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Effect of Vitamin D on Dexamethasone Induced Metabolic Disturbance And Gastric Ulcer

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ABSTRACT

Glucocorticoids are indispensible in medicine but, they have many side effects. Vitamin D is an antioxidant prohormone known by several functions. To detect the protective effect of vitamin D against dexamethasone induced metabolic disturbance and gastric ulceration, 24 adult rats were allocated into 4 groups: group1 (control), group 2 given (vitamin D), group 3 given (Dexamethasone) and group 4 given (vitamin D with dexamethasone). We measured body weight, fasting serum glucose, insulin, Homoeostasis model assessment of both insulin resistance (HOMA-IR), B cell function (HOMA-B) and glucose uptake in muscle. Serum triglycerides (TGs), total cholesterol (TC), low and high density lipoprotein (LDL, HDL) were measured. Gastrocnemius muscle weight and protein content were assessed. Ulcer index (UI), gastric superoxide dismutase (SOD), Glutathione (GSH) and malondialdehyde (MDA) and histopathological examination of stomachs was performed. Dexamethasone caused significant decrease of body weight, significant increase of serum glucose, insulin, HOMA-IR, significant decrease of HOMA-B and glucose uptake. It caused significant increase of serum TG, TC, LDL and significant decrease of HDL. It caused significant decrease of muscle weight and protein content. It caused significant elevation of UI, gastric oxidative stress and mucosal ulcers in histopathology. Giving vitamin D with dexamethasone minimized these effects.

Key words:

Dexamethasone, oxidative stress, vitamin D, insulin resistance, dyslipidemia, gastric ulcer.

Introduction:

Glucocorticoids (GCs) are hormones that can be synthesized and naturally produced in our body from adrenal cortex. They produce many important physiological and biochemical effects on various body tissues. Generally, they regulate metabolism, immunity and inflammatory responses (**1**). Glucocorticoids are used in various diseases including autoimmune diseases, asthma, hematological, allergic and other systemic diseases**(2).** Nowadays, glucocorticoids specially dexamethasone is used in treatment of corona virus infection and was found to decrease corona related mortality rate in intensive care unit patients due to its strong anti-inflammatory properties (**3)**.

In spite of the wide use of glucocorticoids in medicine, they have many side effects which range from cosmetic problems as teleangiectasia to disabling and life-threatening disorders as bleeding peptic ulcer depending on the duration and dose of treatment. Side effects include glaucoma, osteoporosis, Immunosuppression, cardiac problems and cognitive impairment (**4)**.

Metabolic disorders are considered from the most common side effects of glucocorticoids as they cause insulin resistance, diabetes mellitus (DM), dyslipidemia and skeletal muscle atrophy. They can also predispose to gastric mucosal injury and ulceration (**5**). Most of these side effects are due to generation of reactive oxygen species (ROS). Amelioration of GCs side effects with maintaining their wide therapeutic uses will be very useful in the medical field (**6)**.

Vitamin D was known as a vitamin in the 20th century but nowadays it is considered as a prohormone. Vitamin D has two forms, vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol) and. Vitamin D_3 is

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synthesized in the skin from exposure to ultraviolet (UV) rays and is also found in the diet as fish oils, whereas vitamin D_2 is derived mainly from plant origin (**7)**.

Vitamin D was known by its important action in plasma calcium and phosphate homeostasis (**8**). Nowadays, vitamin D has been found to do many other actions as it has strong antioxidant activity. It has many extra skeletal functions as neuroprotection, immunoregulation, antimicrobial defense, anti-inflammatory, and cardiovascular benefits (**9)**. It was proved also to help in the management of corona virus due to its immunomoudulatory effects by enhancing innate immunity (**10)**.

According to the fore mentioned information, we aimedat this work to clarify the beneficial effects of vitamin d on prevention of metabolic disturbance and gastric ulceration induced by dexamethasoneand the possible mechanisms for such effects.

Materials and Methods:

Animals and maintenance:

Twenty four adult male Wistar Albino rats weighing about 200-250gmwere included.The animals were housed six per cage (75x50x25cm) with free access to compact food and water.Animals were kept under suitable laboratory conditions (e.g. humidity, and illumination of 12-h cycles of light and darkness). All experimental protocols described in the present study were approved by the Ethics Review Committee for Animal Experimentation of the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals of Beni-Suef University with approval number (**019_72**) at 13 October 2019.

Chemicals:

Dexamethasone was purchased from Amriyacompany (Cairo, Egypt). 1,25dihydroxycholecalciferol was purchased from Sigma, USA. Glucose colorimetric assay kit was obtained from BioMed Pharmaceutical Industry (Cairo, Egypt). Insulin ELISA kit was purchased from Biosource (San Diego, USA). Total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), SOD, GSH and MDA colorimetric kits were purchased from Bio-Diagnostic (Cairo, Egypt). Bradford protein assay kit was obtained from Biotek instrument (Vermont, United States).

Drugs Administration:

Dexamethasone was purchased from Amriya company in the form of liquid ampoules with concentration of 8ml/2ml. It was injected intraperitoneally as 1ml/Kg/day to groups 3&4and was given daily. Animals were weighed weekly to adjust the dose for each animal.

1,25dihydroxycholecalciferol was purchased from Sigma, USA in the form of powder and was dissolved inparaffin oil. The concentration of vitamin D was arranged as 0.5 µg /ml. It was injected intraperitoneally as 1.25 µg /kg /day to groups 2&4 and was given daily. Animals were weighed weekly to adjust the dose for each animal.

Experimental design:

24 rats were included and were divided into 4 groups (6 rats for each one)**:**

1- **Group 1**: (the control group) which was given distilled water for 2 weeks.
2- **Group 2**: (1,25dihydroxycholecalciferol (Vit D) group) which was given 1,25dihydroxycholecalciferol only as 1.25 µg /kg /dayintraperitoneally(i.p) for 2 weeks (**11, 12**).

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3- **Group 3**: (Dexamethasone (Dex) group) which was given dexamethasone as 1mg/kg/day intraperitoneally (IP) for 2 weeks (**13, 14**).
4- **Group 4**: (Dexamethasone + 1,25dihydroxycholecalciferol (Dex+vit D) group) which was given dexamethasone as 1 mg/kg/day (**13, 14**) combined with 1,25-(OH)2D3 (1.25 µg /kg /day) i.p for 2 weeks (**11, 12**).

After the last dose, rats were fasted for 12 hours and samples were taken for biochemical and histopathological analysis.

Sample preparation:

For biochemical analysis,Blood samples were taken from retro orbital plexus and left to be clotted then centrifuged at 8000rpm for 20 minutes and serum was separated and kept frozen at -80°C till analysis of serum glucose, insulin and lipid profile. Gastrocnemius muscle was excised from both legs and weighed. One of them was homogenized in phosphate buffer saline then centrifuged at 10000 rpm for 20 min then supernatant was kept in -80°C and was used for measurement of total protein. The other was put in ringer's solution with added glucose by concentration of 100mg glucose/ ml solution and incubated at 37°C for 2 hours for measurement for glucose uptake.

Stomachs were dissected, washed with saline and gastric tissues were pinned out flat on a cork board and photographed for lesion assessment with the aid of a magnifier. Small part of it then was excised and was homogenized in phosphate buffer saline then centrifuged at 10000 rpm for 20 min then supernatant was kept in -80°C and was used for measurement of oxidative stress (SOD, MDA, GSH). The other part was kept in formaline 10% for histopathological examination.

Measurements:

1. **Carbohydrate metabolism (fasting serum glucose, insulin, HOMA-IR, HOMA-B and glucose uptale in gastrocnemius muscle):**

- Glucose was measured bycolorimetric assay kit from BioMed Pharmaceutical Industry (Cairo, Egypt) in mmol/L as performed before**(15).**
- Serum insulinwas assessed by ELIZA kits from Biosource (San Diego, USA) in MIu/ml as done before **(16).**
- HOMA-IR, HOMA-B were calculated by the HOMA method according to (**17**).

(HOMA-IR) = (fasting glucose (mmol/l) x fasting insulin (MIu/ml))/22.5

(HOMA-B) = (20 x fasting insulin (MIu/ml))/(fasting glucose (mmol/l) – 3.5)

- Glucose was measured as performed before **(15)**in Ringer's solution which was purchased from AlmotahedoonPharma company (Cairo, Egypt) before and after putting the muscle,then Glucose uptake in muscle was measured in mg/dlas

Glucose uptake in muscle=

Glucose level in fluid before putting the muscle - Glucose level in fluid after incubationof muscle

2. **Lipid metabolism (Serum TG, TC, HDL and LDL):**

- TG, TC, HDL and LDL were measured by colorimetric assay kits from Bio-Diagnostic (Cairo, Egypt) in mg/dl as performed before**(18, 19, 20**).
- LDL was calculatedbyFriedewald equation**(21)**

LDL = total cholesterol - HDL - (TG/5)

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3. **Protein metabolism:**

- Total protein in gastrocnemius musclewas detected by Bradford protein assay kit from Biotek instrument (Vermont, United States) in mg/g.tissue as done before **(22**).

4. **Measurements for gastric ulcer (UI, SOD, GSH, MDA and histopathological examination):**

- For determining ulcer index, gastric tissues were pinned out flat on a cork board and photographed for lesion assessment with the aid of a magnifier, ulcer score was determined by the severity factor which was calculated as performed before (**23)**.
- Preventive index was calculated according to (**24)**

$$\frac{\text{UI of diseased group-UI of pretreated group}}{\text{UI of diseased group}} \text{x } 100$$

- For assessing gastric oxidative stress, SOD in U/gm tissue, GSH in mg/g.tissue and MDA in nmol / g.tissue were determined by colorimetric assay kits from Bio-Diagnostic (Cairo, Egypt) as evaluated before**(25, 26, 27).**
- After macroscopic examination, stomachs were placed in formaldehyde solution 10 % for 24 hours and histopathological examination of stomach**s** was performedwith the routine hematoxylin and eosin stain technique **(28)**.

Statistical analysis:

Data was presentedas**mean ±**standard deviation for quantitative variables. Comparisons between groupsusing analysis of variance (ANOVA) with multiple comparisons post hoc test. Comparisons between basal and final weight in each group were done using paired t test **(29)**. Correlations between quantitative variables were done using Pearson correlation coefficient**(30)**. P-values less than 0.05 were considered as statistically significant.

Results:

a. Body weight:

As shown in table (1), at the beginning of the experiment, there was no significant difference in body weight among all groups**(P>0.05)**.At the end of the experiment (after 2 weeks of drugs administration), there was no significant difference in body weight between the control and Vit D groups (**254.17±14.29, 263.50±11.90**) respectively **(P>0.05)**, while Dex group showed significant decrease of body weight **170.83±8.01** as compared to control and Vit D groups (**P<0.05**). In Dex + Vit D group, there was also significant decrease of body weight as compared to control and Vit D groups with a mean value (**199.33±7.71**) (**P<0.05**). But there was significant increase of body weight in Dex + Vit D group as compared to Dex group**(P<0.05).**

For a comparison of body weight between each group at the beginning and the end of experiment as shown in table (1), there was significant increase in body weight at the end of experiment as compared to at the beginning in control (**254.17±14.29, 209.179.70**) respectively and Vit D groups (**263.50±11.90, 204.17±6.65)**respectively(**P<0.05**).

On the contrary, in Dex group there was significant decrease of body weight at the end of experiment as compared to the basal body weight of it (**200.00 ± 7.07**, **170.83±8.01**) at the beginning and at the end respectively (**P<0.05**). While in Dex+Vit D group there wasn't

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significant change between baseline body weight **200.00±7.07** and final body weight **199.33±7.71 (P>0.05).**

Table (1) Comparison of body weight among all groups

Body weight	Control group	Vit D group	Dex group	Dex+vit D group
Baseline (gm)	**209.17±9.7**	**204.17±6.65**	**200±7.07**	**200±7.07**
Final (gm)	**254.17±14.29@**	**263.50±11.90@**	**170.83±8.01 *#@**	**199.33±7.71*#$**

Values are presented as mean ±SD

*: statistically significant compared to corresponding value in control group (P<0.05)

#: statistically significant compared to corresponding value in Vit D group (P<0.05)

$: statistically significant compared to corresponding value in Dex group (P<0.05)
@: statistically significant between basal and final body weight of the same group (P<0.05)

b. CHO metabolism (Fasting serum glucose, Fasting serum insulin, Homa IR, Homa B and glucose uptake in muscle):

As shown in table (2),administration of Vit D didn't give significant change in fasting serum glucose or insulin as compared to control. The value of serum glucose was (**4.97±0.28**, **4.75±0.37**) for the control and Vit D groups respectively and the value of serum insulin was (**6.54±0.3**, **6.52±0.31**) for the control and Vit D groups respectively (**P>0.05**).

On the other hand, Administration of dexamethasone caused significant increase of both serum glucose and insulin as compared to control and Vit D groups with mean values (**12.68±0.78 ,15±2.09)** for glucose and insulin respectively (**P<0.05**).

Givingvit D with dexamethasone showed a significant increase of serum glucose and insulin levels(**7.04±0.19** ,**9.33±0.58)** respectively as compared to control and Vit D groups (**P<0.05**). However, this group showed a significant decrease of fasting serum glucose and insulin level as compared to Dex group **(P<0.05)**.

Regarding Homa IR, vitamin D administration showed no significant difference in it as compared to control as its was **1.37±0.16** in Vit D group and **1.4±0.09** in control group (**P>0.05**).

On the other hand, dexamethasone administration resulted in a significant increase in Homa IR with a mean value **8.37±0.78** as compared to control and Vit D groups **(P<0.05)**. In Dex+Vit D group, the mean value of Homa IR was **2.9±0.25**showing that it was still significantly increased as compared to the control and Vit Dgroups. But, it showed a significant decrease as compared to Dex group (**P<0.05**).

Concerning Homa B, giving vitamin D showed no significant difference between Vit D and control groups. It was **111.88±31.58** in Vit D group and **92.62±22.69** in control group (**P>0.05**).

However, dex.administration caused a significant decrease in Homa B **33.07±7.02** as compared to control and Vit d groups **(P<0.05**).Combination of vit D with dexshowed a significant decrease of Homa B which was **52.67±2**as compared to the control and Vit Dgroups (**P<0.05**) but showed a significant increase as compared to Dex group **(P<0.05)**.

There was no significant difference in glucose uptake between vit D group which was **90.5±7.52** and control group which was **85.95±12.38 (P>0.05)**. Dex group showed a

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significant impairment of glucose uptake as compared to control and Vit d groups with a mean value (**45.03±2.71**) (**P<0.05**).

Giving vit D with dex enhanced glucose uptake with a mean value (**75.42±2.53)** as there was no significant difference between it and the control group **(P>0.05),** but it was significantly lower thanVit D group **(P<0.05).** Furthermore, glucose uptake in Dex+Vit D group showed a significant increase as compared to Dex group (**P<0.05)**.

Table (2): Comparison of fasting serum (glucose, insulin), Homa IR, Homa B and glucose uptake in muscle among all groups**.**

	Control group	**Vit D group**	**Dex group**	**Dex+vit D group**
Fasting serum glucose (mmol/L)	**4.97±0.28**	**4.75±0.37**	**12.68±0.78 *#**	**7.04±0.19 *#$**
Fasting serum insulin (MIu/ml)	**6.54±0.3**	**6.52±0.31**	**15±2.09 *#**	**9.33±0.58 *#$**
Homa-IR test	**1.4±0.09**	**1.37±0.16**	**8.37±0.78 *#**	**2.9±0.25 *#$**
Homa-B test	**92.62±22.69**	**111.88±31.58**	**33.07±7.02 *#**	**52.67±2 *#$**
Glucose uptake in muscle(mg/dl)	**85.95±12.38**	**90.5±7.52**	**45.03±2.71*#**	**75.42±2.53 #$**

*: statistically significant compared to corresponding value in control group (P<0.05)

#: statistically significant compared to corresponding value in Vit D group (P<0.05)

$: statistically significant compared to corresponding value in Dex group (P<0.05)

c. Lipid metabolism (Serum TGs, TC, LDL and HDL):

As shown in table (3), there wasno significant difference between Vit D group and control group as regards serum TGs, TC, LDL and HDL. Mean values were (**85.25±9.05,131.7±3.89, 53.95±8.26, 60.67±3.87)** in control group versus **(76.63±5.2**, **131.82±3.64,53.52±7.41, 64.32±4.18)** in Vit D grouprespectively **(P>0.05)**.

On the other hand, giving dex caused significant increase in each of TGs, TC and LDL with mean values (**160.52±4.49, 208.43±10.33, 146.62±14.19**) as compared to control and Vit D groups **(P<0.05**) ,while HDL was significantly decreased in Dex group with a mean value **34.18±3.03** as compared to control and Vit D group (**P<0.05)**.

Dex + vit D group showed a significant increase in TGs, TC and LDL with mean values (**109.67±4.26, 83.03±5.97**, **83.03±5.97)** respectivelyas compared to control and Vit D groups (**P<0.05**). However, a significant decrease in this group was observed as compared to Dex group **(P<0.05**). On the contrary, HDL was still decreased significantly in Dex+Vit D group **49.9±3.3** as compared to control and Vit D group (**P<0.05),** while it was increased significantly when compared to Dex group **(P<0.05**).

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Table (3): Comparison of Serum triglycerides, cholesterol, LDL and HDL among all groups**.**

	Control group	Vit D group	Dex group	Dex+vit D group
Serum Triglycerides (mg/dl)	**85.25±9.05**	**76.63±5.2**	**160.52±4.49 *#**	**109.67±4.26 *#$**
Serum Cholesterol (mg/dl)	**131.7±3.89**	**131.82±3.64**	**208.43±10.33 *#**	**154.92±5.19 *#$**
Serum LDL (mg/dl)	**53.95±8.26**	**53.52±7.41**	**146.62±14.19 *#**	**83.03±5.97 *#$**
Serum HDL (mg/dl)	**60.67±3.87**	**64.32±4.18**	**34.18±3.03 *#**	**49.9±3.3 *#$**

*: statistically significant compared to corresponding value in control group (P<0.05)

#: statistically significant compared to corresponding value in Vit D group (P<0.05)

$: statistically significant compared to corresponding value in Dex group (P<0.05)

d. Protein metabolism (Gastrocnemius muscle weight and total protein):

As shown in table (4), there was no significant difference between the control and Vit D group for both muscle weight **(3.93±0.18, 4.32±0.4)** respectively, and total protein **(87.98±3.56, 92.62±5.68)**respectively **(P>0.05)**.

On the other hand, administration of dexresulted in a significant decrease of muscle weight and total protein **(1.62±0.26, 62.17±2.09)** as compared to control and Vit D group **(P<0.05)**.

In Dex+Vit D there was a significant decrease in muscle weight **2.55±0.22** as compared to control and Vit D **(P<0.05)**, while there was no significant difference in total protein between it **80.83±2.80** and control **(P>0.05)**, but it was still significantly decreased as compared to Vit D group **(P<0.05)**.However, the combination resulted in a significant increase of muscle weight and total protein in that group as compared to Dex group (**P<0.05)**.

Table (4): Comparison of gastrocnemius muscle weight and total protein among all groups

	Control group	Vit D group	Dex group	Dex+vit D group
Gastrocnemius muscle weight (gm)	**3.93±0.18**	**4.32±0.4**	**1.62±0.26 *#**	**2.55±0.22 *#$**
Total protein in gastrocnemius muscle(mg/g.tissue)	**87.98±3.56**	**92.62±5.68**	**62.17±2.09 *#**	**80.83±2.80 #$**

*: statistically significant compared to corresponding value in control group (P<0.05)

#: statistically significant compared to corresponding value in Vit D group (P<0.05)

$: statistically significant compared to corresponding value in Dex groupgroup (P<0.05)

e. Results for gastric ulcer:

i. Macroscopic examination, ulcer index and preventive index:

As shown in table (5) andfigure (1), no lesions in control and Vit D groups were found with no significant difference in ulcer index **(P>0.05)**.Administration of dexamethasone resulted in appearance of multiple gastric lesions with ulcer index **6±1.67** which was significantly increased as compared to control and Vit D group **(P<0.05)**. After giving vitamin

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D with dexamethasone, there was still some gastric lesions with ulcer index **1.75±0.61**which was significantly increased as compared to control and Vit D groups **(P<0.05)** but was significantly decreased as compared to Dex group **(P<0.05).** The preventive index which is the percentage of protection from ulcer and determined the effect of vitamin D on dexamethasone induced peptic ulcer was about 70%.

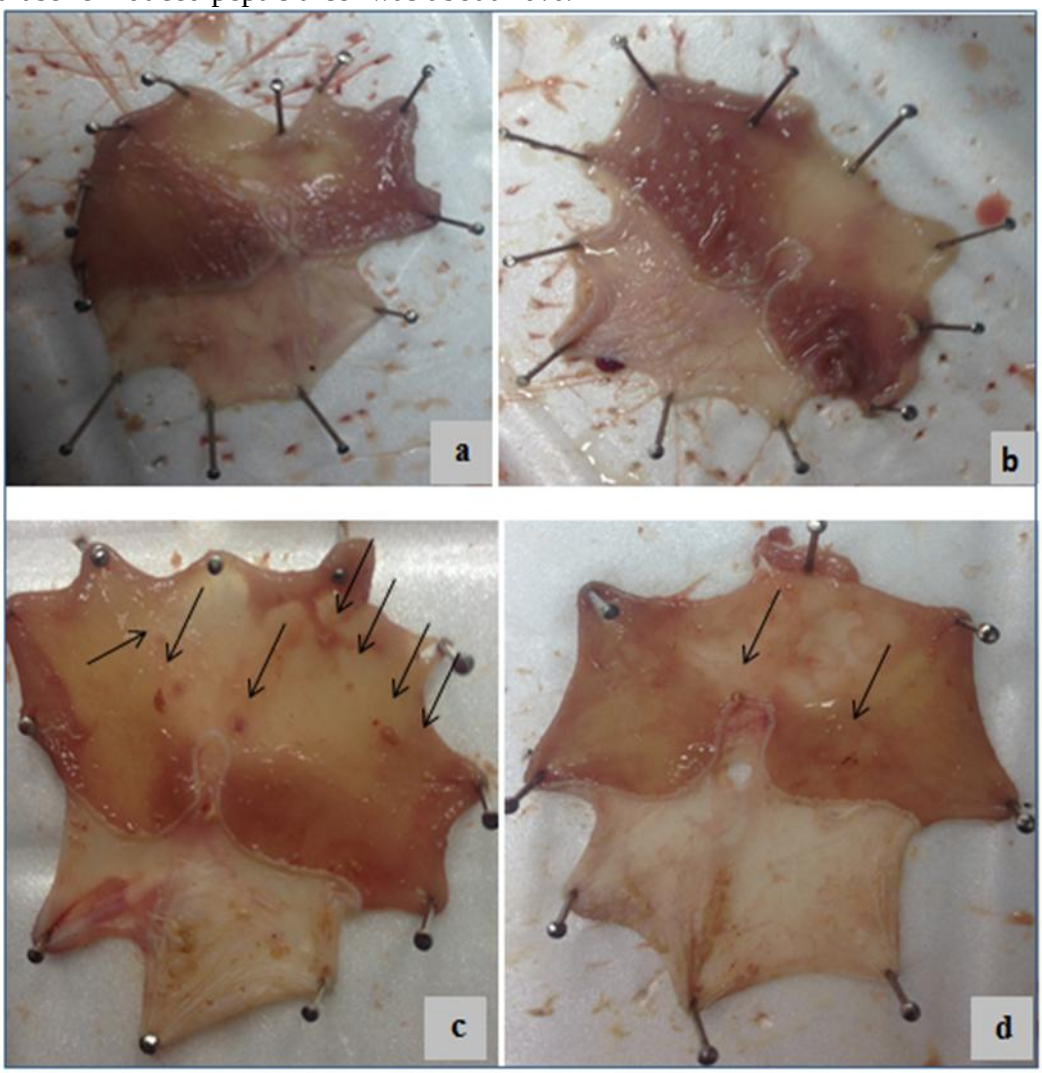

Figure (1): Macroscopic examination of gastric lesionsfor stage 1a) control group with no lesions b) Vit D group with no lesions c) Dex group with multiple lesions d) Dex+Vit D group with some lesions

ii. Oxidant & antioxidant markers in stomach (SOD, GSH, MDA):

As shown in table (5), a significant increase of SOD and GSH in vitamin D group with mean values **(6.94±0.82, 95.28±6.7**) respectively were detected as compared to control group with mean values (**5.65±1.11, 75.2±7.52)** respectively (**P<0.05**), while no significant difference in MDA was found between Vit D group with a mean value **18.08±4.11** and control group with a mean value **22.6±3.91** (**P>0.05**)**.** However, in Dex group the antioxidant markers SOD and GSH were significantly decreased with mean values **(2.17±0.08**, **37.4±2.62**) respectively as compared to control and Vit D groups (**P<0.05**), while MDA was significantly increased with a mean value **95.63±2.5** in Dex group as compared to control and Vit D groups (**P<0.05**).

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No significant difference in the antioxidant markers SOD, GSH between Dex+Vit D groupwith a mean value **(4.42±0.55**, **70.08±3.99)** respectivelyand the control (**P>0.05**), but these markers were significantly decreased in Dex+Vit D group as compared to Vit D groupand significantly increased in Dex+Vit D group as compared to Dex group (**P<0.05).** While MDA was significantly increased in Dex+Vit D group with a mean value **42.82±8.62** as compared to control and Vit D group(**P<0.05**)and significantly decreased as compared to Dex group (**P<0.05**).

Table (5): Comparison of gastric ulcer index, SOD, GSH and MDAamong all groups.

	Control group	Vit D group	Dex group	Dex+vit D group
Ulcer index	**0±0**	**0±0**	**6±1.67 *#**	**1.75±0.61*# $**
SOD in stomach (U/gm tissue)	**5.65±1.11**	**6.94±0.82 ***	**2.17±0.08 *#**	**4.42±0.55 #$**
GSH in stomach(mg/g.tissue)	**75.2±7.52**	**95.28±6.7***	**37.4±2.62 *#**	**70.08±3.99 #$**
MDA in stomach (nmol / g.tissue)	**22.6±3.91**	**18.08±4.11**	**95.63±2.5 *#**	**42.82±8.62 *#$**

*: statistically significant compared to corresponding value in control group (P<0.05)

#: statistically significant compared to corresponding value in Vit D group (P<0.05)

$: statistically significant compared to corresponding value in Dex groupgroup (P<0.05)

Figures (2-4) show a strong negative correlation between Homa IR and both stomach SOD and GSH and a strong positive correlation between Homa IR and gastric MDA.

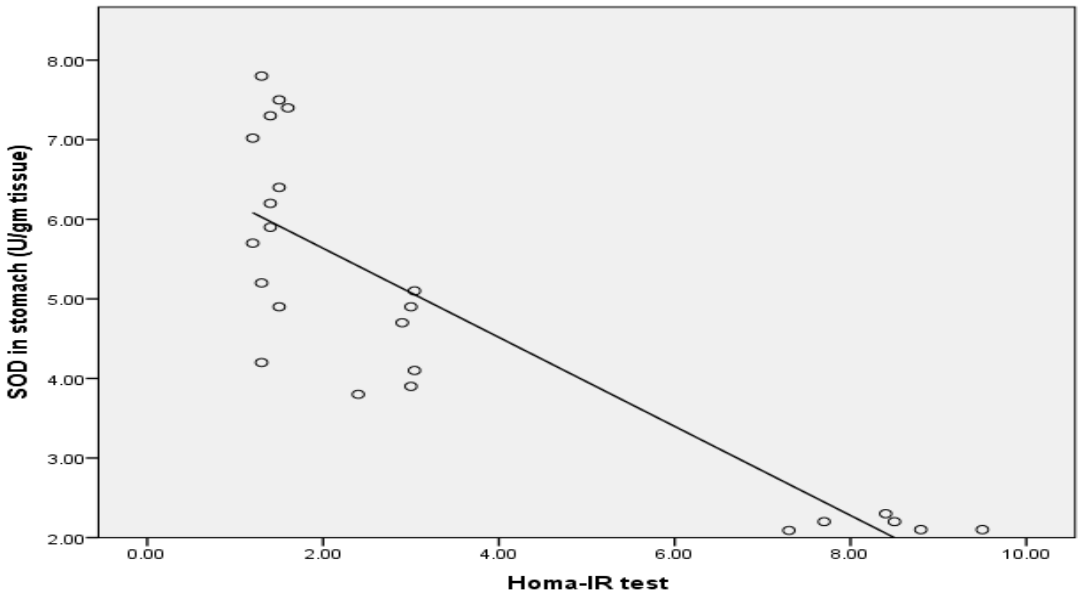

Figure (2): A negative correlation between Homa IR and SOD in stomach

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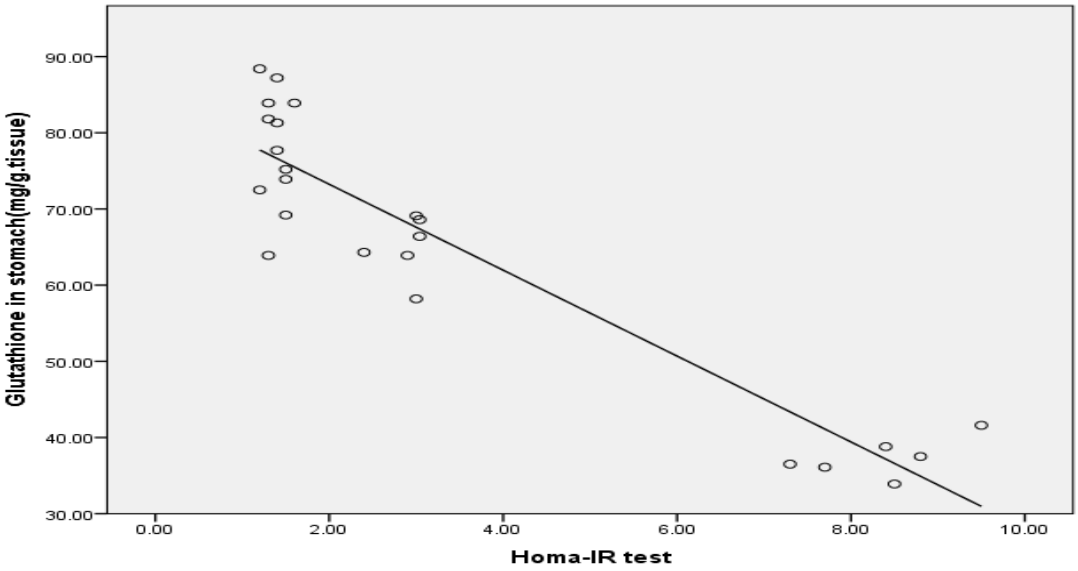

Figure (3): A negative correlation between Homa IR and GSH in stomach

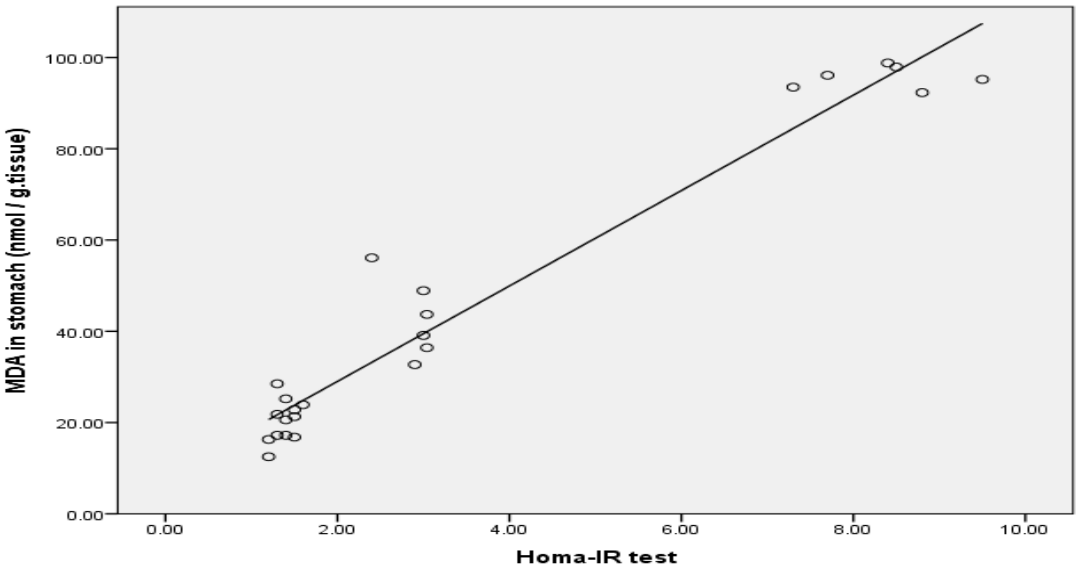

Figure (4): A positive correlation between Homa IR and MDA in stomach

iii. Histopathological examination of stomach:

As observed in figure(5), changes detected by the histological examination of stomach tissues were as the following:

1- **Control group:** Normal gastric mucosa was found as regards glandular architecture and mucin secretion.
2- **Vit D group**: Gastric mucosa showed preserved glandular architecture and mucin secretion.
3- **Dex group**: Focally ulcerated gastric mucosa was detected with granulation tissue formation, the lamina showed congested capillaries.
4- **Dex+Vit D group:** Gastric mucosa showed focal surface erosion, the lamina showed congested capillaries.

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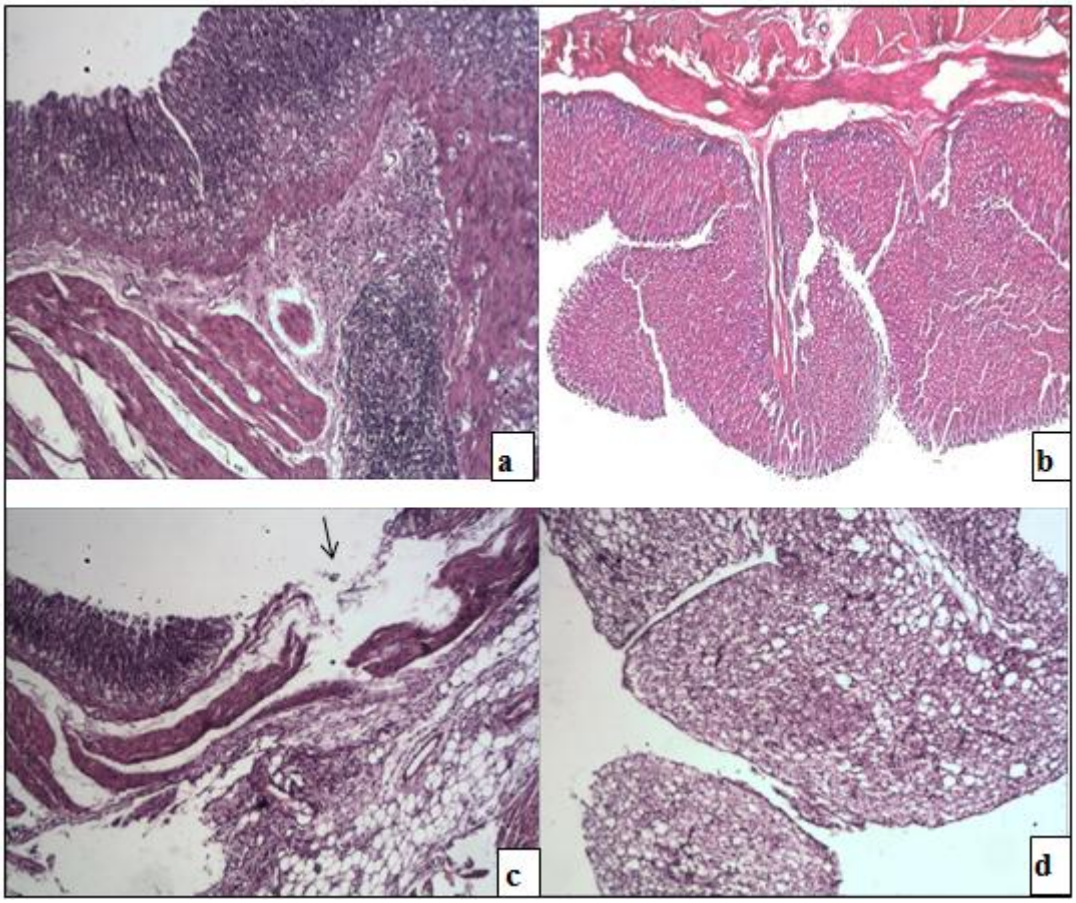

Figure (5): Microscopic examination of gastric tissuefor stage 1. a) Control:normal gastric mucosa b) Vit D group: gastric mucosa with preserved glandular architecture c)Dex group: focally ulcerated gastric mucosa d)Dex+Vit D group: gastric mucosa with focal surface erosion

Discussion:

Glucocorticoids are widely used in almost all medical specialities.However, they have various common side effects including diabetes mellitus (**31)** and peptic ulcer **(32**).Vitamin D is a prohormone, which has long been known in maintaining Ca^{++} homeostasis, but nowadays it has been discovered to have many extra-skeletal effects **(33).**

The present work aimed to study the effect of giving vitamin D in combination with dexamethasone in an attempt to prevent dexamethasone side effects as metabolic disturbance and gastric ulcer.

At the beginning of the present study, there was no significant difference among all groups. At the end of the study, there was also no significant change in body weight between vitamin D group and the control which was in accordance with a study conducted by **Al-Solami et al. (34).**

As a comparison of body weight between each group at the beginning and the end of experiment, we found that there was significant increase in body weight of control group at the end of experiment as compared to at the beginning in accordance with **Andrichet al.** who suggested that it is the normal increase of body weight with respect to time in rats (**35**).

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Our results showed that there was significant increase in body weight in vitamin D group after 2 weeks as compared to basal level in agreement with **Merinoet al. (36).** The significant increase of body weight in healthy rats after vitamin D administration may be due to the normal increase in weight with respect to time in healthy rats generally.

Our results showed that there was significant decrease of body weight at the end of the study between dexamethasone and the control group in agreement with **El-Wakfet al. (37)**.As regards comparison between dexamethasone group at the beginning and at the end of the experiment, the present study revealed that administration of dexamethasone for 2 weeks decreased body weight significantly as compared to basal value in agreement with **Hasonaet al. (38).**

Dexamethasone administration reduces body weight by different mechanisms. It may be due to inhibition of growth hormone as reported by **Hasonaet al. (39**), increasing synthesis and release of leptin, decreasing body weight set point as confirmed by (**D'souza et al.(40**), suppression of protein synthesis and stimulation of its degradation (**41)**.

The present study showed that giving vit D with dexled to prevention of dex induced loss of body weight butwith partial effect. This result was in agreement with **Cheung et al. (42)**.In contrast to our results, **Jin et al.** found that administration of vitD reduced body weight significantly in rats. This difference may be due to vitamin D was given to prevent obesity, so it may work with different mechanism (**43**).

As regards comparison between Dex+ Vit D at the beginning and at the end of the experiment, our results showed there was non-significant change of body weight as compared to basal value.

Vitamin D prevented the decrease in body weight by several mechanisms. It increases GH secretion, decreases leptinas suggested by **Espositoet al. (44**) andhelps muscle anabolism**(45)**.

Concerning fasting serum glucose, insulin and HOMA-IR our results revealed that dex induced a significant increase in serum glucose, insulin and HOMA-IR as compared to control in accordance with **El-Sonbaty et al. (46**). Also, dex caused significant decrease of glucose uptake in gastrocnemius muscle as compared to control in agreement with **Moet al. (47).**

Dexamethasone increases glucose level by many mechanisms. It decreases glucose uptake in muscles by keeping glucose transporter GLUT4 away from the plasma membrane **(48**). It also inhibits glycogen synthesis, increases hepatic gluconeogenesis anddecreases β cell sensitivity to glucose by decreasing the level of GLUT2 (**49**).

Insulin receptor (IR)/Insulin receptor substrate (IRS) /Phosphoinositol3 kinase (PI3K)/Akt is an essential axis for insulin-mediated glucose uptake in tissues. Dexamethasone induces insulin resistance by decreasing expression, activity and affinity of IR, decreasing IRS, PI3K and Aktexpression leading to reducing glucose uptake in tissues (**47)**. Also, it inhibits adenosine monophosphate-activated protein kinase (AMPK) expression which inhibits oxidative stress leading to increased insulin resistance and glucose uptake reduction (**50**).

Our results showed that giving vit D with dex caused significant decrease of glucose, insulin and HOMA-IR as compared to dex group in accordance with **Eltablawy et al. (51)**. On the other hand, **Safarpouret al.** reported non-significant change of serum glucose, insulin and HOMA-IR in diabetic patients received VitD for 8 weeks (**52**), this may be due to

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inadequate duration of treatment in humans.Our results also revealed that administration of vitD with dexresulted in a significant increase of glucose uptake in muscle as compared to dex group in accordance with **Manna et al. (53)**.

Vitamin D maintains glucose homeostasis by different mechanisms. It increases glucose uptake in muscles through increasing GLUT4 expression (**54**). Also it increases hepatic glycogen synthesis, inhibits gluconeogenesis and enhances sensitivity of B cells of pancreas to glucose by increasing the expression of GLUT2 (**55)**.

Vitamin D can decrease insulin resistance by increasing IR, IRS-1, PI3K, Akt and also AMPK expression. It also has strong antioxidant activity resulting in increasing glucose uptake in tissues and insulin sensitivity (**56**).

Our results showed that dex administration caused significant decrease of HOMA-B as compared to control in agreement with **Wallensteenet al. (57)**. In contrast to our study, **Taheriet al.** found significant increase of HOMA-B in subjects given dexamethasone as compared to the same individuals before injection (**58**). The different result may be due to the different dose regimen and timing of the test.

Dex causes insulin resistance and impairment of B cell function at the same time as HOMA-B is mainly determined by insulin/glucose ratio. Beta cells cannot secrete enough insulin able to reduce glucose level **(59**). It induces B cell dysfunction due to affection of closure of ATP-dependent K^+ channels (**60**). It also increases oxidative stress, Bax and caspase-3 activity leading to B cell apoptosis (**61**). It increases alpha2-adrenergic receptor expression in B cells of pancreas leading to decreased insulin secretion (**62)**.

Our study revealed that giving vitamin D with dexamethasone resulted in significant increase of HOMA-B as compared to dex group, but it was still decreased when compared to control in agreement with **Yin et al. (63**).

Vitamin D improves B cell function by several mechanisms. It decreases insulin resistance as it is anti-oxidant leading to better insulin action B cell function **(56)**. It regulates ATP production leading to closure of ATP-dependent K channels and it upregulatesCa^{++} channels in pancreatic B cell and so insulin exocytosis (**64)**. It also downregulates alpha2-adrenergic receptor and upregulates B receptors leading to enhancement of insulin secretion (**65)**.Vitamin D also inhibits apoptosis of pancreatic B cells by inhibition of Bax and caspase-3 expression (**66**).

Regarding lipid metabolism, our results showed that dexamethasone administration caused significant increase in serum triglycerides, cholesterol and LDL and significant decrease of serum HDL as compared to control in accordance with **Mohammedet al. (67**). On the other hand, **El-Sonbaty et al.,** found that dexamethasone administration to rats caused significant increase in serum triglycerides, cholesterol and LDL but didn't affect HDL level (**46**). This may be due to shorter duration of treatment used in that study.

Dexamethasone causes dyslipidemia. It increases serum TGs by stimulating hepatic denovolipogenesis via upregulation of both acetyl-CoA carboxylase, fatty acid synthase and reducing r lipoprotein lipase (LPL) level (**68**). Or it may be also due to dexamethasone induced insulin resistance (**49**).

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Dexamethasone induces hypercholesterolemia by increasing of 3-hydroxy-3-methylglutaryl coenzyme Areductase (HMG-CoA reductase), and the decreased activity of lecithin: cholesterol acyltransferase (**49**).

Dexamethasone also increases LDL level in blood by inhibition of catabolism and uptake of LDL. It also down-regulates LDL receptor, and LPL activity leading to elevation of LDL (**69**). On the other hand, dexamethasone decreases HDL concentration and this is may be due to the presence of Cholesterol Ester Transfer Protein (CETP) enzyme in the circulation which transfers TGs from VLDL to HDL. HDL then becomes enriched with TGs which are rapidly hydrolysed, leading to reduction of HDL (**70**).

Our study revealed that giving vitamin D with dexamethasone resulted in significant decrease of serum TGs, cholesterol and LDL and significant increase of serum HDL as compared to dex group but there was still significant difference in these parameters as compared to control. Our results were in agreement with**Hassan et al. (56**). In contrast to our study, **Adedapo and Ogunfowora,**found that administration of vitamin D didn't cause significant change in serum TGs, cholesterol, LDL and HDL as compared to rats with Triton-X induced hyperlipidemia (**71**). The different results may be due to different method of administration and protocol of treatment.

Vitamin D can prevent dyslipidemia. It prevents TGs elevation via inhibition of hepatic denovolipogenesis by down regulation of both acetyl-CoA carboxylase, fatty acid synthase and activation of LPL (**72**).It also reduces insulin resistance leading to decreased TGs (**56).**

Vitamin D can reduce LDL level by increasing the expression of LDL receptorin liver and upregulating LPL (**73**). On the other hand, vitamin d increases HDLthrough reduction of insulin resistance **(56**).

Concerning protein metabolism, our results showed that dexamethasone decreased gastrocnemius muscle weight significantly as compared to control in agreement with **Fappi et al. (74).**Our results also revealed that administration of dexamethasone led to decreased total protein in gastrocnemius muscle in agreement with **Otsuka et al. (75)**.

Dexamethasone decreases skeletal muscle weight and protein content by different mechanisms. It suppresses muscle anabolism by inhibiting IGF-1 **(74**). It increases muscle catabolism by activating the ubiquitin– proteasome system (UPS) (**76**). It induces forkhead transcription factor O (FOXO), myostatingene expression responsible for muscle atrophy and increases apoptosis**(Langendorf et al., 2020**). It also activates gluconeogenesis (**49**).

Our study revealed that giving vitamin D with dexamethasone leads to significant elevation of gastrocnemius muscle weight as compared to dex group but it didn't reach the control level in accordance with **Yang et al. (77)**.Our results showed that giving vitamin D with dexamethasone resulted in significant elevation of total protein in gastrocnemius muscle as compared to dex group, but improvement was complete in accordance with **Dzik and Kaczor (45)**.

Vitamin D can prevent decreased muscle weight and total protein by several mechanisms. It increases muscle anabolism through activating IGF-1 (**44**). It also inhibits muscle catabolism by suppression of UPS (**45**). It suppresses FOXO, myostatin gene expression preventing protein catabolism and reduces muscle apoptosis (**78**). It also inhibits gluconeogenic enzymes (**55)**.

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Regarding gastric ulcer, our results revealed that dexamethasone caused significant elevation of ulcer index as compared to control in accordance with **Solimanet al. (79)**.

The present study showed that giving vit D with dexamethasone resulted in significant decrease of ulcer index as compared to dex group but it was still elevated as compared to controlin accordance with **Sahinet al. (80)**.

Our results showed that giving dexamethasone resulted in significant decrease of SOD, GSH and increase in MDA in gastric tissue of rats as compared to control in agreement with**Solimanet al. (79).**

In contrast to our result, **Rizk et al. (81)** reported that dexamethasone caused significant elevation of SOD in gastric tissue of rats. The different result was explained by the duration of dexamethasone administration as it was given only for 7 days and SOD may be increased initially as compensation then decreased in longer duration.

Dexamethasone was reported to increase oxidative stress by decreasing the SOD activity **(46)**.It can reduce GSH level by reduction of gamma- glutamylcysteinesynthetase (gamma-GCS) activity (**82**).It increases free radical production leading to increased lipid peroxidation and so increasing MDA level (**83**).

Our study showed that administration of vitamin D only to rats caused significant elevation of SOD, GSH in gastric tissue as compared to control. Also, giving vitamin D with dexamethasone resulted in significant elevation of SOD, GSH in gastric tissue as compared to dex group to a level that reached the control value. MDA level was decreased significantly as compared to dex group but didn't reach the control value. Our results were in agreement with **Sahinet al.(80**).

Vitamin D can activate SOD by stimulating the synthesis of metal ion protein carriers as Zn and Cu which act as cofactors for the activity of SOD **(84)**. It can increase GSH level byactivatinggamma-GCS (**85**). It reduces lipid peroxidation by the interaction between hydrophobic rings leading to accumulation of lipophilic structures in cell membraneto concentrations that inhibit lipid peroxidation and MDA level (**86**).

Our study revealed a strong negative correlation between HOMA-IR and both SOD and GSH level in stomach. Also there is a strong positive correlation between HOMA-IR and gastric MDA indicating that insulin resistance may lead to increased gastric oxidative stress which predisposes to ulcer development.

Insulin resistance leads to disturbance in the insulin signaling pathway increasing NADPH oxidase activity and ROS generation.It also causes hyperglycemia due to decreased glucose uptake in tissues (**50**). Hyperglycemia can directly generate ROS due to auto-oxidation of glucose, increased advanced glycation, products and inhibition of electron transport chain (**87**). ROS in turn cause mucosal damage by degradation of the epithelial basement membrane, damage of DNA**,** apoptosisand reduction of angiogenesis in the gastric mucosa (**88**).

Regarding gastric histopathological examination, our results showed that dexcaused focally ulcerated mucosa in agreement with **El Zahaby et al. (89)**, but givingvit D with dex improved the histopathological picture resulting in formation of onlyfocal mucosal surface erosionin accordance with **Sahinet al. (80**).

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Dexamethasonecauses gastric ulceration by several mechanisms. It causes oxidative stress, hyperglycemia and insulin resistance **(50**) leading to ulcer development.In addition, it stimulates acid secretion and inhibits prostaglandin synthetase activity (**89)**.

Vitamin D prevented gastric ulceration as it is anti-oxidant, prevents hyperglycemia and insulin resistance as illustrated by **Hassan et al. (56)**.In addition, it counteracts hyperacidity (**90**).

Conclusion:

From the present work we conclude that in spite of dexamethasone importance in medicine, it has many side effects. It caused carbohydrate, lipid, protein metabolic disturbance and gastric ulcer. Most of side effects were due to increased oxidative stress.Giving vitamin D with dexamethasone prevented dexamethasone metabolic disturbance and gastric ulceration but the protection was partial in most of the measured parameters. Vitamin D succeeded in protection against dexamethasone induced metabolic disturbance and gastric ulcer due to its antioxidant properties.

Conflict of interest: No conflict of interest

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