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A STUDY REPORT OF REPEATED DOSE 28-DAY ORAL TOXICITY STUDY OF NIRAPARA FRESH WASH IN WISTAR RATS

Medical Science

Dr. Shan Sasidharan* Head, R & D, QA/QC, Solace Research Private Limited, KKR Groups of Companies, Ernakulam, Kerala *Corresponding Author

Dr. Sithara. M. S Toxicologist, Care Keralam ltd,Kinfra small industries park, Koratty,Thrissur,Kerala

ABSTRACT

Nirapara fresh wash is a natural formulation to clean the Vegetables, fruits and non-vegetableitems like fish, meat etc. Nirapara fresh wash help to remove dirt, toxins, microbes, heavy metals, pesticides and other foreign particles from vegetables/fruits and removes toxins, biological fluids ,microbes ,antibiotics, drugs (antibiotics and steroids), poisons,pesticides and other chemicals from non vegetable items. And it offers freshness to vegetables/fruits andnon-vegetable items.The product is not intended for direct oral consumption. It is only used for washing the vegetables /fruits and non-veg items.

Repeated dose 28-day oral toxicity study with 14 day reversal period of *Nirapara Fresh Wash* in Wistar Rats was conducted to evaluate the possible health hazards likely to arise fromrepeated exposure over a relatively limited period of time. The study was conducted in six groups consisting of 52 rats with 26 males and 26 females. The animals were observed for health status, clinical signs of toxicity and mortality. Body weights and food consumptions were recorded at weekly intervals. On completion of 28 days for treatment groups or 42 days for recovery groups respectively, the blood samples were collected from all the animals and subjected to haematological and clinical chemistry evaluation. At termination the animals were humanely sacrificed and subjected to necropsy. Histopathological examination was conducted on the specified list of tissues from the control and the treatment groups.From the above study the product is demonstrated its safety even for consuming directly by oral route.

KEYWORDS

sub acute toxicity, fresh wash, vegetable wash, Nirapara,non vegwash,pesticides, antibiotics scavenger

METHODS

Nirapara Fresh wash formulation

Sl.No	Ingredients	Quantity (%)
1	Curcuma domestica	10
2	Herbal extract 001*	5
3	Natural Vinegar	30
4	Herbal powder 002*	3
5	Herbal extract 003*	2
6	Water	50

***denotes various edible herbs which are completely safe to use and possess sufficient scientific research papers to demonstrate its efficacy and safety**

Accurately weigh all the ingredients and make a fine paste by adding vinegar and finally add water to make up the volume.

Usage directions

A dosage of 10 ml of Nirapara fresh wash should be mixed with 1 litre of tap water in a bowl and dip the required veg/non veg stuffs on it. Wait for 20-30 minutes and strain the liquid out. Wash and rinse thoroughly with fresh tap water and dry.

Animals

8 to 12 Weeks old Wistar albino rats(26 Male and 26 Female Rats) weighing 175 g-300 g (Small animal breeding station, Mannuthy, Thrissur, Kerala) were housed under standard laboratory conditions: air-conditioned environment with adequate fresh air supply with IVC system (Air changes 15 per hour), room temperature 21.0 to 24.0°C, relative humidity 57-65%, with 12 hours light and 12 hours dark cycle. They were offered a standard laboratory diet and water *ad libitum*. All experimental procedures were carried out in accordance with ethical G Institutional Animal EthicsCommittee (IAEC).STUDY NO: CKL/TOX/IAEC/2017-3/86

Experimental procedures

Dose levels selected for the present study was based upon the maximum human dose used in clinical practice. Six groups consisting of equal male and female rats were maintained in the study. Group- I, I R, II, III, IV and IV R served as control, control recovery, low, mid, high dose and high dose recovery group respectively.

Group No.	Group	Dose	No. of rats/ Group	No. of Rats	
				Male	Female
Group-I	Control	Distilled water (1ml/100gbody weight, orally)	6	3	3
Group-I R	Control Recovery	Distilled water (1ml/100gbody weight, orally)	6	3	3
Group-II	Low dose	***Nirapara fresh wash*** (250 mg/kg body weight, orally)	10	5	5
Group-III	Mid dose	***Nirapara fresh wash*** (500 mg/kg body weight, orally)	10	5	5
Group-IV	High Dose	***Nirapara fresh wash*** (1000 mg/kg body weight, orally)	10	5	5
Group-IV R	High Dose Recovery	***Nirapara fresh wash*** (1000 mg/kg body weight, orally)	10	5	5

Experimental animal groups

Freshly prepared ***Nirapara fresh wash*** was administered through oral route by gavage to the animal after formulation preparation using rat gavaging needle fitted to graduated syringe. Theadministration of the test item was done after calculating the dose for each respective group andformulation was made with the dose concentration as mentioned in the study design. Thisprocedure of administration was followed for 28 consecutive days.All the observations made during the study period Clinical Signs and pre-terminal deaths, Body weight, Food and water Consumptionwere recorded and tabulated.(1,2)

Haematology

At the end of treatment (on 28th day) all treatment group animals and on 42nd day allrecovery group animals were fasted overnight. *Ad libitum* water was given during fasting. Bloodsamples were collected from the orbital plexus, under anaesthesia, with EDTA anticoagulant fordetermining the following haematological parameters; Haemoglobin (Hb),Erythrocyte Count (Total RBC),Leukocyte Count (Total WBC),Platelet Count (3,4)

Serum Biochemistry

At the end of treatment (on 28th day) all treatment group animals and on 42nd day allrecovery group animals were fasted overnight. *Ad*

libitum water was given during fasting. Bloodsamples were collected from the orbital plexus, under anaesthesia. The serum was separated bycentrifuging the blood samples at 3000 rpm for 10 mins for determining the following clinicalchemistry parameters; Total Protein Glucose,Alanine aminotransferase (ALT), Aspartate aminotransferase (AST),Alkaline phosphatase (ALP) Creatinine (Creat),Total Cholesterol (TC) Triglycerides,Bilirubin, Urea(5,6)

Tissue collection

All animals were euthanized under anesthesia. The representative tissue samples of heart,liver, spleen and kidneys were collected and preserved in 10% neutral buffered formalin. Thetissues were embedded in paraffin wax, sectioned at five micrometres and stained withhaematoxylin and eosin.(7,8)

Gross and Histopathological Examination

Detailed gross and histopathological examination was performed on the specified list oftissues of all animals from the control and the dosage groups, sacrificed at scheduled termination(9)

STATISTICALANALYSIS

The data on body weight, food intake, water intake, haematology, clinical chemistrygenerated from the present study were subjected to computer statistical analysis using GraphPadPrism software, Version 5.00, USA. 2007.

One way ANOVA with Dunnets post-test was done for different treatment groupscomparing with the Control group data. The unpaired'-test was done for control recovery andhigh dose recovery group data. All analysis and comparisons was evaluated at 5% significancelevel.(10)

RESULTS AND DISCUSSION

Clinical signs and pre-terminal deaths

There were no clinical signs and pre-terminal deaths noticed in any of the doses tested.

Body weights and Body weight changes

None of the animals in treatment groups showed any statistically significant variations inbody weights compared to respective control group indicating that the test item did not had anyeffect on body weights.

Food consumption

There observed slight reduction in the food consumption of males and females duringtreatment period. However there was no statistically significant difference noted in the averageweekly food consumption of animals in treatment and recovery groups compared to the respectivecontrol groups.

Water intake

The average weekly water intake of males and females during treatment and recovery periods were comparable in all the groups.

Haematology

Treatment did not effect on hematological parameters estimated in both the sexes oftreatment and recovery groups when compared with control

GROUPS	WBC (10^6 cells/µl)	HGB (g/dl)	RBC (10^3 cells/µl)	PT (10^3 cells/µl)
Group- I (Control)	7.533 ± 1.106	15.767 ± 0.404	8.363 ± 0.217	658.667 ± 56.757
Group- III (Low dose)	7.200 ± 1.500	14.920 ± 0.303	7.546 ± 0.864	695.200 ± 46.257
Group- IV (Mid dose)	8.000 ± 1.212	14.860 ± 0.428	7.760 ± 0.422	741.800 ± 87.437
Group- V (High dose)	7.760 ± 0.404	15.000 ± 0.828	7.780 ± 0.517	715.400 ± 143.665

Summary of Hematology Parameters of Males during treatment period

GROUPS	WBC (10^6 cells/µl)	HGB (g/dl)	RBC (10^3 cells/µl)	PT (10^3 cells/µl)
Group- I R (Control Recovery)	8.333 ± 1.115	15.033 ± 0.451	8.170 ± 0.386	705.000 ± 217.000
Group- VI R (High dose Recovery)	7.880 ± 0.850	15.020 ± 0.507	7.908 ± 0.242	598.000 ± 94.618

Summary of Hematology of Males in Recovery Groups

GROUPS	WBC (10^6 cells/µl)	HGB (g/dl)	RBC (10^3 cells/µl)	PT (10^3 cells/µl)
Group- I (Control)	5.033 ± 0.586	16.033 ± 2.021	7.550 ± 0.537	578.333 ± 47.385
Group- III (Low dose)	6.340 ± 1.414	14.500 ± 0.324	7.364 ± 0.390	614.600 ± 69.371
Group- IV (Mid dose)	4.940 ± 0.564	14.180 ± 1.026	6.998 ± 0.414	490.800 ± 227.648
Group- V (High dose)	7.320 ± 1.591	14.520 ± 0.377	7.452 ± 0.192	595.200 ± 114.775

Summary of Hematology of Females in Treatment Groups

GROUPS	WBC (10^6 cells/µl)	HGB (g/dl)	RBC (10^3 cells/µl)	PT (10^3 cells/µl)
Group- I R (Control Recovery)	8.300 ± 2.227	14.967 ± 0.551	7.690 ± 0.052	686.333 ± 230.405
Group- VI R (High dose Recovery)	7.560 ± 1.113	14.420 ± 0.763	7.358 ± 0.405	647.200 ± 234.421

Summary of Hematology of Females in Recovery Groups

Serum Biochemistry

Clinical chemistry parameters of the treatment and recovery groups were compared with the control groups. There were no treatment related significant differences in biochemical valuesof study animals

Summary of Serum Biochemistry of Males in Treatment Groups

GROUPS	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Glucose (mg/dl)	TP (g/dl)	Albumin (g/dl)	Bilirubin Total (mg/dl)	Bilirubin Direct (mg/dl)	Uric acid (mg/dl)	Urea (mg/dl)	Creatine (mg/dl)
Group- I Control	78.600 ± 16.372	67.300 ± 3.422	56.200 ± 11.621	183.200 ± 37.414	50.333 ± 2.802	282.300 ± 151.950	97.733 ± 6.216	6.730 ± 0.724	2.000 ± 0.100	0.133 ± 0.058	0.033 ± 0.058	2.660 ± 0.520	34.833 ± 1.305	0.410 ± 0.053
Group- III Low dose	78.080 ± 11.513	74.020 ± 5.569	66.200 ± 13.320	166.160 ± 28.276	50.780 ± 6.659	284.880 ± 30.851	108.660 ± 28.878	6.800 ± 0.305	1.920 ± 0.084	0.180 ± 0.084	0.100 ± 0.071	1.936 ± 0.275	37.900 ± 2.783	0.472 ± 0.051
Group- IV Mid dose	81.640 ± 17.193	64.820 ± 10.393	58.040 ± 15.676	177.040 ± 55.455	43.960 ± 7.987	357.800 ± 88.149	99.000 ± 12.141	6.972 ± 0.436	1.940 ± 0.134	0.160 ± 0.089	0.060 ± 0.089	2.814 ± 1.280	37.300 ± 5.382	0.432 ± 0.044
Group- V High dose	84.120 ± 3.206	63.240 ± 7.162	54.280 ± 8.321	203.580 ± 37.003	47.620 ± 9.646	366.180 ± 76.327	101.900 ± 12.352	7.234 ± 0.166	1.920 ± 0.130	0.080 ± 0.045	0.040 ± 0.055	2.270 ± 0.491	34.680 ± 2.249	0.464 ± 0.034

Summary of Serum Biochemistry of Males in Recovery Groups

GROUPS	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Glucose (mg/dl)	TP (g/dl)	Albumin (g/dl)	Bilirubin Total	Bilirubin Direct	Uric acid (mg/dl)	Urea (mg/dl)	Creatine (mg/dl)
Group- I R Control Recovery	96.900 ± 6.332	75.433 ± 8.056	67.033 ± 12.123	226.767 ± 14.400	51.500 ± 2.787	201.900 ± 77.639	100.333 ± 10.439	7.817 ± 0.540	2.133 ± 0.208	0.100 ± 0.000	0.000 ± 0.000	2.167 ± 0.393	36.867 ± 2.371	0.390 ± 0.104
Group- VI R High dose Recovery	104.940 ± 20.810	75.860 ± 10.948	72.740 ± 15.096	214.360 ± 36.910	57.920 ± 13.706	333.380 ± 161.513	99.860 ± 10.492	6.980 ± 0.833	1.920 ± 0.084	0.080 ± 0.045	0.000 ± 0.000	3.506 ± 3.704	39.740 ± 5.385	0.512 ± 0.140

Summary of Serum Biochemistry of Females in Treatment Groups

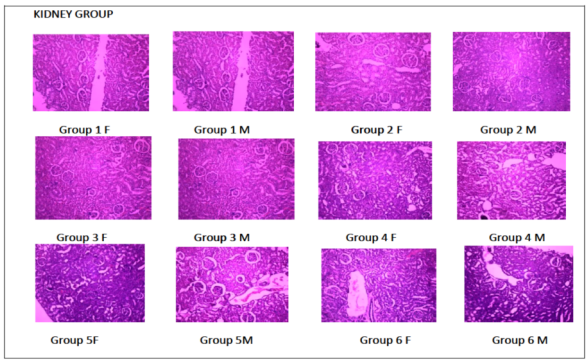
GROUPS	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Glucose (mg/dl)	TP (g/dl)	Albumin (g/dl)	Bilirubin Total (mg/dl)	Bilirubin Direct (mg/dl)	Uric acid (mg/dl)	Urea (mg/dl)	Creatine (mg/dl)
Group- I (Control)	98.667 ± 4.899	87.533 ± 11.347	88.867 ± 1.644	161.667 ± 18.054	43.133 ± 4.737	220.933 ± 38.859	102.467 ± 16.805	7.507 ± 1.365	2.200 ± 0.265	0.100 ± 0.000	0.067 ± 0.058	2.587 ± 0.674	31.333 ± 0.929	0.457 ± 0.006
Group- III Low dose	88.240 ± 13.647	69.000 ± 9.757	64.160 ± 16.015	144.940 ± 13.368	52.360 ± 1.679	271.380 ± 51.905	100.540 ± 19.620	6.348 ± 0.268	1.860 ± 0.114	0.060 ± 0.055	0.020 ± 0.045	2.062 ± 0.712	36.000 ± 1.042	0.412 ± 0.088
Group- IV Mid dose	95.660 ± 25.686	61.200 ± 12.329	67.100 ± 27.206	213.920 ± 48.199	47.640 ± 5.213	276.940 ± 76.645	105.900 ± 17.046	7.170 ± 0.877	1.940 ± 0.167	0.060 ± 0.055	0.000 ± 0.000	2.312 ± 0.718	36.060 ± 6.695	0.444 ± 0.068
Group- V High dose	99.060 ± 12.505	64.320 ± 9.825	80.260 ± 13.986	160.140 ± 19.442	47.780 ± 7.745	251.960 ± 99.900	104.680 ± 10.716	7.228 ± 0.517	2.020 ± 0.148	0.140 ± 0.055	0.020 ± 0.045	2.038 ± 0.243	36.380 ± 2.161	0.520 ± 0.031

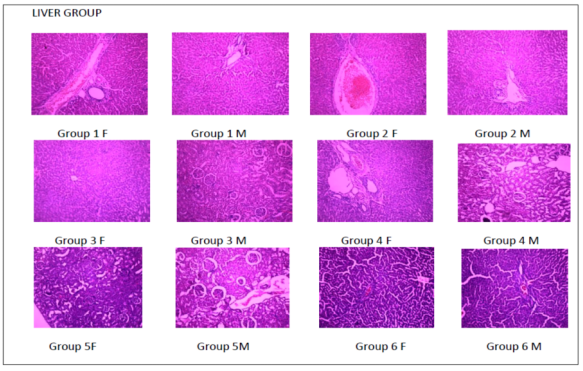
Summary of Serum Biochemistry of Females in Recovery Groups

GROUPS	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Glucose (mg/dl)	TP (g/dl)	Albumin (g/dl)	Bilirubin Total (mg/dl)	Bilirubin Direct (mg/dl)	Uric acid (mg/dl)	Urea (mg/dl)	Creatine (mg/dl)
Group- I R Control Recovery	89.000 ± 5.429	60.433 ± 3.769	72.500 ± 6.102	225.733 ± 5.260	43.833 ± 1.422	200.533 ± 23.548	90.800 ± 5.048	7.377 ± 0.761	2.100 ± 0.173	0.167 ± 0.058	0.000 ± 0.000	3.823 ± 2.722	32.067 ± 1.457	0.373 ± 0.116
Group- VI R High dose Recovery	100.800± 17.352	60.740 ± 12.891	66.020 ± 19.166	237.620 ± 32.452	51.520 ± 5.249	214.440 ± 76.789	114.380 ± 24.950	6.400 ± 1.216	1.860 ± 0.251	0.160 ± 0.055	0.040 ± 0.089	2.744 ± 1.230	35.100 ± 3.687	0.530 ± 0.131

Histopathological Examination

None of the animals in treatment and recovery groups showed any histological changes during evaluation, indicating that the test item did not cause any histological changes in the tissues.





Group 1F : Group-I female, Group 1M : Group-I male/ Group 2F : Group-II female, Group 2 M : Group-II male/Group 3F : Group-III female, Group 3M : Group-III male/Group 4F :Group-IV female, Group 4M : Group-IV male, Group 5F: Group-IV female, Group 5M: Group-IV male/Group 6F: Group-IVR female, Group 6M: Group-IVR male

CONCLUSIONS

No clinical signs and mortality were noticed up to the high dose of 1000mg/kg Body wt. There were no significant treatment related changes in average weekly water intake of malesand females during treatment and recovery period.No effect observed on average daily food consumption was noted in both the sexes up tothe high dose.No differences were noted in body weight and body weightgains of animals across different groups.No effect on hematological parameters of male and female rats treated up to the dose of1000 mg/kg Body wt. was noted.There were no treatments related significant differences in serum biochemical values ofstudy animals treated at and up to the level of 1000 mg/kg Body wt.

REFERENCES

1. Canadian Council on Animal Care (CCAC) guidelines (www.ccac.ca).
2. Heywood, R, and R.W James and R.JSortwell. 1978. Toxicology studies of LAS in rhesus monkeys: (I) simultaneous oral subcutaneous administration for 28 days, Toxicology. 11, pp. 245-250.
3. Human & Environmental Risk Assessment (HERA). 2013. LAS: linear alkyl benzene sulphonate. Revised HERA Report April 2013; accessed June 16, 2014. http://www.heraproject.com/RiskAssessment.cfm.
4. Ito R, H Kawamura, H.S Chang, K.Kudo, S.Kajiwara, S.Toida, Y. Seki, M. Hashimoto and A.Fukushima.Acute, subacute, and chronic toxicity of magnesium LAS (LASMg). J. Med. Soc. Toho Univ. 25.1978. Pp. 850-875.
5. Kay JH, FE Kohn, JC Calandra..Subacute oral toxicity of a biodegradable LAS, Toxicol. Appl. Pharmacol. 7.1965.pp. 812-818.
6. Leuschner, F., R. Poche and C. Gloxhuber. Toxicological examination of a dishwashing agent. Fette ,Seifen , Anstrichm. 7(1),1969.Pp. 570580.
7. OECD Guideline for Testing Chemicals, Section 4. Test No. 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents.
8. Oser BL and K Morgareidge. 1965. Toxicological studies with branched and linear alkylbenzenesulphonates in rats, Toxicol. Appl. Pharmacol. 7: 819-825.
9. Smyth, H.F., Seaton, J. & Fischer, L. 1941. Some pharmacological properties of the "Tergitol" penetrants.J. Ind. Hyg. Toxicol., 23, 478483.
10. Yoneyama&Hiraga 1977. Effect of Linear AlkylbenzeneSulfonate on Serum Lipid in Rats, J Ann Rep Tokyo Metrop Res Lab, Public Health 28(2): 109-111.