

## STUDY ON VARYING LEVELS OF DIETARY VITAMINE AND DEVELOPMENT OF AFLATOXIN (AFB<sub>1</sub>) TOXICITY IN MALE ALBINO RATS

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The term 'tocopherol' was proposed by Evan from the Greek word 'tocos' meaning childbirth or 'offspring' the Greek noun "phero" to bring forth and ol for alcohol.<sup>6</sup> There are eight naturally occurring tocopherol derivatives. Six are toco derivatives and two are toco trienols. Vitamin E function is that the vitamin protect the integrity of cellular membrane against free radical attack. Vitamin C could also protect cells against damage by free radicals from exogenous sources. The most biologically active of these compounds are RRR-  $\mu$  - tocopherol<sup>1</sup>. Vitamin E is on antioxidant and plays a role in preventing the oxidation of carotene and vitamin A in the digestive tract, prevents atherosclerosis, lowers Cholesterol. Vitamin E protects the lungs and other tissues from damage by pollutants and protects RBC against destruction by poisons in the blood stream and helps to form red blood cells and is involved in the productions of energy in the heart and muscle, it also protects white blood cells and participates in body's immune defense. Vitamin E requirement is related to polyunsaturated fatty acids intake. The adult human requirements may vary from 10 - 30 mg per day<sup>15</sup> and an intake of 0.5 mg per day was suggested as a minimum intake for an infant<sup>13</sup>. In many studies it was found that vitamin E has a protective role against liver toxicity caused by carbon tetra chloride, nitrosamine, nitrites etc. Vitamin E might have some sparing effect on AFB<sub>1</sub> induces liver lesions.<sup>17</sup>

**Objective of the study-1.** To observe the role of different levels of dietary vitamin E on aflatoxicosis in male adult albino rats. 2. To observe the normal level of dietary vitamin A on aflatoxicosis in male adult albino rats. 3. To observe the effect of aflatoxin toxicity on the blood haemoglobin and plasma protein, plasma vitamin A and E.

**METHODOLOGY-**The present study was undertaken to study the anticarcinogenic property of vitamin E in rats fed low, normal and excess level of the vitamin E for one month and then injecting aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) to study the effect on various body organs.

Diet Preparation Sago, casein was purchased from the local market. The protein content of casein was estimated and mixed with sago to obtain 10 % protein. Sago, casein, salt and vitamin mixture were a mixed well and for used for feeding the male albino rats for one month.

**ANIMAL EXPERIMENT-**Thirty adult male albino rats of the Charles Foster strain weighing 92.5 to 168.0 gm were taken and divided into three groups. The rates in each group were matched for age and body weight. The animals were caged individually in ordinary galvanized iron cages. The rats were fed daily and the left over was collected everyday in brown bags, dried at 100 C and weighed to determine the actual food intake. Body weights were recorded once in a week. The animals were fed for 28 days, on 29<sup>th</sup> day control group animals were injected with Dimethyl sulfoxide (DMSO) as placebo and the experimental animals from each group were injected AFB<sub>1</sub> (AFB<sub>1</sub> concentration was 20 mg in 100 ml of DMSO or 0.4 mg /ml therefore 0.5 ml / 100 gm of body weight). Animals were sacrificed exactly 24 hours after injection and blood was collected in previously heparinised tube by heart puncture, a small portion of blood was used for haemoglobin estimation and the remaining was centrifuged at 4000 rpm for 15 minutes at 4 C for plasma separation. The liver, kidney, heart and lung were collected and washed with saline to remove blood and blotted on filler paper and weighed for fresh weight (liver, kidney). The known weight of liver, kidney and lung were homogenized in phosphate buffer (p<sup>H</sup> 7.0) within a homogenizer (elteck moter ) at 4000 rpm for 2 min and 10 % tissue homogenate was prepared. This tissue homogenate was used for the analysis of vitamin E and for vitamin A analysis known weight of liver was transferred to 5 % KOH and stored in freezer.

**BIOCHEMICAL ANALYSIS-**The blood haemoglobin was estimated by cyanmethemoglobin method<sup>10</sup> using kit supplied by Oscar biotech pvt. Ltd. New Delhi. Plasma protein was estimated by the Biuret method given by Reinhold (1953). Plasma vitamin A

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was estimated by the method of Neeld and Pearson (1963). Plasma vitamin E was estimated by the method given by Emmerie and Engel (1938) modified by Desai (1984). Liver protein was estimated by the method of Lowery et. al. (1951). Liver, kidney, lung and heart vitamin E was estimated by the method given by Emmerie and Engel (1938) modified by Desai (1984).

**STATISTICAL ANALYSIS**-The results were expressed as mean + standard error of mean (SEM). Data were analyzed with 't' test to determine the significance of difference among individual groups.<sup>9</sup>

**RESULTS AND DISCUSSION**-As mentioned earlier, vitamin E plays a major role in protecting the body particularly the liver in developing cancer. Many studies have been conducted by feeding carcinogenic compounds for long term and therefore in the present study the carcinogenic compound aflatoxin (AFB<sub>1</sub>) was injected in the animals after feeding the animals

low vitamin E (1.88mg), normal vitamin E (12.81mg) and Excess vitamin E (45.6mg) in the diet for four weeks. Animals were fed 10 % casein protein diet Table-1 indicates the vitamin E intake of animals fed low, normal and excess vitamin E in the diet. On an average the low vitamin E fed animals consumed some where around 0.31 mg, normal vitamin E 2.1 mg and excess vitamin E 7.42 mg proportionately. The total food intake of 28 days also increased.

**Table-1 Vitamin E intake (mg) of animals fed low, normal and excess vitamin in experimental diet.**

Group	Tocopherol intake(mg)	
	Total	Perday
Low Vitamin E	8.68+0.09	0.310+0.003
Normal Vitamin E	58.68+0.45	2.100+0.016
Excess Vitamin E	207.75+1.90	7.420+0.068

• Ten animals in each group  
• Values are mean + SEM.

**Table-2 Blood hemoglobin(gm %), Plasma protein (gm %) plasma vitamin A,E(ug %).**

Group	Type	BloodHb. (gm %)	Plasma		
			Protein(gm %)	Vitamin Aug %	Vitamin Eug %
Low Vitamin E	C	13.790+0.212	5.780+0.223	50.040+0.994	0.992+0.072
	E	14.950+0.998	4.560 <sup>b</sup> +0.325	34.290 <sup>b</sup> +3.872	0.748 <sup>b</sup> +0.029
Normal Vitamin E	C	13.210+0.530	5.730+0.209	50.210+2.305	1.511+0.258
	E	16.080 <sup>c</sup> +0.596	4.900 <sup>a</sup> +0.271	36.870 <sup>c</sup> +2.155	1.151+0.154
Excess Vitamin E	C	14.230+1.004	5.620+0.166	47.520+4.066	1.777+0.199
	E	15.880+0.425	5.210+0.387	41.750+3.922	1.407+0.147

• C and E denotes control and experimental group.

• Significantly different at P<0.05<sup>a</sup>, P<0.02<sup>b</sup>, P<0.01<sup>c</sup> compared to their respective control.

The blood haemoglobin was increased by 1.0 gm to 2.5 gm during 24 hours. The maximum blood haemoglobin increase was observed in normal vitamin E fed animals i.e.21.7%, excess vitamin E fed animals showed 11.6 % increase and low vitamin E fed animals showed minimum increase i.e. 8.4%. One study showed that swine fed a diet for 32 days contaminated with AFB<sub>1</sub> have shown increased amount of hemoglobin in their experiment.<sup>17</sup> Plasma protein values decreased by about 0.5 to 1.0 gm in experimental animals compared to normal. The maximum reduction of plasma protein was observed in low vitamin E fed animals i.e.21.1 % and minimum reduction in plasma protein was observed in excess vitamin E fed animals i.e.7.3%. A highly significant difference (P<0.02) in the protein level was seen in the low vitamin E fed animals. A significant different (P<0.05) in a protein level was seen in normal vitamin E fed animal and no significant difference but low values was observed in excess

vitamin E fed animals. effectively decreases the inhibitory action of the toxin on protein metabolism. The protein metabolism is affected and shown a decrease in plasma protein where AFB<sub>1</sub> was injected in rats and Broiler.<sup>2,3</sup> Aflatoxin inhibits protein synthesis and disrupts DNA dependent RNA transcription.<sup>8</sup> As mentioned earlier, the main objective of this study was to study the extent of liver damage caused by injecting AFB<sub>1</sub> at various levels of dietary vitamin E and to find out the amount of vitamin E utilized for protection of liver damage. Plasma was estimated for having properties of protecting the liver from carcinoma therefore these plasma vitamin were studied.(Table-1). The plasma vitamin E value in control animals went on increasing with an increasing amount of vitamin E in the diet. A minimum value of 0.99 mg % was observed in low vitamin E fed animals and a maximum value of .1.8 mg % in the excess vitamin E fed animals where as the normal showed 1.5 mg %

vitamin E in the plasma. The AFB<sub>1</sub> injected group showed a maximum difference in the vitamin E and minimum in the excess vitamin E fed animals. The values in normal and excess vitamin E fed animal injected with AFB<sub>1</sub>, still show higher values than the control animal fed low vitamin E. The low vitamin E fed animals showed a significant difference ( $p < 0.02$ ). The maximum reduction of plasma vitamin E was observed in the vitamin E fed animal (24.5%) while the animals (20%). Roger et.al. (1994) reported that AFB<sub>1</sub> treated swine had less serum tocopherol than control group and suggested that tocopherol concentration decreased by aflatoxin exposure. The obtained result that excess amount of vitamin E protect the liver and still it can resist the higher dose of AFB<sub>1</sub>. As far as vitamin A is concerned that plasma value of vitamin A are quite normal plasma vitamin A was practically same in all the three control groups. A slight decrease was observed in the excess vitamin E fed animals but the values are not statistically significant. The values

of low vitamin E and normal E fed animals showed significant difference ( $p < 0.01$ ) than the excess vitamin E fed animals. The dietary intake of vitamin A was practically same in all the groups. After injecting AFB<sub>1</sub>, maximum reduction was seen in low vitamin E fed animals i.e. 31.4%, comparatively low reduction in normal vitamin E fed animals i.e. 8.6%. This suggests that compared to vitamin E, reduction in vitamin A is much more. This is the similar result which was obtained by Roger et.al. (1994) in AFB<sub>1</sub> treated swine, they had low serum retinol than the control group. Firozi et.al. (1987) also reported that retinol and its physiological derivatives have a great potential to modify carcinogenesis induced by AFB<sub>1</sub> and so depletion of retinol was found in AFB<sub>1</sub> treated animals. It is possible that vitamin A may be playing a role in protecting the liver damage caused by AFB<sub>1</sub> and therefore both vitamin E and vitamin A reduce in the plasma in maximum amount in low vitamin E fed animal where as minimum in excess vitamin E fed animals.

**Table-3 Liver protein (gm%), Liver vitamin A (mg%), Liver, Kidney, Lung and heart vitamin E (mg/g,) of animal fed low, normal and excess tocopherol with and without AFB<sub>1</sub> treatment**

Group	C/E	Liver		Vitamin E (mg/gm)			
		Protein gm%	Vitamin A mg%	Liver	Kidney	Lung	Heart
Low vitamin E	C	12.150 ± 0.727	7.498 ± 1.440	18.350 ± 2.257	16.190 ± 1.075	14.333 ± 0.747	15.530 ± 2.072
	E	10.480 ± 0.274	4.524 ± 0.717	13.320 ± 1.718	19.970 ± 1.311	14.800 ± 2.886	17.060 ± 2.242
Normal Vitamin E	C	11.730 ± 0.350	4.566 ± 0.968	39.050 ± 1.569	23.660 ± 2.869	23.930 ± 0.797	22.230 ± 2.969
	E	11.450 ± 0.516	4.180 ± 0.438	32.066b ± 1.622	20.360 ± 2.869	25.300 ± 1.316	23.130 ± 2.501
Excess Vitamin E	C	12.050 ± 0.408	3.864 ± 1.084	55.990 ± 20276	36.500 ± 4.138	33.340 ± 1.904	31.950 ± 5.400
	E	11.440 ± 0.272	5.220 ± 1.044	50.660 ± 1.602	30.390 ± 4.060	38.550 ± 2.208	26.550 ± 3.369

Significantly different at  $P < 0.02^b$  compared to their respective control.

Liver was analysed for protein vitamin A and vitamin E content. The data of protein vitamin A and vitamin E enumerated in table no 3. All the values of protein were in the normal range but protein content is reduced by around 0.5 to 1.5 gm in animals treated with AFB<sub>1</sub>. This observation suggests that the effect is much more in animals fed a low vitamin E diet. In accordance with Beers et.al. (1992) reported that aflatoxin inhibits protein synthesis and disrupts DNA dependent RNA transcription (Gelboin et.al. 1966). The liver vitamin A values showed a different trend in the control animals though vitamin A in the diet was the same in all the groups. Vitamin A is highest in the animals fed a low vitamin E diet. The value is reduced

where normal vitamin E is given and further reduced when excess vitamin E is given. The observation suggests that vitamin A is sorted or the binding site in a cell is occupied by vitamin E and therefore vitamin A replaced vitamin E in low vitamin E diet where as in excess vitamin E diet maximum amount of vitamin E was stored and therefore less amount of vitamin A was found in the liver. The liver was also analyzed for its vitamin E contents similarly kidney, lung and heart were also analyzed for this vitamin (Table - 3). The maximum amount is deposited in the liver next can be kidney, lung and heart. As expected all the values are low in animals fed low vitamin E diet in and some where higher in the normal vitamin E fed animals and highest

in the excess vitamin E fed animals liver vitamin E decreased in the experiments group their the control group. Maximum reduction in the kidney vitamin E fed was observed in the excess vitamin E fed animals and the maximum reduction was observed in low vitamin E fed animals kidney vitamin E levels decreased in the experimental group composed to control. Lung vitamin E was increased in excess vitamin E fed animals where as in heart vitamin E, a maximum reduction was observed in excess vitamin E fed animals and minimum increase was observed in low vitamin E fed animals. In accordance with Roger et, al. (1994) reported that AFB<sub>1</sub> Dreaded it had depletion in liver vitamin E compared to control. Lathwa and Blum (1989) reported that in

vivo studies clearly indicate that  $\mu$  tocopherol can clearly indicate that alpha tocopherol can directly reduce the corcinogenicity of performed NNC and also successfully inhibit the endogenous conditions. They also suggest that  $\mu$  tocopherol then injected simultaneously with fast may reduce humon exposure to carcinogenic NNC.

**COUCLUSION**-It can be concluded that vitamin E does have an anticarcinogenic property against AFB<sub>1</sub> induced toxicity. Vitamin E greatly reduces the toxicity of AFB<sub>1</sub>, mainly in different body organ like the liver, kidney, lungs. It can also protects the body from cancer, heart disease etc.

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