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Chronic Toxicity Assessment of Lac Dye as Potential Food Colorant

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ABSTRACT

Lac dye is a natural red colored pigment obtained from lac resin which is cultivated on specific host trees. Although, lac dye is being used as colorant in food and pharmaceutical applications, no chronic toxicity assessment for its safety is yet reported. Keeping this in view, long-term dietary toxicity study was carried out on Wistar rats. The dye was orally administered through diet at 0 (control), 250, 500 and 1000 mg/kg body weight, to male and female Wistar rats for 52 weeks. Parameters such as body weight, organ weight ratio, food consumption, urine analysis, biochemical and hematological indices, gross morphology at necropsy and histopathological integrity of organ/tissues were tested. Overall, our results indicated that, repeated dietary exposure of lac dye up to the dose of 1000 mg/kg body weight in diet did not produce any toxic effects in Wistar rats.

Key words *Laccaic acid, lac, long term toxicity, food additive, Natural Red 25.*

Red color has its distinct importance in paintings, printing and food. The role of red colored insect pigments especially kermesic, carminic and laccaic acids for commercial applications has been vital since ancient times (Shamim *et al.,* 2014). Lac dye is one of the most ancient of natural red dyes with laccaic acid as its principal color imparting component. It was commonly used in ancient Chinese and Indian civilizations for dyeing silk and leather and in cosmetics. It is obtained fromsticklac and also known as lac color, lac lake, etc. It is secreted by the tiny insects feeding on the sap of specific host tree, allegedly to protect themselves from harmful UV radiations of the sun[2]. The insects belong to *Kerria* spp. (Family- Tachardiidae, order-Homoptera) feeding on trees and bushes cultivated mostly in India, Thailand and China (Baboo and Goswami, 2010). After maturity of the crop the sticks having lac incrustation are harvested and scraped. The scraped lac is processed (washed and crushed in desired uniform size) to get refined product known as seedlac. During this crushing and washing, the lac dye gets solubilized in water which in turn is processed to obtain the crystallized dye.This dye has multiple applications in food, pharmaceutical, cosmetics and textile industries (Tanaka, 1997). It is also known as Natural Red 25 (CI Number 75450) in international trade (Tanaka, 1997).

According to the national food safety standards use of food additives standards from Health Ministry of China (GB 2760-2011) pure lac dye (obtained from technical grade) is extensively used in number of food products (Ling, 2003; Lu *et al*., 2007; Zang *et al.,* 2003). These products include fruit beverages, vegetable juice (pulp), fruit-flavored beverages, soda pop, compound seasonings, jam, cocoa products, fillings for bakery wares, imitation wine, chocolates and chocolate products.It is in the approved food additive list of China (CNS No. 08.104), Japan (Natural additive 394) and Korea (Natural Additive 13). Lac dye to be used as food additive must conform to the requirements of standard specification of respective countries e.g. GB 2760-2011 in China, Japan external trade organization-specifications and standards for foods, food additives, 2011, and KFDA in Korea (KFDA, 2016). It solubilizes partially in cold water and almost completely in hot water. It gives orange red color in acidic pH and reddish violet in alkaline pH. In alkaline solutions, it decomposes rapidly (Srivastava *et al.,* 2013). Its principal color imparting component are hydroxy-anthraquinone derivatives (Fig. 1) designated as laccaic acid A, B, C, D and E (Chairat, 2009; Pandhare*et al*., 1966; Pandhare *et al*., 1967; Pandhare *et al*., 1969; Rama Rao *et al*., 1968; Bhide *et al*., 1969; Mehandale *et al*., 1968; Venkataraman and Rama Rao, 1972). The major components of lac dye are laccaic acid A, E and B, constituting 40.42%, 20.00% and 17.66% respectively; whereas laccaic acid C and D are minor components contributing 2.54% and 1.51% respectively (Hong *et al*., 2011).

Banerjee and coworkers reported that lac dye was non-mutagenicin the Ames test and no cytotoxicity and mutagenicity was observed in Chinese hamster lungcells in vitro (Banerjee *et al.,* 1984). Dube and coworkers reported that laccaic acid had no mutagenic activity as assessed by two short-termassays (Salmonella/microsome mutagenicity test, and the ϕX fidelity assay); however, laccaic acid did inhibit metabolic cooperation in Chinese hamster V79 cells (Dube *et al.,* 1984).Chakravarty *et al.,* (1982) observed no adverse effects of lac dye in rats (0-20 mg/day/rat) in a three-generation toxicity study. Ninomiya *et al.,* (1973) found no adverse effects of laccaic acid, except for reduced body weight growth in higher dosage groups, in sub-acute toxicity studies in mice (0-2000 mg/kg/day by gavage).

India, at present, produces about 23,000 tons of lac annually. Processing of this raw lac may yield around 200tons of lac dye; majority of which is lost during washing. Some processors use the partially pure form (technical grade) of dye for utilization as textile dye, while other processing industries dispose the sludge of washing as manure to villagers. Thus, an enormous potential exists for utilization of this by-product of lac factories. Even if half of the potential is exploited, then it will be possible to turn trade of lac dye into profitable business of specialty product with assured foreign market.

Furthermore,because of the ban on potentially carcinogenic red dyes Sudan IV, Sudan I and few others, there is a great demand of red color dyes in food industry. The manufacturers are working hard to meet new laws of

safety approval by recognized agencies such as JECFA, EFSA and others, to avoid use of unsafe pigments. Being natural and biodegradable, lac dye has great potential to replace currently used synthetic dyes. In depth toxicity/ safety studies are therefore required to fill the knowledge gap between human experience and the scientifically valid data before regulatory clearance for manufacture and marketing of lac dye. To address this issue, the chronic toxicity study of lac dye was undertaken to further substantiate its safety as food additive.

MATERIALS AND METHODS

Lac dye was obtained from Tajna Shellac Pvt. Ltd., Ranchi, India. The chemical characterization and tests for quality parameters were done in ISO 9000:2008 certified Quality Evaluation Laboratory, ICAR-Indian Institute of Natural Resins and Gums, Ranchi, India. The quality parameter tests of lac dye conform to the GB 2760-2011 (Hygienic standards for uses of food additives of China).

Toxicological studies

To assess the toxicological potential of the lac dye upon repeated dietary exposure to both sexes of rodent species, 52-week dietary toxicity study was conducted at CSIR-Indian Institute of Toxicological Research (IITR), Lucknow. Ethical Committee approval was obtained from Institute Animal Ethics Committee, CSIR-IITR, which followed the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forest and Climate Change, Government of India. Prior undertaking chronic toxicity study,acute and short term oral toxicitystudies of lac dye in albino rats were carried out. For acute oral toxicity test the albino rats were fed with 2000mg/kg body weight as per OECD 401. The lac dye showed no acute toxicity at this dose for the observation period of 14 days. The short term intake of lac dye at test limit dose of 100, 500 and 2500mg/kg body weight in male and female albino rats neither produced any sign of toxicity nor mortality within 90 days. Based on acute and short term oral toxicity studies of lac dye, the test doses for long term dietary toxicity study were chosen as 250, 500 and 1000mg/kg body weight.

The test item was orally administered by mixing in the powdered feed through diet to groups of twenty male and twenty female Wistar rats each, assigned to three dose levels of 250, 500 and 1000mg/kg body weight at a fixed diet of 20 g/rat, once daily for 12 months. This study covered any adverse effect of oral administration of lac dye on the organ systems, dose-effect correlation and consequent health hazards likely to arise from repeated exposure to the test item over a considerable part of the life span of the species used.

Animals and husbandry

Random bred, 6 week old male and female albino Wistar rats (*Rattus norvegicus*) were obtained from Laboratory Animal Facility, CSIR-Indian Institute of Toxicology Research, Lucknow. The rats with body weight range 90-140 g (within ± 20 % of mean weight) were randomly selected for the treatment. All rats, qualifying the veterinary health examination, were acclimatized for 5 days before start of treatment. Total 160 Wistar rats were divided in to four groups comprising 40 animals each (20 males and 20 female animals). The four groups so formed consisted of one control and three treated groups.

The animals were housed individually in standard polypropylene cages, with stainless steel top grill having provision for pelleted food and drinking water in the bottle. Paddy husk was used as bedding material and changed at least twice a week. Identification of animals was done by cage card and picric acid body marking. Certified rodent feed, manufactured by Ashirwad India, Ltd., and reverse osmosis water was provided *ad libitum* to all animals. Animals were maintained and housed at 22± 2 °C, with relative humidity 30-70% and day/night cycles of 12 hour light and 12 hour dark. Adequate fresh air supply with regular air changes was maintained in the experimental room.

Observations

Clinical signs, body weights and food consumption

All treated and control rats were observed once daily for 12 months. The clinical signs of toxicity including changes in fur, eyes, respiration, movements, tremors/ convulsions, salivation, diarrhea, discharges from any orifice and death were recorded during daily observations. The body weights and food consumption of all rats were recorded on test day 1 and then on weekly basis from week 1 to 26 and thereafter on monthly basis up to week 52 (12 months) prior to the sacrifice for necropsy.

Neuro-behavioral toxicity

Observations for neuro-behavioral toxicity symptoms e.g. visual (response to external light source), auditory (response to external noise source), motor response (gait, ataxia) and proprioceptive response (righting reflex, threat perception) were made at six months and after the last dose and prior to the sacrifice for necropsy in treated and control groups.

Hematology

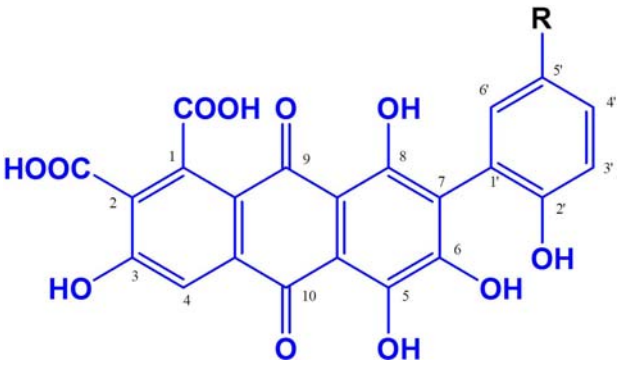
Blood samples from all animals (anesthetized) were collected at 3, 6 and 12 months interval and were screened for hematological abnormalities, if any. Samples were estimated for white blood cells (WBC), red blood cells (RBC), differential leucocytes counts (DLC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV) and platelets (PLT) using a fully automatic hematoanalyzer/ cell counter (MS-9-3 model, France).

Biochemical estimations

Serum was separated from coagulated blood collected from all control and treated rats at 6 and 12 months and analyzed for Glutamic Oxaloacetic Transaminase (GOT), Glutamic Pyruvic Transaminase (GPT), creatinine, Blood Urea Nitrogen (BUN), blood glucose, total protein and albumin through diagnostic kit using a fully automatic analyzer (Awareness Technology, model Chem Well, USA).

Urine analysis

Urine samples were collected twice, at six and twelve months to observe any abnormality in urine from exposure



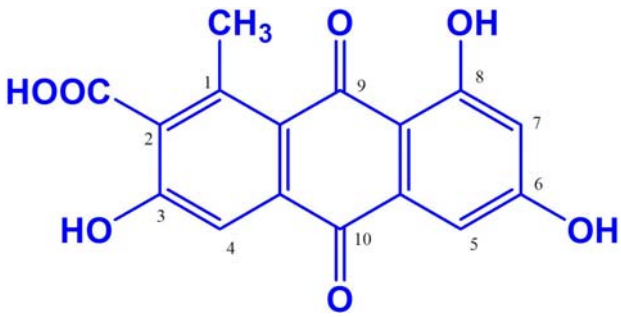
Laccaic acid A, B, C & E

A: R= $CH_2CH_2NHCOCH_3$

B: R= CH_2CH_2OH

C: R= $CH_2CH(NH_2)COOH$

E: R= $CH_2CH_2NH_2$



Laccaic acid D

IUPAC Nomenclature with molecular weight (MW)

1. Laccaic acid A: 7-[5'-[2-(acetylamino)ethyl]-2'hydroxyphenyl]- 9,10-dihydro-3,5,6,8-tetrahydroxy-9,10-dioxo-1,2-anthracenedicarboxilic acid; ($C_{26}H_{19}NO_{12}$; MW=537)
2. Laccaic acid B: 7-[5'-(2-hydroxyethyl)-2'hydroxyphenyl]- 9,10-dihydro-3,5,6,8-tetrahydroxy-9,10-dioxo-1,2-anthracenedicarboxilic acid; ($C_{24}H_{16}O_{12}$;MW=496)
3. Laccaic acid C: 7-[5'-(2-amino-2-carboxyethyl)-2'hydroxyphenyl]- 9,10-dihydro-3,5,6,8-tetrahydroxy-9,10-dioxo-1,2-anthracenedicarboxilic acid; ($C_{25}H_{17}NO_{13}$; MW=539)
4. Laccaic acid E: 7-[5'-(2-aminoethyl)-2'hydroxyphenyl]- 9,10-dihydro-3,5,6,8-tetrahydroxy-9,10-dioxo-1,2-anthracenedicarboxilic acid; ($C_{24}H_{17}NO_{11}$; MW=495)
5. Laccaic acid D: 9,10-dihydro-3,6,8-trihydroxy-1-methyl-9,10-dioxoanthracene-2-carboxilic acid; ($C_{16}H_{10}O_7$; MW=314)

Fig. 1. Structure, molecular formula, molecular weight and IUPAC nomenclature of components laccaic acids

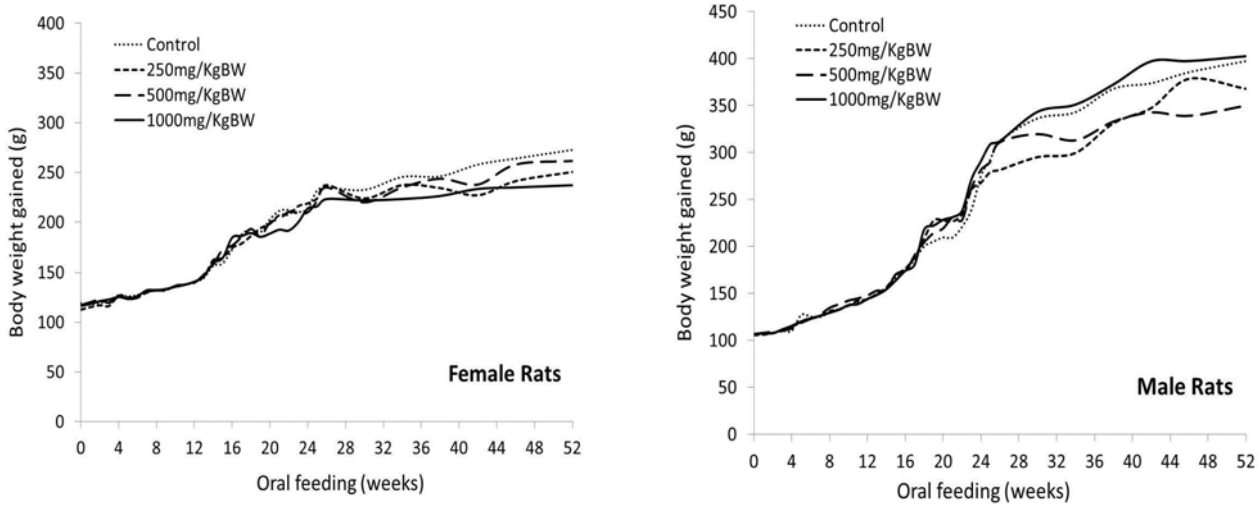
to the test substance. Urine samples from five animals were collected for the qualitative estimation of urobilinogen, protein, pH, blood, specific gravity, ketones, bilirubin and glucose through Urocolor TM 10 SD Standard Diagnostics, INC.

Histopathological evaluation

The tissues were surgically excised from all animals at the time of necropsy and fixed in 10% neutral formalin for subsequent histopathological examination under the supervision of board certified pathologists. The specimen included all gross-lesions in liver, kidney, heart, brain, adrenal, lung, spleen, testes, seminal vesicle, coagulating gland, prostate, epididymis, ovary and uterus.

Necropsy and gross-pathology

Twenty four hours after the last dose, all animals from the control and treatment groups were anesthetized with



Variations were found significant at the level of $P < 0.05$

Fig. 2. Body weight gain by the male and female rats in course of study (52 weeks)

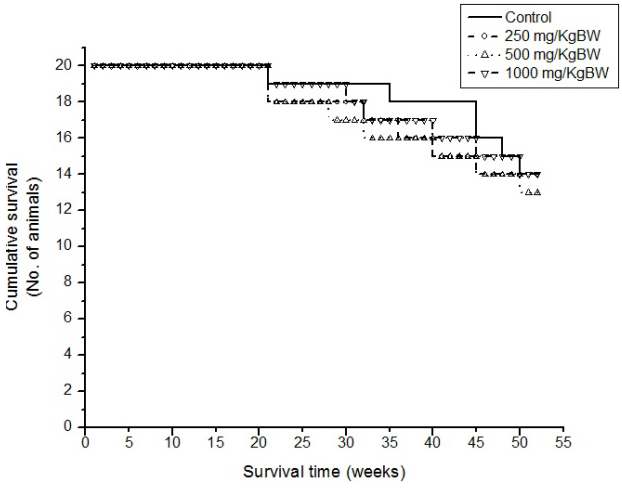


Fig. 3. Survival curves of male rats for all groups

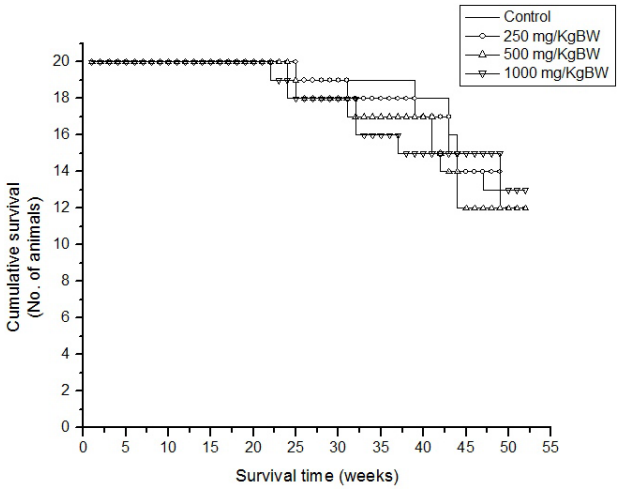


Fig. 4. Survival curves of female rats for all groups

diethyl ether to humanely collect blood samples for hematological, biochemical evaluations. The animals were then killed under deep ether anesthesia and necropsied for gross-pathological examination of external surface, all orifices, cranial, thoracic, visceral cavities and their contents. The liver, brain, kidney, heart, spleen, adrenal, testes, prostate, epididymis, seminal vesicle (male) and uterus, ovary (female) were surgically excised and weighed immediately for absolute weights to calculate their organ body weight ratios.

Statistical analyses

The statistical analysis of the experimental data was carried out and all quantitative variables like laboratory investigations (haematology and clinical chemistry) were subjected to one-way ANOVA test. All analysis and comparisons were evaluated at the 5 % ($P < 0.05$) level of significance.

RESULTS AND DISCUSSION

Body weights, food consumption, toxic signs and pre-terminal deaths

All the rats appeared healthy and gained weight throughout the observation period. The body weight gain of experimental and control animals did not show any significant variation (Fig. 2). The food consumption of experimental and control animals, determined at 1 to 52 weeks of study was found comparable and there was no significant change due to test item exposure. No toxic signs were observed in any animal during 52 weeks dietary exposure.Pre-terminal deaths occurred in male (Fig. 3) and female rats (Fig. 4) of treated groups were comparable to deaths in control group.

Neurobehavioral toxicity

All animals were examined for any signs of abnormality for visual, auditory, motor and proprioceptive response. Neurobehavioral toxicity was scored and recorded as per standard scale (NAD: No abnormality detected, 1: Mild deficit, 2: Moderate deficit, 3: Severe deficit). There were no indications of any test-substance related neurobehavioral toxicity symptoms as no abnormality was detected in animals from any of the four groups.

Hematology

Male rats exposed to the dose of 1000mg/kg body weight at six months dietary exposure showed sporadic decrease in RBC (by 30.91%), Hb (27.9%), HCT (by 28.3%), granulocytes (by 37%) and PLT (by 34.5%) while increases in lymphocytes (by 14.6%). These variations were limited to male rats only that too for six month at 1000 mg/kg body weight dose. These changes did not correlate with dose, sex or other physiological parameters and significant differences were not considered as lac dye exposure related effect (Tables 1&2). No significant changes in hematological parameters were observed in treated rats as compared to respective control animals at three, six and twelve month's dietary exposure.

Biochemical estimations

The activities of serum enzymes like GOT and GPT and other important biochemical constituents like BUN, creatinine, total protein, albumin and glucose, that are indicative of metabolic and pathological abnormalities, showed no significant change in experimental animals as compared to control at six months exposure period. In female rats at all three test doses at 12 months exposure period, mild decrease in GOT activity was detected ranging from 29.6 to 38.93%,while increase in the activity of GPT ranging from 48.15 to 114.4% and level of BUN from 52.4 to 103.2% was observed. The values for these parameters appear to be in normal range. Moreover, in absence of any physiological abnormality, these variations may not be related to the exposure to the test item. The activity of serum enzymes and biochemical constituents in treated male rats at 12 months exposure period were comparable to controls (Tables 3&4).

Urine analysis

Analysis of urine parameters like, urobilinogen, protein, pH, blood, specific gravity, ketones, bilirubin and glucose was carried out. Urobilinogen (60 sec.) of control

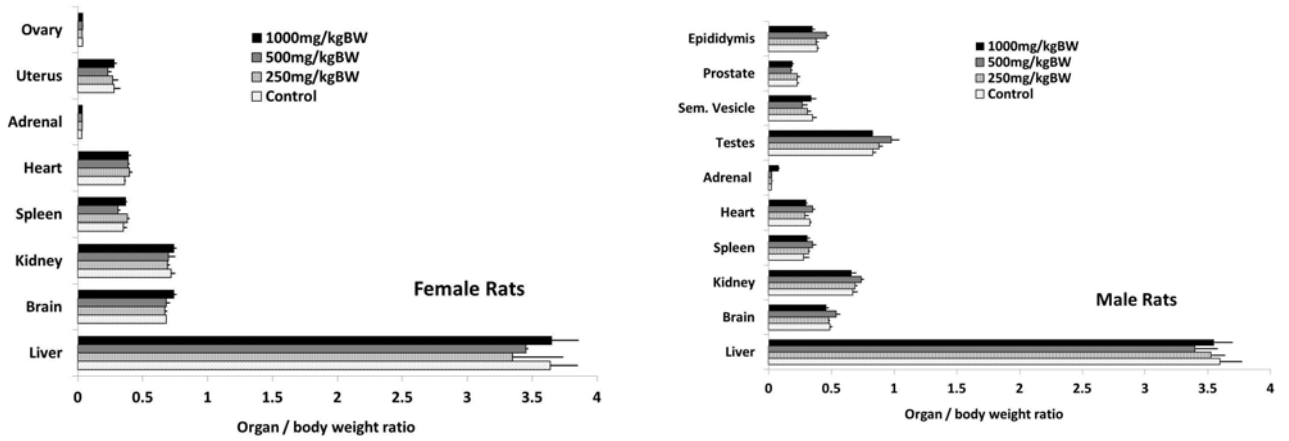


Fig. 5. Effect of long term (52weeks) dietary exposure of lac dye on meanorgan body weight ratio (%) of male and femalerats at necropsy[Organ body weight ratio= (Organ weight x 100)/ Body weight]

male rats gradually decreased from 4 to 1 in 52 week exposure period, similar trend was observed in all treated males; whereas, in control females it was in the range of 0.1-2.0, which was comparable with the urobilinogen of treated females. Specific gravity and pH of urine of all animals from treated and control group was comparable and was in the range of 1.01 - 1.035 and 6 - 8, respectively. Values of other parameters such as protein, blood, ketones, bilirubin and glucose were comparable to their respective controls. Overall urine analysis revealed that, there were no significant changes in urine parameters in experimental animals due to the exposure of the test material for 52 weeks.

Absolute organ weights and organ/body weight ratio

At necropsy after a repeated dietary exposure to lac dye for 52 weeks, the individual absolute weights and organ body weight ratios of vital organs of treated rats did not differ significantly in comparison with controls (Fig.5). Except slight increase in adrenal in male rats at the dose of 1000mg/kg body weight and decreased weight of prostate at the dose of 500 and 1000mg/kg body weight, which was found significant at the level of $P < 0.05$. Decreased relative weight of prostate and increased relative weight of adrenal in male rats is of common occurrence in repeated exposure to xenobiotic. As these values are in the normal range and did not produce any significant physiological outcome, the variations in these parameters can be considered adaptive to treatment.

Gross pathology and histopathology

Observations on liver, brain, kidney, spleen, adrenal, testes, coagulating gland prostate, epididymis, seminal vesicle (male) and uterus, ovary (female) of the treated animals as compared to control animals showed no gross pathological changes. Also no evidences of coloration of any tissue or organs in the animals from the control and treated groups were observed. Histopathological analysis revealed that, liver from one male of control group revealed foci of mononuclear cells and kidney from one male of same group showed multiple cysts. Adrenal, spleen, brain, testes, seminal vesicles, coagulating gland, epididymis and prostate from all the control animals did not revealed any lesions. However, testes from one male of high dose group showed mild interstitial edema. Liver, kidney, brain, adrenal, spleen, seminal vesicles, coagulating gland, epididymis and prostate from all male animals of the high dose group showed normal histology.

Liver, kidney, adrenal, spleen, ovary and uterus from all the control females showed normal histology. Adrenal from one female of high dose group showed medullary hyperplasia. Uterus from one female rat showed hydrometra. Liver, kidney, brain, spleen and ovary from all females of high dose group showed normal histology. Lesions observed in various tissues from above groups in male and female rats appear to be spontaneous in nature. These lesions are incidental in nature, hence changes observed in treated group could not be considered as consequence of the treatment.

As a result of 52 week administration of lac dye to the male and female Wistar rats, no significant treatment related changes in food consumption, body weight gain, organ/ body weight ratio, urine analysis, gross morphology at necropsy and histopathological integrity of organ/tissues were observed in exposed animals as compared to respective controls. Mild variations in hematological and biochemical parameters were observed in random groups of animals which appeared to be in normal range. There were no signs of clinical or neurobehavioral toxicity at any of the three test doses of the lac dye in test animals. Occasional deaths of animals in the all three doses groups were comparable to the deaths in control group. It is therefore, concluded that repeated 52 week dietary exposure of lac dye up to the dose of 1000mg/kg body weight in diet did not produce any significant toxic effects in male and female rats.

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LITERATURE CITED

Table 1.Effect of a long term (3, 6 & 12 months) dietary exposure of lac dye on mean hematology data of male rats

	3 months				6 months				12 months			
Parameter	**Control**	**0.5 % Diet**	**1 % Diet**	**2 % Diet**	**Control**	**0.5 % Diet**	**1 % Diet**	**2 % Diet**	**Control**	**0.5% Diet**	**1 % Diet**	**2 % Diet**
R.B.C. (M/mm^3)	7.18±0.52	7.86±0.54	7.16±0.72	7.37±0.37	7.44±0.22	7.21±0.078	7.04±0.45	5.14±0.46*	6.49± 0.94	5.97± 0.17	6.73±0.2	7.06±0.31
W.B.C. (m/mm^3)	5.15±0.57	4.98±0.53	4.83±0.69	5.19±0.63	9.01±1.14	11.93±1.73	8.88±0.92	10.08±2.42	8.47± 0.4	10.45± 0.57	9.21±0.44	10.43±0.48
Hb (%)	13.5±0.62	14.32±0.37	14.3±0.31	13.06±0.25	11.74±0.33	11.24±0.17	11.28±1.54	8.46±0.67*	12.81 ± 0.52	13.7± 0.75	11.68±0.37	12.56±0.39
Lymphocytes(%)	77.1±1.16	79.68±2.36	76.64±1.85	72.38±0.52	71.52±2.03	77.62±3.17	72.84±1.93	81.98±2.34*	77.3± 1.43	76.2± 1.73	85.5±1.24	81.6±1.7
Monocytes (%)	9.6±1.33	8.26±1.03	8.32±1.25	10.04±1.00	6.94±0.19	6.12±0.64	6.98±0.42	4.48±0.37	0.7± 1.43	0.9 ± 0.27	0.8±0.1	0.6±0.3
Granulocytes(%)	13.3±1.19	12.06±2.05	15.02±2.44	17.58±0.62*	21.5±1.97	16.26±2.54	20.2±1.66	13.54±2.25*	22.0± 1.39	22.9± 1.68	14.2±1.2	17.8±1.6
HCT (%)	42.52±2.11	43.88±2.33	47.02±1.70	42.46±0.91	36.52±1.28	35.04±1.03	34.6±2.38	26.18±2.41*	37.24±3.38	36.31±1.84	38.83±2.56	41.55±0.93
MCV (fl)	50.64±0.61	53.22±0.70	53.66±0.44	51.96±0.77	48.68±0.22	48.62±0.38	49.12±0.27	50.84±0.83	58.25±0.90	61.11±0.52	60.4±1.05	59.75±1.31
PLT (m/mm^3)	477.8±38.18	446.2±27.76	458.8±32.54	446.6±26.12	611.6±32.76	610.6±13.73	592.8±52.97	400.4±25.17*	659.6±13.74	662±13.09	668.2±7.63	571.2±7.97

(Mean ± SE) (* Significant at the level of P < 0.05)

Table 2.Effect of a long term (3, 6 & 12 months) dietary exposure of lac dye on mean hematology data of female rats

	3 months				6 months				12 months			
Parameter	**Control**	**0.5 % Diet**	**1 % Diet**	**2 % Diet**	**Control**	**0.5 % Diet**	**1 % Diet**	**2 % Diet**	**Control**	**0.5% Diet**	**1 % Diet**	**2 % Diet**
R.B.C. (M/mm^3)	6.71± 0.20	6.69± 0.36	6.71± 0.30	6.38± 0.11	5.75±0.67	6.65±0.43	7.18±0.32	7.00±0.42	5.95± 0.25	6.44± 0.18	6.17±0.23	6.07±0.26
W.B.C.(m/mm^3)	5.011± 0.78	5.251± 0.91	8.86± 1.15	5.70± 0.67	7.30±0.74	9.21±0.94	6.50±0.63	8.19±0.40	8.70± 0.7	8.17± 0.40	8.59±0.23	8.41±0.29
Hb (%)	12.181± 0.39	11.46± 0.41	11.78± 0.32	10.38± 0.41	10.76±1.05	11.92±0.75	10.9±0.95	13.16±0.98	13.53± 0.49	12.15± 0.60	13.97±0.47	14.06±0.38
Lymphocytes (%)	79.50± 1.19	79.681± 2.36	77.44±2.05	73.04± 0.81	85±5.05	87.94±1.16	87.46±1.15	85.04±4.21	82.11± 2.15	79.55± 1.7	80.44±1.30	82.44±1.56
Monocytes (%)	7.161± 0.95	6.831± 0.66	5.90± 0.80	6.82± 0.26	4.98±1.61	4.52±1.50	4.32±0.57	4.29±0.27	0.5± 0.24	0.66± 0.37	1.4±0.58	0.6±0.2
Granulocytes(%)	14.22± 0.74	14.021± 2.05	16.66± 1.43	20.18± 0.62*	10.02±3.64	7.54±1.41	8.22±1.21	11.18±4.22	17.33± 2.19	19.77± 1.82	18.11±1.4	17±1.42
HCT (%)	41.38± 3.23	34.8± 1.57	37.82± 2.14	34.64± 1.94	28.58±3.82	33.14±2.21	28.96±2.92	31.82±6.32	39.01±3.11	31.13±4.79	29.65±3.58	28.68±4.31
MCV (fl)	54.861± 2.39	52.161± 0.82	53.16± 0.92	51.44± 0.80	49.34±0.93	54.0±1.06	53.08±2.46	55.46±6.04	58.3±1.03	58.06±1.23	62.3±0.40	60.9±1.06
PLT (m/mm^3)	530.4± 34.89	517.9± 16.84	555±31.99	586± 41.94	786.8±106.97	814.8±101.99	761.2±107.51	644±78.54	656.4±27.13	554.5±54.17	633.8±19.50	644±13.69

(Mean ± SE) (* Significant at the level of P < 0.05)

Table 3. Effect of a long term (6 & 12 months) dietary exposure of lac dye on mean serum biochemical data of male rats

	6 months				12 months			
Parameter	**Control**	**0.5 % Diet**	**1 % Diet**	**2 % Diet**	**Control**	**0.5 % Diet**	**1 % Diet**	**2 % Diet**
GOT(U/L)	220.9±21.75	205.61±12.53	178.96±22.65	186.06±20.75	265.59±21.87	246.34± 15.44	187.90±14.43*	217.18±15.20
GPT(U/L)	58.51±4.59	56.56±5.32	61.35±10.33	58.34±10.24	143.45±11.13	109.93 ± 13.04	108.88±22.98	179.60±31.29
BUN (mg %)	19.93±2.75	23.35±1.24	18.46±2.11	14.41±1.49	17.03± 1.06	16.44 ±0.93	16.74±0.62	20.53±1.22
Total Protein (g%)	7.58±0.27	5.88±0.39	5.64±0.39	5.12±0.34	7.54± 0.42	6.95± 0.94	6.29±0.176	6.02±0.123
Albumin (g %)	3.40±0.17	3.66±0.09	3.58±0.33	3.0±0.24	3.69± 0.043	3.45 ± 0.057	3.26±0.078	3.44±0.065
Creatinine (mg%)	0.48±0.029	0.46±0.033	0.46±0.029	0.41±0.019	0.63 ± 0.047	0.45 ± 0.035	0.64±0.034	0.476±0.035
Glucose (mg %)	95±4.18	90.0±4.11	89.8±3.34	93.8±3.72	129.4± 9.27	150.8 ± 12.30	110.20±10.34	131.62±10.51

(Mean ±SE) (* Significant at the level of P < 0.05)

Table 4. Effect of a long term (6 & 12 months) dietary exposure of lac dye on mean serum biochemical data of female rats

	6 months				12 months			
Parameter	**Control**	**0.5 % Diet**	**1 % Diet**	**2 % Diet**	**Control**	**0.5 % Diet**	**1 % Diet**	**2 % Diet**
GOT(U/L)	135.33±14.7	163.43±18.86	137.32±7.67	136.84±11.25	203.74±13.05	143.40±7.56*	124.41±3.67*	125.55±5.72*
GPT(U/L)	44.82±1.64	49.44±3.31	43.11±1.45	49.43±1.74	110.28±11.02	190.85±19.02*	163.39±17.42*	236.51±27.08*
BUN (mg %)	22.47±1.32	21.0±1.83	16.73±1.22	21.64±0.63	9.07±0.84	13.97±0.81*	13.82±1.58*	18.43±1.10*
Total Protein (g%)	6.08±0.20	5.46±0.19	5.02±0.16	5.41±0.28	8.85±0.65	8.49±0.18	7.01±0.29	7.08±0.18
Albumin (g %)	3.82±0.22	2.94±0.21	2.72±0.21	3.16±0.36	4.18±0.13	4.28±0.13	3.67±0.14	3.66±0.09
Creatinine (mg%)	1.13±0.05	1.29±0.15	0.81±0.05	1.02±0.06	0.39±0.05	0.31±0.01	0.33±0.04	0.26±0.03
Glucose (mg %)	98.6±3.31	97.6±1.43	94.8±1.77	99.2±2.52	157.11±10.92	176.89±11.63	164.0±6.62	171.22±11.18

(Mean ± SE) (* Significant at the level of P < 0.05)

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