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Original Article

Hypervitaminosis -A is associated with increased risk of hepatotoxicity in swiss albino mice

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ABSTRACT

Background: Vitamin A is a fat soluble polyisoprenoid compound with cyclohexenyl ring. It has essential impacts on area of health include vision, cellular differentiation, growth, reproduction, organ development during embryonic and membrane structure and function. Objective: The present study was conducted to evaluate the effect of hypervitaminosis A on biochemical parameters of liver functions & association with increased risk of hepatotoxicity in Swiss albino mice. Methods: Forty Swiss albino mice were randomly divided into four Groups of 10 animals each group and dosed as follows: Group I, which was a control and given only corn oil (2 ml/kg body weight); Group II, III and IV were administered vitamin A orally (retinyl palmitate) 4000 I U/kg body weight/day/mice for four, eight and twelve weeks of experimental period. Fasting blood samples were collected at the end of 4, 8 weeks and finally at the end of the study i.e.12 weeks for estimation of biochemical parameters in all 4 groups. Results: In the present study, the effect of hypervitaminosis A showed a significant decrease ($P<0.05$) in total protein level in the Group IV, compared to I, II, &III Group and no significant change ($P>0.05$) were observed in the Group II as compared to both control and III group. The aspartate transaminase (AST), alanine transaminase(ALT), alkaline phosphatase (ALP) activity, Blood Glucose, serum calcium in the Group IV were significantly higher ($P<0.05$) as compared to Control, Group II and Group III and no significant change ($P>0.05$) were found in the Group II when compared to Control and Group III. Conclusion: It is concluded that chronic intake of vitamin A for twelve weeks or more is associated with increased risk for hepatotoxicity in Swiss albino mice then acute toxicity.

1. Introduction

The incidence of chronic hypervitaminosis A is becoming more frequent problem worldwide due to the to the increasing use of vitamin A supplements in children of developing countries where vitamin A deficiency is commonplace, treatment of skin disorders such as acne and the increased number of people taking excessive doses of vitamin A for supposed beneficial health effects as antioxidant. Vitamin A (retinoid) are fat-soluble vitamins, essential nutrient for human and are involved in a wide variety of biochemical functions including embryogenesis, vision, reproduction, skeletal development, neurodevelopment, growth, maintenance of epithelial tissues, cellular proliferation and differentiation processes. The vitamin is stored primarily in the liver and transported in plasma bound to a specific retinol-binding protein (RBP) [1]. The US Institute of medicine recommended dietary allowance of vitamin A is 1000 IU/day for children aged 1 to 3 years and 1320 IU for aged 4 to 8 years [2]. The ideal way to acquire adequate amounts of vitamin A is to consume a healthy and well-balanced diet. With the exception of sources such as polar bear or chicken liver, which contain approximately 18000 and 16000 to 17000 IU/g of vitamin A, respectively, it is unusual for individuals to develop hypervitaminosis A by consuming natural sources of this vitamin [3,4] or by excessive administration of vitamin A preparations [5]. Toxicity appears to occur only when the amount of vitamin A exceeds the binding capability of RBP. In addition, retinyl palmitate has a long biologic half-life and bioaccumulates. The combination of relatively rapid absorption with a low clearance can produce acute toxicity within hours after a sufficiently high dose (25,000 IU per kg body weight/day) and chronic toxicity after prolonged intake of substantially smaller doses (4,000 IU/kg daily for 3 to 4 months)) [6]. The liver is the

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main organ of vitamin A storage,[7-9] the concentration of vitamin A in liver is usually proportional to its intake [10]. Excessive administration of vitamin A and its derivatives can lead to fibrosis in the disse space and obstruction of sinusoidal blood flow, causing non-cirrhotic portal hypertension, hepatocellular dysfunction [11] , enlargement of the liver, spleen, lymph nodes [12], hepatomegaly and hepatotoxicity have also been reported [13]. A marked elevation in serum levels of aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) also reported [14-16]. some studies suggested that liver damage due to vitamin A and its derivatives occurs mostly after acute poisoning but rarely observed after chronic poisoning [14], while another study [17], showed that chronic intake could be attributed to vitamin A toxicity [13].The serum calcium and phosphorus level are not affected [18], while hypercalemia observed in another studies [19,20]. The aim of the present study was to find out further details, relationship between hypervitaminosis A and cell (hepatocyte) functioning of these tissues and an effort to resolve the controversy about the effect of hypervitaminosis A on biochemical parameters related to liver functioning.

2. Material and Method

2.1. Experimental animals: Forty Swiss albino mice (Wistar strain Mus musculus) 6-8 weeks old, obtained from the animal house of the department of zoology, M.D.S. University, Ajmer, India and weighing about 25-30 grams were selected for study. The animals were housed in standard environmental conditions and animals were exposed to natural day and night cycles. They were randomly allocated to four groups with ten mice each group of either sex. The animals were placed in propylene cages with stainless grill top and bedding of clean paddy husk. Mice were fed standard pelleted rodent feed (manufactured by Lipton Ltd. India) containing 17% protein, 11% fat, 47% carbohydrate, 2.5% minerals, 4.5% fiber,11.5% water, and 6.5% ash, and given filtered water in glass bottles ad libitum. The protocol was approved by the Institutional animal ethical committee. All the animals were taken care of and maintained as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA India). All the cages were marked and labeled appropriately.

2.2. Experimental Design

2.2.1 Study design

The mice were randomly divided into four groups of 10 animals each group and dosed as follows: Group I (control) given only corn oil (2 ml/kg body weight); Group II was administered vitamin A orally (retinyl palmitate, Arovit drops, Roche, Basle, Switzerland) 4000 IU/kg body weight/day/mice [21] , for four weeks; Group III & Group IV received similar dosed (6000 IU/kg body weight/day/mice) for eight and twelve weeks of experimental duration, respectively. At the end of the dosing period, the mice were sacrificed by severing the jugular vein, after light ether anesthesia, and 2.5 ml blood sample was collected from each animal into plain test tube and was incubated for 30 minutes before being centrifuged at 1000 rpm for 10 minutes. Thereafter, the serum was separated and collected in a clean, dry test tube for the estimation of serum biochemical parameters.

2.3. Evaluation of Biochemical Parameters

Serum was used for determination of biochemical parameters, at 37 °C by Semi autoanalyzer (Transasia, ERBA Chem-5 Plus, Transasia Bio-Medicals Ltd.) using Diagnostic reagent kits supplied by the same manufracturer. The parameters and the respective methods applied are the following: aspartate aminotransferase (AST) - Henry method (modified IFCC); alanine aminotransferase (ALT) - Henry method (modified IFCC); urea - enzymatic colorimetric, urease method; blood glucose – GOD/POD method; Total protein- Biurate method; Total bilirubin – Diazo method; Alkaline phosphatase – AMP method; serum calcium - OCPC/ Arsenazo method.

2.4. Statistical Analysis

The data were statistically analyzed and expressed as mean ± SD. Statistical analysis of the variance between control and experimental values was done using Student's-t test. Values of $P < 0.05$ were considered significant.

3. Results

3.1. Effect of Hypervitaminosis A on body weight changes

The Groups-III and Groups-IV showed a consistently decrease in the body weight over the experimental period. The mean body weight of Group III and IV at termination of experimental period were significantly decreased ($P <0.05$) as compared to the control group. Mice in Group II showed a comparatively less decrease in body weight over the four-week period, and the mean body weight at termination was not significantly different ($P > 0.05$) as compared to control and group-III. Group-IV showed a comparative progressive decrease in their body weight over the twelve week period and the values obtained at termination of the study was significantly higher ($P < 0.05$) as compared to Group-II and Group-III.

3.2. Effects of hypervitaminosis A on serum biochemical parameters

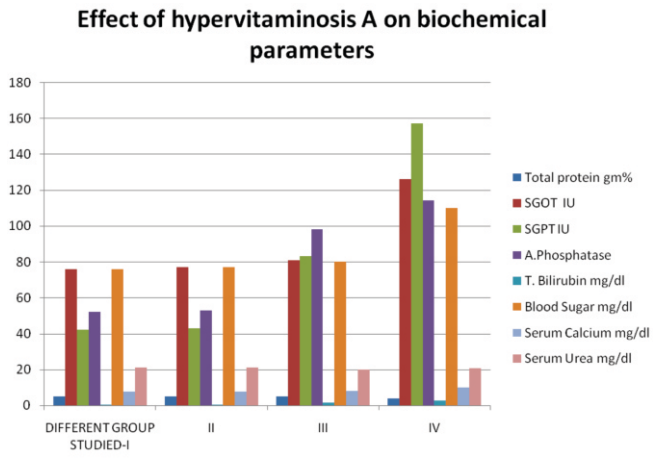
The Total Proteins (TP) concentration in the Group IV was very significantly Lower ($P<0.05$) when compared to Control, II, III Group. There was no significant change ($P>0.05$) in the TP concentration of mice in the Group II as compared to Control and III Group and also no significant decrease ($P>0.05$) in Group III as compared to the Group-I was present. The AST activity, Blood Glucose and serum calcium in the Group IV were significantly higher ($P<0.05$) when compared to Control, II, III Group. There were no significant change ($P>0.05$) in the AST activity, Blood Glucose and serum calcium of mice in the Group II as compared to Control and group III and also no significant increase ($P>0.05$) in Group III as compared to the Group-I. A non-significant ($P>0.05$) change in the urea concentration was observed when the values were compared among all Groups (Table 1).The ALT activity was significantly higher ($P<0.0001$) in the Group IV as compared to Control, II or III Group. The ALT activity in the Group III was significantly higher ($P<0.0001$) when compared to the Control and Group II but there was no significant change ($P>0.05$) in the Group II when compared to control Group. The ALP activity and serum Total Bilirubin was significantly higher in the IV group relatively to the Control ($P<0.0001$), II ($P<0.0001$) Group. The Group III showed significantly higher ($P<0.0001$) ALP activity and

Table-1: Effect of hypervitaminosis A on biochemical parameters

Biochemical Parameters	Groups			
Parameters	**Groups-I**	**Groups-II**	**Groups-III**	**Groups-IV**
Total protein gm%	5.06 ± 0.86	5.05 ± 0.73	4.81 ± 0.62	4.05 ± 0.38**
AST IU	76.4 ± 11.18	77.4 ± 11.73	81.5 ± 31.41	126.4 ±22.57**
ALT IU	42.7 ± 9.73	43.7 ± 10.99	83.9 ± 19.23**	157.7 ± 27.51**
A.Phosphatase	52.5 ± 10.16	53.6 ± 10.96	98.2 ± 19.70**	114.1 ± 26.73**
T.Bilirubin mg/dl	0.46 ± 0.20	0.52 ± 0.20	1.74 ± 0.76*	2.50 ± 0.86**
Blood Sugar mg/dl	76.16 ± 11.91	77.0 ± 12.0	80.05 ± 14.08	110.1 ± 16.05**
Serum Calcium mg/dl	7.62 ± 0.72	7.82 ± 0.98	8.01 ± 1.15	10.06 ± 1.33**
SerumUrea mg/dl	21.15 ± 3.29	21.14 ± 3.26	20.89 ± 3.22	20.85 ± 3.23

Values are expressed as mean ± SD, n=10, animals in each group. *p<0.05, (significant) **p<0.001 (highly significant) when compared to control Groups.

Figure



serum total bilirubin when compared to the control and Group II. There was no significant change (P>0.05) in the ALP activity in the Group IV compared to Group III but serum total bilirubin level significantly higher (P<0.001) in the Group IV as compared to Group III.

4. Discussion

Hypervitaminosis A is a hidden common problem in developing countries due to high rates of illiteracy, untrained or less trained paramedical staff or half knowledge about the antioxidants due to exaggerated publicity of various products of antioxidants available in market. The picture of chronic hypervitaminosis A is highly variable, but anorexia, dry itchy skin, alopecia, bone pain, increased intracranial pressure, and hepatosplenomegaly are the most common manifestations [22,23]. The present study reporting that prolonged high dose of Vitamin A (retinyl palmitate) administration causes significantly decrease in total body weight due to decrease food intake as a result of lack of appetite comparable to the control group .This finding agreed with what had been reported in earlier studies [24]. In the present study, there was significant decrease in the total proteins concentration in the Group IV mice who were received vitamin A for prolonged period (12 weeks) as compared to I (control), II (4 weeks), & III (8 weeks) Group mice who were received no or less vitamin A during the experimental duration. Similar results have been reported in previous studies after chronic intoxication of vitamin A [15]. Enlargement of the liver, spleen and lymph nodes have been reported in adults due to hypervitaminosis A [12]. Liver damage due to vitamin A occurs after acute poisoning [14]. Excessive administration of vitamin A can lead to fibrosis in the Disse space and obstruction of sinusoidal blood flow, causing non-cirrhotic portal hypertension and hepatocellular dysfunction [11]. Elevated serum concentrations of aspartate transaminase (AST), alanine transaminase (ALT) and occasionally lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) have been reported after chronic intoxication of vitamin A due to cellular likage [14,15,16]. Our result in accordance to the above findings. In the present study, Blood Glucose and serum calcium in the group IV were significantly increased when compared to Control, II and III group. Similar results supported by pervious study [19,20,12]. Hypercalemia due to vitamin A suppressed serum parathyroid hormone and direct stimulation of bone resorption i.e. increase osteoclastic activity [25]. The serum total bilirubin was significantly increases due to hepatotoxicity with the length of experimental period. The chronic toxicity of vitamin A is accumulative. When administration of excess vitamin A is prolonged, the limits of hepatic storage and RBP binding capacity are exceeded. Retinyl ester, rather than retinol, is mobilized from the liver and made far more available to membranes than would be usual. It has been considered that the amphiphatic nature of retinyl ester and detergent-like action may be responsible for the labialization of various organelle membranes, resulting in the release organelle contents or alterations in membrane function. However, it is generally accepted that surfactant detergent-like activity is probably relevant only at extremely high concentrations. The more recent concept is that most retinoid-induced toxicities result from nuclear receptor mediated interaction and ensuing altered gene expression [26].

5. Conclusion

In conclusion, the present study demonstrates that intake of excessive vitamin A has deleterious effects on liver that depends on the dose and duration of intake. Therefore we recommend that vitamin A preparation should not be taken excessively as dietary supplement to prevent vitamin A deficiency, as antioxidant and in the self treatment of various skin disorders.

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