Effect of Vitamin A on Biochemical Parameters of Adrenal Gland of Swiss Albino Mice

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Effects of high doses of vitamin A on the biochemical parameters of the adrenal gland of 10 d to 40 d old Swiss albino mice were studied by measuring total protein and cholesterol content, acid phosphatase and alkaline phosphatase enzyme levels. The animals were divided into four different age groups for both control and test groups. Control groups did not show any significant change in total protein amount, cholesterol content, acid phosphatase and alkaline phosphatase during the entire period of study. Total protein and cholesterol content of test groups decreased significantly at higher dose levels of vitamin A. Acid phosphatase and alkaline phosphatase contents were elevated at each dose level. The result of this study revealed that vitamin A is responsible for stimulation of adrenal gland activity through increased corticoid synthesis and membrane permeability.

Vitamin A is an important regulator of various vital processes such as cell proliferation, differentiation[1], cell permeability and enzyme activities in the body[2]. It has been recognized that vitamin A is essential for normal development and maintenance of many epithelial tissues including liver, gut and adrenal gland[3,4]. Vitamin A also serves as a potent antiinflammatory and antioxidant in human diseases. Vitamin A can also delay malignant transformation of cells[5,6].

The absorption of vitamin A in the body is almost complete, while very small quantities are excreted through faeces and urine. Vitamin A is essential for normal development of body, but large doses may cause side effects on biological systems[7-9]. Vitamin A supplementation with measles vaccines has a long-term effect on measles specific antibody concentrations and it improves measles control[10]. The endocrine system is an extremely sensitive system of body and vitamin A is an important factor for proper functioning of various endocrine glands[11].

Protective functions of adrenal gland during stress conditions help in maintaining the overall metabolism of the body. It also influences reproductive system[12]. Administration of high doses of vitamin A produces degenerative changes both in cortex and medulla[13]. In the present work, an attempt has been made to evaluate the effects of overdoses of vitamin A on biochemical parameters of adrenal gland.

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MATERIALS AND METHODS

Swiss albino mice of different age groups (10 to 40 d old) originally procured from All India Institute of Medical Sciences, New Delhi, were used for the study. The animals were housed in the departmental animal house under optimum conditions such as air-cooled rooms under controlled conditions of temperature (25±3°) and relative humidity (60±5%) with a 12 h light/dark cycle. The animals were maintained on standard mice feed (obtained from Hindustan Levers, New Delhi and Lipton Tiger Ltd., Delhi) and tap water (boiled water cooled to room temperature) was provided *ad libitum* to the animals. Soaked black gram and wheat were also given as a supplement to standard mice feed. The animals were divided into four test groups on the basis of their age. Group I consisted 10 d old mice, group II consisted 20 d old mice, group III consisted 30 d old mice and group IV 40 d old mice. For each test group corresponding age-matched control groups were also maintained. All experimental protocols were reviewed and accepted by the Institutional Animal Ethics Committee (IAEC) prior to the initiation of the experiment. Vitamin A used in the experiment was manufactured by USV Ltd, Mumbai using Aquasol technology. Each 2 ml ampoule contained 1 00 000 IU of retinyl palmitate in water for injection.

Experimental protocol:

Injections of 5 000 IU (0.1 ml) and multiples of 5 000 IU up to 25 000 IU (0.1 to 0.5 ml) of vitamin A were administered to animals of each age test group with a minimum of 10 animals/ dose. These doses were injected intraperitonialy with a micro syringe. Six animals were used for each age-matched control group. All age-matched control groups received only vehicle (olive oil and normal saline). The autopsy interval was 10 d after dosing for both test and age-matched control groups.

Biochemical evaluation:

Total protein content of the adrenal gland was determined in terms of nitrogen by Folin-Farmer's microkjeldahl method[14]. Total cholesterol of the adrenal gland was determined by the method reported by Zlatkis *et al*. using glacial acetic acid as solvent and ferric chloride as color reagent[15]. The amount of cholesterol was expressed in mg/g of tissue. Acid phosphatase and alkaline phosphatase activities of adrenal glands were determined by the method of Fiske and Subbarow, using buffer at 5.0 pH and 9.0 pH, respectively[16]. The phosphatase activity was expressed in Bodoansky units in terms of mg Pi/g/h.

Statistical analysis:

All results were expressed as mean±SEM. Student's't' test was applied to determine significance of the difference between the data obtained from the age-matched control and test groups. Multiple comparisons to elicit the significant difference between various groups were performed. The significance of difference was accepted at $p<0.05$.

RESULTS AND DISCUSSION

The experiments demonstrated that in the first group (10 d old mice) there was 100% mortality after administration of 5 000 IU dose of vitamin A. Therefore, all experiments for that group were abandoned. Observations revealed, variations in total protein content for all other test groups at various dose levels of vitamin A. Total protein content was reduced in all age groups. The reduction of total protein content was found non-significant at low dose level (5 000 IU) for second group. However, the reduction was found to be highly significant at 10 000 IU and 15 000 IU ($p<0.05$) dose levels, when compared to that in age-matched control group. The results presented in Table 1 indicate that as the dose of the vitamin A increases in third and fourth test groups the total protein content decreases. The data obtained from the biochemical analysis of total protein content for different experimental groups revealed that reduction occurs due to its rapid metabolism and increased biosynthesis to meet the condition of stress.

Cholesterol level in adrenal gland decreased non-significantly (Table 2) at 5 000 IU dose of vitamin A for all test groups. However, the reduction of cholesterol level was observed to be significant ($p<0.05$) at 10 000 IU and 15 000 IU dose levels for the second group, at 15 000 IU and 20 000 IU dose levels for the third group, at 15 000 IU, 20 000 IU and 25 000 IU dose levels for the fourth group. It has been observed that hypervitaminosis induced a decline in cholesterol amount. It is reported in several studies that a reduction of cholesterol level was observed in adrenal gland of mice, which is associated with an increase in levels of corticosteroids[17]. Therefore, it can be inferred that decrease of cholesterol amount during hypervitaminosis could be due to its conversion to corticosteroids.

Analysis of results presented in Table 3 and Table 4 indicate that as the dose of vitamin A increases in second, third and fourth test groups the activity of acid phosphatase and alkaline phosphatase increases significantly ($p<0.05$). The adrenal gland of mice showed enzymatic changes during various stages of development.

TABLE 1: EFFECT OF DIFFERENT DOSES OF VITAMIN A ON TOTAL PROTEIN CONTENT OF ADRENAL GLAND OF MICE

Dose IU	Protein content (mg/g)		
	Group II[b]	Group III[b]	Group IV[b]
Control[a]	60.41±2.051	68.89±1.843	70.50±1.999
5000	59.29±0.983*	63.27±0.927*	70.36±1.210*
10000	48.18±0.209**	60.44±1.229**	66.01±0.631*
15000	42.92±0.837**	59.14±0.510**	62.73±1.422**
20000	-	50.23±0.012**	59.94±0.587**
25000	-	-	52.33±0.794**

IU=International Unit, n[a]= mean of 06 replicate readings, n[b]= mean of 10 replicate readings, Group II, Group III and Group IV= test groups, *= not significant, **= significant (p<0.05)

TABLE 2: INFLUENCE OF DIFFERENT DOSES OF VITAMIN A ON CHOLESTEROL LEVEL OF ADRENAL GLAND OF MICE

Dose IU	Cholesterol Level (mg/g)		
	Group II[b]	Group III[b]	Group IV[b]
Control[a]	17.26±1.013	15.42±0.867	14.31±0.770
5000	17.18±0.410*	14.87±0.323*	14.26±0.491*
10000	14.07±0.202**	15.17±0.935*	12.56±0.613*
15000	9.90±0.279**	10.85±0.594**	10.32±0.359**
20000	-	7.54±0.214**	7.29±0.178**
25000	-	-	6.89±0.01**

IU=International Unit, n[a]= mean of 06 replicate readings, n[b]= mean of 10 replicate readings, Group II, Group III and Group IV= test groups, *= not significant, **= significant (p<0.05)

TABLE 3: EFFECT OF DIFFERENT DOSES OF VITAMIN A ON ACID PHOSPHATASE ACTIVITY OF ADRENAL GLAND OF MICE

Dose IU	Acid phosphatase activity (mgPi/g/h)		
	Group II[b]	Group III[b]	Group IV[b]
Control[a]	1.62±0.251	1.80±0.192	2.0±0.309
5000	1.70±0.180*	1.84±0.393*	2.18±0.210*
10000	1.97±0.037**	1.97±0.027*	2.31±0.139*
15000	2.69±0.056**	2.70±0.146**	2.77±0.101**
20000	-	3.39±0.059**	3.45±0.112**
25000	-	-	4.79±0.081**

IU=International Unit, n[a]= mean of 06 replicate readings, n[b]= mean of 10 replicate readings, Group II, Group III and Group IV= test groups, *= not significant, **= significant (p<0.05)

TABLE 4: INFLUENCE OF DIFFERENT DOSES OF VITAMIN A ON ALKALINE PHOSPHATASE ACTIVITY OF ADRENAL GLAND OF MICE

Dose IU	Alkaline phosphatase activity (mgPi/g/h)		
	Group II[b]	Group III[b]	Group IV[b]
Control[a]	5.96±0.183	6.14±0.145	7.15±0.162
5000	5.82±0.120*	6.25±0.091*	7.26±0.051*
10000	6.49±0.060**	6.33±0.112**	7.21±0.143*
15000	7.58±0.049**	6.99±0.133**	7.55±0.199**
20000	-	7.36±0.204**	8.49±0.125**
25000	-	-	8.68±0.010**

IU=International Unit, n[a]= mean of 06 replicate readings, n[b]= mean of 10 replicate readings, Group II, Group III and Group IV= test groups, *= not significant, **= significant (p<0.05)

The phosphatases are associated with the transport of substances across the cell membrane. It has been reported that increased acid phosphatase activity is responsible for elevation of lysosomal activity in the cells, which may lead to increase in degradation of matrix[18,19]. Therefore, hypervitaminosis has been implicated for damages at cellular level and it activates lysosomes to release proteases, which could degrade structural protein of adrenal gland. The alkaline phosphatase activity was reported to be associated with differentiation of cell and transport of metabolites across the cell membrane[20].

On the basis of results obtained in present study, it may be concluded that high doses of vitamin A stimulate adrenal gland activity. Vitamin A is responsible for increase in enzyme activity causing changes in membrane permeability, which may lead to biochemical changes in adrenal gland[21].

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