European Review for Medical and Pharmacological Sciences 2023; 27: 11445-11456

The potential therapeutic effect of metformin in type 2 diabetic patients with severe COVID-19

H.M. AL-KURAISHY[1], A.I. AL-GAREEB[1], A.A. EL KHOLY[2], E. EL-KHATEEB[3,4], A. ALEXIOU[5,6], M. PAPADAKIS[7], E. ELEKHNAWY[8], N. ALSUBAIE[9], R.S. HAMAD[10,11], G.E. BATIHA[12]

[1]Department of Clinical Pharmacology and Medicine, College of Medicine, Al-Mustansiriyia University, Baghdad, Iraq
[2]Department of Clinical Pharmacy, Faculty of Pharmacy, Ain-Shams University, Cairo, Egypt
[3]Department of Clinical Pharmacy, Faculty of Pharmacy, Tanta University, Tanta, Al Gharbia, Egypt
[4]Certara UK Limited (Simcyp Division), Sheffield, United Kingdom
[5]Department of Science and Engineering, Novel Global Community Educational Foundation, Hebersham, Australia
[6]AFNP Med, Wien, Austria
[7]Department of Surgery II, University Hospital Witten-Herdecke, University of Witten-Herdecke, Wuppertal, Germany
[8]Pharmaceutical Microbiology Department, Faculty of Pharmacy, Tanta University, Tanta, Egypt
[9]Department of Pharmacy Practice, College of Pharmacy, Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia
[10]Biological Sciences Department, College of Science, King Faisal University, Al Ahsa, Saudi Arabia
[11]Central Laboratory, Theodor Bilharz Research Institute, Giza, Egypt
[12]Department of Pharmacology and Therapeutics, Faculty of Veterinary Medicine, Damanhour University, Damanhour, AlBeheira, Egypt

Abstract. – OBJECTIVE: Type 2 diabetes mellitus (T2DM) is regarded as a chief risk factor for(coronavirus disease 2019 (COVID-19) owing to dysregulation of the expression of angiotensin-converting enzyme 2 (ACE2) and chronic low-grade inflammatory disorders. Metformin, an insulin-sensitizing agent for managing T2DM, has pleiotropic anti-inflammatory and oxidant potentials, which may lessen the risk of diabetic complications. So, we aimed to reveal the potential role of metformin monotherapy in treating T2DM patients with COVID-19.

PATIENTS AND METHODS: In this prospective cohort study, 60 hospitalized T2DM patients with COVID-19 on metformin plus standard anti-COVID-19 treatments compared to 40 hospitalized T2DM patients with COVID-19 on other diabetic pharmacotherapy like insulin and sulfonylurea, were recruited. Inflammatory and oxidative stress biomarkers and radiological and clinical outcomes were assessed at admission time and at the time of discharge.

RESULTS: The results of this study illustrated that metformin treatment in T2DM patients with COVID-19 was more effective in reducing inflammatory and oxidative stress biomarkers with significant amelioration of radiological scores and clinical outcomes compared to T2DM patients with COVID-19 on another diabetic pharmacotherapy.

CONCLUSIONS: Our findings highlighted that metformin efficiently managed T2DM patients with COVID-19 by reducing inflammatory and oxidative stress with mitigating effects on the radiological scores and clinical outcomes.

Key Words:
T2DM, Metformin, SARS-CoV-2.

Introduction

COVID-19 is a worldwide pandemic triggered by severe acute respiratory coronavirus type 2 (SARS-CoV-2)[1]. Patients having COVID-19 might be asymptomatic or have mild flu-like sickness in 85% of cases. Nevertheless, 10-15% of the instances could suffer from moderate symptoms like fever, sore throat, headache, sweating, anosmia, dry cough, myalgia, and dyspnea. Severe cases (5% of the COVID-19 patients) could have severe symptoms, such as dyspnea, severe fever,

Corresponding Authors: Engy Elekhnawy, MD; e-mail: Engy.ali@pharm.tanta.edu.eg
Gaber El-Saber Batiha, MD; e-mail: dr_gaber_batiha@vetmed.dmu.edu.eg

tachypnea, and signs of hypoxemia owing to the progress of acute lung injury (ALI) as well as acute respiratory distress syndrome (ARDS)[2]. It could progress to developing a critical case requiring admission to the intensive care unit (ICU) and mechanical ventilation. Most COVID-19 patients recovered with a low mortality rate (2-3%) without complications. Nevertheless, hospitalized severely COVID-19 patients in the ICU have high mortality (up to 50%). In addition, if recovered, most of them could develop long-term complications called post-COVID-19. The underlying reasons for this high mortality rate in critically COVID-19 patients are attributed to the cytokine storm and its linked complications, such as ARDS, respiratory failure, multi-organ failure (MOF), as well as shock[3].)

Thousands of SARS-CoV-2 variants are grouped into monophyletic groups known as clades, which are divided SARS-CoV-2 variants into five groups, alpha, beta, gamma, delta, and omicron[4] (Table I).

A downregulation usually occurs after the interaction between SARS-CoV-2 and angiotensin-converting enzyme receptor-2 (ACE2) receptors. ACE2 participates in the regulation of the renin-angiotensin system (RAS), and it transforms the pro-inflammatory angiotensin II (AngII) to the anti-inflammatory Ang1-7. Thus, when ACE2 is downregulated, a rise in the circulating AngII will be induced with a decrease in the Ang1-7. Such alterations would augment the discharge of the pro-inflammatory cytokines as well as tissue injury[5].

Also,(SARS-CoV-2 can attach cluster differentiation 147 (CD147) that is significantly expressed in the erythrocytes, endothelial, epithelial, neuronal, and lymphocyte cells. CD147 could induce various inflammatory diseases *via* prompting the discharge of the inflammatory cytokines and platelet activation[6]. Likewise, SARS-CoV-2 can bind dipeptidyl peptidase 4 (DPP4), but to a lower degree than ACE2[7]. DPP4 is central to glucose metabolism by degrading incretins like glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1(GLP-1). Over-expression of DPP4 in obesity and type II diabetes mellitus (T2DM) is linked with the development of hyperglycemia[8]. Also, DPP4 has a non-enzymatic activity, as it activates T cell *via* the production of co-stimulatory signals after binding to adenosine deaminase[9]. Herein, DPP4 is implicated in the development of immunoinflammatory disorders. Consequently, DPP4 might be a potential connection between COVID-19 and DM, which could explain the vulnerability of diabetic patients to the influence of COVID-19.

The probable risk factors for(COVID-19 are linked to many factors, including gender, age, and co-morbidities like T2DM[10]. T2DM is regarded as a metabolic disorder and may present with acute and chronic complications like diabetic ketoacidosis and chronic renal failure[11]. It has been shown that obesity, gestational diabetes, and lack of exercise increase the hazard for the progression of T2DM[12]. T2DM is typically started in the middle age group, though it may begin at the young age group called maturity-onset diabetes of youth (MODY)[13,14]. The pathophysiology of hyperglycemia in T2DM is linked to peripheral insulin resistance (IR) as well as relative insulin deficiency[15]. T2DM is regarded as a potential risk factor for COVID-19 severity owing to dysregulation of ACE2, DPP4, and CD147, as well as chronic low-grade inflammatory disorders[16,17].

T2DM is a mainly preventable disease by regular exercise and dietary changes with reduced body weight in obese patients[18]. Metformin is an insulin-sensitizing compound utilized as a first-line for T2DM. Metformin is an oral drug absorbed from the small intestine by the plasma membrane monoamine transporter (PMAT) that is highly expressed in the enterocytes. Its uptake is undertaken by the organic cation transporter 2 (OCT2) expressed on the brush border of enterocytes[19]. Metformin has a positive charge, and it is highly accumulated in cells with a negative charge, like mitochondria[20,21]. Metformin

Table I. SARS-CoV-2 variants.

Variants	Lineage	Country	Date	Transmission
Alpha	B.1.1.7	United Kingdome	2020	High
Beta	B.1.351	South Africa	2020	High
Gamma	P.1	Brazil	2020	High
Delta	B.1.617.2	India	2020	High
Omicron	B.1.1.529	Botswana	2021	Low

hinders the production of ATP by inhibiting the mitochondrial complex I, causing an increase in the adenosine monophosphate protein kinase (AMPK)[22]. AMPK decreases gluconeogenesis, fat synthesis, and hepatic fat storage, improving insulin sensitivity[23]. In addition, metformin stimulates the utilization of glucose by the microbiota in the gut by activating the discharge of the GLP-1 from L cells in the intestine[24]. Moreover, metformin improves peripheral glucose utilization by increasing the expression of glucose transporter type 4 (GLUT4) with subsequent improvement of insulin sensitivity[25]. Also, metformin has pleiotropic characteristics, including anti-inflammatory and oxidant properties, thus decreasing the complications of diabetes[26,27].

Recently, it has been shown that metformin was efficient against the proliferation of SARS-CoV-2 as documented in *in silico* studies[28,29]. Metformin in combination with DPP4 inhibitors was also efficient in the control of T2DM patients with COVID-19. Therefore, we aimed to elucidate the possible role of metformin monotherapy in managing T2DM patients with COVID-19.

Patients and Methods

Study Design

In this prospective cohort study, 60 hospitalized T2DM patients (45 men and 15 women) with COVID-19 aged 45-61 years on metformin therapy 850 mg twice daily plus standard anti-COVID-19 treatments compared to 40 hospitalized T2DM patients (35 men and five women) with COVID-19 aged 46-63 years on other diabetic pharmacotherapy like insulin and sulfonylurea were recruited. The study continued for 21 days. This study was conducted in the Al-Atah Medical Center, a specialized center for treating COVID-19, from March to June 2020 in Baghdad, Iraq. Furthermore, all methods were accomplished in agreement with the applicable guidelines and regulations of the Helsinki Declaration[30]. Written consent was obtained from all hospitalized patients or their relatives. This study was approved by a scientific jury and the editorial board in the College of Medicine, Al-Mustansiriyah University, Iraq, Baghdad, according to reference No. 34MTR on 20/2/2020. T2DM patients aged > 40 years on metformin and/or other diabetic pharmacotherapy were included in this study. Nevertheless, T2DM patients with complications on metformin in combination with other anti-diabetic pharmacotherapy were excluded from this study. The hospitalized patients were prospectively evaluated at hospitalization time, during hospitalization time, and at the time of release for clinical and radiological outcomes. Past medical history, demographic data, and clinical and radiological outcomes were collected.

Study Outcomes

The main results were the consequence of metformin monotherapy in T2DM patients having COVID-19 on the inflammatory and oxidative stress biomarkers as well as radiological and clinical outcomes. The secondary outcomes were hospital stay and mortality.

Anthropometric Profile

Body mass index (BMI) was detected as follows:

$$BMI = \text{weight (kg)}/\text{height (m}^2\text{)}^8$$

Both systolic blood pressure (SBP) and diastolic blood pressure (DBP) of patients and controls were detected by an automated digital sphygmomanometer at the supine position from the left arm repeated twice for more precision values. Other pressure indices were estimated indirectly as follows:

Mean arterial pressure (MAP) = (SBP+2DBP)/3

Pulse pressure (PP) =SBP-DBP or MAP-DBP

The assessment of the biochemical profile, the radiological profile, and clinical outcomes are shown in the supplementary file.

Statistical Analysis

SPSS (IBM, Chicago, IL, USA) was used in the current study. The results were presented as percentages (%), numbers (n), and mean ± standard deviation (SD). The ANOVA test revealed the statistical difference between more than three groups at p-value < 0.05.

Results

Demographic Features

In Table II, the demographic features showed that 100 T2DM male patients with COVID-19 aged 48.82±5.09 years. The duration of T2DM was 4.96±2.53 years, and 60 (60%) T2DM patients with COVID-19 were on metformin therapy compared to 40 (40%) of T2DM patients with COVID-19 on other diabetic medicine. Associat-

Table II. Demographic characteristics of the patients.

Variables	Number* (%)
Number	100
Age (years)	48.82 ± 5.09
Male sex	80 (80%)
Duration of T2DM (years)	4.96 ± 2.53
Diabetic therapy	
Metformin monotherapy	60 (60%)
Other	40 (40%)
Associated treatments	
Statins	42 (42%)
Fenofibrate	39 (39%)
Anti-platelets	23 (23%)
Anticoagulants	4 (4%)
Antihypertensive	52 (52%)
Angiotensin-converting enzyme inhibitors	22 (42.30%)
Angiotensin receptor blockers	20 (38.46%)
Calcium channel blockers	10 (19.23%)
Montelukast	5 (5%)
In-hospital treatments	
Oxygen supplements	89 (89%)
Antiviral drugs	98 (98%)
Prophylactic antibiotics	29 (29%)
Comorbidities	
Hypertension	52 (52%)
Dyslipidemia	81 (81%)
Ischemic heart disease	11 (11%)
Mild asthma	5 (5%)

*Data expressed as mean ± SD.

ed treatments in T2DM patients with COVID-19 were statins, fenofibrate, anti-hypertensive medications, anti-platelets, anticoagulants, and montelukast. In-hospital treatments of T2DM patients with COVID-19 were oxygen supplements, antiviral, prophylactic antibiotics, and other components of anti-COVID-19 standard treatments. In addition, T2DM patients with COVID-19 were related to other comorbidities like hypertension, ischemic heart disease (IHD), dyslipidemia, and mild asthma.

Clinical Presentations

T2DM patients with COVID-19 on metformin as a single therapy presented with less fever, GIT disorders, shock, cardiovascular disorders and dysglycemia compared to other treatments for diabetes ($p<0.05$). However, hypoxemia was more prevalent in T2DM patients with COVID-19 on metformin than other diabetic treatments ($p=0.005$). The remaining clinical outcomes had no considerable difference (Table III).

Anthropometric, Cardiometabolic and Inflammatory Profiles

Blood pressure profile and BMI were not considerably different in T2DM patients with COVID-19 on metformin monotherapy (Group I) in relation to T2DM patients with COVID-19 on other diabetic therapy (Group II) ($p>0.05$). Glycemic indices except for HbA1c were ameliorated at the time of discharge (D) in comparison with the admission time (A) in both groups ($p<0.05$). Besides, lipid profiles were ameliorated at the time of discharge (D) in relation to the time of admission (A) in both groups ($p<0.05$). Cardiac indices AI and CVRI were lessened, while AC and CRR were not alleviated at discharge (D) compared with the admission time (A) in both

Table III. Clinical presentations of T2DM patients with COVID-19.

Features	I (n = 60)	II (n = 40)	*p*-value
Fever	49 (81.66)	38 (95.00)	0.007*
Headache	51 (85.00)	36 (90.00)	0.38
Sweating	56 (93.33)	38 (95.00)	0.66
Dry cough	60 (100.00)	40 (100.00)	-
Dyspnea	51 (85.00)	39 (97.50)	0.05
Hypoxemia	49 (81.87)	34 (56.67)	0.005*
Anosmia	51 (85.00)	37 (92.50)	0.16
Asthenia	50 (83.33)	38 (95.00)	0.08
Myalgia	41 (68.33)	33 (82.50)	0.11
GIT disorders	23 (38.33)	29 (72.50)	0.0009*
Shock	3 (5.00)	9 (22.50)	0.01*
Cardiovascular disorders	7 (11.67)	11 (27.50)	0.04*
Oxygen requirement	51 (85.00)	40 (100.00)	-
Diabetic complications			
Diabetic ketoacidosis	7 (11.67)	9 (22.50)	0.14
Hyperglycemia	23 (38.33)	33 (82.50)	0.0001*
Hypoglycemia	1 (1.67)	11 (27.50)	0.0001*

groups. Regarding the inflammatory biomarker levels, CRP was reduced from 23.90±4.66 mg/L at the time of A to 9.06±3.72 mg/L at the time of D (p<0.0001) in Group I. However, it reduced from 25.09±3.61 mg/L to 13.51±3.92 mg/L in Group II. The reduction was more in Group I in relation to Group II (p=0.04). Likewise, other inflammatory biomarkers were also ameliorated in both groups, though this difference was more in Group I in regard to Group II (p<0.001). These changes were also revealed in WBC count, lymphocyte %, neutrophils %, and NLR in Group I in relation to Group II (p=0.01). CT scan and clinical scores were more ameliorated in Group I in relation to Group II at the time of D (p=0.01) (Table IV).

Oxidative Stress Biomarkers

TOS was higher in Group I at A time of 16.73±4.72 µmol/L, which reduced to 6.92±2.82 µmol/L (p=0.004). Nevertheless, TOS was reduced insignificantly in Group II from 18.55±5.41 µmol/L at A time to 16.95±5.71 µmol/L at D time (p=0.06). The difference in TOS was significant at D time in Group I in relation to Group II (p=0.006). TAS was low in

Table IV. Anthropometric, cardiometabolic and inflammatory profile in T2DM patients with COVID-19 on metformin monotherapy in relation to other diabetic pharmacotherapy.

Parameters	Group I		p	Group II		p
	A (n = 60)	D (n = 57)		A (n = 40)	D (n = 31)	
BMI (kg/m^2)	29.68 ± 2.91	29.51 ± 2.01	ns	30.38 ± 3.89	30.67 ± 3.11	ns
SBP (mmHg)	133.81 ± 10.93	132.78 ± 11.79	ns	135.21 ± 10.53	133.04 ± 10.73	ns
DBP (mmHg)	79.03 ± 8.05	78.04 ± 8.44	ns	78.99 ± 7.93	77.83 ± 7.51	ns
FBG (mg/dL)	123.61 ± 9.41	120.97 ± 9.51	ns	120.63 ± 8.42	122.94 ± 8.93	ns
FSI (µIU/mL)	19.05 ± 7.66	12.85 ± 4.52	0.0001	24.99 ± 9.01	16.94 ± 6.31	0.0001
HOMA2-IR	2.61 ± 1.05	1.77 ± 1.01	0.01	3.37 ± 1.91	2.33 ± 1.10	0.01
IS (%)	38.3 ± 9.52	56.5 ± 11.41	0.0001	29.7 ± 4.61	43.00 ± 9.27	0.0001
HbA1c (%)	6.93 ± 1.41	6.93 ± 1.45	ns	7.05 ± 2.93	7.05 ± 2.94	ns
TG (mg/dL)	190.78 ± 11.83	147.33 ± 12.41	0.0001	213.63 ± 13.91	154.88 ± 9.31	0.0001
TC (mg/dL)	119.68 ± 9.32	150.27 ± 11.91	0.0001	112.04 ± 7.94	145.94 ± 10.51	0.0001
HDL (mg/dL)	33.94 ± 8.05	40.73 ± 5.47	0.0001	31.61 ± 4.91	36.01 ± 5.91	0.01
non-HDL	85.74 ± 9.73	109.54 ± 10.81	0.0001	81.85 ± 9.31	109.73 ± 10.04	0.0001
VLDL (mg/dL)	38.15 ± 4.11	29.40 ± 9.44	0.0001	42.60 ± 4.02	31.06 ± 3.05	0.0001
LDL (mg/dL)	47.6 ± 6.51	86.9 ± 10.09	0.0001	37.7 ± 6.05	79.00 ± 8.26	0.0001
AC	2.5 ± 1.56	2.68 ± 1.22	ns	2.5 ± 1.02	3.05 ± 1.93	ns
AI	0.39 ± 0.06	0.19 ± 0.01	0.0001	0.47 ± 0.08	0.27 ± 0.04	0.0001
CVRI	5.6 ± 2.54	3.6 ± 1.99	0.01	6.7 ± 3.11	4.30 ± 2.56	0.01
CRR	3.52 ± 1.16$^{\#}$	3.68 ± 1.22	ns	3.5 ± 1.56	4.9 ± 1.41	ns
CRP (mg/L)	23.90 ± 4.66$^{\#}$	9.06 ± 3.72*	0.0001	25.09 ± 3.61	13.51 ± 3.92	0.0001
D-dimer (ng/mL)	314.95 ± 14.04$^{\#}$	172.02 ± 10.99*	0.0001	397.31 ± 19.37	292.88 ± 16.11	0.0001
Ferritin (ng/mL)	314.01 ± 17.11$^{\#}$	211.01 ± 9.91*	0.0001	398.54 ± 19.02	398.54 ± 19.02	0.0001
LDH (U/L)	229.63 ± 11.41$^{\#}$	177.81 ± 10.02*	0.0001	311.84 ± 19.05	217.89 ± 16.55	0.0001
PCT (ng/mL)	0.20 ± 0.04$^{\#}$	0.15 ± 0.01*	0.01	0.23 ± 0.03	0.19 ± 0.08	0.04
SaO_2 (%)	92.07 ± 1.16$^{\#}$	98.07 ± 1.99*	0.0001	90.02 ± 1.22	96.04 ± 1.01	0.03
WBC (10^3/µL)	11.06 ± 3.05	8.91 ± 2.33	0.04	12.17 ± 3.81	9.61 ± 3.95	0.03
Lymphocytes (%)	17.81 ± 3.38	26.17 ± 5.53	0.01	14.03 ± 3.64	22.19 ± 4.21	0.001
Neutrophils (%)	82.73 ± 8.11	73.22 ± 6.03	0.003	85.93 ± 9.81	77.91 ± 8.11	0.005
NLR	4.64 ± 1.71$^{\#}$	2.79 ± 1.99*	0.03	6.12 ± 1.29	3.51 ± 1.78	0.02
CT scan score (%)	50.68 ± 3.92$^{\#}$	4.68 ± 1.92*	0.0001	59.21 ± 3.03	29.33 ± 4.09	0.0001
Clinical score (%)	–	1.74 ± 1.03*	–	–	2.94 ± 1.88	0.0002
Mortality rate (%)	–	5.0*	–	–	22.5	0.0001

Data expressed as mean ± SD, Group I: T2DM patients with COVID-19 on metformin monotherapy, Group II: T2DM patients with COVID-19 on other anti-diabetic agents, A: Admission time, D: Discharge time, BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, FBG: Fasting blood glucose, FSI: Fasting serum insulin, HOMA2-IR: a homeostatic model for assessment of type 2 IR, IS: Insulin sensitivity, HbA1c: glycated hemoglobin, TG: triglyceride, TC: total cholesterol, HDL: high-density lipoprotein, LDL: low-density lipoprotein, VLDL: very low-density lipoprotein, AC: atherogenic coefficient, AI: atherogenic index, CVRI: cardiovascular risk index, CRR: cardiac risk ratio, CRP: C-reactive protein, LDH: lactate dehydrogenase, PCT: procalcitonin, NLR: Neutrophil: lymphocyte ratio, ns: not significant, *p < 0.05 compared to the values at the time of D. $^{\#}p$ < 0.05 compared to the values at the time of A.

Group I (1,145.86±130.94 µmol/L) and Group II (1,123.72±190.03 µmol/L); these were increased to 1,246.21±180.55 µmol/L and 1,143.91±190.44 µmol/L respectively. Therefore, OSI was ameliorated from 1.46±0.97 to 0.55 ±0.068 (p=0.0001) in Group I, while it was reduced insignificantly from 1.65±0.81 to 1.48±0.85 (p=0.07) (Figure 1).

Discussion

It is observed that patients with T2DM have a greater incidence of COVID-19, which could be explained by the relatively higher ACE2 expression in patients having T2DM[10,31]. In addition, the higher expression of ACE2 in the pancreatic β cells may augment the risk of acute pancreatic injury induced by SARS-CoV-2 with the progression to a new-onset DM[32]. The high pro-inflammatory cytokines and the extravagant immune response could persuade IR. Therefore, API and IR may cause new-onset DM in COVID-19 with the progression of hyperglycemia and blood glucose variability. Emerging data from Chinese studies involving 2,209 COVID-19 patients showed that 11% had T2DM[33].

Similarly, a systematic review by Yanga et al[34] demonstrated that 8% of DM patients had COVID-19. Likewise, T2DM COVID-19 patients are associated with high severity and mortality[35]. These observations suggest that T2DM patients are at high risk for developing COVID-19 severity and mortality compared to the general population.

This study revealed that COVID-19 could induce more complications due to inflammatory and oxidative stress disorders. However, T2DM patients with severe COVID-19 on metformin monotherapy develop fewer complications compared to other diabetic pharmacotherapy. Demographic characteristics of recruited patients showed that most of the patients were of male sex and had associated co-morbidities like obesity and hypertension that may affect the clinical course and final clinical outcomes. Male sex is considered a possible risk factor for increased severity of COVID-19, as documented

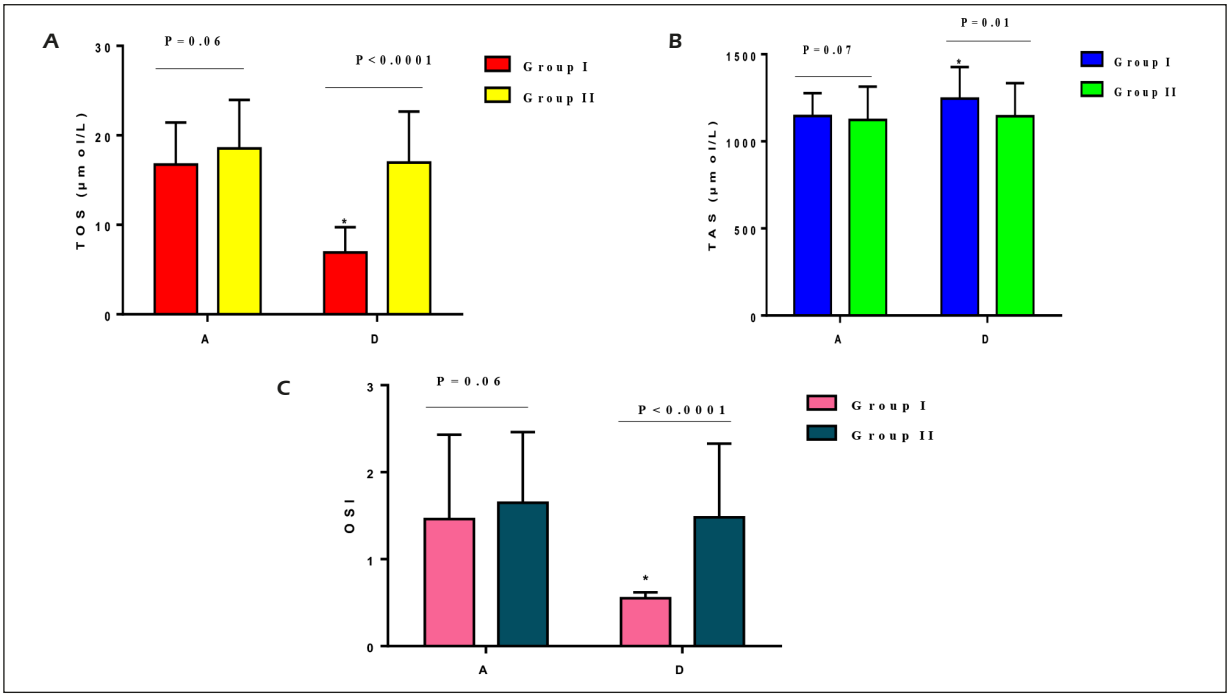

Figure 1. Oxidative stress in T2DM patients with COVID-19: Group I: T2DM patients with COVID-19 on metformin monotherapy, Group II: T2DM patients with COVID-19 on other anti-diabetic agents. **A**, Total oxidant status (TOS) was reduced significantly p=0.004 in Group I, while it was decreased insignificantly in Group II p=0.06. **B**, Total antioxidant status (TAS) was markedly increased by p=0.009 in Group I, though it was increased insignificantly by p=0.07 in Group II. **C**, Oxidative stress index (OSI) was reduced significantly p=0.0001 in Group I, though it was reduced insignificantly p=0.07 in Group II. A: Admission time, D: Discharge time.

by longitudinal and retrospective studies[36,37]. Expression of ACE2 and hormonal differences in both sexes might be the possible mechanisms through which the female sex was more protected against COVID-19 severity[38,39]. Studies included 8,108 COVID-19 patients, 50% of the female sex and 47% of the male sex, illustrated that male COVID-19 patients were in greater danger of hospitalization, ICU transfer, the requirement of endotracheal intubation and mortality[40,41]. Besides, both obesity and hypertension reduce SARS-CoV-2 clearance with succeeding prolonged immune stimulation and overstated immune response[42]. It was revealed that SARS-CoV-2 infection correlates with reduced cholesterol, HDL, and LDL[43]. SARS-CoV-2 exploits HDL and HDL receptor scavenger receptor beta 2 (SR-B2) to facilitate its entry. Hypolipidemia is also present in other viral infections; however, triglyceride level is augmented in SARS-CoV-2 infection[44,45]. Hypolipidemia is mainly associated with mild-moderate COVID-19 and tends to escalate and normalize at the time of recovery[46]. Masana et al[44] revealed that high triglyceride and low HDL at admission predict COVID-19 severity and mortality. These outcomes align with this study's results as most COVID-19 patients with associated dyslipidemia and other co-morbidities at the time of admission develop COVID-19 severity.

The recruited T2DM patients with severe COVID-19 were on other treatments, including statins, fenofibrate, anti-platelets, anticoagulants, ACE1s, ARBs, CCBs, and montelukast that may affect COVID-19 clinical course. Therefore, these treatments and other confounding factors were adjusted to ensure the therapeutic effect of metformin on COVID-19 compared to other diabetic pharmacotherapy.

At A time, Group I patients had fewer inflammatory biomarkers and better SaO_2% and CT scan scores compared to Group II patients. Likewise, patients had fewer inflammatory biomarkers, better SaO_2%, CT scan and clinical scores at D time compared to Group II. However, oxidative stress biomarkers did not significantly differ at A time, but they were more ameliorated at D time in Group I patients in relation to Group II patients. Remarkably, the mortality rate was 5% in Group I in relation to 22.5% in Group II. These novel findings may explain metformin's pleiotropic anti-inflammatory, antioxidant, and pulmoprotective effects against the development and progression of COVID-19 severity.

Different clinical studies emphasized the protective role of metformin in the control of COVID-19. For example, a cohort study comprising 31966 T2DM patients with COVID-19 revealed that metformin treatment decreased the hazard of in-hospital stay, ICU admission and mortality[47,48]. A retrospective cohort study performed by Bramante et al[49] indicated that metformin treatment effectively reduced mortality in hospitalized T2DM patients with COVID-19. An observational study exposed that metformin reduced COVID-19 severity and mortality in women with T2DM or obesity[50], suggesting a gender-specific effect of metformin. A meta-analysis study involving nine studies with 10233 COVID-19 patients revealed that COVID-19 patients administering metformin were associated with a low mortality rate in the pooled adjusted and non-adjusted models[51,52]. A meta-analysis involving 17 clinical studies displayed that metformin decreased the severity and mortality of T2DM patients with COVID-19[53]. In addition, a systematic review and meta-analysis revealed that metformin treatment improves clinical outcomes in mild-moderate COVID-19[54,55].

In T2DM patients, a higher binding affinity of SARS-CoV-2 to the overexpressed ACE2, T cell dysfunction, and reduction of SARS-CoV-2 clearance augment the susceptibility for the progression of hyperinflammation as well as cytokine storm[56,57]).

As well, hyperglycemia in T2DM patients increases the expression of the mechanistic target of the rapamycin (mTOR) pathway with subsequent discharge of the pro-inflammatory cytokines and T-cell dysfunction[58,59]. Interestingly, through activating AMPK or inhibiting the mTOR pathway, metformin can attenuate the release of pro-inflammatory cytokines and COVID-19 severity[56,60]. Notably, metformin was initially used as an antiviral agent mainly against influenza virus infection, and reduction of blood glucose was the reported side effect[61]. Consequently, metformin may have probable effects against SARS-CoV-2 infection. Metformin has been noticed to reduce the binding of SARS-CoV-2 to their receptors, ACE2, by induction of its phosphorylation. Metformin can also reduce the activity of DPP4 and CD147[62,63]. DPP4 and CD147 are engaged with the entry and severity of SARS-CoV-2 infection[64,65]. Therefore, inhibition of the expression of DPP4 and CD147 by metformin may reduce tropism of SARS-CoV-2 infection with subsequent attenuation of COVID-19 severity.

In addition, metformin regulates the immune response by stimulating the development of anti-inflammatory regulatory T cells (Treg) and alternative macrophages[66,67]. Furthermore, metformin hinders the expression of the different inflammatory signaling pathways like NLRP3 inflammasome, NF-κB which are triggered in COVID-19[68,69]. Likewise, metformin prohibits macrophage and neutrophils in hyperoxia-induced ALI. The experimental study confirmed that metformin can hinder the release of SARS-CoV-2 from the endosome by increasing endosomal pH[56,70]. These annotations support the notion that COVID-19 patients on metformin may develop fewer complications during SARS-CoV-2 infection.

These results showed that T2DM patients on metformin therapy are linked with lower severity and mortality during SARS-CoV-2 infection. The proposed mechanism of metformin against COVID-19 is wide).

Notably, metformin has anti-inflammatory and antioxidant properties[71], which may decrease SARS-CoV-2-induced hyperinflammation and oxidative stress. Coll et al[72] revealed that metformin increases the expression and release of anti-inflammatory GDF15. Remarkably, growth differentiation factor 15 (GDF15) is considered a predictive and prognostic biomarker linked with COVID-19 severity[73,74]. Increased expression of GDF15 in COVID-19 could compensate for the counterbalance of SARS-CoV-2-induced hyperinflammation. Therefore, metformin may protect against COVID-19 through the GDF15-dependent pathway. Unfortunately, the GDF15 serum level was not detected in this study.

Therefore, metformin's anti-inflammatory and antioxidant effects have led to decreasing the inflammatory and oxidative biomarkers in this study with further improvement of the radiological and clinical scores).

Metformin, in the present study, reduced oxidative stress burden and decreased COVID-19-induced oxidative stress, which links with the development of hyperinflammation and critical complications[75]. It has been shown that antioxidants like vitamin D and flavonoids were efficient in COVID-19 by reducing oxidative stress. A cohort study[76] illustrated that metformin was effective in lessening nitrosative and oxidative stress biomarkers in T2DM patients through the restoration of antioxidant status[76]. These findings suggest that metformin might be effective in managing COVID-19 *via* modification of the development and progression of oxidative stress.

Furthermore, different preclinical studies observed that metformin could attenuate parquet and LPS-induced ALI/ARDS. Tsaknis et al[77] observed that metformin decreased ventilator-induced ALI by restoring alveolar capillary permeability in rabbits. Excitingly, metformin improves agmatine production from gut microbiota that inhibits the production of advanced glycation end-products (AGEs)[78]. Amplified AGEs expression is related to the severity of COVID-19 in elderly T2DM patients. Also, an experimental study showed that the administration of agmatine can attenuate ALI in rats[79]. These findings may clarify the enhancement effect of metformin on the oxygenation percentage in T2DM patients having a severe COVID-19 disease.

Moreover, metformin decreased D-dimer serum levels considerably in T2DM patients with severe COVID-19 at discharge. Investigation of hospitalized T2DM COVID-19 patients revealed that using metformin has lower thrombotic complications, as evidenced by low D-dimer serum levels[80]. Also, metformin can attenuate thrombosis risk by impeding platelet activation and the release of mitochondrial DNA in rats[81]. Furthermore, metformin and metformin derivatives have pro-fibrinolytic properties, thereby limiting fibrinolytic failure during thrombotic events. Interestingly, metformin can lessen the expression of tissue plasminogen activator (tPA) with significant inhibition of plasminogen activator inhibitor 1 (PAI-1)[81,82]. This could clarify the possible decrease of metformin on D-dimer levels in T2DM COVID-19 patients.

Conclusions

Metformin was reported to have an inhibitory potential against SARS-CoV-2, as documented in *in silico* studies. Also, metformin combined with DPP4 inhibitors was found to be efficient in the control of T2DM patients with COVID-19. Thus, this perspective aimed to reveal the probable role of metformin in managing T2DM patients with COVID-19. Treatment of T2DM patients with COVID-19 using metformin showed a more efficient potential in reducing the inflammatory and oxidative stress biomarkers with a substantial improvement of the radiological scores and clinical outcomes compared to T2DM patients with COVID-19 on other diabetic pharmacotherapy. Therefore,

clinical trials and prospective studies are needed to endorse the possible mechanistic role of metformin treatment against the development and progression of COVID-19.

Conflict of Interest
The authors declare that they have no conflict of interests.

Acknowledgements
Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2023R120), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Ethics Approval
This study was approved scientific jury and the editorial board in the College of Medicine, Al-Mustansiriayiah University, Iraq, Baghdad (reference No. 34MTR on 20/2/2020).

Informed Consent
Informed consent was obtained from all individual participants included in the study.

Availability of Data and Materials
All data available in the manuscript.

Funding
None.

Authors' Contribution
HMA and AAE performed the study, edited the main text, and approved the final edition of the manuscript. EEK, AA, MP, EE and GE-SB prepared the tables, wrote the main text, and approved the final edition of the manuscript. NA and RSH revised the manuscript. All authors approved the final edition of the manuscript.

References

1) Onohuean H, Al-Kuraishy HM, Al-Gareeb AI, Qusti S, Alshammari EM, Batiha GES. Covid-19 and development of heart failure: mystery and truth. Naunyn-Schmiedebergs Arch Pharmacol 2021; 394: 2013-2021.
2) Al-Kuraishy HM, Al-Gareeb AI, Al-Hussaniy HA, Al-Harcan NAH, Alexiou A, Batiha GES. Neutrophil Extracellular Traps (NETs) and Covid-19: A new frontiers for therapeutic modality. Int Immunopharmacol 2022; 10: 85-96.
3) Al-Kuraishy HM, Al-Gareeb AI, Kaushik A, Kujawska M, Batiha GES. Hemolytic anemia in COVID-19. Ann Hematol 2022; 2: 1-9.
4) Shrestha LB, Foster C, Rawlinson W, Tedla N, Bull RA. Evolution of the SARS-CoV-2 omicron variants BA. 1 to BA. 5: Implications for immune escape and transmission. Rev Med Virol 2022; 32: e2381.
5) Batiha GE, Al-Kuraishy HM, Al-Gareeb AI, Elekhnawy E. SIRT1 pathway in Parkinson's disease: a faraway snapshot but so close. Inflammopharmacol 2023; 31: 37-56.
6) Bao W, Min D, Twigg SM, Shackel N, Warner FJ, Yue DK, Mclennan SV. Monocyte CD147 is induced by advanced glycation end products and high glucose concentration: possible role in diabetic complications. Amer J Phys Cell Phys 2010; 299: 1212-1219.
7) Du H, Wang DW, Chen C. The potential effects of DPP-4 inhibitors on cardiovascular system in COVID-19 patients. J Cell Mol Med 2020; 24: 10274-10278.
8) Abdul-Hadi MH, Naji MT, Shams HA, Sami OM, Al-Harchan NAA, Al-Kuraishy HM, Al-Gareeb AI. Oxidative stress injury and glucolipotoxicity in type 2 diabetes mellitus: The potential role of metformin and sitagliptin. BBRJ 2020; 4: 166.
9) Alomair BM, Al-Kuraishy HM, Al-Buhadily AK, Al-Gareeb AI, De Waard M, Elekhnawy E, Batiha GES. Is sitagliptin effective for SARS-CoV-2 infection: false or true prophecy? Inflammopharmacol 2022; 30: 2411-2415.
10) Singh AK, Gupta R, Ghosh A, Misra A. Diabetes in COVID-19: Prevalence, pathophysiology, prognosis and practical considerations. Diabetes & Metabolic Syndrome. Clin Res Rev 2020; 14: 303-310.
11) Rasheed HA, Al-Kuraishy HM, Al-Gareeb AI, Hussien NR, Al-Nami MS. Effects of diabetic pharmacotherapy on prolactin hormone in patients with type 2 diabetes mellitus: Bane or Boon. J Adv Pharm Technol Res 2019; 10: 163.
12) Al-Nami MS, Al-Kuraishy HM, Al-Gareeb AI, Al-Mamoori F. Metabolic profile and prolactin serum levels in men with type 2 diabetes mellitus: Old-new rubric. Inter J Crit Illness Injury Sci 2019; 9: 120.
13) Alotaibi B, Negm WA, Elekhnawy E, El-Masry TA, Elharty ME, Saleh A, Abdelkader DH, Mokhtar FA. Antibacterial activity of nano zinc oxide green-synthesised from Gardenia thailandica triveng. Leaves against Pseudomonas aeruginosa clinical isolates: in vitro and in vivo study. Art Cells Nanomed Biotechnol 2022; 50: 96-106.
14) Attallah NG, Elekhnawy E, Negm WA, Hussein IA, Mokhtar FA, Al-Fakhrany OM. In vivo and in vitro antimicrobial activity of biogenic silver nanoparticles against Staphylococcus aureus clinical isolates. Pharm 2022; 15: 194.
15) Nadwa EH, Al-Kuraishy HM, Al-Gareeb AI, Elekhnawy E, Albogami SM, Alorabi M, Bati-

ha GE, De Waard M. Cholinergic dysfunction in COVID-19: frantic search and hoping for the best. Naunyn-Schmiedeberg's Arch Pharmacol 2023; 396: 453-68.

16) Al-Kuraishy HM, Al-Fakhrany OM, Elekhnawy E, Al-Gareeb AI, Alorabi M, De Waard M, Albogami SM, Batiha GES. Traditional herbs against COVID-19: back to old weapons to combat the new pandemic. Europ J Med Res 2022; 27: 1-11.
17) Batiha GES, Al-Gareeb AI, Elekhnawy E, Al-Kuraishy HM. Potential role of lipoxin in the management of COVID-19: a narrative review. Inflammopharmacol 2022; 30: 1993-2001.
18) Abdul-Hadi M H, Naji MT, Shams HA, Sami OM, Al-Kuraishy HM, Al-Gareeb AI. Erectile dysfunction and type 2 diabetes mellitus: a new twist. Inter J Nut Pharmacol Neurol Dis 2020; 10: 43.
19) Koepsell H. Role of organic cation transporters in drug–drug interaction. Expert Opin Drug Metab Toxicol 2015; 11: 1619-1633.
20) Viollet B, Guigas B, Garcia NS, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: an overview. Clin Sci 2012; 122: 253-270.
21) Vial G, Detaille D, Guigas B. Role of mitochondria in the mechanism (s) of action of metformin. Front Endocrinol 2019; 10: 294.
22) Fontaine E. Metformin-induced mitochondrial complex I inhibition: facts, uncertainties, and consequences. Front Endocrinol 2018; 9: 753.
23) Fontaine E. Metformin and respiratory chain complex I: the last piece of the puzzle? Biochem J 2014; 463: e3-e5.
24) He L. Metformin and systemic metabolism. Trends Pharmacol Sci 2020; 41: 868-881.
25) LaMoia TE, Shulman GI. Cellular and molecular mechanisms of metformin action. Endocr Rev 2021; 42: 77-96.
26) Lv Z, Guo Y. Metformin and its benefits for various diseases. Front Endocrinol 2020; 11: 191.
27) Flory J, Lipska K. Metformin in 2019. JAMA 2019; 321: 1926-1927.
28) Parthasarathy H, Tandel D, Siddiqui AH, Harshan KH. Metformin suppresses SARS-CoV-2 in cell culture. Virus Res 2023; 323: 199010.
29) Cory TJ, Emmons RS, Yarbro JR, Davis KL, Pence BD. Metformin suppresses monocyte immunometabolic activation by SARS-CoV-2 spike protein subunit 1. Front Immunol 2021; 12: 733921.
30) Assoc GA. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. J Amer Collage Dentists 2014; 81: 14-18.
31) Al-Kuraishy HM, Al-Gareeb AI, Alblihed M, Guerreiro SG, Cruz-Martins N, Batiha GES. COVID-19 in relation to hyperglycemia and diabetes mellitus. Front Cardio Med 2021; 8: 644095.
32) Attallah NG, El-Kadem AH, Negm WA, Elekhnawy E, El-Masry TA, Elmongy EI, Altwaijry N, Alanazi AS, Al-Hamoud GA, Ragab AE. Promising Antiviral Activity of Agrimonia pilosa Phytochemicals against Severe Acute Respiratory Syndrome Coronavirus 2 Supported with In Vivo Mice Study. Pharm 2021; 14: 1313.
33) Singh AK, Gupta R, Misra A. Comorbidities in COVID-19: Outcomes in hypertensive cohort and controversies with renin angiotensin system blockers. Diabetes Metab Syndr 2020; 14: 283-287.
34) Yang J, Zheng Y, Gou X, Pu K, Chen Z, Guo Q, Ji R, Wang H, Wang Y, Zhou Y. Prevalence of comorbidities and its effects in patients infected with SARS-CoV-2: a systematic review and meta-analysis. Int J Infect Dis 2020; 94: 91-95.
35) Attallah NG, Al-Fakhrany OM, Elekhnawy E, Hussein IA, Shaldam MA, Altwaijry N, Alqahtani MJ, Negm WA. Anti-biofilm and antibacterial activities of Cycas media R. Br secondary metabolites: In silico, in vitro, and in vivo approaches. Antibiot 2022; 11: 993.
36) Pivonello R, Auriemma RS, Pivonello C, Isidori AM, Corona G, Colao A, Millar RP. Sex disparities in COVID-19 severity and outcome: are men weaker or women stronger? Neuroendocrinol 2021; 111: 1066-1085.
37) Alwani M, Yassin A, Al-Zoubi RM, Aboumarzouk OM, Nettleship J, Kelly D, AL-Qudimat AR, Shabsigh R. Sex-based differences in severity and mortality in COVID-19. Rev Med Virol 2021; 31: e2223.
38) Abdelaziz A, Sonbol F, Elbanna T, El-Ekhnawy E. Exposure to sublethal concentrations of benzalkonium chloride induces antimicrobial resistance and cellular changes in Klebsiellae pneumoniae clinical isolates. Microb Drug Resis 2019; 25:631-638.
39) Al-Kuraishy HM, Al-Gareeb AI, Albogami SM, Jean-Marc S, Nadwa EH, Hafiz AA, A. Negm W, Kamal M, Al-Jouboury M, Elekhnawy E, Batiha GE. Potential therapeutic benefits of metformin alone and in combination with sitagliptin in the management of type 2 diabetes patients with COVID-19. Pharm 2022; 15: 13-61.
40) Gomez JMD, Du-Fay-de-Lavallaz JM, Fugar S, Sarau A, Simmons JA, Clark B, Sanghani RM, Aggarwal NT, Williams KA, Doukky R. Sex differences in COVID-19 hospitalization and mortality. J Wom Health 2021; 30: 646-653.
41) Negm WA, El-Aasr M, Kamer AA, Elekhnawy E. Investigation of the Antibacterial Activity and Efflux Pump Inhibitory Effect of Cycas thouarsii R. Br. Extract against Klebsiella pneumoniae Clinical Isolates. Pharm 2021; 14: 756.
42) Sanoudou D, Hill MA, Belanger MJ, Arao K, Mantzoros CS. Editorial: Obesity, metabolic phenotypes and COVID-19. Met Clin Exp 2022; 128: 155121.
43) Kočar E, Režen T, Rozman D. Cholesterol, lipoproteins, and COVID-19: Basic concepts and clinical applications. Biochimica et Biophysica Acta (BBA). Mol Cell Biol Lip 2021; 1866: 158849.

44) Masana L, Correig E, Ibarretxe D, Anoro E, Arroyo JA, Jericó C, Guerrero C, Miret M, Näf S, Pardo A. Low HDL and high triglycerides predict COVID-19 severity. Sci Rep 2021; 11: 7217.

45) Elekhnawy E, Negm WA. The potential application of probiotics for the prevention and treatment of COVID-19. Egypt J Med Hum Genet 2022; 23: 36.

46) Abdelkader DH, Negm WA, Elekhnawy E, Eliwa D, Aldosari BN, Almurshedi AS. Zinc Oxide Nanoparticles as Potential Delivery Carrier: Green Synthesis by Aspergillus niger Endophytic Fungus, Characterization, and In Vitro/In Vivo Antibacterial Activity. Pharm 2022; 15: 1057.

47) Ojeda-Fernández L, Foresta A, Macaluso G, Colacioppo P, Tettamanti M, Zambon A. Metformin use is associated with a decrease in the risk of hospitalization and mortality in COVID-19 patients with diabetes: A population-based study in Lombardy. Diab Obes Met 2022; 24: 891-898.

48) Khudhair HD, Al-Gareeb AI, Al-Kuraishy HM, El-Kadem AH, Elekhnawy E, Negm WA, Saber S, Cavalu S, Tirla A, Alotaibi SS. Combination of vitamin C and curcumin safeguards against methotrexate-induced acute liver injury in mice by synergistic antioxidant effects. Front Med 2022; 9: 866343.

49) Bramante CT, Ingraham NE, Murray TA. Metformin and risk of mortality in patients hospitalised with COVID-19: a retrospective cohort analysis. Lancet Health Long 2021; 2: e34-e41.

50) Bramante CT, Ingraham NE, Murray TA, Marmor S. Observational study of metformin and risk of mortality in patients hospitalized with Covid-19. MedRxiv [Preprint] 2020; 23: 1-15.

51) Zhang J, Dong J, Martin M, He M, Gongol B, Marin TL, Chen L, Shi X, Yin Y, Shang F. AMP-activated protein kinase phosphorylation of angiotensin-converting enzyme 2 in endothelium mitigates pulmonary hypertension. Am J Respir Crit Care Med 2018; 198: 509-520.

52) Attallah NG, Negm, WA, Elekhnawy E, Altwaijry N, Elmongy EI, El-Masry TA, Alturki EA, Yousef DA, Shoukheba M. Antibacterial activity of Boswellia sacra Flueck. Oleoresin extract against Porphyromonas gingivalis periodontal pathogen. Antibiot 2021; 10: 859.

53) Yang W, Sun X, Zhang J, Zhang K. The effect of metformin on mortality and severity in COVID-19 patients with diabetes mellitus. Diabetes Res Clin Pract 2021; 178: 108977.

54) Zangiabadian M, Nejadghaderi SA, Zahmatkesh MM, Hajikhani B, Mirsaeidi M, Nasiri MJ. The efficacy and potential mechanisms of metformin in the treatment of COVID-19 in the diabetics: a systematic review. Front Endocrinol (Lausanne) 2021; 12: 645194.

55) Almukainzi M, El-Masry TA, Negm WA, Elekhnawy E, Saleh A, Sayed AE, Ahmed HM, Abdelkader DH. Co-delivery of gentiopicroside and thymoquinone using electrospun m-PEG/PVP nanofibers: In-vitro and In vivo studies for antibacterial wound dressing in diabetic rats. Intern J Pharm 2022; 625: 122106.

56) Kamyshnyi O, Matskevych V, Lenchuk T, Strilbytska O, Storey K, Lushchak O. Metformin to decrease COVID-19 severity and mortality: Molecular mechanisms and therapeutic potential. Biomed Pharmacother 2021; 144: 112230.

57) Al-kuraishy HM, Al-Gareeb AI, Elekhnawy E, Batiha GE. Dipyridamole and adenosinergic pathway in Covid-19: a juice or holy grail. Egyp J Med Hum Gen 2022; 23:140-150.

58) Pollizzi KN, Patel CH, Sun IH, Oh MH, Waickman AT, Wen J, Delgoffe GM, Powell JD. mTORC1 and mTORC2 selectively regulate CD8+ T cell differentiation. J Clin invest 2015; 125: 2090-2108.

59) Al-Kuraishy HM, Al-Gareeb AI, Elekhnawy E, Batiha GES. Nitazoxanide and COVID-19: a review. Mol Biolog Rep 2022; 49: 11169-11176.

60) Sonbol FI, El-Banna TE, Abd El-Aziz AA, El-Ekhnawy E. Impact of triclosan adaptation on membrane properties, efflux and antimicrobial resistance of Escherichia coli clinical isolates. J App Microbiol 2019; 126: 730-739.

61) Salvatore T, Pafundi PC, Morgillo F, Di Liello R, Galiero R, Nevola R, Marfella R, Monaco L, Rinaldi L, Adinolfi LE. Metformin: An old drug against old age and associated morbidities. Diabetes Res Clin Pract 2020; 160: 108025.

62) Cuthbertson J, Patterson S, O'Harte F, Bell P. Investigation of the effect of oral metformin on dipeptidylpeptidase-4 (DPP-4) activity in Type 2 diabetes 1. Diabet Med 2009; 26: 649-654.

63) Floch RL, Chiche J, Marchiq I, Naiken T, Ilc K, Simon MP, Roux DI, Pouyssegur J. Growth inhibition of glycolytic tumors by targeting basigin/lactate-H+ symporters (MCTs): metformin sensitizes MCT inhibition. Cancer Res 2012; 72: 3225-3225.

64) Radzikowska U, Ding M, Tan G, Zhakparov D, Peng Y, Wawrzyniak P, Wang M, Li S, Morita H, Altunbulakli C. Distribution of ACE2, CD147, CD26, and other SARS-CoV-2 associated molecules in tissues and immune cells in health and in asthma, COPD, obesity, hypertension, and COVID-19 risk factors. Allergy 2020; 75: 2829-2845.

65) Andrews MG, Mukhtar T, Eze UC, Simoneau CR, Ross J, Parikshak N, Wang S, Zhou L, Koontz M, Velmeshev D. Tropism of SARS-CoV-2 for human cortical astrocytes. Proceed Nation Acad Sci 2022; 119: e2122236119.

66) Samuel SM, Varghese E, Büsselberg D. Therapeutic potential of metformin in COVID-19: reasoning for its protective role. Trends Microbiol 2021; 29: 894-907.

67) Attallah NG, El-Sherbeni SA, El-Kadem AH, Elekhnawy E, El-Masry TA, Elmongy EI, Altwaijry N, Negm WA. Elucidation of the metabolite profile of Yucca gigantea and assessment of its cytotoxic, antimicrobial, and anti-inflammatory activities. Mol 2022; 27: 1329.

68) Lukito AA, Pranata R, Henrina J, Lim MA, Lawrensia S, Suastika K. The effect of metformin consumption on mortality in hospitalized COVID-19 patients: a systematic review and meta-analysis. Diabetes Metab Syndr Clin Res Rev 2020; 14: 2177-2183.

69) Alherz FA, Negm WA, Elekhnawy E, El-Masry TA, Haggag EM, Alqahtani MJ, Hussein IA. Silver Nanoparticles Prepared Using Encephalartos laurentianus De Wild Leaf Extract Have Inhibitory Activity against Candida albicans Clinical Isolates. J Fungi 2022; 8: 1005.

70) Alotaibi B, El-Masry TA, Elekhnawy E, El-Kadem AH, Saleh A, Negm WA, Abdelkader DH. Aqueous core epigallocatechin gallate PLGA nanocapsules: Characterization, antibacterial activity against uropathogens, and in vivo reno-protective effect in cisplatin induced nephrotoxicity. Drug Deliv 2022; 29: 1848-1862.

71) Dehkordi AH, Abbaszadeh A, Mir S, Hasanvand A. Metformin and its anti-inflammatory and anti-oxidative effects; new concepts. J Renal Injury Preven 2018; 8: 54-61.

72) Coll AP, Chen M, Taskar P, Rimmington D, Patel S, Tadross JA, Cimino I, Yang M, Welsh P, Virtue S. GDF15 mediates the effects of metformin on body weight and energy balance. Nature 2020; 578: 444-448.

73) Rochette L, Zeller M, Cottin Y, Vergely C. GDF15: an emerging modulator of immunity and a strategy in COVID-19 in association with iron metabolism. Trends Endocrinol Metab 2021; 32: 875-889.

74) Elekhnawy EA, Sonbol FI, Elbanna TE, Abdelaziz AA. Evaluation of the impact of adaptation of Klebsiella pneumoniae clinical isolates to benzalkonium chloride on biofilm formation. Egyp J Med Hum Gen 2021; 22: 1-6.

75) Paul AK, Hossain MK, Mahboob T, Nissapatorn V, Wilairatana P, Jahan R, Jannat K, Bondhon TA, Hasan A, de Lourdes Pereira M. Does oxidative stress management help alleviation of COVID-19 symptoms in patients experiencing diabete. Nutrients 2022; 14: 321.

76) Chakraborty A, Chowdhury S, Bhattacharyya M. Effect of metformin on oxidative stress, nitrosative stress and inflammatory biomarkers in type 2 diabetes patients. Diabetes Res Clin Pract 2011; 93: 56-62.

77) Tsaknis G, Siempos II, Kopterides P, Maniatis NA, Magkou C, Kardara M, Panoutsou S, Kotanidou A, Roussos C, Armaganidis A. Metformin attenuates ventilator-induced lung injury. Crit Care 2012; 16: R134.

78) Kotagale N, Rahangdale S, Borkar A, Singh K, Ikhar A, Takale N, Umekar M, Taksande B. Possible involvement of agmatine in neuropharmacological actions of metformin in diabetic mice. Eur J Pharmacol 2021; 907: 174255.

79) Liu G, Mei H, Chen M, Qin S, Li K, Zhang W, Chen T. Protective effect of agmatine against hyperoxia-induced acute lung injury via regulating lncRNA gadd7. Biochem Biophys Res Commun 2019; 516: 68-74.

80) Usman A, Bliden KP, Cho A, Walia N, Jerjian C, Singh A, Kundan P, Duhan S, Tantry US, Gurbel PA. Metformin use in patients hospitalized with COVID-19: lower inflammation, oxidative stress, and thrombotic risk markers and better clinical outcomes. J Thromb Thrombolysis 2022; 53: 363-371.

81) Xin G, Wei Z, Ji C, Zheng H, Gu J, Ma L, Huang W, Morris-Natschke SL, Yeh JL, Zhang R. Metformin uniquely prevents thrombosis by inhibiting platelet activation and mtDNA release. Sci Rep 2016; 6: 36222.

82) Markowicz-Piasecka M, Huttunen KM, Mateusiak Ł, Mikiciuk-Olasik E, Sikora J. Sulfenamide and sulfonamide derivatives of metformin can exert anticoagulant and profibrinolytic properties. Chem Biol Interact 2018; 284: 126-136.