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Sub-chronic (90 day) oral toxicity study in rats with lutein diacetate

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Abstract

The purpose of this study was to investigate the safety of Lutein diacetate (nutraceutical) by determining its potential toxicity following 90 days sub chronic administration in Sprague Dawley rats. The Lutein diacetate was administered orally by gavage as a suspension in corn oil at the graded doses of 2.1, 22.5 and 210 mg/kg b.wt./day for 90 consecutive days. Animals in the control recovery and high dose recovery groups were further observed for 28 days without any treatment. The changes observed were stained (light brown coloured) faeces at 210 mg/kg body weight/day dose during the 90 day treatment period and normal coloured faeces were observed during the recovery period. No treatment related effects were seen in any of the parameters monitored in rats given 2.1, 22.5 and 210 mg/kg body weight/day of Lutein diacetate. Results indicate that oral dose of Lutein diacetate is relatively safe in rats up to 210 mg/kg body weight/day.

Keywords: Lutein diacetate, 90 day exposure, NOAEL (No Observed Adverse Effect Level), Sprague Dawley rats, toxicity

Introduction

Lutein is a naturally occurring carotenoid and is widely distributed in fruits and vegetables, particularly concentrated in flowers. Epidemiological studies suggest that a high Lutein intake (6 mg/day) is associated with a lower risk of cataract and age related macular degeneration (Richer *et al.*, 2004; Samimi, 2005). Long term antioxidant supplementation with Lutein and α-tocopheral not only improved serum lutein levels but also improved visual acuity in clinical studies (Olmedilla *et al.*, 2003). Many of the clinical studies suggest that Lutein has potential antioxidant activity (Duncan *et al.*, 2002; Kruger *et al.,* 2002). The safety profile of Lutein as a dietary ingredient has been determined in experimental animals (Duncan *et al.*, 2002). The product investigated in this project was lutein concentrate (Soybean oil dispersion at 20% activity) which is extracted from marigold flowers (*Tagetes erecta*) purified and refined in crystalline form. Lutein is an OTC (Over The Counter) product in US and other international markets for past several years. The evidence of its use in clinical studies has been documented (Richer *et al.*, 2004; Olmedilla *et al.*, 2003; Duncan *et al.*, 2002; Kruger *et al.,* 2002). This product can be used as a lifetime nutraceutical substitute. Very limited information is available on the toxic manifestations of Lutein diacetate; further no clear guidelines for assessing toxicity of nutraceuticals are available. Hence, the present study was designed based on WHO guidelines (2000) and available literature (Spainhour, 2001). The present 90 day study was designed and conducted in accordance with OECD Guideline (1998).

The objective of this 90-day oral toxicity study was to assess the systemic toxic potential of the Lutein diacetate in Sprague Dawley rats when administered by oral gavage and also to obtain an estimation of No Observed Adverse Effect Level. This study may provide a rational basis for human health risk assessment.

Materials and Methods

Test Chemical

The test article Lutein diacetate was manufactured and supplied by Industrial Organica,

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S.A. de C.V.Ave. Almazan No. 100, Col. Topo Chico, Monterrey N.L.64260, Mexico. It was an oil suspension with a purity of 20.60%. The Lutein diacetate was mixed with corn oil and the stability and homogeneity in the formulation mixture were confirmed using HPLC with a UV detector.

Laboratory Animals and Care

Young adult, healthy, Sprague Dawley rats of 7 to 8 weeks, weighing between 211-250 g (males) and 152-190 g (females) at the start of the study were used for this experiment. These animals were bred and maintained at Advinus Therapeutics Private Limited, Bangalore. Rats selected for this study were checked for health status and were maintained under standard laboratory conditions, air conditioned with adequate fresh air supply (12-15 air changes/hour), environment: with temperature 20-24°C, relative humidity 30-70% with 12 hours light and 12 hours dark cycle. The rats were housed 2 per cage in sterilized suspended polysulphone cages. The rats were given pelleted feed (Ssniff Spezialdiaten GmbH., Germany) and deep bore well filtered water was given *ad libitum*. The animals were acclimatized for 5 days prior to administration of Lutein diacetate.

Treatment

Groups of 10 male and 10 female rats were administered Lutein diacetate formulations in corn oil by gavage at the graded doses of 2.1 (low), 22.5 (mid) and 210 (high) mg/kg/day once daily for 90 consecutive days. The concurrent vehicle control group rats received corn oil alone. In addition, we had two separate groups of rats at the control and the high dose which were treated like the test of the group for 90-days followed by observation for a 28-day recovery period without any treatment.

In-life Observations

All rats were observed for clinical signs, mortality, eye abnormalities, functional (neurological) observations and change in weekly body weights and feed intake. Detailed neurological examination was conducted. During neurological examination, UGO Basile Multiple Activity Cage Model No. 7445 was used to study the locomotor activity of rats. The apparatus Chatillon DFIS 2 MODEL described by was used to measure fore limb and hind limb grip strength sequentially in each rat.

At the end of treatment and recovery period, haematology, clinical chemistry, parameters were monitored. Haematology, clinical chemistry and prothrombin time analysis were determined by using instruments ADVIA 2120 haematology system (Bayer Health Care LLC, USA), Roche/ Hitachi 902 Analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan) and ST-art-4 coagulation analyzer (Diagnostica stago, 92600 Asnieres, France), respectively.

At study termination, detailed necropsy and organ weights were carried out on all animals. The tissues were processed by routine paraffin embedding and 5μ sections were stained with Mayer's Hematoxylin-Eosin stain. Histopathological evaluation was carried out on full set of tissues collected from 210 mg/kg b.wt./day (high dose) and vehicle control group. All gross lesions from all dose rats were also evaluated histologically.

Statistical Analysis

The data of body weights, net body weight gains, feed intake, neurological observations, haematology and clinical chemistry were compared by Bartlett's test and then subjected to one-way analysis of variance (ANOVA – Snedecor and Cochran, 1987). When F was found significant, Dunnett's Pair wise comparison (Scheffe, 1953) was done individually. Organ weight and organ weight ratio data was analyzed by Student's t-test.

Results and Discussion

In the present investigation, the toxicity of Lutein diacetate in Sprague Dawley rats has been studied in order to demonstrate the safety profile of Lutein diacetate in case of long-term consumption. There were no treatment related mortalities, clinical signs of toxicity or ophthalmological changes throughout the course of the study at 2.1 and 22.5 mg/kg/day dose rats. Animals treated at 210 mg/kg b.wt./day exhibited stained (light brown coloured) faeces during the treatment period. Stained (light brown coloured) faeces were also observed during first 4 days after

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withdrawal treatment however, during subsequent days of recovery period normal coloured faeces was observed, indicating reversibility of the finding. The colouration of the faeces in rats at 210 mg/kg b.wt./day is probably due to the presence of carotenoids (Lutein). It can be corroborated that the presence of carotenoids in the faeces is due to the excretion of excessive dose of carotenoid. This observation was reversible as normal coloured faeces were observed during the recovery period. Functional observational battery tests including grip strength and motor activity measures performed on test item treated group rats up to the highest dose (210 mg/kg b.wt./day) did not reveal any treatment related abnormalities (Table 1). Also, the test item did not affect the mean body weights, net body weight gains (Table 2) and food consumption (data not presented) at any of the doses tested. Laboratory investigations showed no treatment related changes in the haematological (Table 3) and clinical chemistry (Table 4) parameters. There were no treatment related changes in terminal fasting body weights, absolute organ weights, relative organ weights (Tables 5 & 6) and gross or histopathological changes (data not presented). Gross and histological examination, did not reveal any treatment related lesions in any organs.

The safety of Lutein in the past was demonstrated by feeding whole plant materials containing Lutein (Kruger et al, 2002). It is generally accepted that a greater intake of fruit and vegetables can help prevent heart diseases and mortality, implying indirectly the impact of Lutein in these materials. Since fruits and vegetables are

Table 1. Effect of Lutein diacetate on functional observation battery.

Dose (mg/kg/day)		Motor activity		Grip strength (kg)			
		Males	Females	Males		Females	
				Fore limbs	Hind limbs	Fore limbs	Hind limbs
Vehicle control	Mean	1764	2114	1.026	0.537	1.012	0.500
	SD	393.2	291.5	0.071	0.068	0.062	0.060
	N	10	10	30	30	30	30
2.1	Mean	1585	2050	1.023	0.552	0.968	0.526
	SD	441.2	330.0	0.069	0.073	0.071	0.058
	N	10	10	30	30	30	30
22.5	Mean	1887	2265	1.020	0.546	1.004	0.521
	SD	277.6	215.6	0.069	0.060	0.063	0.056
	N	10	10	30	30	30	30
210	Mean	1586	2041	1.002	0.529	0.988	0.508
	SD	370.7	195.6	0.069	0.064	0.064	0.052
	N	10	10	30	30	30	30
Vehicle control recovery	Mean	1768	2158	0.993	0.538	0.992	0.502
	SD	545.5	205.9	0.076	0.075	0.070	0.063
	N	10	10	30	30	30	30
210 recovery	Mean	1929	1890[a]	0.971	0.515	0.969	0.507
	SD	627.3	168.7	0.045	0.061	0.050	0.054
	N	10	10	30	30	30	30

a: significantly lwer than the vehicle control recovery group. Values are mean ± S.D, N: No. of observations

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Table 2. Body Weights and Cumulative Net Body Weight Gains in rats following consecutive daily oral administration for 90 days.

Dose (mg/kg/day)		Males Initial Bwt	Males 13 week-Bwt	Males 13 week Net Bwt Gains	Females Initial Bwt	Females 13 week-Bwt	Females 13 week Net Bwt Gains
Vehicle control	Mean	246	437	191	175	269	94
	SD	9.52	33.66	29.17	7.64	21.22	15.89
	N	10	10	10	10	10	10
2.1	Mean	244	455	211	175	270	94
	SD	10.04	29.27	22.95	12.62	16.06	7.36
	N	10	10	10	10	10	10
22.5	Mean	244	445	201	174	271	97
	SD	10.81	28.18	21.94	10.23	14.34	10.54
	N	10	10	10	10	10	10
210	Mean	245	449	204	176	274	98
	SD	10.02	37.98	29.84	8.77	9.07	6.49
	N	10	10	10	10	10	10
Vehicle control recovery	Mean	244	450	206	173	266	93
	SD	8.26	19.29	15.41	8.85	10.30	6.10
	N	10	10	10	10	10	10
210 recovery	Mean	244	455	211	171	266	95
	SD	12.11	34.09	23.67	8.42	18.00	12.10
	N	10	10	10	10	10	10

Values are mean ± S.D, N: No. of rats, Bwt: Body weight

Table 3. Effect of Lutein diacetate on haematological parameters.

Dose (mg/kg Bwt/day)		Males WBC G/l	Males RBC T/l	Males Hb g/l	Males Hct l/l	Males P.T. s	Females WBC G/l	Females RBC T/l	Females Hb g/l	Females Hct l/l	Females P.T. s
Vehicle control	Mean	10.90	9.54	172	0.520	15.7	7.28	8.27	153	0.464	16.1
	SD	1.86	0.25	6.43	0.01	0.76	2.30	0.39	5.86	0.02	0.56
	N	10	10	10	10	10	10	10	10	10	10
2.1	Mean	10.41	9.31	170	0.510	15.3	8.10	8.50	157	0.471	15.3
	SD	1.79	0.48	7.99	0.03	0.94	2.98	0.29	4.46	0.01	1.08
	N	10	10	10	10	10	10	10	10	10	10
22.5	Mean	10.86	9.53	172	0.521	14.9	7.87	8.37	153	0.469	14.8[a]
	SD	2.62	0.31	7.52	0.02	0.77	2.01	0.29	3.86	0.01	1.56
	N	10	10	10	10	10	10	10	10	10	10
210	Mean	10.13	9.65	173	0.530	14.3[a]	8.14	8.39	155	0.476	15.0
	SD	1.51	0.32	4.24	0.01	0.41	1.36	0.26	4.78	0.02	0.73
	N	10	10	10	10	10	10	10	10	10	10
Vehicle control recovery	Mean	10.84	9.82	174	0.521	16.8	6.57	8.94	171	0.485	15.0
	SD	1.71	0.35	5.09	0.02	1.46	1.31	0.36	4.27	0.02	1.61
	N	10	10	10	10	10	10	10	10	10	10
210 recovery	Mean	10.88	9.78	171	0.519	16.3	7.28	8.77	170	0.480	14.6
		2.12	0.48	6.46	0.02	1.54	1.64	0.32	5.38	0.01	1.72
	N	10	10	10	10	10	10	10	10	10	10

Values are mean ± S.D, N: No. of rats, a: Significantly lower than the vehicle control group

Table 4. Effect of Lutein diacetate on clinical chemistry parameters.

Dose		Males							Females						
(mg/kg Bwt/day)		Glu mmol/l	BUN mmol/l	T.Pro g/l	AST U/l	ALT U/l	Creat μmol/l	Chol mmol/l	Glu mmol/l	BUN mmol/l	T.Pro g/l	AST U/l	ALT U/l	Creat μmol/l	Chol mmol/l
Vehicle control	Mean	6.37	1.98	68.2	75	66	48	2.27	6.33	1.72	67.2	72	55	51	2.24
	SD	1.49	0.28	3.43	7.91	12.83	3.37	0.36	0.71	0.24	2.78	7.45	7.07	3.28	0.20
	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
2.1	Mean	6.81	1.71	68.6	73	69	48	2.14	6.05	1.62	68.6	75	54	51	2.13
	SD	1.11	0.25	1.44	11.79	20.00	2.22	0.26	0.59	0.19	2.97	5.52	8.61	1.10	0.23
	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
22.5	Mean	7.23	1.89	69.7	83	67	49	2.22	6.41	1.62	67.1	70	59	51	2.00
	SD	0.92	0.30	2.01	14.33	10.32	3.50	0.30	0.78	0.28	2.24	5.65	9.70	3.61	0.25
	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
210	Mean	6.95	2.14	70.0	74	68	48	2.17	6.24	1.62	65.9	73	60	50	2.20
	SD	1.09	0.59	1.69	8.46	13.24	3.13	0.28	0.53	0.20	2.22	8.53	13.72	2.37	0.28
	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Vehicle control	Mean	6.37	2.69	69.5	76	63	45	2.50	5.78	2.94	70.2	65	43	49	2.40
recovery	SD	1.12	0.27	3.01	54.98	35.88	2.36	0.35	0.75	0.39	2.27	8.27	6.63	3.14	0.44
	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
210 recovery	Mean	6.58	2.54	70.5	64	56	46	2.70	5.88	2.95	70.2	75[a]	51	47	2.51
	SD	0.76	0.24	2.21	7.68	7.37	3.53	0.32	0.86	0.31	2.76	12.25	10.13	4.61	0.30
	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10

Values are mean ± S.D, N: No. of rats, a: Significantly higher than the vehicle control recovery group

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Table 5. Effect of Lutein diacetate on Terminal Fasting Body Weights, Organ Weights and Organ Weight Ratios - Males

Group No.		Fasting	Organ weights (g)						Organ weight ratios (%)					
Dose (mg/kg Bwt/day)		Bwt(g)	Adrenals	Kidneys	Liver	Spleen	Heart	Brain	Adrenals	Kidneys	Liver	Spleen	Heart	Brain
Vehicle control	Mean	423	0.060	2.550	12.090	0.776	1.436	1.907	0.014	0.603	2.858	0.183	0.341	0.452
	SD	30.88	0.006	0.237	1.311	0.078	0.081	0.062	0.001	0.028	0.218	0.009	0.024	0.026
	N	10	10	10	10	10	10	10	10	10	10	10	10	10
2.1	Mean	441	0.057	2.569	11.885	0.767	1.486	1.999[a]	0.013	0.583	2.696	0.174	0.338	0.455
	SD	28.86	0.007	0.206	1.014	0.117	0.107	0.100	0.002	0.027	0.134	0.021	0.019	0.027
	N	10	10	10	10	10	10	10	10	10	10	10	10	10
22.5	MeanSDN	433	0.057	2.570	11.958	0.748	1.429	1.987[a]	0.013	0.595	2.761	0.172	0.331	0.460
		27.15	0.008	0.142	1.155	0.147	0.092	0.076	0.002	0.028	0.164	0.026	0.019	0.028
	N	10	10	10	10	10	10	10	10	10	10	10	10	10
210	Mean	436	0.059	2.608	12.256	0.771	1.493	2.006[a]	0.014	0.599	2.811	0.177	0.343	0.462
	SD	36.39	0.006	0.218	1.170	0.079	0.094	0.067	0.002	0.026	0.131	0.011	0.014	0.034
	N	10	10	10	10	10	10	10	10	10	10	10	10	10
Vehicle control	Mean	442	0.055	2.590	10.830	0.786	1.432	2.016	0.013	0.587	2.452	0.178	0.325	0.457
recovery	SD	17.92	0.009	0.145	0.837	0.067	0.071	0.063	0.002	0.026	0.137	0.012	0.018	0.020
	N	10	10	10	10	10	10	10	10	10	10	10	10	10
210 recovery	Mean	449	0.057	2.604	11.689	0.851	1.445	1.975	0.013	0.581	2.601	0.189	0.322	0.442
	SD	34.82	0.007	0.190	1.408	0.141	0.140	0.060	0.001	0.024	0.192	0.024	0.019	0.031
	N	10	10	10	10	10	10	10	10	10	10	10	10	10

Values are mean ± S.D, N: No. of rats, a: Significantly higher than the vehicle control group

Table 6. Effect of Lutein diacetate on Terminal Fasting Body Weights, Organ Weights and Organ Weight Ratios - Females.

Group No.		Fasting	Organ weights (g)						Organ weight ratios (%)					
Dose (mg/kg Bwt/day)		Bwt(g)	Adrenals	Kidneys	Liver	Spleen	Heart	Brain	Adrenals	Kidneys	Liver	Spleen	Heart	Brain
Vehicle control	Mean	261	0.065	1.523	7.677	0.596	1.026	1.810	0.025	0.585	2.942	0.228	0.394	0.698
	SD	21.10	0.009	0.115	0.908	0.081	0.092	0.039	0.004	0.022	0.195	0.023	0.017	0.053
	N	10	10	10	10	10	10	10	10	10	10	10	10	10
2.1	Mean	260	0.063	1.560	7.338	0.588	0.999	1.811	0.024	0.600	2.824	0.226	0.384	0.698
	SD	13.80	0.007	0.112	0.788	0.055	0.073	0.062	0.003	0.031	0.284	0.019	0.022	0.035
	N	10	10	10	10	10	10	10	10	10	10	10	10	10
22.5	Mean	261	0.061	1.537	7.389	0.615	0.975	1.787	0.023	0.589	2.833	0.236	0.375	0.686
	SD	14.06	0.006	0.144	0.684	0.071	0.067	0.068	0.003	0.031	0.205	0.023	0.029	0.027
	N	10	10	10	10	10	10	10	10	10	10	10	10	10
210	Mean	265	0.061	1.523	7.134	0.586	0.972	1.852	0.023	0.574	2.689[a]	0.221	0.366[a]	0.699
	SD	9.79	0.005	0.084	0.450	0.049	0.057	0.070	0.002	0.026	0.151	0.019	0.018	0.026
	N	10	10	10	10	10	10	10	10	10	10	10	10	10
Vehicle control	Mean	258	0.064	1.534	6.153	0.606	0.971	1.823	0.025	0.594	2.385	0.235	0.377	0.707
recovery	SD	10.13	0.010	0.081	0.445	0.059	0.066	0.061	0.005	0.027	0.181	0.027	0.032	0.034
	N	10	10	10	10	10	10	10	10	10	10	10	10	10
210 recovery	Mean	258	0.065	1.451	6.147	0.585	0.969	1.833	0.025	0.562[b]	2.382	0.227	0.377	0.714
	SD	19.22	0.011	0.148	0.659	0.062	0.081	0.021	0.004	0.038	0.183	0.019	0.030	0.053
	N	10	10	10	10	10	10	10	10	10	10	10	10	10

Values are mean ± S.D, N: No. of rats, a: Significantly lower than the vehicle control group, b: Significantly lower than the vehicle control recovery group.

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chemically complex foods, it is difficult to pinpoint any single nutrient that contributes the most to the cardioceptive effects. Studies with natural products do not prove the safety of the components, since contents of the active ingredients are relatively low and also the complicating factors of other ingredients. Our study with pure Lutein diacetate when administered at sufficiently high dose clearly shows that the pure Lutein does not have any deleterious effect.

In conclusion, the present investigation demonstrates that the only effects observed were stained faeces in rats at 210 mg/kg/day during the treatment period that reversed after the fourth day of recovery period; this was not considered as an adverse effect. The No Observed Adverse Effect Level (NOAEL) in Sprague Dawley rats for Lutein diacetate on repeated oral gavage administration for 90 consecutive days is 210 mg/kg b.wt./day.

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