BIOCHEMICAL AND IMMUNOLOGICAL RESPONSES TO USE DEXAMETHASONE IN RATS

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Abstract
This study was conducted to determine the side effects of dexamethasone n some physiological and immunological criteria, which included, Measuring sugar level, liver (ALT, AST, LDH, Total protein) and kidney function (urea, createnine), total and differential count of W.B.C,CRP, TNF-α ,IL6,IL10. thirty rats were divided into two groups (15\ rat). G1: This group was considered negative control injection with normal saline, G2: This group was injected with 2 mg\kg dexamethasone. The results showed a significant increase in the level of enzymesALT, AST and sugar levels and decreased level of total protein in the group treated with the drug, also showed, a significant decrease in the total number of W.B.C and a significant decreased in the level CRP, TNF-α, IL-6 and IL-10 in treated group as compared with control .
Keywords: Dexamethasone, Fasting blood glucose, Monocyte.

INTRODUCTION

Corona disease is one of the serious respiratory diseases caused by the Covid-19 virus. It was first discovered in December 2019 in Wuhan Province, China. [1], It spread very quickly to many parts of the world and turned into a dangerous pandemic, leading to infection50,446,517 individuals and death 1,256,869 people as of November 9th, 2020 [2].

The Corona virus is of a single-stranded type and consists of two secondary subunits, the first of which is responsible for attaching the virus to cell receptors and the second of which is responsible of fusion the virus with the cell'smembrane[3] .

The genome of this RNA virus is the biggest of all known RNA viruses, spanning from 26 to 32 kb [4]. The Covid virus, like other viruses, has the ability to constantly change through many random mutations, and it also has the ability to improve or decrease virulence. . Furthermore, mutations can improve the virus's ability to alter adaptive immune responses resulting from previous infection or vaccination

, thereby increasing the risk of reinfection or lowering medication or vaccine efficacy [5]. Some CoV-2 variations have already been shown to impair sensitivity to plasma from previously infected or immunized patients, as well as the ability to select appropriate treatment [6]

The coronavirus takes 3–7 days to incubate, although it can take up to 14 days in certain situations[7]. Fever, dry cough, and anosmia are the most typical symptoms of infection, raised white cell count and elevated CRP are the primary laboratory results [8]. As a result, an effective treatment. There is an urgent need not only to treat those infected or showing symptoms, but also to reduce the duration of transmission of the virus between individuals in order to reduce its spread in the community. Among the potential treatments for COVID-19 old drugs were used because information of safety profile, side effects, posology, and medication interactions is well understood [9,10].The mild condition can be managed at home by informing patients about the danger symptoms, continued hydration, nourishment, fever and cough management are the conventional treatments. Oxygen therapy may be required in hypoxic persons by administering nasal prongs, face mask, high-flow nasal cannula, or non-invasive breathing [11,12].

Furthermore administering anti -CoV-2 medicines or activating ACE2 could be potential therapeutic methods for treating this illness, medications that could improve multi-organ decline in severe or critical patients are urgently needed. corticosteroids are the most typically used in the treatment of a wide range of different inflammatory conditions, as well as autoimmune diseases. The primary justification for using glucocorticoids in clinical practice is that they may be useful in reducing damage to tissues, such as the lungs in coronavirus infection by decreasing cytokine generation [13].The goal of this current work was to estimate the effect of dexamethasone 2 mg\kg on ALT, AST LDH, fasting blood sugar, total protein, total and differential counts of WBC ,the level of CRP, TNF--α, IL-6 and IL-10 in rats.

MATERIALS AND METHODS

1. Experimental design

Thirty healthy male rats weighing 280±10 grams divided into two groups;G1: This healthy group was considered negative control was injected with normal saline.G2: This group was injected with 2 mg\kg dexamethasone.

2. Biochemical analysis

The rats were fasted overnight for blood collection than, the serum was separated immediately (3000 r.p.m\15 min) and store at 4 C for further biochemical test, the level of ALT, AST, LDH were estimated by using kit provided from (Randox\British), blood glucose was measured by kit provided from (Biolab Company Germany), total protein was done by the colorimetric

test \Biuret method by using human total (proteinliquicolor\Germany) WBC were calculated by automated digital counter machine. . The values of CRP,IL-6 and IL-10 were estimated by using (BioSystem S,A.Costa Brava,30.08030 Barcelona\Spain) and TNF-α value was evaluated by using(TNF-α ELISA Kit , Elabscience,China).

RESULTS

The results obtained a significant increase in ALT and AST activity in group treated with 2 mg\kg of dexamethasone (40.33 ±1.20, 45.67 ±1.20)I U/L respectively compared to control (25.26 ±1.8, 26.00 ±1.3 2) U/L, LDH activity show significant decrease in group treated with 2 mg\kg of dexamethasone (144.05 ±3.67) compared to control (144.05 ±3.67) U/L. results were relieved significant increased in fasting blood glucose in dexamethasone group (128.43±5.35) mg\dl compared to control(85.43±3.65) mg\dl,also the result show no significant decreased in the level of total protein in group treated with 2 mg\kg of dexamethasone (4.26±0.86)g\dl compared to control(5.94±1.57)g\dl. .

Table1: The effect of dexamethasone on ALT, AST , LDH, fasting blood glucose and total protein in rats

Group	Mean ± SE				
	ALT (U/L)	**AST (U/L)**	**LDH (U/L)**	**Fasting glucose mg\dl**	**Total protein g\dl**
Control	25.26 ±1.8 b	26.00 ±1.3 2 a	164.32±8.42 a	85.43±3.65 b	5.94±1.57 a
Dexamethasone 2 mg\kg	40.33 ±1.20 a	45.67 ±1.20 b	144.05 ±3.67 b	128.43±5.35 a	4.26±0.86 a

The values of total WBC show high significant decreased in groups treated with 2 mg\kg of dexamethasone (5.63±1.2) cells X 10^3 compared with control (3.06 ±1.67) cells X 10^3 , results obtained significant increase in neutrophil in treated group (60.50 ±5.32) % and decreased in Lymphocyte (33.11 ±3.65)%Compared to control (50.42 ±4.60)(42.26 ±3.31) %respectively , The percentage of,Monocyte, Basophil, Eosinophil obtained no significant change in dexamethasone group compared to control.

Table 2: The effect of dexamethasone on total and differential count of WBC in rats

Groups	**Mean ± SE**					
	WBC Cellx10^3	**Neutr.%**	**Lymph.%**	**Mono.%**	**Baso. %**	**Eosino%**
Control	5.63±1.2 a	50.42 ±4.60 b	42.26 ±3.31 a	2.39 ±0.37 a	1.93 ±0.35 a	2.43 ±1.43 a
Dexamethasone 2 mg\kg	3.06 ±1.67 b	60.50 ±5.32 a	33.11 ±3.65 b	2.96 ±0.73 a	1.53±0.07 a	2.15 ±0.14 a

The results in Table No. 3 showed a significant decrease in the level of CRP level in dexamethasone group (4.32±0.93) compared with control (6.8 0 ±1.73), TNF-α significantly decreased in dexamethasone group (48.32±9.10) pg/ml as compared with control (60.8.65±0.54) pg/ml, the results of IL-6 ,IL10 were significant decreased in dexamethasone (5.32±0.75, 70.26±2.98) pg/ml respectively as compared with control group(8.32±1.640, 94.44±5.57) respectively,

Table3 : The effect of dexamethasone on CRP, TNF-α, IL-6 and IL-10 in rats

Group	**Mean ± SE**			
	CRP mg\L	**TNF-α (pg/ml)**	**IL-6 pg\ml**	**IL-10 pg\ml**
Control	6.8 0 ±1.73 a	60.8.65±0.54 a	8.32±1.64 a	94.44±5.57 a
Dexamethasone 2 mg\kg	4.32±0.93 b	48.32±9.10 b	5.32±0.75 b	70.26±2.98 b
Differences small letters are significant (P<0.05) as compression between columns				

DISCUSSION

The current data indicated that , there was a significant increase in the level of ALT, AST, fasting blood glucose and significant increase in the level of LDH and total protein in groups treated with 6 mg\kg of dexamethasone as compared with control (table 1). SARS-CoV-2 infection could affect liver function as a result of excessive production of inflammatory cells, this is especially important in the treatment of COVID-19 since the use of medications with a strong immunosuppressive impact may raise the risk of severe viral reactivation, as a result, it's a good idea to check the serology of all COVID-19 patients according to the state of infection, the immunosuppressant drugs used in the

treatment of COVID-19 also may contribute to evaluate liver enzyme [14]. COVID-19-positive critically sick individuals may show signs of liver dysfunction [15] , as well as patients with COVID-19 and cirrhosis are more likely to suffer acute or chronic liver failure (ACLF) [16].After infection, the immune system can be rapid, and lead to acute complication in different organ , intravascular coagulation , respiratory distress ,multi-organ failure and death. The overproduction of early-response inflammatory mediators such as IL-6 and IL-10 may be a sign of cytokine storm condition that includes excessive or unregulated of cytokine synthesis and secretion resulting in endothelial damage in many organs including the liver. Angiotensin-converting enzyme 2 (ACE2), is a major enzyme that contribute in the internalization of COVID-19 infection .ACE2 is expressed in pancreas, leading to insulin resistance or decrease insulin secretion [17,18]. In addition, infection with SARS-CoV-2 can produce hyperglycemia in patients who do not have diabetes. This observation, together with the presence of ACE2 in the pancreas, suggests that coronaviruses may harm islets selectively, perhaps leading to hyperglycemia [19]

Glucocorticoids suppress the growth and differentiation of all WBC subtypes as well as the quantity of macrophages, eosinophils, and basophil granulocytes (table 2) [20].Glucocorticoids increase the number of neutrophils produced from the bone marrow and the amount of IL-10 generated by dendritic cells [21], stimulate the synthesis of anti-inflammatory protein as well as decrease the production of pro-inflammatory proteins, they also diminish the membrane expression of MHC class II and suppress antigen presentation to T cells. Glucocorticoids bind to the glucocorticoid receptor (GR) located in the cytoplasm of almost cells, after binding with glucocorticoids, the GR dissociates from immunophilin and heatshock proteins 70 which affect the cell immune response and produce anti-inflammatory effect by releasing of anti-inflammatory protein . Corticosteroids that used in the treatment of coronavirus complication such as acute respiratory infection, septic shock, inflammatory cytokine storms such as IL-6, IL-8 and IL-10, lungs inflammation, are caused excessive decreasing in all types of WBC counts [22,23]

According to the results presented in table 3 ,there was a significant decrease in the level of CRP, TNF-α, IL-6 and IL-10 in group treated with 6 mg\kg of dexamethasone as compared with control ,this might be due to that dexamethasone has a strong anti-inflammatory and immunosuppressive properties and is commonly used as an adjunctive treatment for different viral pneumonia. It has a 25-fold higher activity and longer duration of action than other corticosteroid drug such as ibuprofen, and its similar to that of the substances produced naturally by the body to modulate viral infection[24,25,26],as a result of COVID-19 infection, the airway macrophages release IL-6, IL-8, IL-10, and TNF-α and all of them are represent maladaptive forms of immune response to infection, the cytokines storm lead to lungs destruction and affect several body system. Corticosteroids can be used to reduce the cytokine storm because of their ability to decrease the gene transcription of pro-inflammatory cytokines, inhibit cytokine generation and inhibit cytokine destructive effect [27,28,29].The level of IL-6 is the primary signal for the production of CRP and TNF-α in patient with COVID-19 and pneumonia, this may be related to that after COVID-19 infection the IL-6 attract monocytes and inflammatory CD14+/CD16+ monocytes that express high levels of IL-6,these cells play an important role in cytokine storm which leads to further lung damage[30,31]. The current study investigated the beneficial and positive effect of dexamethasone(6 mg\kg) in the treatment of COVID-19 infection.

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