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ASSESSMENT OF THE IMPACT OF THE EGYVIR ON RATS EXPERIMENTAL ANIMALS; A PRECLINICAL STUDY FOR SARS-COV-2 TREATMENT

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ABSTRACT

Background: The SARS-COV-2 is a worldwide pandemic problem. We developed a herbal extract with potent in-vitro virucidal, anti-inflammatory and immunomodulatory effects called EGIVIR. Our aim is to assess the bioavailability and cytotoxicity of EGYVIR on different organs and biological systems in Sprague Dawley rats as a model of experimental animals.
Methods: 128 rats were divided into 16 groups (8 rats each), where Egyvir was assessed in oral doses of 20, 30, and 40 mg/kg body weight, and by inhalation in 0.2, 0.3, and 0.4 mg/kg body weight, four times/day, compared to the control groups.
Results: The Egyvir had no significant effect on the blood pressure, pulse, motor activity, histological, hematological, and coagulation profiles. Also, the blood levels of triglycerides, cholesterol, blood glucose, lactate dehydrogenase (LDH), and creatine phosphor kinase (CPK) were not significantly affected. Egyvir had no harmful effect on the kidney and liver functions, blood electrolytes levels and urinary levels of sodium, potassium, and chloride. There was no significant effect on the serum levels of interleukin-1β (IL-1β), IL-2, IL-4, IL-6, IL-10, interferon- γ (IFN-γ), and tumor necrosis factor- α (TNF-α). Additionally, there was no significant change in the levels of Superoxide dismutase (SOD), catalase, reduced glutathione (GSH), and malonaldehyde (MDA) in comparison to the control groups ($P<0.05$).
Conclusion: Egyvir is considered a safe antiviral natural drug. It could be used for the treatment of SARS-COV-2 without any adverse effects when used with the recommended doses. However, these data are a preliminary step for validation in a clinical setting.

Keywords: Egyvir, SARS-COV-2, Antiviral, Curcumin

INTRODUCTION

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) or COVID-19 viral infection becomes now a worldwide life-threatening pandemic disease **(Lu *et al.*, 2020)**. It had been estimated that the total number of confirmed COVID-19 cases all over the world was 297,912,706 till 5th January 2022, with 5,466,645 global deaths **(John Hopkins University, 2022)**.
Patients infected with SARS-CoV-2 can be presented with several symptoms varying from a mild degree of upper respiratory tract affection to a severe acute respiratory distress syndrome (ARDS), that may lead to multiple organ failures and eventually death **(Bakhiet and Taurin, 2021)**. This variability of disease severity is strongly correlated to the patients' age, accompanied comorbidities, and other conditions associated with dysregulated immune system as in cancer patients **(Huang *et al.*, 2020; Liu *et al.*, 2020; Miyashita *et al.*, 2020)**. SARS-CoV-2 is a cytopathic virus that kills the host cells as a part of its replication cycle **(Chu *et al.*, 2020)**. It enters the cells through binding to the Angiotensin-Converting Enzyme 2 (ACE2) receptor, which is highly expressed on the alveolar epithelial type II cells **(Zhuang *et al.*, 2020)**. These alveolar epithelial cells are critical for the gas exchange function in the lung and consequently prevent the alveoli from collapsing **(Dobbs, 1989)**. Additionally, ACE2 receptors are expressed on the surface of other cells including type I pneumocytes, endothelial cells, heart, intestine, blood vessels, kidneys, and urinary bladder **(Monteil *et al.*, 2020; Zou *et al.*, 2020)**. The binding of the virus to these cells results in cell damage and death with the release of pro-inflammatory mediators including IL-1β, IL-6, IL-8, TNF-α, IP10, MCP-1, and RANTES **(Huang *et al.*, 2020; Li *et al.*, 2020)**. If this inflammatory response is not controlled by a potent immune system, it will lead to a severe disease course and death **(Huang *et al.*, 2020)**.
Worldwide, a large number of studies had been developed to produce an effective treatment specific for SARS-COV-2 virus. There are about 3947 clinical trials registered in clinicaltrials.gov till November 19, 2020 **(Bakhiet and Taurin, 2021)**, and other medications were repurposed for the treatment of SARS-CoV2, including lopinavir-ritonavir, favipiravir, remdesivir, arbidol, hydroxychloroquine, and azithromycin **(Cao *et al.*, 2020; Gautret *et al.*, 2020; Million et al., 2020; Zhou *et al.*, 2020)**. In addition to the recent advancement in immunotherapy using mRNA SARS-CoV-2, and adenovirus type-5 vectored vaccines **(Ahn *et al.*, 2020; Zhu *et al.*, 2020)**. However, till now there was no effective specific treatment developed for the SARS-COV-2 infection according to the WHO **(Bakhiet and Taurin, 2021; Lima *et al.*, 2021)**. On the other side, although there were many vaccines were developed against SARS-COV-2, only three vaccines, Sputnik V, BNT162b2, and mRNA-1273, have proved up to 90 % efficacy **(Bakhiet and Taurin, 2021)**. To date, still social distancing and isolation with supportive management for the patients are the most effective measures used all over the world especially in developing countries **(Zhai *et al.*, 2020)**. Therefore, searching for another modality for the treatment of SARS-COV-2 infection is highly required.
We developed an antiviral herbal extract with a potent immunomodulatory effect called EGIVIR, which is formed of a combination of curcumin extract with black pepper extract **(Roshdy *et al.*, 2020)**. Curcumin is a hydrophobic polyphenol, which constitutes the main active ingredient of the rhizomes of turmeric (Curcuma longa) **(Akbar *et al.*, 2018; Soleimani *et al.*, 2018)**. It is used as a spice in foods as well as for cosmetic and pharmaceutical purposes in many countries **(Hosseini *et al.*, 2018)**. Curcumin is characterized by its potent anti-inflammatory, antioxidant, and anticancer activities **(Catanzaro *et al.*, 2018)**. It suppresses the release of the pro-inflammatory cytokines including IL-6, TNF-α, and IL-1β through inhibiting the NF-κB and MAPK signalling pathways **(Cho *et al.*, 2007; Jin *et al.*, 2007)**. Also, it inhibits cyclooxygenase-2 (COX-2), and STAT signalling pathways **(Ghosh *et al.*, 2015)**. In addition, many studies had reported the antiviral effect of curcumin against different viruses including influenza virus, human papillomavirus (HPV), human immunodeficiency virus (HIV), herpes simplex virus−2 (HSV2), adenovirus, hepatitis, and Zika viruses **(Dai *et al.*, 2018; Ferreira *et al.*, 2015; Mounce *et al.*, 2017; Yang *et al.*, 2016)**. Different mechanisms had been proposed to explain the antiviral effect of the curcumin. These mechanisms

are varying from interfering with the entry of the virus into the host cells, inhibiting virus encapsulation and replication, as well as targeting cellular transcription **(Obata *et al*., 2013; Zahedipour *et al*., 2020)**. Moreover, many recent reports had studied the potential role of curcumin in the treatment of SARS-COV-2 infection **(Babaei *et al*., 2020; Soni *et al*., 2020; Zahedipour *et al*., 2020)**. They reported that curcumin can inhibit ACE2 receptors, and viral protease **(Hoffmann *et al*., 2020)**. Additionally, it stimulates the host's innate immunity through increasing interferon production **(Mrityunjaya *et al*., 2020)**.

Piperine is the major active constituent of black pepper, it is characterized by its potent anti-inflammatory and antioxidant action **(Mittal and Gupta. 2000; Vaibhav *et al*., 2012)**. Therefore, it was considered for trial in COVID-19 treatment to inhibit the hyper inflammation reaction induced during SARS-COV-2 infection **(Mrityunjaya *et al*., 2020)**. Also, it was reported to improve the bioavailability of curcumin by 2000 times **(Prasad *et al*., 2014)**.

Hence, depending upon our previous results regarding the efficiency of Egyvir as a potent immunomodulator and effective treatment against SARS-COV-2 infection in-vitro. The aim of the current study was to assess the bioavailability, action, and different side effects that could be produced by EGYVIR on different organs and biological systems in rats as a model of experimental animals. This will allow for applying this drug for a clinical trial to be used as an effective antiviral drug against SARS-COV-2 infection.

MATERIAL AND METHODS

Preparation of the EGYVIR

The EGYVIR was prepared from healthy disease-free Curcuma longa (Turmeric) root and Piper nigrum seeds extraction, which were purchased from a local market in Egypt. The plant materials were dried and ground into powder, of which a weight of 40 mg of the well dried air Curcuma longa roots powder was infused in 100 ml of aqueous Piper nigrum seed extract until completely exhausted. Then it was filtered through a four-layered muslin cloth to prepare a total concentration of 40 mg/Liter, which was stored at 4 C until further use, according to our previous study **(Roshdy *et al*., 2020).**

The chemical composition of the prepared EGYVIR extract was determined by Gas Chromatography–Mass Spectrometry (GC-MS) using the trace GC-TSQ Evo 8000 mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25µm film thickness). The temperature of the column oven was initially adjusted at 50 ˚C and raised by 5 ˚C /min to reach 200 ˚C for 2 min, then raised to the final required temperature of 300 ˚C by 25 ˚C /min and kept for 2 min. The injector and mass spectrometry transfer line temperatures were held at 270 and 260 ˚C respectively. Helium gas was used as a carrier with a constant flow rate of 1 ml/min. Electron ionization (EI) mass spectra were collected at 70 eV ionization voltage over the range of m/z 50–650 in full scan mode. The components were identified by comparing their retention times and mass spectra with mass spectral libraries **(Fiehn, 2016)**.

Experimental Animals

The current study was performed on 128 Sprague Dawley rats, sixty-four males (weight, 200 ± 3.9 gm) and 64 females (weight, 136 ± 16 g) obtained from the animal house colony, National Research Centre, Cairo, Egypt. All animals were acclimated to a colony room with an ambient temperature of 22±1°C, a humidity of 50±10%, and a 12 h light/dark cycle for at least 10 days, in metal cages before the start of the experiment. During this period, the rats were examined for any abnormalities suggestive of health problems. Rats were allowed to freely access food and water, where the body weights were recorded regularly. All experiments were carried out according to the ethical guidelines for the care and use of experimental animals, and the research protocol was approved by the Ethical Committee of the National Research Centre, Cairo, Egypt (approval no: CU/F/16/21).

Experimental design

The included 128 rats were divided into 16 groups (8 rats each); six groups (3 male groups and 3 female groups) were supplemented orally with EGYVIR in three different doses 20, 30, and 40 mg/kg body weight according to the performed cytotoxicity assay (Roshdy et al., 2020). The other 6 groups (3 male groups and 3 female groups) were inhaled EGYVIR in three different doses 0.2, 0.3, and 0.4 mg/kg body weight, divided four times/day via nebulizer (Roshdy et al., 2020). The remaining four groups represented the control groups. All groups were assigned as follows; G1and G2: Control male and female rats which were administered 0.9% normal saline solution daily orally for two consecutive weeks. G3 and G4: male and female rats which were supplanted with a low dose of EGYVIR 20 mg/ kg body weight orally daily for two weeks. G5 and G6: male and female rats were supplemented orally daily with a moderate dose of 30 mg/ kg body weight of EGYVIR for two weeks. G7 and G8: male and female rats which were supplemented orally daily with a high dose of 40 mg/ kg body weight of EGYVIR for two weeks. While G9 and G10: control male and female rats were inhaled daily with 0.9% normal saline solution in dose of 0.2-0.4 mg/ kg body weight for two weeks. G11 and G12: male and female rats which were inhaled daily of EGYVIR in a low dose of 0.2 mg/ kg body weight for two weeks. G13 and G14: male and female rats were daily inhaled daily of EGYVIR in a moderate dose of 0.3 mg/ kg body weight for two weeks. G15 and G16: male and female rats were daily inhaled EGYVIR in a high dose of 0.4 mg/ kg body weight for two weeks.

Management of the Assessed Animals

The bodyweight of the experimental rats had been recorded at least two times prior to the randomization and at the end of the experiment. Animals were observed twice daily, in the morning and afternoon with a minimum of 6 hours interval for mortality, morbidity, and clinical signs of toxicity. Detailed clinical examinations were conducted weekly outside the home cage. The blood pressure, pulse, behavioral and motor activity including object recognition, rotarod and activity cage were conducted on the animals prior to and at the end of the experiment.

Sample Collection

At the end of the two weeks of the experiment, the urine samples were collected on clean cups for urine analysis. Fasting blood samples were collected through the jugular vein under light anesthesia for assessment of the blood glucose level and hematological profile including hemoglobin concentration (Hb), red blood cells count (RBCs), white blood cells count (WBCs), platelets count, hematocrit ratio and reticulocytes count. Another blood sample was taken for the assessment of coagulation blood functions including Fibrinogen, prothrombin time (PT), and partial thromboplastin time (PTT). All hematological analysis was performed using the hematology analyzer (Sysmex, Germany). The separation of the serum was done through the collection of the blood in plain test tubes, and it was left for clotting, then centrifuged at 3000 rpm for 15min to separate the serum. The serum was stored into sterile Cryotubes at - 20 °C to be used for the biochemical analysis. After that, all animals were sacrificed by decapitation under anesthesia with an intraperitoneal injection of ketamine 40 mg/kg.

Organ weight and histopathological study

After scarification of the rats, a wide laparotomy was performed, and the different body organs were weighed, paired organs were weighed together and the organ-to-whole body weight percentages were recorded (**Supp. 1**). Tissue preservation was performed in 10% neutral-buffered formalin for twenty-four hours, followed by decalcification in formic acid. Washing was done in tap water and then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were performed for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 °C in a hot air oven for twenty-four hours. Paraffin beeswax tissue blocks were prepared for sectioning at 4 microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, and stained by Hematoxylin & Eosin stain for examination through the light microscope **(Abdelhameed *et al*., 2021)**.

Biochemical Assessment

The stored serum was analyzed for liver function tests including glutamic oxaloacetic transaminase (GOT), glutamic pyruvate transaminase (GPT), alkaline phosphatase (ALP), albumin, and total protein (TP). Kidney function tests including serum urea and creatinine. Determination of serum creatinine phosphokinase (CPK), lactate dehydrogenase (LDH), low-density lipoprotein (LDL), cholesterol (TC), triglycerides (TG), blood minerals including calcium (Ca), phosphorus (P), sodium (Na), potassium (K), and chloride (Cl), urinary content of Na, K, and Cl levels were done according to the manufactures' instructions (all tests were performed using the fully automated system, ADVIA 1800, Siemens Healthineers).

Determination of the cytokine levels

The serum cytokine levels including interleukin-1β (IL-1β), IL-2, IL-4, IL-6, IL-10, interferon- γ (IFN-γ) and tumor necrosis factor-α (TNF-α) were assessed using the enzyme-linked immunosorbent assay (ELISA) technique according to the manufacturers' instructions (Biotrak ELISA System, GE Healthcare Life Sciences, Pittsburgh, PA).

Determination of the serum antioxidant levels

The serum levels of Superoxide dismutase (SOD), catalase, reduced glutathione (GSH), and malonaldehyde (MDA) were estimated according to the manufacturers' instructions (Ransod Randox Lab, Antrim, UK) For the measurement of SOD, catalase, and GSH. While the Biodiagnostic, Egypt was used for the measurement of the serum level of MDA.

Statistical analyses

Data were expressed as a mean ± standard deviation after testing for normality distribution using GraphPad Prism 7 (GraphPad Software Inc, San Diego, CA, USA). Comparisons between the different groups of animals were performed using the two-way analysis of variance (ANOVA) and followed by Tukey's multiple comparison test to assess the significance between-group differences. P-value was considered significant at P< 0.05.

RESULTS

Histopathological Analysis

During the complete gross necropsy analysis, there were no macroscopic pathological changes were observed in the examined organs in all studied groups in relation to the control groups, either treated with oral (Figure 1) or inhalation (Figure 2) routes at any of the examined doses. Regarding, the liver and kidney which are the key metabolic organs, the livers of all groups showed normal lobular structure, normal hepatic parenchymal cell histology as well as normal portal areas and central veins. The kidney tissue showed normal histological features of the glomeruli and renal tubules. The heart in all groups revealed a normal structure of the cardiac muscles which appeared with clear cross striations and centrally located nuclei without any evidence of necrobiotic changes. Normal alveolar and bronchiolar structure was a common finding in the lungs of all groups, apart from mild congestion in two animals of the medium dose with the oral route group. The tracheal sections of all groups were within normal structure, with healthy and intact ciliated pseudostratified columnar epithelium, as well as the mucosal and fibroelastic layers were normally seen. Regarding the brain, the cerebrum and cerebellum areas appeared with normal histology. Generally, during the histopathological analysis of all tissues, there were no obvious significant changes among the assessed tissue groups.

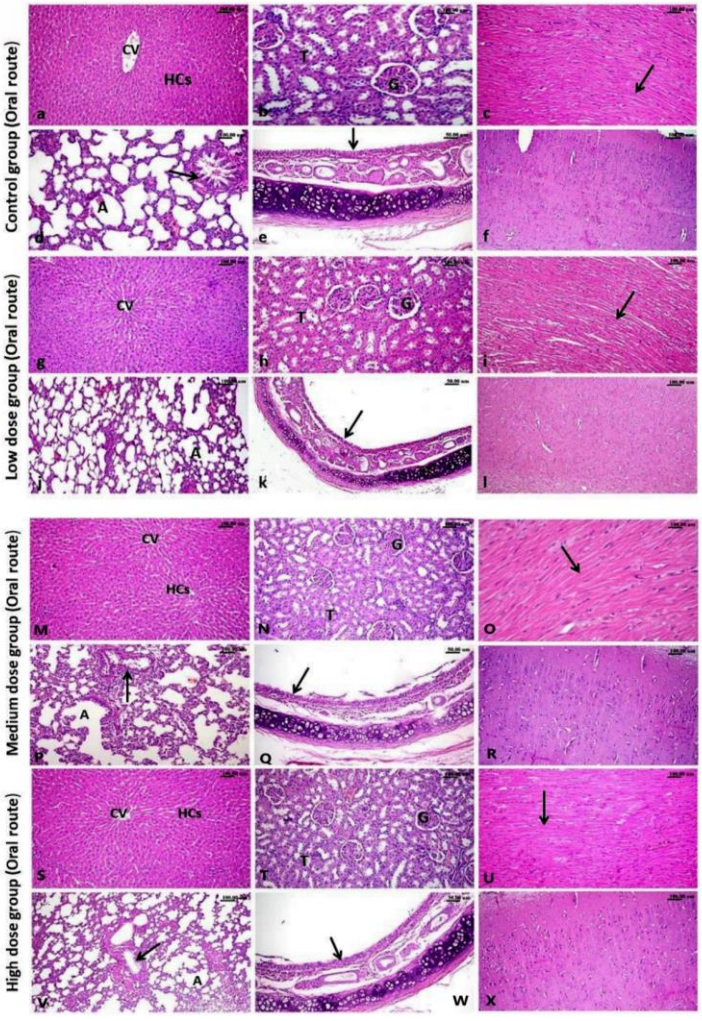

Figure 1 Histopathological assessment of the different doses of orally administrated Egyvir on the liver (A, G, M, S), kidney (B, H, N, T), heart (C, I, O, U), lung (D, J, P, V), trachea (E, K, Q, W), and the brain (F, L, R, X) of the tested rats' groups.

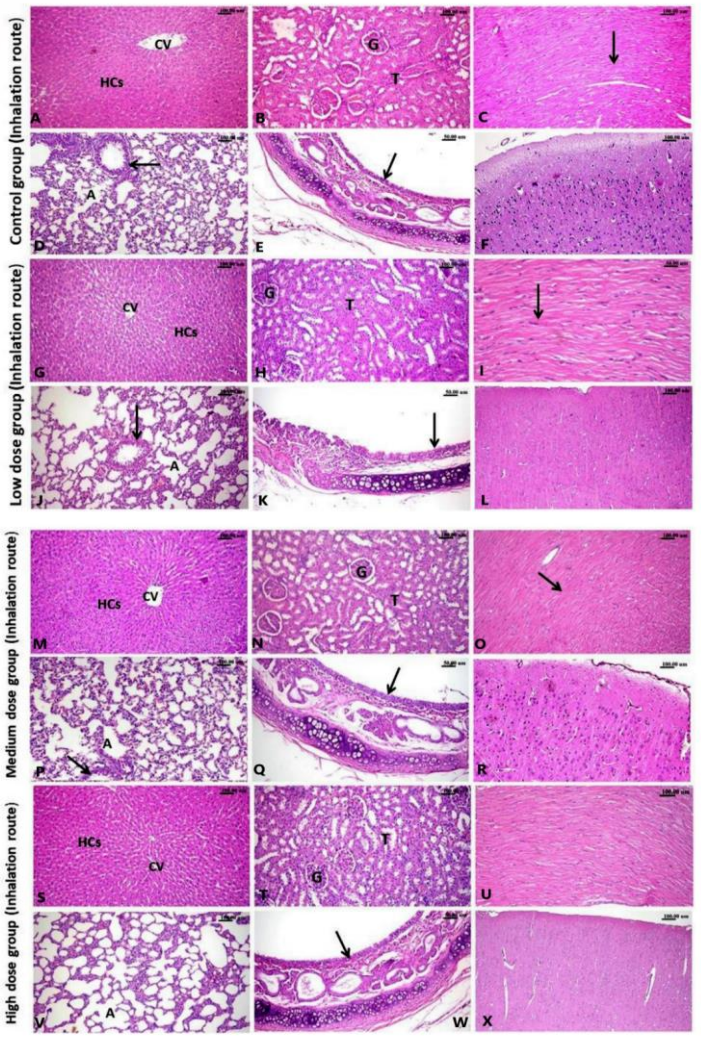

Figure 2 Histopathological assessment of the different doses of administrated Egyvir by inhalation route on the liver (A, G, M, S), kidney (B, H, N, T), heart (C, I, O, U), lung (D, J, P, V), trachea (E, K, Q, W), and the brain (F, L, R, X) of the tested rats' groups.

Assessment of the body weight, blood pressure, pulse rate, motor activity and coordination in the assessed rats

There was no significant change in the body weight between the treatment and the control groups after inhalation or oral administration of Egyvir with the assigned tested doses (P>0.05, for all, Figure 3 a,b).

There was no significant change in the arterial blood pressure level after 2 weeks of inhalation or oral administration of Egyvir with its different doses in the tested female rats compared to the control (P<0.05, for all). However, in the male rats, there were significant changes in the arterial blood pressure regarding the low and high doses of Egyvir compared to the control group after two weeks of oral administration (P=0.009 and P=0.012; respectively). Similarly, there were significant changes in the blood pressure regarding the low and high doses of Egyvir compared to the control group after two weeks of inhalation of Egyvir (P=0.006 and P=0.002; respectively, Figure 3 c,d).

In addition, there was no significant change detected in the pulse rate between the treatment and the control groups after inhalation or oral administration of Egyvir with the assigned tested doses (P>0.05, for all, Figure 3 e,f).

Finally, clinical observation of the experimental animals regarding the motor activity and coordination revealed non-significant records in both motor activity count/5min and motor coordination/sec for all treatment/route groups (P>0.05, for all, Figure 3 g,h).

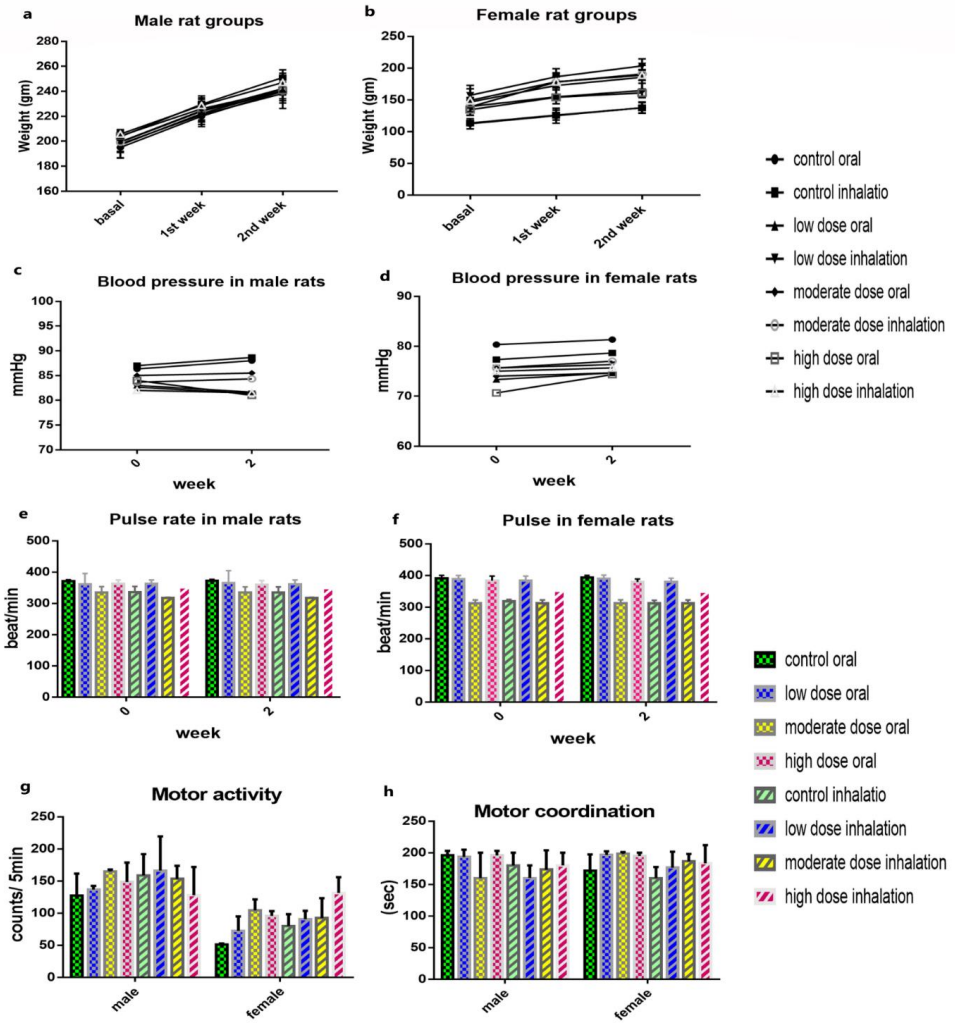

Figure 3 The effect of the Egyvir on a) male and b) female rats' weight, c) male and d) female rats' blood pressure, e) male and f) female rats' pulse rates, g) motor activity and h) motor coordination.

Determination of the Effect of Egyvir on the Hematological and Coagulation Profile of the Assessed Animals

There was no significant effect of the Egyvir with its assigned different doses either administrated with oral or inhalation routes on the hematological or the coagulation profile. There was no significant difference between the examined groups in comparison to the control groups regarding the RBCs count, WBCs count, Hb concentration, platelets count, hematocrit ratio, reticulocytes ratio, Fibrinogen, PT, and PTT ($P<0.05$). However, there was a significant difference between males and females regarding the RBCs count, WBCs count, and fibrinogen concentration ($P=0.026$, $P<0.001$, and $P=0.005$; respectively, Figure 4).

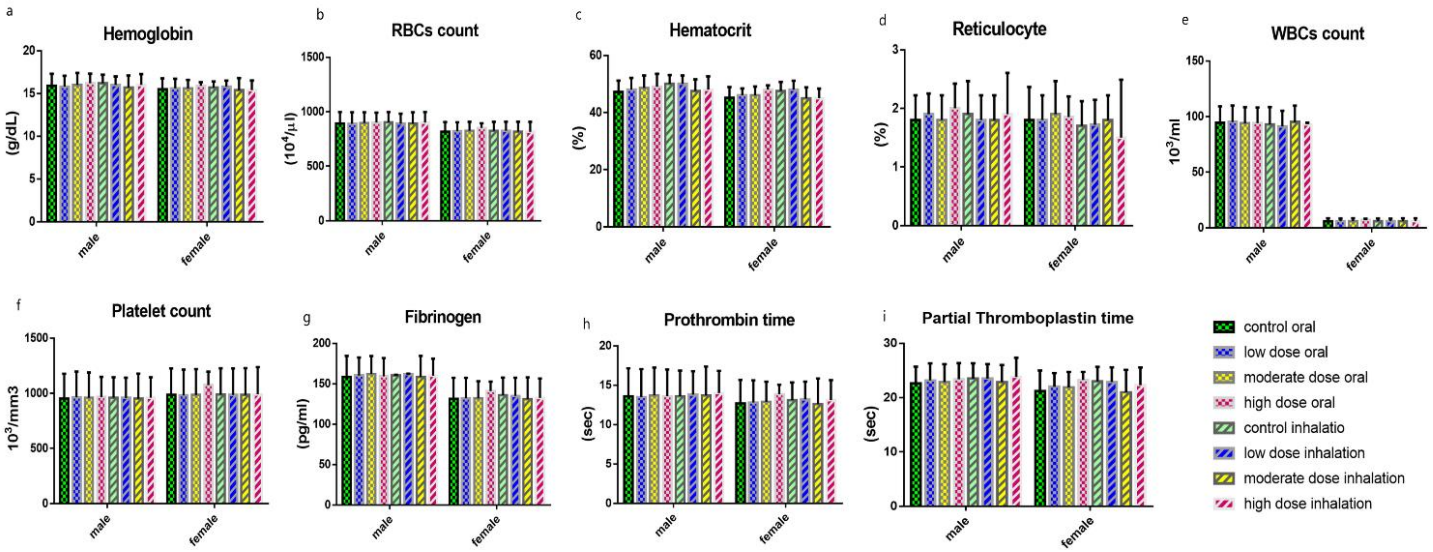

Figure 4: The effect of the Egyvir on a) the hemoglobin concentration, b) red blood cells count, c) hematocrit ratio, d) reticulocytes count, e) white blood cells count, f) platelets count, g) Fibrinogen, h) prothrombin time (PT), and i) partial thromboplastin time (PTT).

Determination of the effect of Egyvir on the lipid profile, kidney, and liver function tests

There was no significant effect of the different doses of Egyvir [20-40 mg/kg orally, and 0.2-0.4 mg/kg by inhalation] on the blood levels of triglycerides, cholesterol, blood glucose. Also, there was no significant effect of the different doses of Egyvir on the serum levels of LDH and CPK (P<0.05 for all). However, there was a significant increase in the serum levels of triglycerides in the assessed male rates compared to the female rats (P=0.009). While there was a significant increase in the blood cholesterol levels in the female rats compared to the assessed male rats (P=0.006, Figure 5 A-E). In addition, there was no significant effect of Egyvir on the kidney function tests in the form of serum urea and creatinine (P<0.05 for all, Figure 5 F,G).

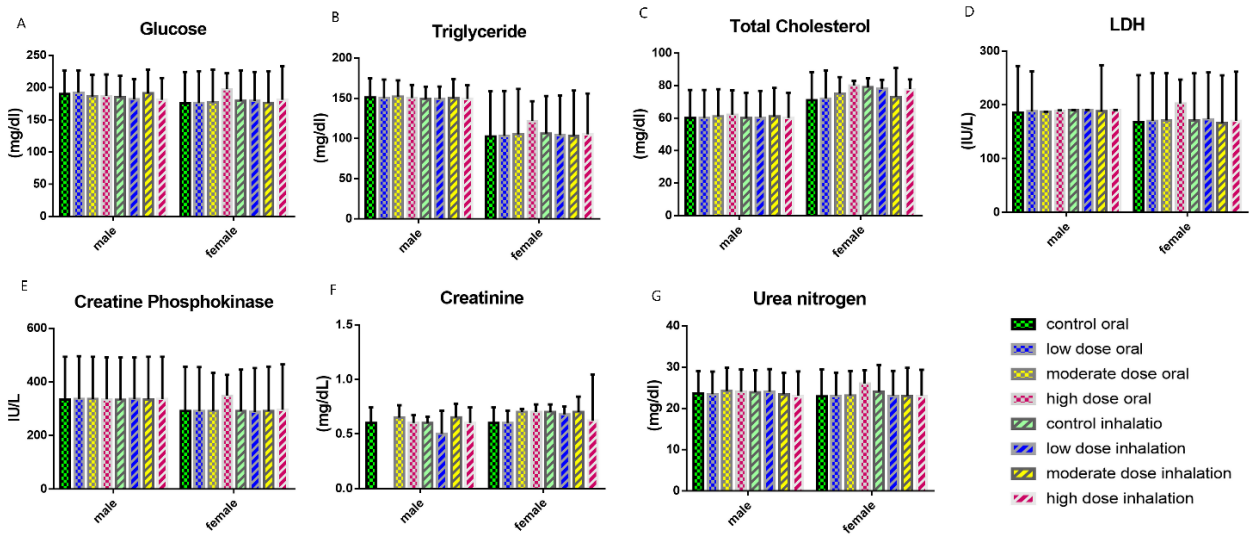

Figure 5 The effect of the Egyvir on the serum levels of a) blood glucose, b) triglycerides, c) total cholesterol, d) lactate dehydrogenase (LDH), e) creatine phosphokinase, f) creatinine and g) urea nitrogen.

Regarding the liver function tests, there were no significant changes in the serum levels of GPT, GOT, alkaline phosphatase, albumin, and total proteins between the assessed groups compared to the control groups (P<0.05 for all, Figure 6 a-e).

Urine analysis showed that the urinary levels of sodium, potassium, and chloride were not significantly affected by the different doses of Egyvir (oral and inhalation), in comparison to the control group (P<0.05 for all, Figure 6 f-h).

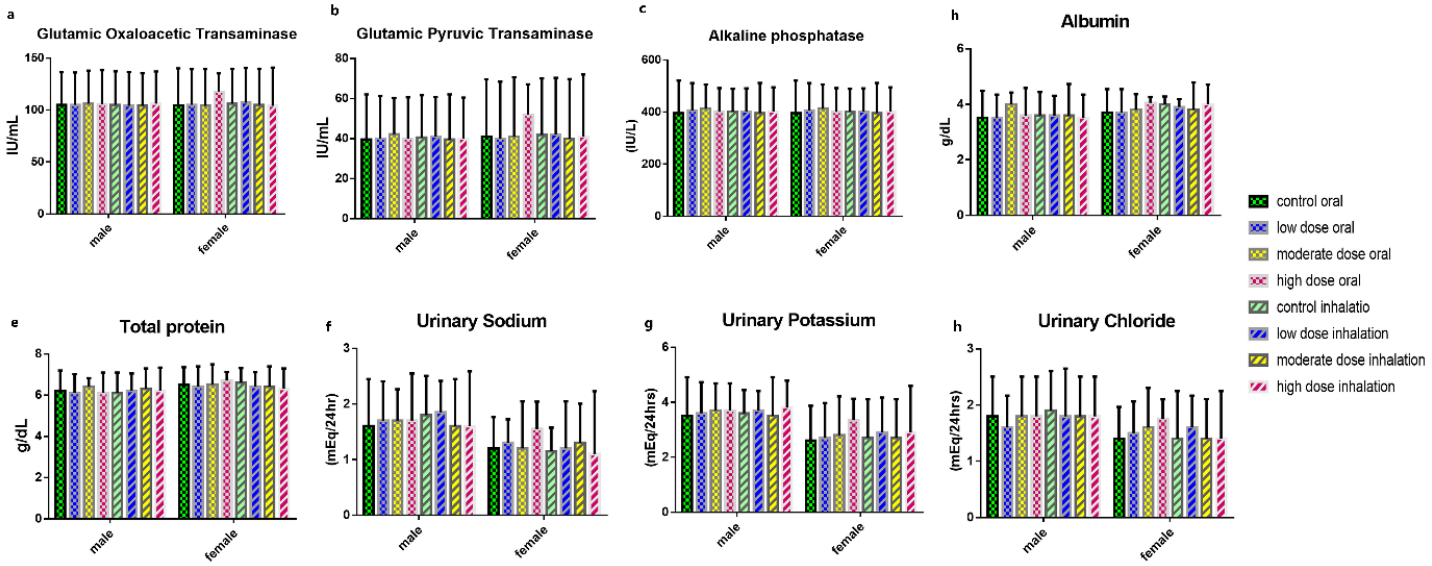

Figure 6 The effect of the Egyvir on a) glutamic oxaloacetic transaminase (GOT), b) glutamic pyruvate transaminase (GPT), c) alkaline phosphatase, d) albumin, and e) total proteins, f) urinary sodium, g) urinary potassium and h) urinary chloride.

Assessment of the effect of the Egyvir on the serum antioxidant levels

There were no significant changes in the expression levels of SOD, catalase, GSH, and MDA in the tested groups in relation to the control groups when Egyvir was administrated with its different doses, either by oral or inhalation routes (P<0.05 for all, Figure 7 a-d).

Assessment of the effect of the Egyvir on the blood electrolytes levels

There was no significant impact of the different doses of Egyvir [20-40 mg/kg orally, and 0.2-0.4 mg/kg by inhalation] on the blood levels of sodium, potassium, chloride, calcium, and inorganic phosphorus in comparison to the control rat groups (P<0.05 for all, Figure 7 e-i).

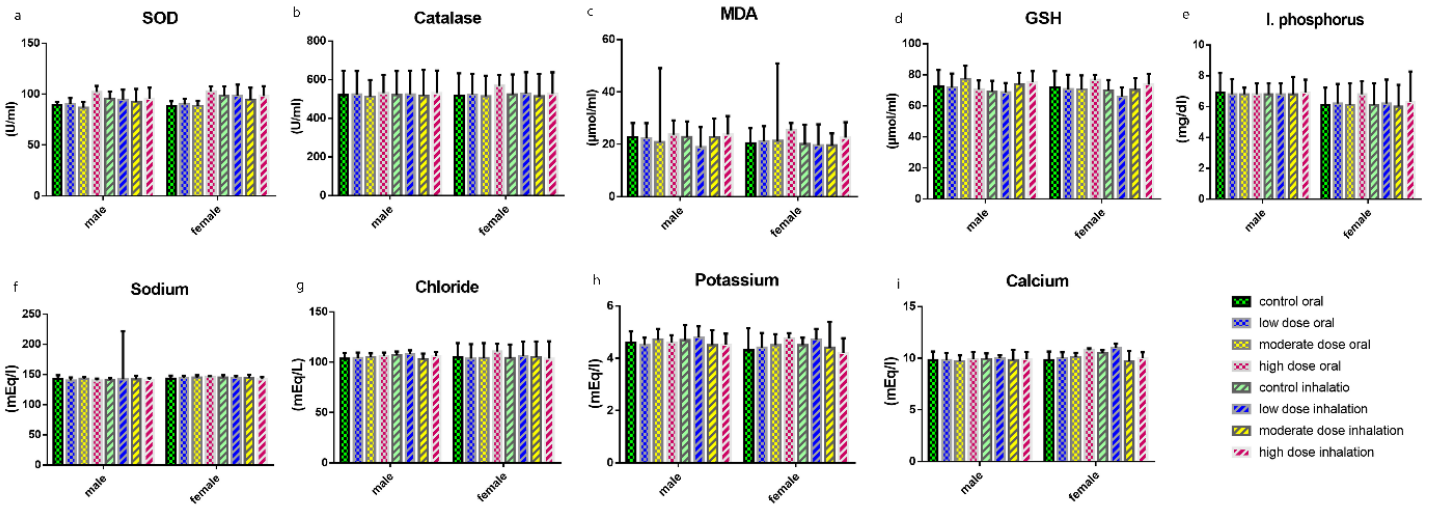

Figure 7 The effect of the Egyvir on the serum levels of a) Superoxide dismutase (SOD), b) catalase, c) malonaldehyde (MDA), d) reduced glutathione (GSH), e) inorganic phosphorus, f) Sodium, g) Chloride (Cl), h) Potassium (K), and i) Calcium (Ca).

Assessment of the effect of the Egyvir on the cytokine levels in the experimental animals

The impact of the Egyvir on the immunological function of the body was assessed by measuring the cytokine levels in the serum of the examined animals with different tested doses in both oral and inhalation routes. There was no significant effect of the Egyvir on the expression levels of IL-1β, IL-2, IL-4, IL-6, IL-10, IFN-γ and TNF-α in the tested groups in comparison to the control groups (P<0.05 for all). However, there was a significant difference between males and females regarding the IL-2 and IL-6 expression (P=0.012 and P<0.001; respectively, Figure 8).

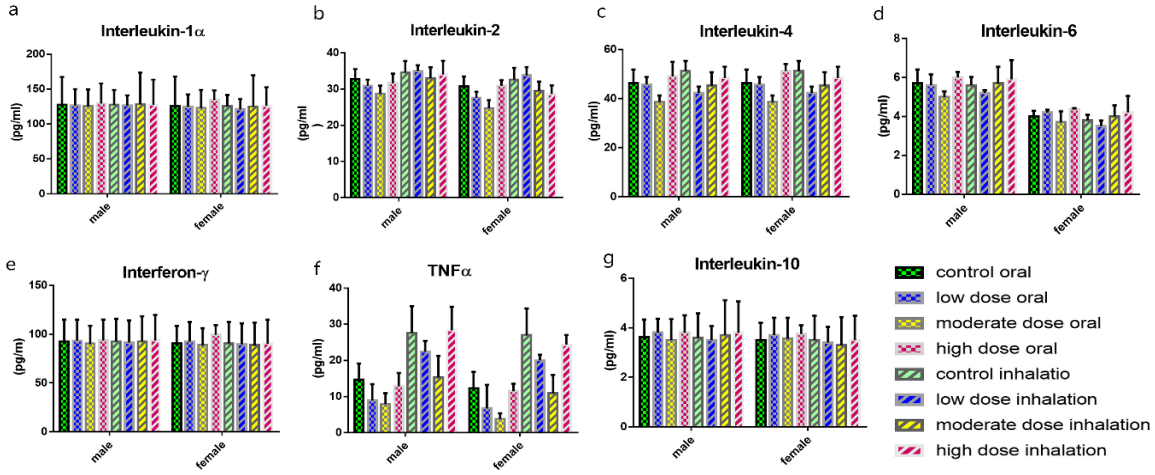

Figure 8 The effect of the Egyvir on the cytokines levels of a) Interleukin-1α, b) Interleukin-2, c) Interleukin-4, d) Interleukin-6, e) Interferon-γ, f) Tumor necrosis factor-α (TNF-α), and g) Interleukin-10.

DISCUSSION

Nowadays, the world is facing a pandemic of SARS-COV-2 infection, which results in nearly more than five million deaths globally, and still, the mortality rate is increasing. Therefore, efforts all over the world are directed towards finding a specific treatment for the SARS-COV-2 virus. We have developed a promising herbal extract called EGYVIR as a potential antiviral drug against SARS-CoV-2 **(Roshdy *et al.*, 2020).** It is formed mainly of a combination of black pepper extract and curcumin extract. The Egyvir showed a virucidal effect through antagonizing the NF-kβ pathway as proved by the in-silico as well as the in-vitro assessment. Consequently, it inhibits the release of IL-6 and TNFa that play an important role in the cytokine storm, which is responsible for the inferior outcome of the SARS-CoV-2 infection.

Here in the current study, we tried to assess the bioavailability, action, and cytotoxicity of Egyvir in Sprague Dawley rats as a model of in vivo study. The cytotoxicity of the EGYVIR was evaluated on Vero-E6 cells using MTT assay. The data showed that the cytotoxic concentration 50 (CC50) value of EGYVIR was 0.57 µg. Hence, we selected a concentration of 0.05–0.4 µg/mL for the in vivo assay of the drug. We tested Egyvir in 20, 30, and 40 mg/kg body weight orally, and in 0.2, 0.3, and 0.4 mg/kg body weight by inhalation, divided four times/day via nebulizer.

The present data showed that the treatment of both male and female rats daily with EGYVIR by both routes either oral or inhalation for two weeks did not show any signs of toxicity including mortality, hair loss, diarrhoea, and patches of yellow colour appearance. In addition, abnormalities in behaviour, motor activity, health status, food and water intake among the treated animals were not observed. Also, blood pressure and pulse were monitored before the beginning of the experiment and at the end of study after two weeks of Egyvir administration. The results showed non-significant changes between the treated groups and the control groups. To the best of our knowledge, the Egyvir is a herbal extract that is formed of fifty-three natural ingredients including mainly Pentatricontane (41.04%), Amyrin (9.49%), Lupeol (8.86%), Turmerone (8.13%), Sitosterol (7.61%), Bisdemethoxycurcumin (6.8%), Piperine (4,6%), Vitamin D3 (1.76%), and Curcumin (1.3%). All these components are well known for their beneficial effects on the body including their anti-inflammatory, antioxidant, and immunopotentiation properties **(Arbab *et al*., 2016; Ghosh *et al*., 2015; Siddique and Saleem 2011; Vaibhav *et al*., 2012; Yang *et al*., 2016).** In line with these studies, our data revealed that Egyvir had no adverse effect on the haematological and coagulation profiles of the experimental rats in the form of RBCs count, WBCs count, Hb concentration, platelets count, hematocrit ratio, reticulocytes ratio, Fibrinogen, PT, and PTT. Similarly, there was no significant effect of Egyvir on the blood levels of triglycerides, cholesterol, blood glucose, LDH, and CPK. In addition, Egyvir had no harmful effect on the kidney and liver functions together with the blood electrolytes levels and urinary levels of sodium, potassium, and chloride. Therefore, Egyvir is considered a very safe natural drug that could be used effectively without any adverse effects with the recommended doses.

In regard to the antioxidant effect of the Egyvir, the administrated drug did not show any significant changes in the expression levels of SOD, catalase, GSH, and MDA in the tested groups in relation to the control groups**.** These data are consistent with many other studies proposed that curcumin is a potent antioxidant due to increasing the synthesis of the antioxidant enzymes and neutralizing the produced free radicals in the body **(Agarwal *et al*., 2010; Barclay *et al*., 2000; Biswas *et al*., 2005; Menon and Sudheer. 2007).** Moreover, Mittal and Gupta (2000) reported that Piperen which is an important component of Egyvir, can

protect against oxidative stress by antagonizing reactive oxygen species (ROS), free radicals, and hydroxyl radicals. Similarly, Lupeol which represents 8.86% of EGYVIR extract has a potent antioxidant activity. It decreases the ROS level and promotes the antioxidant enzyme activities in chemical-induced oxidative stress conditions **(Siddique and Saleem, 2011).**

The current study also showed that Egyvir had no significant harmful impact on the immunological function of the body regarding the cytokine levels of IL-1β, IL-2, IL-4, IL-6, IL-10, IFN-γ, and TNF-α. These data are in agreement with many published studies reported that curcumin has potent anti-inflammatory and immunomodulatory functions through inhibiting NF-κB, MAPK, cyclooxygenase-2 (COX-2), and STAT signalling pathways that result in interfering with the production of the pro-inflammatory cytokines such as IL-1, IL-2, IL-6, IL-10, transforming growth factor (TGF)-β, IFN, and TNF-α. The blockade of these cytokines results in inhibiting the cytokine storm responsible for the adverse events of SARS-COV-2 infection **(Cho *et al*., 2007; Ghosh *et al*., 2015; Hirano and Murakami 2020; Vardhana and Wolchok. 2020).** Additionally, our previous docking work showed that EGYVIR had a good binding affinity with p50 subunit of NF-κB which attenuates NF-κB pathways **(Roshdy *et al*., 2020).** Moreover, β-sitosterol enhances the mitochondrial glutathione redox mechanism which can decrease the cytokine storm **(Zhao *et al*., 2015).** Similarly, the other components of Egyvir namely piperine, turmerone, and lupeol were also reported to inhibit the NF-κB signalling pathway and consequently IL-6 expression **(Derosa *et al*., 2016; Pradeep and Kuttan, 2004; Reuter *et al*., 2009).**

CONCLUSION

In conclusion, the present study showed that Egyvir is a safe drug that did not induce any sign of toxicity or harmful effect on the different body organs and systems of the experimental animals. The recommended administered dose is 20-40 mg/kg body weight orally, or 0.2-0.4 mg/kg body weight by inhalation. However, this study introduced preliminary data to be transferred to the clinical setting. As Egyvir is a herbal extract formed of many beneficial components, which achieved potent virucidal, anti-inflammatory, antioxidant, and immunomodulatory functions against SARS-COV-2 infection. Therefore, it will be a potential new modality for the treatment of SARS-COV-2 patients especially those with immunodeficiency or other comorbidities.

Compliance with ethical standards should be placed before the References

Conflict of interest: All authors declare that there is no possible conflict of interest.

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Ethical approval: The study followed the Helsinki Declaration, and the protocol was approved by the Egypt's National Research Center Committee (approval no: CU/F/16/21).

REFERENCES

Abdelhameed, MF., Asaad, GF., Ragab, TIM., Ahmed, RF., El Gendy, AEG., Abd El-Rahman, SS., Elgamal, AM., Elshamy, AI., 2021. Oral and Topical Anti-Inflammatory and Antipyretic Potentialities of Araucaria bidiwillii Shoot Essential Oil and Its Nanoemulsion in Relation to Chemical Composition. Molecules. 26(19):5833. https://doi.org/10.3390/molecules26195833

Agarwal, R., Goel, SK., Behari, JR., 2010. Detoxification and antioxidant effects of curcumin in rats experimentally exposed to mercury. J Appl Toxicol. Jul;30(5):457-68. https://doi.org/10.1002/jat.1517

Ahn, DG., Shin, HJ., Kim, MH., Lee, S., Kim, HS., et al. 2020. Current Status of Epidemiology, Diagnosis, Therapeutics, and Vaccines for Novel Coronavirus Disease 2019 (COVID-19). J Microbiol Biotechnol. 30(3):313-324. https://doi.org/10.4014/jmb.2003.03011

Akbar, MU., Rehman, K., Zia, KM., Qadir, MI., Akash, MSH., Ibrahim, M., 2018. Critical Review on Curcumin as a Therapeutic Agent: From Traditional Herbal Medicine to an Ideal Therapeutic Agent. Crit Rev Eukaryot Gene Expr. 28(1):17-24. https://doi.org/10.1615/CritRevEukaryotGeneExpr.2018020088

Arbab, AH., Parvez, MK., Al-Dosari, MS., Al-Rehaily, AJ., Ibrahim, KE., Alam, P., Alsaid, MS., Rafatullah, S., 2016. Therapeutic efficacy of ethanolic extract of Aerva javanica aerial parts in the amelioration of CCl4-induced hepatotoxicity and oxidative damage in rats. Food Nutr Res. 60:30864. https://doi.org/10.3402/fnr.v60.30864

Babaei, F., Nassiri-Asl, M., Hosseinzadeh, H., 2020. Curcumin (a constituent of turmeric): New treatment option against COVID-19. Food Sci Nutr. 8(10):5215-5227. https://doi.org/10.1002/fsn3.1858

Bakhiet, M., Taurin, S., 2021. SARS-CoV-2: Targeted managements and vaccine development. Cytokine Growth Factor Rev. 58:16-29. https://doi.org/10.1016/j.cytogfr.2020.11.001

Barclay, LR., Vinqvist, MR., Mukai, K., Goto, H., Hashimoto, Y., Tokunaga, A., Uno, H., 2000. On the antioxidant mechanism of curcumin: classical methods are needed to determine antioxidant mechanism and activity. Org Lett. 2(18):2841-3. https://doi.org/10.1021/ol000173t

Biswas, SK., McClure, D., Jimenez, LA., Megson, IL., Rahman, I., 2005. Curcumin induces glutathione biosynthesis and inhibits NF-kappaB activation and interleukin-8 release in alveolar epithelial cells: mechanism of free radical scavenging activity. Antioxid Redox Signal. 7(1-2):32-41. https://doi.org/10.1089/ars.2005.7.32

Cao, B., Wang, Y., Wen, D., Liu, W., Wang, J., et al. 2020. A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. N Engl J Med. 382(19):1787-1799. https://doi.org/10.1056/NEJMc2008043

Catanzaro, M., Corsini, E., Rosini, M., Racchi, M., Lanni, C., 2018. Immunomodulators Inspired by Nature: A Review on Curcumin and Echinacea. Molecules. 23(11):2778. https://doi.org/10.3390/molecules23112778.

Cho, JW., Lee, KS., Kim CW. Curcumin attenuates the expression of IL-1beta, IL-6, and TNF-alpha as well as cyclin E in TNF-alpha-treated HaCaT cells; NF-kappaB and MAPKs as potential upstream targets. Int J Mol Med. 2007 Mar;19(3):469-74. https://doi.org/10.3892/ijmm.19.3.469.

Chu, H., Chan, JF., Yuen, TT., Shuai, H., Yuan, S., et al. 2020. Comparative tropism, replication kinetics, and cell damage profiling of SARS-CoV-2 and SARS-CoV with implications for clinical manifestations, transmissibility, and laboratory studies of COVID-19: an observational study. Lancet Microbe. 1(1):e14-e23. https://doi.org/10.1016/S2666-5247(20)30004-5.

Dai, J., Gu, L., Su, Y., Wang, Q., Zhao, Y., et al. 2018. Inhibition of curcumin on influenza A virus infection and influenzal pneumonia via oxidative stress, TLR2/4, p38/JNK MAPK and NF-κB pathways. Int Immunopharmacol. 2018 54:177-187. https://doi.org/10.1016/j.intimp.2017.11.009.

Derosa, G., Maffioli, P., Simental-Mendı'a, LE., Bo, S., Sahebkar, A., 2016. Effect of curcumin on circulating interleukin-6 concentrations: A systematic review and meta-analysis of randomized controlled trials. Pharmacol Res, 111: 394-404. https://doi.org/10.1016/j.phrs.2016.07.004.

Dobbs, LG., 1989. Pulmonary surfactant. Annu Rev Med. 40:431-46. https://doi.org/10.1146/annurev.me.40.020189.002243.

Ferreira, VH., Nazli, A., Dizzell, SE., Mueller, K., Kaushic, C., 2015. The anti-inflammatory activity of curcumin protects the genital mucosal epithelial barrier from disruption and blocks replication of HIV-1 and HSV-2. PLoS One. 10(4):e0124903. https://doi.org/10.1371/journal.pone.0124903.

Fiehn, O., 2016. Metabolomics by Gas Chromatography-Mass Spectrometry: Combined Targeted and Untargeted Profiling. Curr Protoc Mol Biol. 114:30.4.1-30.4.32. https://doi.org/10.1002/0471142727.mb3004s114.

Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. Int J Antimicrob Agents. 2020 Jul;56(1):105949. https://doi.org/10.1016/j.ijantimicag.2020.105949.

Ghosh S, Banerjee S, Sil PC. The beneficial role of curcumin on inflammation, diabetes and neurodegenerative disease: A recent update. Food Chem Toxicol. 2015 Sep;83:111-24. https://doi.org/10.1016/j.fct.2015.05.022.

Hirano, T., Murakami, M., 2020. COVID-19: a new virus, but a familiar receptor and cytokine release syndrome. Immunity 52 (5), 731-733. https://doi.org/10.1016/j.immuni.2020.04.003.

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., et al. 2020. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. 181(2):271-280.e8. https://doi.org/10.1016/j.cell.2020.02.052.

Hosseini, A., Hosseinzadeh, H., 2018. Antidotal or protective effects of Curcuma longa (turmeric) and its active ingredient, curcumin, against natural and chemical toxicities: A review. Biomed Pharmacother. 99:411-421. https://doi.org/10.1016/j.biopha.2018.01.072.

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., et al. 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 395(10223):497-506. https://doi.org/10.1016/S0140-6736(20)30183-5.

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., et al. 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 395(10223):497-506. https://doi.org/10.1016/S0140-6736(20)30183-5.

Jin, CY., Lee, JD., Park, C., Choi, YH., Kim, GY., 2007. Curcumin attenuates the release of pro-inflammatory cytokines in lipopolysaccharide-stimulated BV2 microglia. Acta Pharmacol Sin. 28(10):1645-51. https://doi.org/10.1111/j.1745-7254.2007.00651.x.

John Hopkins University. "Coronavirus resource center." (2022). https://coronavirus.jhu.edu/.

Li, S., Jiang, L., Li, X., Lin, F., Wang, Y., et al. 2020. Clinical and pathological investigation of patients with severe COVID-19. JCI Insight. 5(12):e138070. https://doi.org/10.1172/jci.insight.138070.

Lima, WG., Brito, JCM., da Cruz Nizer, WS., 2021. Bee products as a source of promising therapeutic and chemoprophylaxis strategies against COVID-19 (SARS-CoV-2). Phytother Res. 35(2):743-750. https://doi.org/10.1002/ptr.6872.

Liu, Y., Mao, B., Liang, S., Yang, JW., Lu, HW., et al. 2020. Association between age and clinical characteristics and outcomes of COVID-19. Eur Respir J. 55(5):2001112. https://doi.org/10.1183/13993003.01112-2020.

Lu, H., Stratton, CW., Tang, YW., 2020. Outbreak of pneumonia of unknown etiology in Wuhan, China: The mystery and the miracle. J Med Virol. 92(4):401-402. https://doi.org/10.1002/jmv.25678.
Menon, VP., Sudheer, AR., 2007. Antioxidant and anti-inflammatory properties of curcumin. Adv Exp Med Biol. 595:105-25. https://doi.org/10.1007/978-0-387-46401-5_3.
Million, M., Lagier, JC., Gautret, P., Colson, P., Fournier, PE., et al. 2020. Early treatment of COVID-19 patients with hydroxychloroquine and azithromycin: A retrospective analysis of 1061 cases in Marseille, France. Travel Med Infect Dis. 35:101738. https://doi.org/10.1016/j.tmaid.2020.101738.
Mittal, R., Gupta, RL., 2000. In vitro antioxidant activity of piperine. Methods Find Exp Clin Pharmacol. 22(5):271-4. https://doi.org/10.1358/mf.2000.22.5.796644.
Miyashita, H., Mikami, T., Chopra, N., Yamada, T., Chernyavsky, S., Rizk, D., Cruz, C., 2020. Do patients with cancer have a poorer prognosis of COVID-19? An experience in New York City. Ann Oncol. 31(8):1088-1089. https://doi.org/10.1016/j.annonc.2020.04.006.
Monteil, V., Kwon, H., Prado, P., Hagelkrüys, A., Wimmer, RA., et al. 2020. Inhibition of SARS-CoV-2 Infections in Engineered Human Tissues Using Clinical-Grade Soluble Human ACE2. Cell. 181(4):905-913.e7. https://doi.org/10.1016/j.cell.2020.04.004.
Mounce, BC., Cesaro, T., Carrau, L., Vallet, T., Vignuzzi, M., 2017. Curcumin inhibits Zika and chikungunya virus infection by inhibiting cell binding. Antiviral Res. 142: 148-157. https://doi.org/10.1016/j.antiviral.2017.03.014.
Mrityunjaya, M., Pavithra, V., Neelam, R., Janhavi, P., Halami, PM., Ravindra, PV., 2020. Immune-Boosting, Antioxidant and Anti-inflammatory Food Supplements Targeting Pathogenesis of COVID-19. Front Immunol. 11:570122. https://doi.org/10.3389/fimmu.2020.570122.
Obata, K., Kojima, T., Masaki, T., Okabayashi, T., Yokota, S., et al. 2013. Curcumin prevents replication of respiratory syncytial virus and the epithelial responses to it in human nasal epithelial cells. PLoS One. 8(9):e70225. https://doi.org/10.1371/journal.pone.0070225.
Pradeep, CR., Kuttan, G., 2004. Piperine is a potent inhibitor of nuclear factor-kappaB (NF-kappaB), c-Fos, CREB, ATF-2 and proinflammatory cytokine gene expression in B16F-10 melanoma cells. Int Immunopharmacol, 4: 1795-1803. https://doi.org/10.1016/j.intimp.2004.08.005.
Prasad, S., Tyagi, AK., 2014. Aggarwal BB. Recent developments in delivery, bioavailability, absorption and metabolism of curcumin: the golden pigment from golden spice. Cancer Res Treat. 46(1):2-18. https://doi.org/10.4143/crt.2014.46.1.2.
Reuter, S., Charlet, J., Juncker, T., Teiten, MH., Dicato, M., et al. 2009. Effect of curcumin on nuclear factor kappaB signaling pathways in human chronic myelogenous K562 leukemia cells. Ann N Y Acad Sci, 1171: 436-447. https://doi.org/10.1111/j.1749-6632.2009.04731.x.
Roshdy, WH., Rashed, HA., Kandeil, A., Mostafa, A., Moatasim, Y., et al. 2020. EGYVIR: An immunomodulatory herbal extract with potent antiviral activity against SARS-CoV-2. PLoS One. 15(11):e0241739. https://doi.org/10.1371/journal.pone.0241739.
Siddique, HR., Saleem, M., 2011. Beneficial health effects of lupeol triterpene: a review of preclinical studies. Life Sci. 88(7-8):285-93. https://doi.org/10.1016/j.lfs.2010.11.020.
Soleimani, V., Sahebkar, A., Hosseinzadeh, H., 2018. Turmeric (Curcuma longa) and its major constituent (curcumin) as nontoxic and safe substances: Review. Phytother Res. 32(6):985-995. https://doi.org/10.1002/ptr.6054.
Soni, VK., Mehta, A., Ratre, YK., Tiwari, AK., Amit, A., et al. 2020. Curcumin, a traditional spice component, can hold the promise against COVID-19? Eur J Pharmacol. 886:173551. https://doi.org/10.1016/j.ejphar.2020.173551.
Vaibhav, K., Shrivastava, P., Javed, H., Khan, A., Ahmed, ME., et al. 2012. Piperine suppresses cerebral ischemia-reperfusion-induced inflammation through the repression of COX-2, NOS-2, and NF-κB in middle cerebral artery occlusion rat model. Mol Cell Biochem. 367(1-2):73-84. https://doi.org/10.1007/s11010-012-1321-z.
Vardhana, SA., Wolchok, JD., 2020. The many faces of the anti-COVID immune response. J Exp Med. 217(6):e20200678. https://doi.org/10.1084/jem.20200678.
Yang, M., Lee, G., Si, J., Lee, SJ., You, HJ., Ko, G., 2016. Curcumin Shows Antiviral Properties against Norovirus. Molecules. 21(10):1401. https://doi.org/10.3390/molecules21101401.
Zahedipour, F., Hosseini, SA., Sathyapalan, T., Majeed, M., Jamialahmadi, T., et al. 2020. Potential effects of curcumin in the treatment of COVID-19 infection. Phytother Res. 34(11):2911-2920. https://doi.org/10.1002/ptr.6738.
Zhao, L., Feng, C., Hou, C., Hu, L., Wang, Q., Wu, Y., 2015. First discovery of acetone extract from cottonseed oil sludge as a novel antiviral agent against plant viruses. PLoS One. 10(2):e0117496. https://doi.org/10.1371/journal.pone.0117496.
Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., et al. 2020. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 395(10229):1054-1062. https://doi.org/10.1016/S0140-6736(20)30566-3.
Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., et al. 2020. China Novel Coronavirus Investigating and Research Team. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 382(8):727-733. https://doi.org/10.1056/NEJMoa2001017.
Zhuang, MW., Cheng, Y., Zhang, J., Jiang, XM., Wang, L., Deng, J., Wang, PH., 2020. Increasing host cellular receptor-angiotensin-converting enzyme 2 expression by coronavirus may facilitate 2019-nCoV (or SARS-CoV-2) infection. J Med Virol. 92(11):2693-2701. https://doi.org/10.1002/jmv.26139.
Zou, X., Chen, K., Zou, J., Han, P., Hao, J., Han, Z., 2020. Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. Front Med. 14(2):185-192. https://doi.org/10.1007/s11684-020-0754-0.

Supp. 1: The wight of different body organs in relation to the body weight after administration of Egyvir

organ	sex	Oral route				Inhalation route			
		control	Low dose	Moderate dose	High dose	control	Low dose	Moderate dose	High dose
		Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
Heart	Male	0.49	0.34	0.48	0.45	0.38	0.47	0.41	0.38
	female	0.43	0.45	0.43	0.43	0.46	0.43	0.45	0.41
Lung	Male	0.65	0.57	0.58	0.66	0.62	0.58	0.71	0.86
	female	0.69	0.78	0.62	0.81	0.69	0.91	1.09	1.19
Liver	Male	5.11	3.60	3.41	3.62	5.21	3.63	4.07	3.60
	female	4.82	4.17	3.58	4.85	4.81	5.37	5.98	5.65
Kidney	Male	0.8	0.71	0.84	0.91	0.96	0.82	0.93	0.97
	female	0.95	1.39	0.93	1.42	0.99	1.46	1.43	1.24
Brain	Male	1.13	0.8	0.92	0.70	1.12	0.63	0.83	0.84
	female	1.04	1.23	1.02	1.19	1.04	1.36	1.27	1.21
Spleen	Male	0.66	0.46	0.5	0.62	0.62	0.30	0.40	0.41
	female	0.57	0.37	0.44	0.74	0.61	0.74	0.67	0.64
Thymus	Male	0.26	0.22	0.2	0.2	0.18	0.19	0.18	0.22
	female	0.27	0.25	0.21	0.31	0.22	0.31	0.27	0.27
Pancreas	Male	0.20	0.29	0.41	0.15	0.29	0.29	0.30	0.21
	female	0.25	0.37	0.32	0.44	0.33	0.45	0.48	0.4
Supra-renal	Male	0.04	0.04	0.03	0.04	0.03	0.04	0.04	0.04
	female	0.04	0.04	0.04	0.06	0.04	0.06	0.06	0.05
Salivary glands	Male	0.23	0.27	0.24	0.23	0.28	0.27	0.27	0.26
	female	0.38	0.26	0.22	0.36	0.40	0.42	0.39	0.4
Ovary	female	0.18	0.11	0.11	0.15	0.17	0.17	0.13	0.15
Uterus	female	0.23	0.11	0.10	0.14	0.23	0.16	0.14	0.17
Testes	Male	1.62	1.42	1.22	0.79	1.38	1.52	1.56	1.53
Epididymis	Male	0.37	0.34	0.3	0.34	0.27	0.41	0.49	0.41
Vas def.	Male	0.07	0.07	0.06	0.05	0.08	0.07	0.1	0.09
Acss.gl.	Male	0.29	0.21	0.29	0.24	0.26	0.23	0.2	0.26