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BIOCHEMICAL ALTERATIONS OF EXPERIMENTALLY INDUCED HYPOMAGNESAEMIA IN MALE BALADI GOATS.

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A B S T R A C T

This study was performed to investigate the effect of experimental hypomagnesemia on glutathione redox cycle, nitric oxide, serum, glucose, calcium, inorganic phosphorus, magnesium, cortisone and insulin in baladi goats. Two groups of twelve healthy Baladi male goats were used; the first one contained 5 animals and kept as control group. The second group includes 7 animals administrated with potassium chloride and citric acid daily until the development of the characteristic signs of hypomagnesaemia observed. Blood samples were collected at 6, 12, 18 and 24 days of administration. The obtained results revealed that decreases in serum magnesium, calcium, inorganic phosphorus, insulin and nitrate concentration and erythrocytes glutathione peroxidase, reduced glutathione, glutathione reductase, glutathione-S-transferase and total superoxide dismutase activities were decreased whereas erythrocytes catalase activity, serum cortisone and glucose concentration were significantly increased in hypomagnesemic induced group when compared with the control group. From these results it could be conclude that nitric oxide release, glutathione redox cycle activities, insulin and cortisone levels were markedly affected by hypomagnesaemia. Such alterations may be considered as predisposing factors responsible for the tissue injury and vascular changes observed in hypomagnesaemia.

KEY WORDS: Goat, Hypomagnesemia, Glutathione redox cycle

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1. I N T R O D U C T I O N

Magnesium influences physic-chemical properties of cellular membranes, thus is involved in establishing and maintaining intracellular electrolytes content. Also, it acts as cofactor for multiple enzymes and its deficiency induces oxidative damage by increasing the production of reactive oxygen species (ROS) and enhancing susceptibility to oxidative stress and changes in antioxidant status [1]

Extracellular magnesium plays a major role in acetylcholine production and destruction; also it is found in neuromuscular junction Therefore hypomagnesemia is accompanied with neuromuscular disturbances and tetany [2]. Nitric oxide (NO) induces vasodilatation, regulates normal vascular tone and inhibits platelets aggregation [3]. Moderate magnesium deficiency during pregnancy affects blood pressure [4]. Also impairment of glutathione peroxidase inhibits NO production by endothelial cells in hypomagnesaemia [5]. Alteration in antioxidant enzymes activities was suggested to be responsible for cardiac muscle lesions observed in hypomagnesemia [7]. In hypomagnesemia catalase activity in cardiac muscle was increased, while the activity of GSH-Px was decreased significantly [6]. However, erythrocytic magnesium was a better indicator of the prolonged sub-clinical magnesium deficiency and this has been reported to be associated with low milk

production and low milk fat content in dairy cows [8]. **T**herefore this study was designed to follow up the possible effects of hypomagnesemia on NO release, activities of antioxidant enzymes of glutathione redox cycle, insulin, glucose, cortisone, calcium, inorganic phosphorus and magnesium of experimentally induced Hyperomagnesmia in goats.

2. MATERIAL AND METHODS

The present study was carried out on a private Farm in Siwa Oasis on twelve adult male Baladi goats, 9-12 months old and with body weight ranged from 28-32 kg. animals were fed on a ration composed of barseem and concentrate mixture consisted of the following ingredients: wheat bran 35% , yellow corn 22%, cotton seed cake 35%, calcium carbonate 2% and sodium chloride 1%, rice polish 4 % and molasses 3 %. The amount of ration given to the animals was 1.00 kg concentrate mixture and 0.50 kg barseem/head/day twice daily and water was available *ad- libitum*. The chemical composition of the concentrate mixture and barseem was performed according to AOAC (2000) [40] as following:

Feed stuff	Concentrate Mix.	Barseem
Moist. %	9	82
Ash %	10.4	1.8
Prot. %	15.8	5
E.E %	5	0.5
Fibers %	14	2.8
N.F.E %	45.8	7.9

Before the beginning of the experiment all goats were examined clinically for parasitic infestation. Each of the animals groups was kept in a separate pen provided with feeding trough and watering buckets.

Experimental design

Animals were divided into two groups:
Group A: include five animals were used as control healthy group.

Group B: comprised seven goats were used as experimental hypomagnesaemia group. The experimental induction of hypomagnesaemia in goats was performed by oral daily dose administration of 1.39 g/kg potassium chloride *(MW 74.55)* and 1.19 g/kg body weight citric acid *(MW 192.10)* for 24 days by using the stomach tube according to [9, 10].

Blood samples and biochemical analysis:

Blood samples were collected at 6, 12, 18 and 24 days from the onset of administration of Kcl and citric acid. Two blood samples were collected; the first one was collected in tubes without anticoagulant for serum separation, which were used freshly for the quantitative determination of nitric oxide [11]. Cortisone and insulin concentrations using immunoradiometric assay [12], calcium [13], inorganic phosphates [4], glucose [15] and magnesium [16] as it was indicated before. The second blood samples was collected in tubes contained 20 IU heparin/1 ml blood; and used for preparation of hemolysate as described by [17]. This hemolysate was subjected for determination of Erythrocyte Glutathione peroxidase (GSH-Px) [18]; Reduced glutathione (GSH); glutathione reductase GR-ase and glutathione – S – transferrase (GST) [19]; total superoxide dismutase (t-SOD)[20]and Catalase (CAT) [21].

Statistical analysis:

Statistical analysis was done by student *t*-test according to [22]**.**

3. RESULTS

The recorded data demonstrated in Tables 1, 2 and 3 revealed that the experimental induction of hypomagnesaemia in male goats resulted in decreases of serum Mg, Ca, P, insulin and NO levels and erythrocytes GSH-Px, GST, GSH and t-SOD activities and increased values of

serum glucose and cortisone level and erythrocytes CAT activity when compared with the values of control healthy group.

4. DISCUSSION

Magnesium plays a fundamental role in many functions of the cell including (utilization, transport and storage of energy); metabolism of carbohydrates, protein and lipids, maintenance of normal cell membrane function; and the regulation of parathyroid hormone *(PTH)* secretion [23]. In this study, hypomagnesaemia was induced by administration of potassium chloride and citric acid resulted in decreases of serum calcium and inorganic phosphorus levels this due to the excessive amount of potassium chloride and citric acid in the rumen levels to retarded Mg, Calcium and Phosphorus absorption from the reticulum as stated by [10 and 24]. The transitory decline in plasma calcium concentration in response to magnesium deficiency may have resulted from increased urinary calcium excretion with a coincident decrease in calcium resorption from bone and a similar change in phosphorus metabolism followed changes in magnesium [25].This is confirmed by the opinion of [26] who suggested that potassium administration directly depressed the circulating levels of divalent cations (calcium, phosphorus and magnesium) because of the increased intra cellular potassium level increased excretion of magnesium or increased cellular uptake of it.

Table 1 Serum magnesium, calcium and inorganic phosphorus concentrations in control and hypomagnesaemia induced goats

Blood parameter	Control group	Hypomagnesaemia group			
		6 days	12 days	18 days	24 days
Magnesium (mg/dl)	4.39±0.29	2.65±0.34*	2.22±0.48**	1.12±0.29**	1.01±0.08***
Calcium (mg/dl)	9.99±0.64	8.97±0.38	7.10±0.55*	5.93±0.61*	4.41±0.70**
Phosphorus (mg/dl)	5.94±0.37	3.69±0.21*	3.42±0.39*	3.10±0.41*	2.39±0.52*

S.E.: Standard error, *: Significant at (P<0.05), **: Highly significant at (P<0.01), ***: Very highly significant at (P<0.001).

Table 2 Serum Glucose, insulin, cortisone and nitrate concentrations in clinical healthy control and hypomagnesemic induced goats .

Blood parameter	Control group	Hypomagnesemic group			
		6 days	12 days	18 days	24 days
Glucose (mg/dl)	80.33±2.49	97.15±3.01	118.77±4.16	129.79±3.33*	141.90±4.70*
Insulin (µU/dl)	11.33±0.37	10.11±0.49	8.39±0.53	7.17±0.42*	4.98±0.25*
Cortisone (ng/dl)	4.83±0.12	7.97±0.29	11.75±0.41**	21.25±1.11**	31.80±2.75**
Nitrate (µmol/L)	58.93±1.19	41.11±1.21	20.16±2.12**	17.11±2.09**	8.77±1.17***

S.E.: Standard error, *: Significant at (P<0.05), **: Highly significant at (P<0.01), ***: Very highly significant at (P<0.001)

Table 3 Erythrocytes GSH-PX, GR-ase, GST, GSH, t-SOD and CAT levels for the clinical healthy control and the hypomagnesaemia induced goats

Blood Parameter	Control group	Hypomagnesaemia group			
		6 days	12 days	18 days	24 days
GSH-PX (U/g Hb)	4.18±0.79	3.16±0.81	2.25±0.39**	1.21±0.41**	1.03±0.09***
GR-ase (U/g Hb)	0.95±0.11	0.77±0.12	0.59±0.09*	0.47±0.10**	0.35±0.12**
GST (U/g Hb)	0.49±0.09	0.38±0.08	0.29±0.07*	0.18±0.03*	0.11±0.01**
GSH (U/g Hb)	1.08±0.11	0.79±0.21	0.68±0.11*	0.51±0.12*	0.39±0.11**
t-SOD (U/g Hb)	14.97±1.62	9.25±0.78	8.97±0.73	7.25±0.72*	6.23±0.81**
CAT (U/g Hb)	35.89±3.11	42.15±2.75	69.75±3.98*	81.75±4.75**	96.81±5.81**

S.E.: Standard error, *: Significant at (P<0.05), **: Highly significant at (P<0.01), ***: Very highly significant at (P<0.001)

Also, the decreases phosphorus level may be attributed to that, the first response of the parathyroid gland to the fall in the plasma magnesium level was increasing the secretion of PTH for mobilizing of both calcium and magnesium from bone and increasing magnesium reabsorption by the renal tubules for rising the plasma magnesium level

The obtained results revealed marked decrease in NO concentration in hypomagnesemic goats. These results are in accordance with [4] who observed that moderate Mg deficiency during pregnancy adversely decreased NO production and blood pressure.

The recorded decreased production of NO could be attributed to the decreased insulin level in hypomagnesemic goats as suggested by [28] who reported that insulin activities NO synthase, which is the key enzyme for NO synthesis.

Insulin level showed significant decrease in hypomagnesemic goats, these results are similar to that reported by [29] who found that Mg deficiency aggravated insulin resistance and its supplementation improve insulin sensitivity and secretion. These results due to that magnesium activate insulin receptors for its action so the hypomagnesaemia retarded its action as stated by [30] who found that, magnesium was exhibit some insulin-like activities and inhibit insulin-stimulated lipogenesis in rat adipose tissue. The increases in oxidative stress followed magnesium deficiency probably due to the abnormal metabolic milieu such as hyperglycemia, dyslipidemia, and elevated free fatty acids (FFA), which commonly occur in patients have diabetes and in insulin resistant state [31]. In this respect, [32] reported that the hypomagnesaemia accompanied have hyperglycemia without increase in insulin level. The severity of hypoinsulinemia and hyperglycemia in diabetic patients are correlated with hypomagnesaemia [33].

The decreased activity of GSH-Px in hypomagnesemic goats might be another explanation for the decrease in NO production. This suggestion was confirmed by [5] who stated that impairment of GSH-Px resulted in inhibition of NO production from endothelial cells.

The observed increased serum cortisone in the present study is in agreement with the data of [34] who noticed that Mg deficiency induced vascular damage, increased blood pressure and cortisone level. These elevated values could be related to the stressful conditions followed hypomagnesaemia. This is because Mg normally affects the limbic-hypothalamus and pituitary-adrenocortical axes reducing the release of adrenocorticotropic hormone (ACTH) and affecting the adrenocortical sensitivity to ACTH [35].

The presented data exhibited that the experimental hypomagnesaemia accompanied by significant decrease in erythrocytes activities of t-SOD, GSH-Px, GSH, GR-ase and GST, while catalase activity was markedly increased; These results are in accordance with [36] who reported that hypomagnesaemia is stressful syndrome exhausted the antioxidant enzymes .This due to the oxidative stress induced by hypomagnesaemia includes a disturbance between the pro-oxidant and antioxidant balance in favor of the former, which contributed to the developed pathologic effects observed in hypomagnesaemia [37].

Catalase enzyme showed a highly significant increased indicates adaptation changes in response to large amount of hydrogen peroxide, which decomposed by catalase [38]. So, the rise in CAT activity may be a response to the need for further enzymatic capacity to deal with the production of H_2O_2.The significant decrease in the activity of t-SOD, GSH-px, GSH, GR-ase and GST reported in the present study leads to depletion of antioxidant defense mechanism in RBCS, which cause inability of RBCs to remove harmful effects of arising O_2 in hypomagnesaemia [39].

From these results, we can conclude that: hypomagnesaemia is stressful syndrome resulted in exhaustion and disturbance in glutathione redox cycle and antioxidant because Mg is important Co-factor for many enzymes of normal healthy life chemical reactions and major factors for defense against oxidant radicals.

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مجلة بنها للعلوم الطبية البيطرية
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التغيرات الكيميائية الحيوية فى نقص الماغنسيوم المحدث تجريبيا فى ذكور الماعز البلدى

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الملخص العربى

تم إجراء هذا البحث لدراسة تأثير نقص الماغنسيوم على أكسيد النيتريك، نظام الجلوتاثيون ريدوكس، مستوى الكورتيزون، الأنسولين، جلوكوز الدم، الكالسيوم والفسفور غير العضوي فى الماعز البلدى. وقد استخدم لهذا الغرض اثنا عشر من الذكور تم تقسيمهم إلى مجموعتين الأولى الضابطة تحتوى على خمسة حيوانات سليمة بلا تدخل فى غذائها. والمجموعة الثانية تحتوى على سبعة حيوانات تم إحداث نقص الماغنسيوم فيها تجريبيا بتجريعهم كلوريد البوتاسيوم وحمض الستريك (1.39 جم/كجم و1.19 جم/كجم بالترتيب) – بعد ظهور الأعراض المميزة لهذا المرض تم تجميع عينات الدم بعد 6، 12، 18 و 24 يوما من إحداث هذا النقص وتم قياس مستوى النيترات، الكورتيزون، الأنسولين، الماغنسيوم، الكالسيوم والجلوكوز فى مصل الدم والجلوتاثيون بيرواكسيديز، الجلوتاثيون المختزل، الجلوتاثيون ريدكتيز، الجلوتاثيون–S–ترانسفيريز، السوبر أكسيد ديسميوتيز الكلى والكتاليز فى خلايا الدم الحمراء. وقد أظهرت النتائج نقصا معنويا فى نشاط الجلوتاثيون بيرواكسيديز، الجلوتاثيون المختزل، والجلوتاثيون ريدكتيز، الجلوتاثيون–S–ترانسفيريز والسوبر أكسيد ديسميوتيز الكلى بينما ازداد نشاط الكتاليز زيادة معنوية – كما أظهرت النتائج انخفاض فى مستوى الأنسولين، والماغنسيوم، الكالسيوم والفسفور غير العضوي وارتفاع فى مستوى الكورتيزون وسكر الدم فى المجموعة المحدث فيها نقص الماغنسيوم بالمقارنة بالمجموعة الضابطة. ومن هذه النتائج يمكن استخلاص أن نقص الماغنسيوم قد اثر فى نشاط نظام الجلوتاثيون ريدوكس، إنتاج أكسيد النيتريك، مستوى الأنسولين، الكورتيزون وسكر الدم.

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