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ORIGINAL ARTICLE

Role of Active Vitamin D in Protection Against Glucocorticoids-Induced Metabolic Syndrome and Gastric Lesion

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ABSTRACT

Background: Glucocorticoids are used in the treatment of several diseases, but they have many side effects. Many actions for vitamin D have been discovered due to its strong antioxidant properties. **Objective:** The aim of the present study was to detect the protective effect of vitamin D against glucocorticoid-induced metabolic syndrome and gastric lesion. **Materials and methods:** 24 adult male albino rats were included. Rats were randomly allocated into 4 groups (6 rats/each group), group1 (control), group 2 (vitamin D treated), group 3 (Dexamethasone treated) and group 4 (dexamethasone with vitamin D treated). Drugs were given intraperitoneally daily for 14 days. Blood pressure (BP) was measured before and after the experiment. Serum glucose, insulin, Lipid profile were measured. Also, angiotensin receptor 1 (AT1) gene expression in aortic tissue was detected. Histopathological Analysis for stomach was performed. **Results:** Dexamethasone resulted in a significant increase of BP, glucose, insulin and dyslipidemia with an increase of AT1 gene expression, and significant hypocalcemia was detected besides gastric lesions. Vitamin D with dexamethasone reversed these results. **Conclusion**: Vitamin D prevented dexamethasone-induced metabolic syndrome and gastric lesions due to its antioxidant properties besides inhibition of AT1 gene expression.

Keywords: Dexamethasone, vitamin D, oxidative stress, dyslipidemia AT1 gene expression

INTRODUCTION

Glucocorticoids are used in acute conditions including acute gout, and bacterial meningitis and for fetal lung maturation **[1].**

Chronic glucocorticoid therapy can be used in many diseases like pulmonary diseases such as idiopathic interstitial pneumonia ; neurologic diseases such as multiple sclerosis, and also organ transplantation [**2**]**.** Glucocorticoids have many side effects like diabetes mellitus (DM), dyslipidemia, and hypertension besides gastric ulceration **[3].**

Recent researches have discovered that vitamin D is not only involved in Ca homeostasis, but also has a role in modulation of other different diseases [**4**]. The aim of this work is to clarify the role of vitamin D on prevention of metabolic syndrome and gastric lesions induced by glucocorticoids therapy.

MATERIALS AND METHODS

Animals and maintenance:

Twenty four adult male albino rats weighing about 200-250gm were involved in this experiment. The animals were housed six per cage with free access to food and water. Animals were kept under constant laboratory condition of 12-h cycles of light and darkness. The study protocol was approved by ethical and scientific committee of Beni-Suef University, approval number (**019_72**) at 13 October 2019.

All animal experiments comply with the ARRIVE guidelines and should be carried out in accordance with the U.K. Animals.

Chemicals:

Dexamethasone was purchased from Amriya company (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). Ca^{++} colorimetric assay kit was purchased from Biovision company (Cairo, Egypt). Angiotensin receptor 1 (AT1) -qPCR kits were purchased from Qiagen, Germantown, USA.

Experimental design:

Twenty four adult male albino Rats were included and were divided into 4 groups (6 rats for each one)**:**

1- **Group 1**: the control group

2- Group 2: (1,25dihydroxycholecalciferol (Vit D) group) which was given 1,25dihydroxycholecalciferol only as 1.25 µg /kg /day i.p for 2 weeks **[5].**

3- **Group 3**: (Dexamethasone (Dex) group) which was given dexamethasone as 1mg/kg/day i.p for 2 weeks[**6**]

4- Group 4: (Dexamethasone + 1,25dihydroxycholecalciferol(Dex+vit D) group) which was given dexamethasone as 1 mg/kg/day combined with 1,25-(OH)2D3 (1.25 µg /kg /day) i.p for 2 weeks**[5,6].**

After the last dose, rats were fasted for 12 hours then sacrified under ether anesthesia. Blood samples were taken for biochemical and histopathological analysis.

Sample preparation:

Blood samples were taken from retro orbital plexus by capillary tube and left to be clotted then centrifuged at 8000rpm for 20 minutes, serum was separated and kept frozen at -80°C till analysis of serum glucose, insulin, lipid profile, Ca^{++} besides SOD, MDA. Aorta was excised and homogenized in phosphate buffer saline then centrifuged at 10000 rpm for 20 min, the supernatant was kept in -80°C for measurement of (AT1) gene expression.

Measurement of fasting serum glucose, insulin, HOMA-IR:

- Glucose was measured by colorimetric assay kit from BioMed Pharmaceutical Industry (Cairo, Egypt) in mmol/L as performed before**[7].**
- Serum insulin was assessed by ELIZA kits from Biosource (San Diego, USA) in MIu/ml as done before[**8**]**.**
- HOMA-IR was calculated by the HOMA method according to[**9**].

(HOMA-IR) = (fasting glucose (mmol/l) x fasting insulin (MIu/ml))/22

Measurement of 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 **[10, 11,12]**.
- LDL was calculated by Friedewald equation[**13**]

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

Measurement of BP:

It was measured by noninvasive tail cuff method in conjunction with a PowerLab system from ADInstruments company (Australia) . The noninvasive blood pressure (NIBP) System output the pressure and pulse signals to a PowerLab via a Bayonet Neill–Concelman (BNC) connection**[14]**.

Measurement of SOD activity (U/ml) and MDA(nmol/ml) :

SOD and MDA colorimetric kits were purchased from Biodiagnostic company (Cairo, Egypt). Measurement was performed as described previously **[15,16, 17]**.

Measurement of AT 1 gene expression:

Quantitative real time PCR:

1. ***RNA extraction:***

Qiagen tissue extraction kit **(Qiagen, USA)** was used for isolation of total RNA according to manufacture instructions.

2. ***cDNA synthesis:***

Using high capacity cDNA reverse transcription kits (Fermentas, USA), The total RNA (0.5–2 µg) was used for cDNA conversion**.**

Real-time qPCR using SYBR Green I:

An applied Biosystem with software version 3.1 (StepOne™, USA) was used for amplification and analysis of rt-q PCR. The qPCR assay with the primer sets were optimized at the annealing temperature. The primer sequences are as the following: For AT1, Forward primer : 5-GTGGGAGAAAGTTTGCCAGG-3, Reverse primer:5- GTAGGAAGAGAGGGAAGAGG-3 and for Beta actin gene, Forward 5′-ATCACCATCTTCCAGGAGCG-3′, Reverse 5′-CCTGCTTCACCACCTTCTTG-3′

Calculation of Relative Quantification (RQ) (relative expression):

According to applied Bio system software, the relative quantitation was calculated using the following equation:

Δ Ct = Ct gene test – Ct endogenous control

$\Delta\Delta$Ct = ΔCt sample1 – ΔCt calibrator

RQ = Relative quantification = $2^{-\Delta\Delta Ct}$

The RQ is the fold change compared to the calibrator (untreated sample)

Measurement of serum Ca^{++} (mg/dl):

Calcium colorimetric assay kit was purchased from Bio vision company, Cairo, Egypt). As manufacture instructions[**18**].

Histological examination:

Stomachs were dissolved and washed with saline. 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) . The other part was kept in formaline 10% for histopathological examination.

Determination of ulcer index and histopathological examination for stomach

Gastric tissues were pinned out flat on a cork board and photographed for lesion assessment with the aid of a manifestations to detect ulcer index, ulcer score was determined by the severity factor which was calculated as performed before [**19**].

- After macroscopic examination, stomachs were placed in formaldehyde solution 10 % for 24 hours and histopathological examination of stomach**s** was performed with the routine hematoxylin and eosin stain technique[**20**].

Statistical analysis:

Data was presented as mean ± standard deviation, using analysis of variance (ANOVA) with multiple comparisons post hoc test. Comparisons between basal and final BP and weight were performed using paired t test [**21**]. P-values < 0.05 were considered as statistically significant.

RESULTS

Effect of active vitamin D and dexamethasone on fasting serum glucose and insulin:

As shown in table (1), Administration of dexamethasone caused significant increase of both serum glucose and insulin as compared to control and vitamin D groups(**P<0.05**). Administration of vitamin D with dexamethasone showed significant increase of serum glucose and insulin levels as compared to control and vitamin D groups (**P<0.05**),but showed a significant decrease of fasting serum glucose and insulin level as compared to dexamethasone group **(P<0.05)**.

Dexamethasone administration resulted in a significant increase in Homa IR as compared to control and vitamin D groups **(P<0.05)**. When vitamin D was given with dexamethasone, Homa IR significantly decreased as compared to dexamethasone group (**P<0.05**). (Table 1)

Effect of vitamin D and dexamethasone on serum triglycerides, total cholesterol, LDL and HDL:

As observed in table (2), administration of dexamethasone caused significant increase of triglycerides, total cholesterol and LDL as compared to control and vitamin D groups **(P<0.05**) ,while HDL was significantly decreased in dexamethasone group as compared to control and vitamin D groups (**P<0.05)**.

In dexamethasone + vitamin D group, a significant decrease was observed as compared to dexamethasone group **(P<0.05**). On the contrary, HDL was decreased significantly in this group as compared to control and vitamin D groups (**P<0.05),** while it was increased significantly when compared to dexamethasone group **(P<0.05**).

Effect of vitamin D and dexamethasone on Blood pressure(BP):

NO significant difference in blood pressure was detected among all groups at the beginning of the experiment. At the end of the experiment, as shown in table 3 there was no significant difference in BP between the control and vitamin D groups **(P>0.05)**. However, administration of dexamethasone resulted in a significant increase in BP as compared to control and vitamin D groups **(P<0.05)**.

After combination of vitamin D with dexamethasone, BP was significantly decreased as compared to Dexamethasone group (**P<0.05)**.

Effect of vitamin D and dexamethasone on AT1 expression and serum Ca ++ level :

Rats given dexamethasone showed a significant increase of AT1 gene expression as compared to control and vitamin D groups **(P<0.05)**. Administration of vitamin D with dexamethasone caused partial down regulation of AT1 **(P<0.05)**, However showed a significant decrease as compared to Dexamethasone group **(P<0.05)**. Table (3)

Administration of dexamethasone caused a significant decrease of serum Ca^{++} as compared to control and vitamin D groups **(P<0.05)**. Combination of vitamin D with dexamethasone resulted in a complete protective effect against dexamethasone induced hypocalcaemia **(P>0.05)**.There was a significant increase of serum Ca^{++} in Dexa+Vit D group as compared to dexamethasone group **(P<0.05)**. Table (3)

Effect of vitamin D and dexamethasone on macroscopic examination of the stomach, ulcer index:

As shown in table (4),figure (1), administration of dexamethasone caused appearance of multiple gastric lesions with ulcer index which was significantly increased as compared to control and vitamin D groups **(P<0.05)**. Administration of vitamin D with dexamethasone caused some gastric lesions with ulcer index which was significantly increased as compared to control and vitamin D groups **(P<0.05)** but was significantly decreased as compared to dexamethasone group **(P<0.05).**

Effect of vitamin D and dexamethasone on SOD, GSH and MDA in stomach :

As shown in table (4), in dexamethasone group, the antioxidant markers SOD and GSH were significantly decreased as compared to control and vitamin D groups (**P<0.05**), while MDA was significantly increased in dexamethasone group as compared to control and vitamin D groups (**P<0.05**).

Vitamin D with dexamethasone protected against oxidative stress and no significant difference in the SOD, GSH was reported in comparison to control rats (**P>0.05**). However a significant increase of theses markers was found as compared to dexamethasone group (**P<0.05).** However, MDA was significantly increased in this group as compared to control and vitamin D groups (**P<0.05**) and it was significantly decreased in as compared to Dexamethasone group (**P<0.05**).

Histopathological examination of stomach:

As shown in figure 2, **Control, vitamin D groups showed** normal gastric mucosa and mucin secretion, while **Dexamethasone group** showed focal ulceration of gastric mucosa with congested capillaries. **Adminestration of vitamin D with dexamethasone improved g**astric mucosa lesion and showed minimum erosion.

Table 1: Comparison of fasting serum (glucose, insulin) and Homa IR among all groups

	Control group	Vit D group	Dex group	Dex+vit D group
Fasting serum glucose (mmol/L)	**4.64±0.2**	**4.65±0.23**	**14.55±1.11 *#**	**7.06±0.23 *#$**
Fasting serum insulin (MIu/ml)	**7.33±0.48**	**7.14±0.5**	**18.82±0.87 *#**	**8.78±0.44* #$**
Homa-IR test	**1.52±0.17**	**1.45±0.16**	**12.02±1.06 *#**	**3.11±0.20 *#$**

*: significant as compared to control group (P<0.05)
#: significant as compared to Vit D group (P<0.05)
$: significant as compared to Dex group (P<0.05)

Table 2: 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)	**97.9±2.72**	**89.67±8.24**	**172.28±8.79 *#**	**124.53±5.64 *#$**
Serum Cholesterol (mg/dl)	**136.15±3.14**	**127.17±3.17**	**221.07±10.41 *#**	**157.65±8.05 *#$**
Serum LDL (mg/dl)	**51.79±6.07**	**42.62±4.5**	**168.36±7.6 *#**	**82.29±7.41 *#$**
Serum HDL (mg/dl)	**63.45±3.60**	**71.3±3.2**	**23.62±5.15 *#**	**55.24±4.47 *#$**

*: significant as compared to control group (P<0.05)
#: significant as compared to Vit D group (P<0.05)
$: significant as compared to Dex group (P<0.05)

Table 3: Comparison of BP among all groups at the beginning and the end of the experiment

	Control group	Vit D group	Dex group	Dex+vit D group
Basal BP (mmHg)	**104.83±2.93**	**101.83±5.49**	**101.67±1.75**	**102.17±5.42**
Final BP (mmHg)	**115.73±2.3**	**115.47±4.53**	**168.5±3.62 *#**	**122.17±1.23 *#$**
AT1 gene expression	**1.01±0.01**	**0.74±0.31**	**4.79±1**	**4.95±1**
Ca++(mg/ dl)	**10.4±0.37**	**10.38±0.31**	**8.62±0.43**	**10.11±0.4**

*: significant as compared to control group (P<0.05)
#: significant as compared to Vit D group (P<0.05)
$: significant as compared to Dex group (P<0.05)

Table 4: Comparison of ulcer index, SOD, GSH and MDA among all groups:

	Control group	Vit D group	Dex group	Dex+vit D group
Ulcer index	**0.0±0.0**	**0.0±0.0**	**5±2.07*#**	**1.4±0.52*# $**
SOD in stomach (U/gm tissue)	**6.78±0.82**	**8.04±0.72 ***	**2.33±0.37 *#**	**6.09±0.23 #$**
GSH in stomach (mg/g.tissue)	**86.42±1.95**	**101.83±5.42 ***	**37.22±5.79 *#**	**78.27±7.59 #$**
MDA in stomach (nmol / g.tissue)	**12.43±1.75**	**10.23±0.39**	**86.6±10.37 *#**	**23.47±3.63 *#$**

*: significant as compared to control group (P<0.05)
#: significant as compared to Vit D group (P<0.05)
$: significant as compared to Dex group (P<0.05)

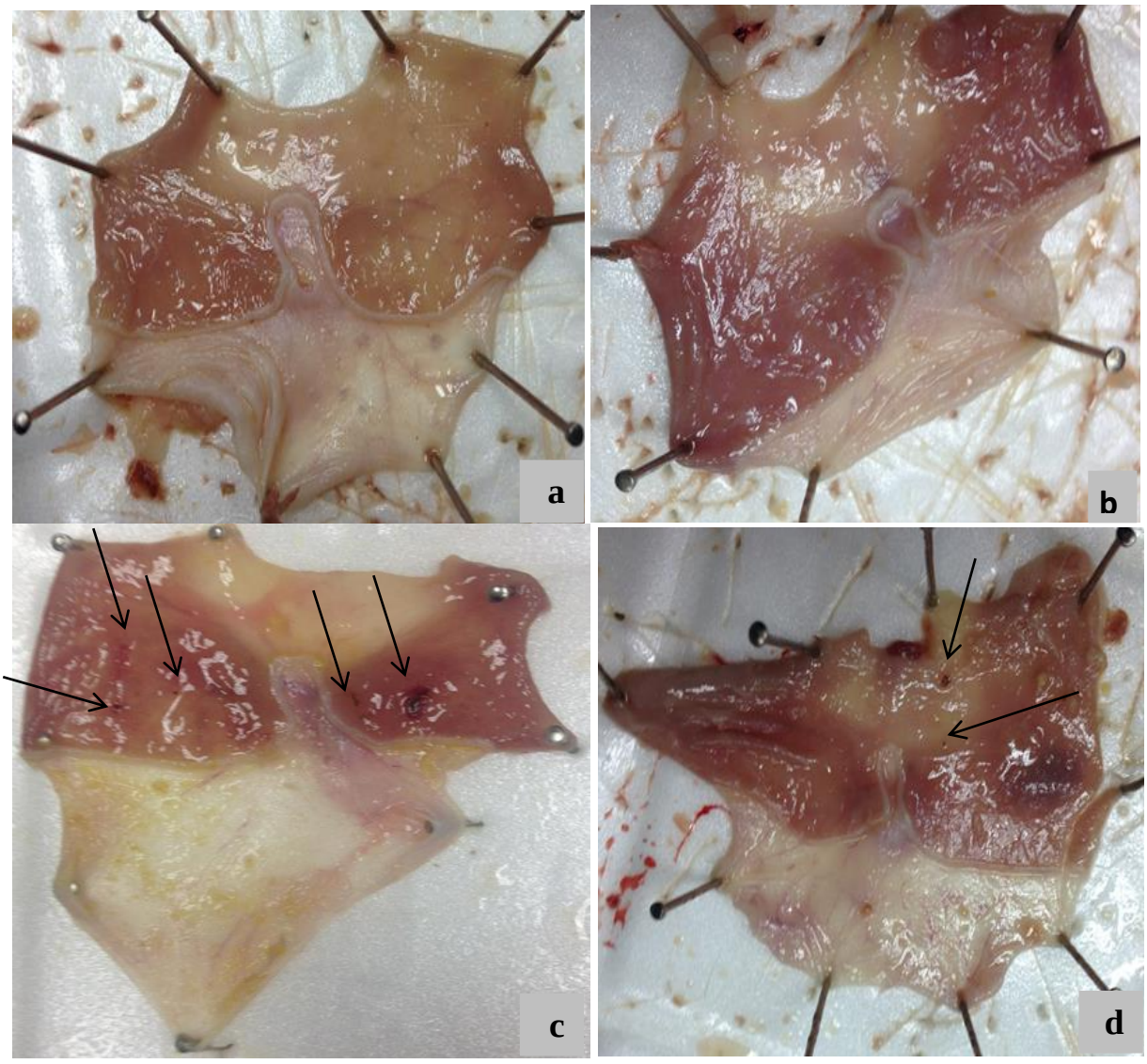

Figure 1: Gross picture of gastric lesions a) control group with no lesions b) Vitamin D group with no lesions c) Dexamethasone group with numerous lesions d) Dexamethasone vitamin D group with few lesions

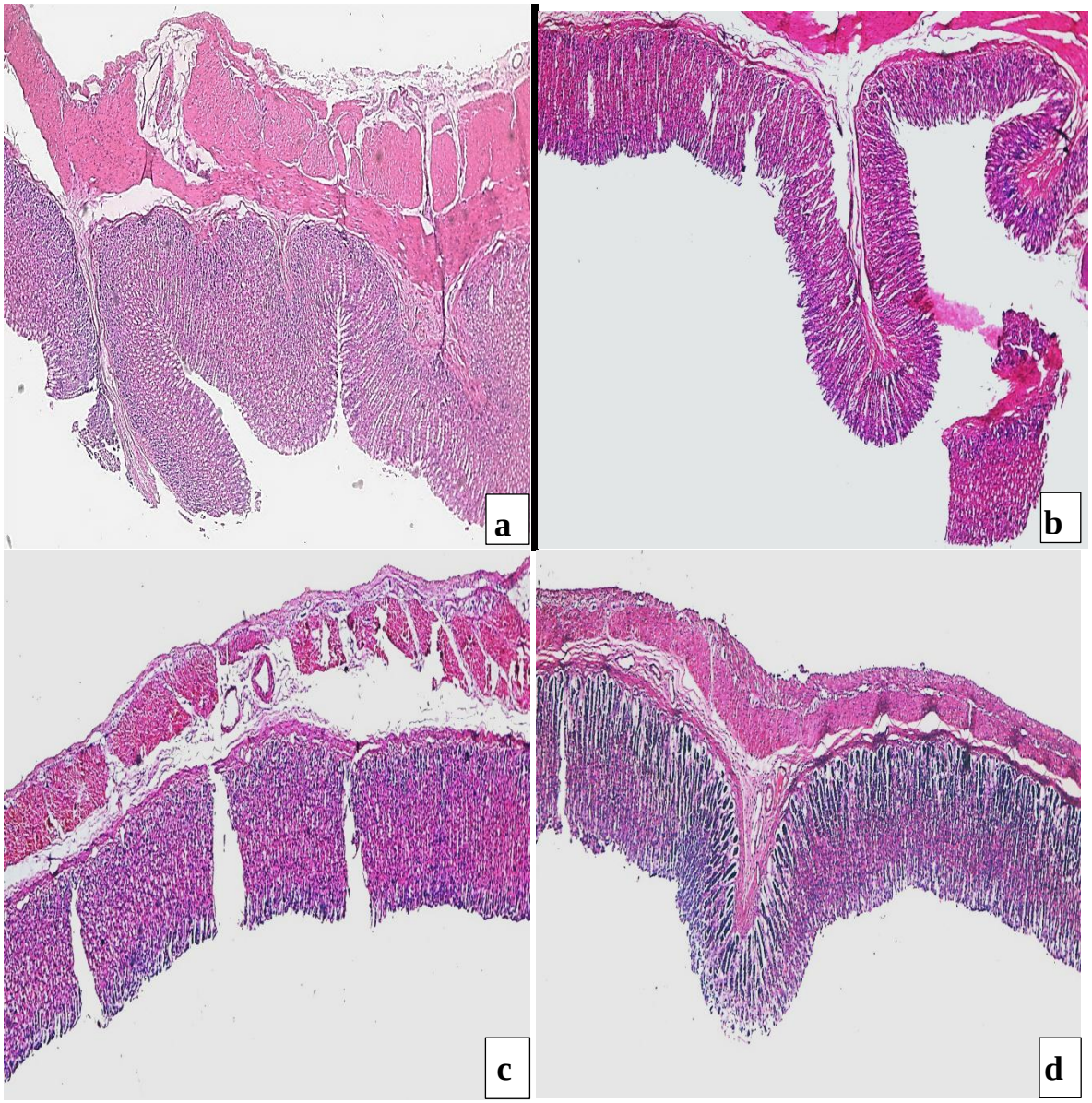

Figure 2: Microscopic examination of gastric tissue. a, b) Control, vitamin D : normal mucosa. c) Dexamethasone group: ulceration of gastric mucosa d) Dexamethasone +Vitamin D group: gastric mucosa with surface erosion.

DISCUSSION

Glucocorticoids have multiple side effects, despite of their huge indications. Vitamin D has many functions rather than Ca homeostasis **[4].** The present work aimed to study the protective effect of active form of vitamin D against dexamethasone induced metabolic disturbance, hypertension and gastric ulcer.

In the current study, dexamethasone induced a significant increase in serum glucose, insulin and HOMA-IR as compared to control rats. Vitamin D prevented these disorders as reported by **Eltablawy et al.[22].** Dexamethasone has been documented to decrease insulin receptor substrate (IRS), phosphatidyl inositol triphosphate (PI3K) expression with subsequent development of insulin resistance [**23**]. On the other hand, vitamin D has been found to up regulate the expression of IRS-1, PI3K. Also, it has strong antioxidant activity resulting in increasing glucose uptake in tissues and insulin sensitivity [**24**].

In the present study, dexamethasone administration caused significant increase in serum triglycerides, cholesterol and LDL and significant decrease of serum HDL **[25]**,while vitamin D prevented these results [**24**]. Dexamethasone causes Dyslipidemia through stimulation of hepatic denovo lipogenesis [**26**]. Vitamin D has been reported to down regulate of both acetyl-CoA carboxylase, fatty acid synthase and consequent inhibition of lipogenesis [**27**]. Moreover, vitamin d

increases HDL through reduction of insulin resistance[**24**].

In the present study, a significant elevation of BP was developed in dexamethasone group with up-regulation of AT1 expression **[28].** Administration of vitamin D with dexamethasone decreased BP **[29].** Dexamethasone causes an increase of GR nuclear translocation and GR binding glucocorticoid response Element (GREs) at AT1R[**30**].

In the present study, vitamin D caused a significant down-regulation of AT1 expression[**31**]. Also, vitamin D contributes in suppression of renin resulting in down - regulation of AT1R [**32**].

In the current work, dexamethasone caused significant decline in serum Ca^{++} level [**33**]. Dexamethasone causes hypocalcaemia through inhibition of the absorption from the intestinal and stimulation of excretion. Dexamethasone inhibits the epithelial Ca channel TRPV5 expression in the duodenum besides suppression of active calcium-transporting proteins CaBP-9k and PMCA1. On contrary, dexamethasone down regulates TRPV5, TRPV6, CaBP-9k and PMCA1 in the kidney leading to enhancement of Ca excretion[**34].**

Vitamin D administration with dexamethasone resulted in a significant increase in serum Ca^{+} to correct hypocalcemia resulted from dexamethasone **[35] .**

Ca^{++} homeostasis is known to be the main action for vitamin D, vitamin D works on intestine, kidney and bone. It increases Ca^{++} absorption through increased ATP-dependent calcium pump besides up-regulation of TRPV6 and calbindin D [**36**].

In the present work, dexamethasone caused a significant elevation of ulcer index as compared to control **[37]**, while vitamin D resulted in a significant decrease of ulcer index **[38]**. Also, showed dexamethasone resulted in a significant decrease of SOD, GSH and increase in MDA in gastric tissue of rats **[37],** but vitamin D reversed this action**[38].**

Gastric histological examination showed that dexamethasone caused focally ulcerated mucosa **[39],** but administration of vitamin D attenuated these abnormalities **[38]**. This ulceration may be due to oxidative stress, or insulin resistance[**40**]. Further more, dexamethasone stimulates acid secretion and inhibits prostaglandin synthetase activity [**39**] Vitamin D protected against gastric ulceration anti-oxidant and anti hyperglycemic properties **[24]**.

CONCLUSION

Dexamethasone caused hyperglycemia and hyperinsulinemia, hypertension and gastric ulceration . These actions may be due to development of oxidative stress and increased activity of renin angiotensin system. Vitamin D administration prevented dexamethasone induced these abnormalities. This may be explained due to its antioxidant properties and through inhibition of renin angiotensin system. Other studies can be performed to continue the results of this study, through increasing the dose of vitamin D or the duration of administration to reach to a complete protection.

- **This work didn't receive any fund**
- **This study took approval from ethical and scientific committee of Beni-Suef University.**
- **All authors declared (NO conflict of interest**

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