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EFFECT OF MATERNAL VITAMIN E + SELENIUM STATUS DURING THE PERINATAL PERIOD IN TISSUES ANTIOXIDANTS OF RAT PUPPIES AND THEIR DAMS

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ABSTRACT

The first days of life represent a critical period for the new born, the present study aimed to investigate tissue antioxidant of rats puppies during the whole suckling period in response to maternal prenatal injection with Vit. E + Selenium. Twenty female albino rats at breeding age were divided into two groups: 1- control group, comprised 10 animals maintained on basal diet and not supplied by any additives. 2- Experimental group consists of 10 animals maintained on basal diet and injected with vitamin E + Selenium at a dose of 20 mg/kg.b.wt. and 150 µg/kg.b.wt., respectively, through I.M. weekly for 3 successive weeks. Both groups bred and the injection were repeated during pegnancy period in the experimental one. After labor, rat puppies were killed at intervals of one, 4, 9, 14 and 21st days whereas dams were scarified at 21st. Liver, heart and lung tissues of puppies and their dams were used for determination of glatothione peroxidase (GSHpx) Cu/Zn superoxide dismutase (SOD) and Catalase (CAT) activities in addition to reduced gluthione (GSH) concentration. The obtained data revealed significant increase in hepatic GSHpx, Cu/Zn SOD activities and GSH concentration in addition to lung Cu/Zn SOD of experimental group of dams compared to control group. Concerning rat puppies, there were significant increase in GSHpx, Cu/Zn SOD activities in heart, lung and liver of puppies obtained from injected dams at one day followed by high significant increase at 4th day that increase become non significant at 9, 14 and 21st day compared to puppies obtained from control group as well as GSH concentration showed significant increase in liver at one day and in heart, lung and liver at 4th day. These results may attributed to the transfer of selenium from

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placenta to newly borned and through colostrums similar to vitamin E that transferred via mammary glands.

Key Words: Vit. E, selenium, prentatal, tissues antioxidants, puppies.

INTRODUCTION

Vitamin E has been reported to be scavenger of oxygen free radicals which initiate and propagate chain oxidation (Guesta *et al.*, 1995). It was recorded that its concentration in heart and lung was higher in dams compared with their puppies (Buttris and Diplock, 1988). In addition Pazak and Scolz (1996) found an increase in liver concentration of vitamin E in rat puppies of dams with high vitamin E concentration as well as significant increase in heart and lung vitamin E concentration of rat offspring obtained from dams fed vitamin E + Selenium containing diet.

Selenium is an essential trace elements that has a role in preventing peroxidation injuries in tissues. It exerts role probably via the seleno enzyme glutathione peroxdase (Zikic *et al.*, 1998). It has been showed improvement in selenium status of goat kid following supplementation of their dams with selenium during breeding season (Wichtel *et al.*, 1996). Also, Seaz *et al.* (1996) Also, Harmili *et al.* (1990) showed that injection of pregnant ewes with selenium for one mouth before parturition raised blood selenium of both ewes and lambs for several months. Moreover, Elsy and Lance (1983) recorded significant difference in hepatic selenium concentration of selenium administered group and reach to about two fold than control group.

Accordingly the present study was under taken to determine the activities of GSHpx, Cu/Zn SOD and catalase. In addition to GSH concentration of liver, heart and lung tissues of female rats and their puppies at brith and post partum intervals through the whole suckling period 21 days of age in response to maternal prenatal injection of vitamin E + Selenium.

MATERIALS AND METHODS

A total number of 20-female mature rats at breeding age weighed 250 g. They are classified into 2 groups control group comprised 10-animals maintained throughout the experimental period on basal diet.

Experimental group consisted of 10-animals maintained on basal diet and injected intramuscularly with Vit. E and selenium at a dose of 20

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mg/kg.b.wt. and 150 μ g/kg.b.wt., respectively, weekly for 3 weeks before mating.

Then mature male rats were introduced for both group as 3-femal/male and the injection still in the experimental group until labour.

7-puppies were scarified after birth before suckling the others still with their dams and 6-puppies scarified at 4, 9, 14 and 21 days. Finally 10-dams also scarified at 21 days.

The heart, lung and liver tissues of puppies and their dams were rapidly removed perfused with 0.9% NaCl followed by homogenization and processing for determination of glutathione peroxidase (GSHpx) activity (EC 1. 11. 1.9) according to method of (Avissar *et al.*, 1991), Cu/Zn superoxide dismutase (Cu/Zn SOD) activity (EC 1. 15. 1.1) using the method of Nebot (1993), catalase (CAT) activity (EC 1. 11. 1.6) according to the method described by Sinha (1972), in addition to GSH concentration using method of Butler *et al.* (1963). Protein concentration were measured as described by Doumas and Biggs. The data were statistically analyzed using student t test explained by Kempthorn (1969).

RESULTS

Table (1) revealed significant increase in hepatic GSHpx, Cu/Zn SOD and lung Cu/Zn SOD activities in maternal group injected with vitamin E + Selenium compared with control group. As well as a high significant increase in GSH concentration was recorded.

Table (2 and 3) indicated that there were significant increase in liver, heart and lung GSHpx and Cu/Zn SOD activities of rat puppies obtained from dams injected with vit E + Se at one day followed by a high significant increase at 4 days, that increase become non significant at 9, 14 and 21 days compared with puppies obtained from control group.

Table (4) showed a non significant increase in liver, heart and lung catalase activity of rat puppies obtained from dams injected with vit E + Se compared with those obtained from control group.

Table (5) revealed significant increase in liver, GSH concentration of rat puppies obtained from dams injected with vit E + Se at one day and in liver, heart and lung at 4 day compared with puppies obtained from control dams.

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		GSHpx u/mg protein	SOD u/mg protein	Catalase u/mg protein	GSH u mol/mg protein
Liver	Control	102.5±6.21	11.54 ± 1.12	22.8±1.30	66.63±4.16
	Vit E + Se	126.63±5.94*	19.31±2.47*	26.66±1.73	97.13±6.5**
Heart	Control	14.47±1.93	23.00±2.25	13.49±2.63	7.09±0.94
	Vit E + Se	19.05±2.17	27.11±3.00	19.84±3.11	9.79±0.82
Lung	Control	6.99±0.97	12.99±1.02	18.66±1.91	10.21±1.02
	Vit E + Se	7.42±1.13	20.11±2.15*	20.00±2.43	12.76±1.54

 Table (1): Effect of vitamin E + Selenium on maternal liver, heart and lung antioxidant enzyme activities.

Table (2): Liver, heart and lung GSHpx activity (μ /mg protein) of rat puppies at different ages under the effect of maternal vit E + Se status.

		One day	4 day	9 day	14 day	21 day
Liver	Control	51.86±3.70	59.15±5.10	55.16±4.76	51.00±3.99	56.11±4.32
	Vit. E + Se	70.23±5.02*	113.88±8.89**	69.60±6.14	53.13±4.48	59.94±5.12
Heart	Control	66.66±5.61	70.32±4.09	63.17±4.88	64.00±3.9	66.11±5.81
	Vit. E + Se	93.33±6.43	100.54±5.12**	70.44±5.73	67.60±4.02	67.90±6.13
Lung	Control	43.01±3.25	41.70±3.60	48.43±4.10	46.70±3.97	40.28±4.06
	Vit. E + Se	60.28±4.49*	67.76±4.16**	60.01±5.21	53.50±4.66	51.12±5.19

Table (3): Liver, heart and lung Cu/Zn SOD activity (μ /mg protein) of rat puppies at different ages under the effect of maternal vit E + Se status.

		One day	4 day	9 day	14 day	21 day
Liver	Control	6.07±0.98	9.05±0.88	6.98±0.95	7.13±1.27	5.24±0.56
	Vit. E + Se	11.32±1.44*	15.73±1.32**	7.66±0.76	9.60±1.43	6.68±0.91
Heart	Control	4.21±0.86	5.00±0.42	5.28±0.99	6.43±0.89	4.49±0.42
	Vit. E + Se	8.32±0.94*	9.91±1.11**	7.94±1.12	8.04±1.87	5.68±0.57
Lung	Control	6.13±1.5	5.41±1.10	6.00±0.79	7.94±1.13	5.11±0.99
	Vit. E + Se	16.10±2.77*	18.19±2.22**	8.19±0.96	8.04±1.27	6.35±1.04

Results are presented mean ± S.E. * Significant at (P<0.05) ** High significant at (P<0.01).

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Table (4): Liver, heart and lung catalase activity (μ /mg protein) of rat puppies at different ages under the effect of maternal vit E + Se status.

		One day	4 day	9 day	14 day	21 day
Liver	Control	10.81±1.43	14.97±2.20	13.99±1.27	10.13±1.20	11.25±1.47
	Vit. E + Se	15.19±1.81	21.05±3.01	20.02±2.25	11.20±1.50	12.40±1.27
Heart	Control	12.00±1.23	10.14±1.07	10.50±1.16	11.43±1.30	9.43±0.98
	Vit. E + Se	16.83±2.12	13.02±1.21	12.88±2.00	15.04±2.22	9.93±1.56
Lung	Control	8.33±1.11	9.93±1.15	8.93±0.99	7.63±0.91	6.11±0.82
	Vit. E + Se	12.51±2.25	14.89±2.03	9.00±0.87	8.14±0.97	8.00±0.87

Table (5): Liver, heart and lung GSH concentration (µmol/mg protein) of rat puppies at different ages under the effect of maternal vit E + Se status.

		One day	4 day	9 day	14 day	21 day
Liver	Control	51.06±5.77	56.46±5.15	64.13±6.31	68.18±5.50	61.06±5.14
	Vit. E + Se	85.11±7.23*	99.29±7.85**	76.01±6.80	68.59±5.71	69.66±6.30
Heart	Control	45.00±4.12	35.77±3.40	39.60±3.80	35.53±3.16	36.18±2.79
	Vit. E + Se	49.39±5.00	51.58±4.20*	41.98±4.11	42.11±4.99	41.23±4.61
Lung	Control	46.74±3.42	41.39±3.7	45.46±4.13	39.58±3.88	31.06±3.00
	Vit. E + Se	56.79±5.58	60.14±4.49*	51.60±4.20	47.80±4.50	38.66±4.11

Results are presented mean ± S.E.

* Significant at (P<0.05)

** High significant at (P<0.01).

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DISCUSSION

The presented data in Table (1) were agreed with those recorded by Kakker et al. (1996) who suggested that Se act by strengthing defense system. Also, Turan et al. (1997) found that liver GSHpx activity of female rabbit were significantly increased with dietary selenium and vitamin E supplementation. Moreover, a high significant increase in hepatic, cardiac and renal GSHpx activity of stressed rat by selenium treatment (Manal and Soad, 1999) who contributed this results to the protective effect of selenium on antioxidant defense system. It act also as a cofactor for GSHpx which is acytoprotective enzyme as well as vit E that has been reported to be scavenger of oxygen free radicals (O₂, HO and peroxyl radicals) which initiate and propagate chain oxidation (Othman and El-Meseri, 1997). Moreover, Samy and Safinaz (2004) concluded that, the naturally occurring antioxidant agent as vit. E can minimize tissue injury and slow the degenerative process via neutralizing the oxidative stress induced changes. Also, Stajn et al. (1997) showed that selenium induced significant increase in hepatic and renal Cu/Zn SOD of rat. It could be explained the prophylactic role of selenium through stimulation of free radical scavenger, thus protecting glutathione which is naturally occurring antioxidant (Abd El-Magid, 2000).

In the present study, GSH content was similar to those of Hashem (1998), as selenium might have a role in activiation of gama glutamyl cysteine synthase and GR enzymes that responsible for reduction of GSSG to GSH.

Concerning puppies, our data were came in accordance to those reported by Bik *et al.* (1994) who found that GSHpx activity was high in ewes and lambs when ewes were injected with selenium. Also, selenium concentration was higher in liver of rats feed in different dietary level of selenium compared with those fed on a basal diet. Thus there was a good correlation between GSHpx activity and selenium in all tissues of rats supplemented with selenium including liver, kidney, muscles and lung while the greatest amount of selenium associated GSHpx was in liver and lung (Whanger and Butler, 1987).

Moreover, Pazak and Scholz (1996) recorded an increase in liver vitamin E of rats puppies nursed dams with high vitamin E level approximately 30 fold within 4 days post partum as well as a significant increase in heart and lung vitamin E concentration of rat offspring from dam fed vitamin E + Selenium diet.

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Thus, in the present study the higher GSHpx activity at one and 4 day could be attributed to transfer of essential trace element specially selenium from placenta to newly borned and through colostrums (Guesta, 1995). As well as, the increase in serum vitamin E concentration in newly born lambs of ewes, supplemented with vitamin E via mammary gland transfere (Jerne *et al.*, 1994), and as the protective effect of selenium on the antioxidant defense system via activity as cofactor for GSHpx which is cytoprotective enzyme and so enhance the endogenous antioxidant capacity (Othman and El-Mesiri, 1997).

Moreover, vitamin E has been reported to be scavenger of oxygen free radically which initiate and propagate chain oxidation. Also, help to maintain the concentration of GSH and enhance glutathione recycling. This synergism between vitamin E and selenium significantly increase GSHpx activity (Milad *et al.*, 2001) as well as modulate the suppression of SOD activity and GSH concentration induced by oxidative stress Abd Elsalam (2002) and induced by Aging Mahfouz *et al.* (2003).

Conclusion:

It was concluded that administration of vitamin E + Selenium to females in their breeding season is benefit to modulate maternal and their offspring tissue antioxidant system during the critical suckling period specially 1^{st} four days.

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تعتبر الأيام الأولى من العمر من أهم المراحل الحرجة فى حياة الصغار لذا استهدفت هذه الدراسة معرفة تأثير حقن فيتامين هـ ± السيلينيوم فى إناث الفئران البيضاء قبل وأثناء الحمل على نشاط بعض الأنزيمات المضادة للأكسدة فى أنسجة الكبد والقلب والرئة للصغار وأمهاتهم عند الولادة وخلال فترة الرضاعة وقد استخدمت لهذه الدراسة 20 من إناث الفئران البيضاء البالغة فى مرحلة التزاوج وصغارهم. تم تقسيم هذه الإناث إلى مجموعتين: المجموعة الأولى تتكون من 10 حيوانات استخدمت كمجموعة ضابطة تم تغذيتها

على عليقة حافظة ولم تحقن.

المجموعة الثانية تتكون من 10 حيوان تم تغذيتها على نفس العليقة وحقنت بفيتامين (هــ) ± سيلينيوم فى العضل بجرعة 20 مجم، 150 ميكروجرام/كجم من وزن الجسم على التوالى أسبوعيا لمدة ثلاثة أسابيع قبل التزاوج بين الفئران ثم تكرر حقن فيتامين هــ ± سيلينيوم أثناء فترة الحمل.

تم ذبح صغار الفئران بعد الولادة عند عمر يوم واحد، 4، 9، 14 و 21 يوم من الولادة ثم أخذت عينات من نسيج الكبد والقلب والرئة للصغار وذلك لقياس نشاط أنزيمات الجلوتاثيون بيروكسيديز والسوبر أكسيد ديسميوتيز والكتاليز بالإضافة إلى تركيز الجلوتاثيون المختزل وقد تم ذبح الأمهات بعد 21 يوم من الولادة وأخذت عينات من القلب والرئة والكبد وقياس الأنزيمات السابقة.

أظهرت النتائج زيادة معنوية فى نشاط إنزيمات الجلوتاثيون بيروكسيديز والسوبر أكسيد ديسميوتيز والكتاليز وأيضا تركيز الجلوتاثيون المختزل فى نسيج الكبد بالإضافة إلى زيادة معنوية فى نشاط أنزيم السوبر أكسيد ديسميوتيز فى نسيج الرئة للأمهات المحقونة بفيتامين هـ ± السيلينيوم بالمقارنة بالمجموعة الضابطة.

أما بالنسبة لصغار الفئران قد سجلت النتائج زيادة معنوية فى نشاط إنزيمات الجلوتاثيون بيروكسيديز والسوبر أكسيد ديسميوتيز والكتاليز فى أنسجة الكبد والقلب والرئة لصغار فئران المجموعة المحقونة عند عمر يوم متبوعة بزيادة عالية المعنوية عند عمر أربعة أيام هذه الزيادة أصبحت غير معنوية عند عمر 9، 14، 21 يوم بالمقارنة بصغار فئران المجموعة الضابطة. كما أظهرت النتائج زيادة معنوية فى تركيز الجلوتاثيون المختزل فى نسيج الكبد عند عمر يوم واحد وفى نسيج القلب والرئة والكبد عند أربعة ألمجموعة المحترف المجموعة المحقونة فيتامين (هـ) ± سيلينيوم بالمقارنة بصابطة. نسيج الكبد عند عمر يوم واحد وفى نسيج القلب والرئة والكبد عند أربعة أيام لصغار فئران المجموعة المحقونة فيتامين (هـ) ± سيلينيوم بالمقارنة بصغار المجموعة الضابطة.

تستنيج من هذه الدراسة أن حقن فينامين هـ ± السيلينيوم للأمهات في قتره ما قبل الحمل وخلالها مهم لزيادة أنشطة الأنزيمات المضادة للأكسدة في أنسجة الصغار وأمهاتهم. وخاصة في المرحلة الحرجة والتي تتمثل في الأيام الأولى من العمر.