed of COVID-19 patients (*n* = 30).

Parameters	**Variables**	**COVID-19 patients (*n*= 30) First day**	**COVID-19 patients (*n* = 30) After 7 day**	**COVID-19 patients (*n* = 30) After14 day**	**COVID-19 patients (*n* = 30) After21 day**	**COVID-19 patients (*n* = 30) After 28 day**	***p* valu**
Antioxidant vitamins	**Vitamin A (µg /dL)**	**24.5 ±2.1**	**24.1 ±1.9**	**23.6 ±1.8**	**23.9 ±1.7**	**24.0 ±1.8**	**<0.0**
	VitaminC (mg/dL)	**0.31±0.41**	**0.28±0.40**	**0.29±0.38**	**0.30±0.38**	**0.30±0.37**	>0.0
	VitaminE (mg/dL)	**0.61±0.03**	**0.57±0.02**	**0.58±0.03**	**0.59±0.03**	**0.58±0.04**	**<0.0**
Antioxidant enzymes	**GSH (µmol/l)**	**1.9±0.37**	**1.8±0.34**	**1.9±0.33**	**1.9±0.33**	**2.0±0.32**	>0.0
	GPx (U/g)	**30.1±2.0**	**29.9±1.9**	**30.0±2.0**	**30.0±2.1**	**29.9±1.9**	>0.0
	SOD (U/ml)	**1.71±0.37**	**1.70±0.36**	**1.69±0.35**	**1.67±0.36**	**1.7±0.35**	**<0.0**
	Catalase (MU/l)	**111.5 ±2.8**	**111.2 ±1.8**	**111.1 ±1.9**	**111.4 ±1.9**	**111.5 ±1.9**	**<0.0**
Oxidative stress markers	**MDA (mmol/l)**	**4.7±0.48**	**4.8±0.47**	**4.8±0.46**	**5.0±0.44**	**4.9±0.49**	**<0.0**

The results showed the effect of honey consumed over time on COVID-19 patients are shown in table (5).When honey is given to patients three times a day and every times 12g and the patients are examined byPCRtechnique showed Positive. When honey is given to patients three times a day and every times 12g , after 7 day 252g and the patients are examined byPCRtechniqueshowed Positive.When honey is given to patients three times a day and every times 12g , after 14 day 504g and the patients are examined byPCRtechniqueshowed Negative.When honey is given to patients three times a day and every times 12g , after 21 day 756g and the patients are examined byPCRtechniqueshowed Negative.When honey is given to patients three times a day and every times 12g, after 28 days 1008g and the patients are examined byPCRtechniqueshowed Negative.

Table 5. Examination results of the effect of honey consumed ofCOVID-19patients over time and examined by PCR technique.

COVID-19 patients (*n*= 90) Over time	**COVID-19 patients (*n*= 90) First day**	**COVID-19 patients (*n* = 90) After 7 day**	**COVID-19 patients (*n* = 90) After 14 day**	**COVID-19 patients (*n* = 90) After 21 day**	**COVID-19 patients (*n* = 90) After 28 day**
Honey consumed of COVID-19 patients	**(36 g)**	**(252 g)**	**(504 g)**	**(756 g)**	**(1008 g)**
PCR test of COVID-19 patients	**Positive**	**Positive**	**Negative**	**Negative**	**Negative**

The results showedon without honey consumed COVID-19 patients are shown in table (6).When the patients are examined first day by PCRtechnique showed Positive.When the patients are examinedafter 7 day by PCRtechnique showed Positive.When the patients are examined after 14 day by PCRtechnique showed Positive.When the patients are examined after 21 day by PCRtechnique showed Negative.When the patients are examined after 28 day by PCRtechnique showed Negative.

Table 6. Examination results without honey consumed of COVID-19 patients and examined by PCR technique.

COVID-19 patients (*n*= 30) Over time	**COVID-19 patients (*n*= 30) First day**	**COVID-19 patients (*n* = 30) After 7 day**	**COVID-19 patients (*n* = 30) After 14 day**	**COVID-19 patients (*n* = 30) After 21 day**	**COVID-19 patients (*n* = 30) After 28 day**
Without honey consumed of COVID-19 patients	**(0 g)**	**(0 g)**	**(0 g)**	**(0 g)**	**(0 g)**

als of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 8961 - 8974
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PCR test of COVID-19 patients	**Positive**	**Positive**	**Positive**	**Negative**	**Negative**

Discussion

This study examined the levels of antioxidants of vitamins (vitamins A, C and E), enzymes (GSH, GPx SOD and Catalase), and some oxidative stress markers (MDA) in COVID19 patients and controls group.The effect of COVID 19 on serum levels of vitamins A, C and E has been investigatedof the current study.Vitamins A, C and E showed significantly lower in COVID 19patients when compared with control group.Vitamin Ashowed the decrease in COVID 19 patients compared to controls;these findings are similar to other reports[18]. Our results showed low levels of vitamin C in COVID-19 patients,this results also supports by other report[19],that antioxidants vitamins levels are decrease in SARS-COV-2 infection due to their scavenging effect on ROS.Also other reported showed the low levels of antioxidant vitamins in COVID 19 patients, and hence suggested the aim lead to improving the levels of these vitamins the useful in these patients[20]. Our study showed lower values of antioxidant vitamins in COVID 19 patients may be due overproduction of the ROS and a deprived antioxidant system. The increase the oxidative stress by typically activating phagocytes which produce the ROS[21].Vitamin C and E being renowned antioxidants and to some extent when vitamin A scavenge for the ROS and counteract their effects, in this process of scavenging lead to the level of these vitamins in the body becomes depleted[22]. Vitamins C and E are found in the body and lead to helpful in cytokine storms and cellular injury and these vitamins are the strong antioxidants and decrease the ROS and these vitamins as helpful in SARS-Cov-2 and other viral infections [23].The decrease level of these vitamins are showed in this study is owing to their role in scavenging ROS as they are being used up in the process and findings are similar to other reports[24].The antioxidant defenses was detected in the COVID-19 patients as evidenced by the levels of vitamin A, C and E that were the largely interval and all the in COVID-19 patients, their median value in the vitamin C corresponds to the hypovitaminosis C[25]. Our results that vitamin C,E and Adecrease in COVID-19 patients and similar to other reported[26,27].Infact, the intravenous administration only has able torestore high-level ascorbic acid plasma concentration[28].The high doses of vitamin C can help to decrease the "cytokines storm"[29].Our results also showed lower level the GSH,GPx, SOD and catalase in COVID-19 patientscompere with controls group andsimilar to other study, the reports work on patients with Epstein–Barr virus, decreased activities of GSH, SOD and GPx[30]. Also other reported similar to our study decreases in the activities of these antioxidant in patientswith viral disease[31].Our results showed the higher levels of catalase in COVID-19 compared to control group , the study has also demonstrated that thepartial reduction of O2 in oxidative processes generates superoxide which is acted upon by the SOD converting it to hydrogen peroxide H2O2 which may

ıls of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 8961 - 8974
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subsequently react forming hydroxyls (OH) through the Fenton reaction , GSH/GPX and CAT are important antioxidant components in neutralizing these oxidative processes. Our result supported by other reports work on SARS-COV-2 infection activates the phagocytic cells which cause the ROS to be excessively produced, while the antioxidant enzymes are insufficiently present leading to a weakened antioxidant system in the face of increased ROS production[32].The GPx system allows reduced GSH to bind to free radicals giving oxidized GSH which lead to regenerated into GSH through this system[33].Other study suggested work on SARS-COV-2 similar to other RNA viruses can trigger an oxidative stress leading to a weakened antioxidant system, infection with COVID19 patients leads to heightened oxidative stress overwhelming the body's antioxidant mechanisms as shown by the decreased levels of SOD, GSH and GPx in our study and supported by other report work on SARS-COV-2 infection[34]. On other hand ourresults the increase level of oxidative stresssuch as MDAin COVID-19 patients and compere with controls groups,COVID-19 is associated with increased oxidative processes , the increased oxidative stress causes increased lipid peroxidation and our result supported by other report[35].The another study on factors which increase production of the ROS such as malaria[36].Other reported increased lipid peroxidation and increase level of MDA are Similar to our results the increased levels of MDA in COVID-19[37].Our result is given honey to patients three times a day and every times 12g till 28 day and the patients are examined by PCRtechnique and showed effect the honey on change levels variables over time.Thesereports showed honey consumed that effect on immune system in COVID-19 patients over time because honey work similar to antioxidant.The several enveloped viruses killed by the virucidal ingredients of honey thereforealso have a potent suppressive effect on COVID-19 , cell death are triggered by viral infection through draining the lymphocytes which can be tackled by antioxidants , That proves are relation between antiviral and antioxidant actions [38].Alsothe honey provides a favorable environment lead to promotes healing quickly , At the same time are the antibacterial properties of honey can speed up the healing process by producing white blood cells WBC to the increase the pro-inflammatory cytokines including TNF-α, IL-1β, and IL-6 [39]. In some reports suggested the honey has proved its potency against several RNA and DNA viruses, influenza virus, rubella, herpes simplex virus , varicella-zoster virus and has proved that it can be a potential antiviral agent[40].The another reports shown the honey has that may affect the pharmacokinetics of some drugs,in vivo honey interferes with the activity of cytochrome p450 (CYP450) isozymes , it was also shown that increased CYP3A4 activity requires regular ingestion of honeywhile occasional ingestion is unlikely to significantly affect drug plasma concentrations, the honey may cause altered response to drugs metabolized by CYP3A4[41]. Another human study reported that daily consumption of the honey does not affect hepatic and intestinal CYP3A and P-glycoprotein activities but affectimmune system[42]. Our result smiler to other report that honey work similar to antioxidant and effect on immune system in COVID-19 patients[43].

Conclusion

This present research evaluated the levels of antioxidant vitamins and enzymes among COVID-19 patients compared with control group.From our findingswe can conclude that COVID-19 patients are the prone to depleted the levels of antioxidant substances due to their increased ofutilization in the counterbalancing the negative effect of free radicals. In addition to the deficiency of antioxidants and increased oxidative stressin COVID-19 lead to a gap in understanding thespecific micronutrient deficient in COVID-19which can distort body's immune function while the increasing susceptibility to other infectious disease, also honey consumed that effect immune system in COVID-19 patients over time because honey work similarto antioxidant. Honey can be considered a serine potential natural antioxidant medicine. The honey acts through a modulatory road of multiple signaling pathways and molecular targets,it may interfere with multiple targets in cell signaling pathways such as induction of caspases in apoptosis, stimulation of TNF-α, IL-1β, IFN-γ, IFNGR1, p53, and immune cells, inhibition of cell proliferation, cell cycle arrest, inhibition of lipoprotein oxidation, IL-1, IL-10, COX-2, LOXs, and PGE2, and modulation of other diverse targets.

References

1. World Health Organization. Novel Coronavirus (2019-nCoV).*WHO Bulletin*, (2020).
2. Wu F, Zhao S, Yu B, et al. A new coronavirus associated withhuman respiratory disease in China. *Nature* (2020); 579(7798):265–269.
3. Kong R, Yang G, Xue R, et al. COVID-19 Docking Server:an interactive server for docking small molecules, peptidesand antibodies against potential targets of COVID-19, (2020).
4. Wong SH, Lui RN and Sung JJ. Covid-19 and the digestivesystem. *J Gastroenterol Hepatol* (2020); 35: 744–748.
5. Dietz W and Santos-Burgoa C. Obesity and its implicationsfor COVID-19 mortality. *Obesity* (2020); 28(6): 1005.
6. Ntyonga-Pono MP. COVID-19 infection and oxidative stress:an under-explored approach for prevention and treatment *Pan African Med J* (2020); 35(Suppl. 2): 12.
7. A.F. Gombart, A. Pierre, S. Maggini, A review of micronutrients and the immune System–working in harmony to reduce the risk of infection, Nutrients 12 (1) (2020) 236.
8. Jones. D.P. Redefining oxidative stress. Antioxid. Redox Signal (2006), 1865–1879.
9. Ito, F.; Sono, Y.; Ito, T. Measurement and Clinical Significance of Lipid Peroxidation as a Biomarker of Oxidative Stress: OxidativeStress in Diabetes, Atherosclerosis, and Chronic Inflammation. Antioxidants (2019), 8, 72.
10. P.C. Calder, Nutrition, immunity and Covid-19, BMJ Nutr. Prev. Health (2020) .
11. Editorial: Honey: sweet and dangerous or panacea?," SouthAfrican Medical Journal, (1994)vol. 48, no. 56, p. 2300.
12. Miguel, M.G.; Antunes, M.D.; Faleiro, M.L. Honey as a Complementary Medicine. Integr. Med. Insights (2017),12.

13. 13.Kek, S.P.; Chin, N.L.; Yusof, Y.A.; Tan, S.W.; Chua, L.S. Total phenolic contents and colour intensity ofMalaysian honeys from the Apis spp. and Trigona spp. bees. Agric. Agric. Sci. Procedia (2014), 2, 150–155.
14. Albaridi, N.A. Antibacterial Potency of Honey. Int. J. Microbiol. (2019), 246–507.
15. 15.Boyer R.Mordent Experimental Biochemistry, 3[ed], San Francisco, Addisoi Wesley Longman(2000).
16. 16.Al Zamely O. M. Y"Ischemic heart disease via oxidative hypothesis", Ph.D. thesis, Mustansiriya University, Iraq (2001).
17. Cruz,M.C., Castano,I., Helguera, O. A. and Penas, A.D..Oxidative stress response to menadione and cumene hydroperoxide in the opportunistic fungal pathogen *Candida glabrata*. Mem Inst Oswaldo Cruz, Rio De, (2009) 104(4): 649-654.
18. Care C, Chiscano-Camón L, Ruiz-Rodriguez JC, et al. Vitamin C levels in patients with SARS-CoV-2-associated acute respiratory distress syndrome. Crit Care (2020); 24: 522.
19. Chris P. COVID-19 infection and oxidative stress: an underexplored approach for prevention and treatment. PAMJ (2020); 35(Supp2): 12–13.
20. Shakoor H, Feehan J, Al AS, et al. Immune-boosting role of vitamins D, C, E, zinc, selenium and omega-3 fatty acids: Could they help against COVID-19? Maturitas (2020); 143: 1–9
21. Derbyshire E and Delange J. COVID-19: is there a role for immunonutrition, particularly in the over 65s? BMJ Nutr Prevent Health (2020); 3: 071.
22. Delgado-Roche L and Mesta F. Oxidative stress as key player in severe acute respiratory syndrome coronavirus (SARSCoV) infection. Arch Med Res (2020); 51(5): 384–387.
23. Jan H, Usman H and Zainab R. COVID-19: a brief overview on the role of vitamins specifically vitamin C as immune modulators and in prevention and treatment of SARS-Cov-2 infections. Biomed J Sci Tech Res (2020); 28(3): 21580–21586.
24. Lindblad, M.; Tveden-Nyborg, P.; Lykkesfeldt, J. Regulation of Vitamin C Homeostasis during Deficiency. Nutrients (2013), 5, 2860–2879.
25. Chiscano-Carnon, L.; Ruiz-Rodriguez, J.C.; Rulz-Sanmartin, A.; Roca, O.; Ferrer, R. Vitamin C levels in patients with SARS-CoV-2- associated acute respiratory distress syndrome. Crit. Care (2020), 24, 1–3.
26. Singer, P.; Blaser, A.R.; Berger, M.M.; Alhazzani, W.; Calder, P.C.; Casaer, M.P.; Hiesmayr, M.; Mayer, K.; Montejo, J.C.; Pichard, C.; et al. ESPEN guideline on clinical nutrition in the intensive care unit. Clin. Nutr. (2019), 38, 48–79.

27. Carr, A.C.; Rosengrave, P.C.; Bayer, S.; Chambers, S.; Mehrtens, J.; Shaw, G.M. Hypovitaminosis C and vitamin C deficiency in critically ill patients despite recommended enteral and parenteral intakes. Crit. Care (2017).

28. Boretti, A.; Banik, B.K. Intravenous vitamin C for reduction of cytokines storm in acute respiratory distress syndrome. PharmaNutrition (2020), 12, 100190.

29. Dworzanski J, Strycharz-Dudziak M, Kliszczewska E, et al. Glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity in patients with diabetes mellitus type 2 infected with Epstein-Barr virus. PLoS ONE (2020); 15(3): e0230374.

30. Strycharz-dudziak M. Total antioxidant status (TAS), superoxide dismutase (SOD), and glutathione peroxidase (GPx) in oropharyngeal cancer associated with EBV infection. Viruses (2019): 5832410.

31. Qin M, Cao Z, Wen J, et al. An antioxidant enzyme therapeutic for COVID-19, (2020) .

32. Chris P. COVID-19 infection and oxidative stress: an underexplored approach for prevention and treatment. PAMJ (2020); 35(Supp2): 12–13.

33. Derouiche S. Oxidative stress associated with SARS-Cov-2 (COVID-19) increases the severity of the lung disease—a systematic review. J Infect Dis Epidemol (2020); 6(3): 1–6.

34. Nour Eldin EEM, Nour Eldein MM, El-Readi MZ, et al. Evaluation of the diagnostic and predicative values of 8-isoprostaglandin F2α as a biomarker of breast cancer. Oncol Res Treat (2020); 43(10): 506–517.

35. Muhammad Y, Aminu YK, Ahmad AE, et al. An elevated 8-isoprostaglandin F2 alpha (8-iso-PGF2α) in COVID-19 subjects co-infected with malaria. Pan Afr Med J (2020); 37: 78.

36. E. Peterhans, Oxidants and antioxidants in viral diseases: disease mechanisms and metabolic regulation, J. Nutr. 127 (5) (1997) 962S–965S.

37. A.J. Tonks, E. Dudley, N.G. Porter, J. Parton, J. Brazier, E.L. Smith, et al., A 5.8- kDa component of manuka honey stimulates immune cells via TLR4, J. Leukoc. Biol. 82 (5) (2007) 1147–1155

38.] B. Zeina, O. Othman, S. Al-Assad, Effect of honey versus thyme on Rubella virus survival in vitro, J. Alternative Compl. Med. 2 (3) (1996) 345–348.

39. N.S. Al-Waili, Topical honey application vs. acyclovir for the treatment of recurrent herpes simplex lesions, Med. Sci. Mon. 10 (8) (2004) MT94–98.

40. J. Horn and P. Hansten, “The sweet effect of honey on drug metabolism,” Pharmacy Times, (2007) vol. 73, no. 9, p. 48.

41. L. Fetzner, J. Burhenne, J. Weiss et al., "Daily honey consumption does not change CYP3A activity in humans," The Journal of Clinical Pharmacology, vol. 51, no. 8, pp. (2020) 1223– 1232, 2011.

42. H . Shakoor et al., Immune-boosting role of vitamins D, C, E, zinc, selenium and omega-3 fatty acids: Could they help against COVID-19 , Maturitas 143 (2021) 1–9

43. K. S. Hossain, G. Hossain, A. Moni Prospects of honey in fighting against COVID-19: pharmacological insights and therapeutic promises, heliyon 6 (2020).

44. Jalil AT. COVID-19 most affected age groups and lethality in Europe, Glob. J. Public Health Med. 2020;2:179-84.

45. Saleh, Marwan Mahmood, Abduladheem Turki Jalil, R. A. Abdulkareem, and Ahmed Aduljabbar Suleiman. "Evaluation of immunoglobulins, CD4/CD8 T lymphocyte ratio and interleukin-6 in COVID-19 patients." (2020).