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EVALUATION OF SAFETY OF ARIFLEX TABLET - A POLYHERBAL FORMULATION IN SPRAGUE DAWLEY RATS

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ABSTRACT

Objectives: Ayurvedic plants are known for many years to have anti-inflammatory and anti-arthritic effects. Ariflex tablet is a polyherbal formulation intended for treatment of osteoarthritis, rheumatoid arthritis, gout, lumbago, sciatica, spondylitis etc., Acute and repeated dose 90-days studies were conducted to evaluate the safety profile of Ariflex tablet in rats. **Materials and Methods:** In acute study, Ariflex tablet was finely powdered, suspended in water and then administered orally to Sprague Dawley rats at 2000 mg/kg. In repeated dose study, Ariflex tablet was administered to rats at 250, 500 and 1000 mg/kg by oral gavage for 90 days and assessed for treatment related changes in body weight, feed consumption, hematological, biochemical and pathological parameters. Histopathological examination was conducted for tissues from control and the high dose groups and was extended to target organs from the lower dose, intermediate dose and recovery groups. **Results:** In acute study, the test item did not produce any mortality or adverse clinical signs. In the 90-days oral toxicity study, animals did not exhibit any toxicity related symptoms and no mortality was observed. No significant changes were found in hematological and biochemical parameters. Also, significant abnormal changes were not observed in relative organ weights. Even microscopic findings did not show any changes when compared with control group animals and animals in recovery group. **Conclusions:** Based on the findings of the study, the median lethal dose of Ariflex tablet was found to be more than 2000 mg/kg. The No Observed Adverse Effect Level (NOAEL) of Ariflex tablet can be considered as 1000 mg/kg in both male and female rats, under the

experimental conditions and doses employed. The results suggest that Ariflex tablet is well tolerated antiarthritic formulation.

KEYWORDS: Acute, polyherbal, sub chronic, toxicity, Ariflex tablet.

INTRODUCTION

Arthritis is a debilitating disease that severely affects the quality of life of patient. It is an inflammatory disease, affecting mainly joints. It affects globally about 1-2 % of the population. Conventional modern medicine is devoid of satisfactory treatment to severe cases of these diseases. To a large extent, these diseases are treated symptomatically and the drugs used in the treatment have varying levels of toxic side effects. Long term usage of NSAIDs leads to development of hyperacidity, gastric ulcers and duodenal ulcers. Long term corticosteroid use results in hyperglycemia, weight gain and osteoporosis. DMARDs used in the treatment of Rheumatoid arthritis are discontinued by patients either because of lack or loss of efficacy or clinically apparent or subjective side effects (signs and symptoms related to therapy), and laboratory abnormalities (as surrogate markers of organ damage). Therefore patients seek alternative therapies especially herbal medicine as a safer and effective option. Complementary and Alternative Medicine (CAM)[1,13] is rapidly becoming a viable option to modern pharmacotherapy for a multitude of chronic ailments.

These include degenerative and inflammatory bone and joint conditions such as osteoarthritis and rheumatoid arthritis. However their efficacy and safety are not clear. It is only in recent times that some of the single/multiple herbs have been tested for acute, subs chronic and chronic toxicity by modern testing methods.[14,16] These plants usually consist of many active ingredients.[2, 11, 18, 22, 27, 36, 37] Hence evaluation of these multicomponent herbal products using conventional toxicological assessment methods is difficult.

Ari Healthcare Pvt. Ltd. has conceptualized and developed innovative polyherbal formulation i.e. Ariflex Tablet for the effective management of osteoarthritis, Rheumatoid arthritis, Gouty arthritis, Sciatica and Spondylosis. Ariflex tablet is a herbal formulation composed of extracts of established medicinal plants such as *Boswellia serrate*[3,4,12,17,25,35], *Commiphora mukul*[5,19,20,21,29,30,32,33,34], *Pluchea lanceolata, Withania somnifera*[6,7,10], *Vitex negundo*[24], *Tinospora cordifolia*[8,9,26,31], *Ricinus communis*[28], and *Zingiber officinale*.[38,39,40]

These plants have been indicated for the treatment of osteoarthritis, rheumatoid arthritis,

lumbago, spondylitis, etc. Organ protective activity of these plants is also demonstrated by many research workers. The doses and ratios of each plant were selected from large spectrum of plants known for their anti-inflammatory and immunomodulatory properties. Although herbal entities are believed to be relatively safe, the toxicity characteristics of the test materials need to be confirmed prior to human clinical trials. Generally this is accomplished by conducting general preclinical safety studies to uncover potential toxic effects of drug in question. Our modest attempt was to demonstrate that the combination semi purified plant extracts can be tested along similar lines as prescription drug. With the above considerations, the present study was aimed to assess the single dose acute oral toxicity and 90-days repeated dose toxicity of novel herbal formulation Ariflex tablet.

MATERIALS AND METHODS

Preparation of herbal formulation

Ariflex Tablets were manufactured and supplied by **Ari Healthcare Pvt. Ltd.,** R & D Center, Unit No.401, International Biotech Park, BTS 2 Building, Chrysalis Enclave,4th Floor, Plot No.2, MIDC Phase II, Hinjewadi, Pune-411057. India. The polyherbal formulation **Ariflex Tablet** comprises of standardized extracts of the Ayurvedic plants, namely Shallaki extract (*Boswellia serrata*) - 110 mg, Guggulu extract (*Commiphora mukul*) - 100 mg, Rasna extract (*Pluchea lanceolata*) – 65 mg, Ashwagandha extract (*Withania somnifera*) - 65 mg, Nirgundi extract (*Vitex negundo*) - 60 mg, Guduchi extract (*Tinospora cordifolia*) – 55 mg, Eranda extract (*Ricinus communis*) - 50 mg and Shunthi extract (*Zingiber officinale*) - 20 mg.

The above composition was approved by FDA, Maharashtra State and also by FDA, Uttarakhand State as an Ayurvedic Proprietary Medicine.

Animals

Female Sprague Dawley (SD) rats of 8-12 week's age were used for the acute oral toxicity and SD rats of either sex of 7-8 weeks age were used for the 90-days repeated dose toxicity. All animals were bred and reared at animal house of TOXINDIA; Pune, India. The females used were nulliparous and non-pregnant. All animals were acclimatized and maintained under standard housing conditions (temperature: 21±2^{0}C, relative humidity: between 55±5% with 12 -15 air changes per hour and 12 h light-12 h dark cycle).

All animals were provided with purified water and Nutrimix brand pelleted standard rat and

mice feed Manufactured by Nutrivet Life Sciences, Panchal Nivas, Uruli Devachi Fata, Saswad Road, Pune, was provided *ad libitum.*

All experiments were carried out after the approval of Institutional Animal Ethics Committee (IAEC) and in accordance with the OECD Guidelines for Testing of Chemicals, Section 4, No. 423 - Acute Oral Toxicity - Acute Toxic Class Method, adopted 17 December, 2001 and "Repeated Dose (90 day) Oral Toxicity Study with 28 Day Recovery Period" OECD 408 adapted on 21 September, 1998.

Study design

Acute oral toxicity study

The study was conducted in accordance with OECD guidelines for the testing of chemicals, Section 4, No. 423 - Acute Oral Toxicity - Acute Toxic Class Method. Animals were fasted overnight and 4 hr. after test substance administration but water was provided *ad libitum*. The sighting study was conducted by dosing group of three animals at 2000 mg/kg body weight (p.o.). The treated animals were observed carefully for presence of adverse clinical signs and mortality at 10 min, 30 min, 1, 2, 4 and 6 h after dosing and once daily for 14 days.

90-days repeated dose oral toxicity study

The study was conducted in accordance with the OECD guideline 408 (Repeated Dose 90-day Oral Toxicity Study in Rodents).[11] One twenty Sprague Dawley rats of either sex were equally divided into six groups (each consisting of 10 males and 10 females).

G1 and G5R (R - Recovery) served as vehicle controls and the animals were daily administered with normal saline by oral gavage. Groups G2, G3 and G4/G6R were administered daily at the dose of 250, 500 and 1000 mg/kg of **Ariflex Tablet** by oral gavage once daily for consecutive 90 days, respectively. The dose volume was maintained at 10 ml/kg. Vehicle control recovery (G1R) and high dose recovery (G4R) groups were maintained for a further 28 days period without administration of either vehicle or test item.

Throughout the study period, the animals were observed for clinical signs of toxicity and mortality/morbidity on daily basis. Detailed veterinary examination, body weight and feed consumption (weekly), functional observation test were done in week 13.

Ophthalmoscopic examination was done initially and in week 13. Hematological, clinical chemistry, urinalysis, gross pathology and target organ weighing were performed at

termination. Histopathological examination was conducted on the specified list of tissues from the control and the high dose groups and was extended to target organs from the lower dose and recovery groups.

Statistical analysis

The raw data was subjected to statistical analysis. The data on body weight and weight gain, feed intake, organ weights and ratios, hematological and clinical chemistry estimations, urine analysis were analyzed statistically.

One way ANOVA with Dunnet test was used for different treatment groups comparing with the control group and the unpaired *t*-test was done for comparing control recovery and high dose recovery group data. All analyses and comparisons were evaluated at the 95% level of confidence ($P < 0.05$).

RESULTS

Acute oral toxicity study

No mortality, abnormal clinical signs, or any gross pathology findings were observed in animals treated with 2000 mg/kg body weight. Body weight gain in all animals found to be normal during the experiment period

90-days repeated dose oral toxicity study

No clinical signs of toxicity or mortality were noted upon sub chronic exposure of the test item. **Ariflex Tablet** did not induce any remarkable and significant alteration in mean body weight of the male and female rats (Fig.1 and Fig. 2). The feed consumption of different groups of rats was found to be comparable to control groups.

The feed consumption of recovery groups of control and high dose rats also found to be consistent with the main groups. Functional observational tests (open field observations, locomotor activity, and grip strength) and Ophthalmoscopy examination revealed no abnormal findings. Treatment of **Ariflex tablet** did not exhibit any remarkable changes in hematological (Tables 1 and 2), clinical chemistry (Tables 3 and 4) and in urine analysis when compared to control group.

The group means values of absolute and relative weights of kidneys; liver, adrenals, testes/ovaries, spleen, brain, epididymis/uterus, thymus and hearts of male and female rats treated with **Ariflex Tablet** were found to be comparable to those of the respective control

group rats at termination of the treatment and reversal periods [Tables 5 and 6].

Different doses of **Ariflex Tablet** did not induce any remarkable and treatment related gross pathological alterations in any of the organs/tissues of treated rats and that of reversal group. All the microscopic changes noticed in this study appeared to be incidental, as their frequency and severity remained identical for the control and the treated animals.

Ariflex Tablet at and up to the dose level of 1000 mg/kg body weight did not produce any histopathological effects in rats under the said experimental conditions.

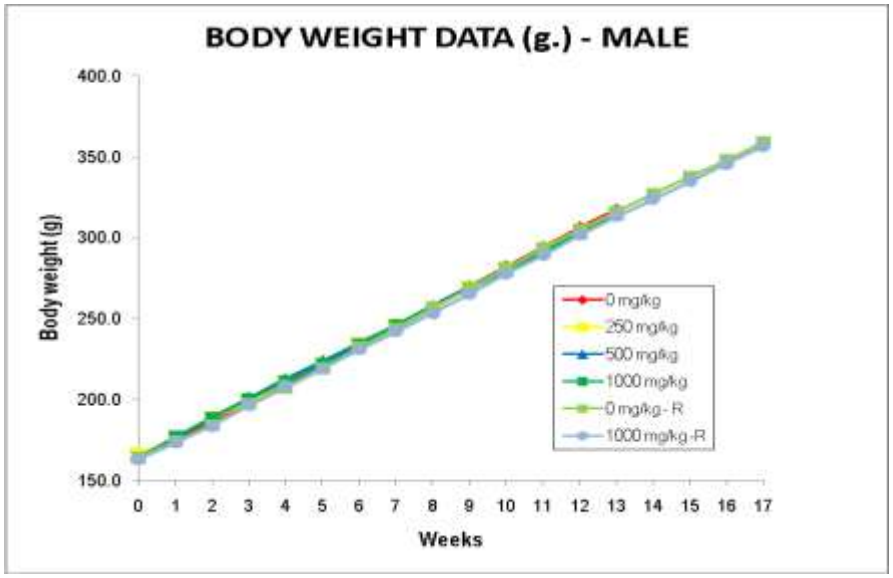

FIG.1: EFFECT OF ARIFLEX TABLET ON BODY WEIGHTS (g)

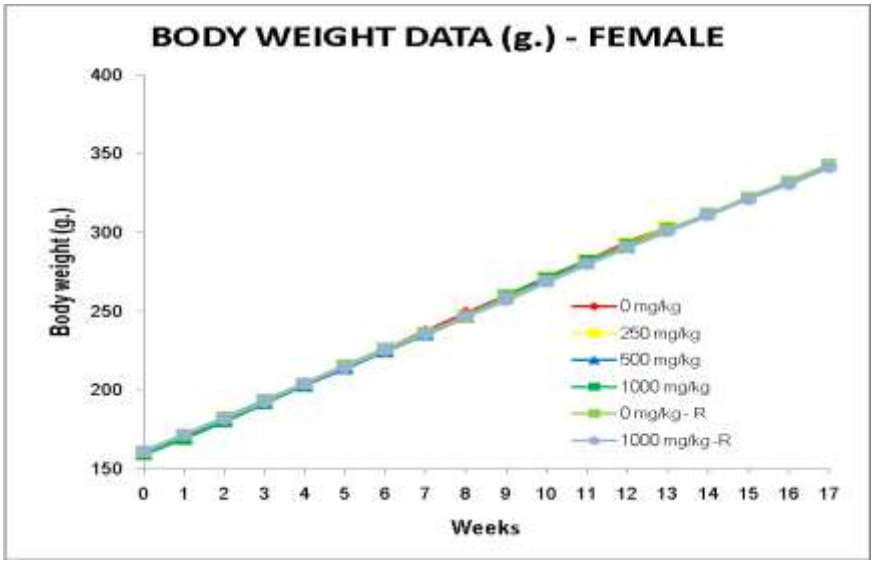

FIG.2: EFFECT OF ARIFLEX TABLET ON BODY WEIGHTS (g)

Table 1: effect of ariflex tablet on haematological parameters- male rats

Group & Dose (mg/kg)		Hb (g/dl)	PCV (%)	Total RBC ($x10^6$/ cmm)	RBC Indices			Total WBC ($x10^3$/ cmm)	Differential WBC (%)				Platelets ($x10^3$/ cmm)
					MCH (pg)	MCV (fl)	MCHC (g/dl)		N	L	E	M	
G1 0	Mean	13.97	39.73	8.20	17.20	48.74	35.25	14.05	27.40	68.90	2.30	1.40	839.40
	± SD	1.18	2.05	0.77	2.48	4.06	3.56	1.00	4.01	4.63	1.34	1.07	51.32
G2 250	Mean	14.06	39.84	8.61	16.43	46.66	35.43	13.97	29.20	67.30	2.10	1.40	848.80
	± SD	1.08	2.35	0.78	1.71	5.29	3.81	1.17	2.70	2.54	1.20	0.84	57.14
G3 500	Mean	13.98	40.76	8.33	16.93	49.47	34.37	13.87	27.60	68.80	2.00	1.60	849.60
	± SD	1.16	2.19	1.00	1.81	5.46	3.09	1.17	3.66	3.97	1.25	0.97	61.04
G4 1000	Mean	13.70	39.58	8.44	16.43	47.25	34.73	13.88	28.00	68.00	2.20	1.80	835.70
	± SD	1.16	1.93	0.83	2.59	4.47	3.97	1.43	3.65	4.47	1.23	1.23	55.88
G5(R) 0	Mean	13.78	40.44	8.13	17.05	50.19	34.20	13.78	27.20	69.20	2.20	1.40	840.40
	± SD	1.16	2.16	0.72	1.86	6.11	3.70	0.97	3.77	3.82	1.23	0.84	61.50
G6(R) 1000	Mean	14.09	39.98	8.36	16.90	48.30	35.48	14.11	28.00	68.10	2.10	1.80	837.90
	± SD	1.06	2.61	0.72	1.11	6.54	4.57	0.98	2.91	2.42	1.10	1.03	57.09

Table 2: Effect Of Ariflex Tablet On Haematological Parameters- Female Rats.

Group & Dose (mg/kg)		Hb (g/dl)	PCV (%)	Total RBC ($x10^6$/ cmm)	RBC Indices			Total WBC ($x10^3$/ cmm)	Differential WBC (%)				Platelets ($x10^3$/ cmm)
					MCH (pg)	MCV (fl)	MCHC (g/dl)		N	L	E	M	
G1 0	Mean	13.94	39.96	8.24	17.08	49.03	34.98	13.90	26.70	69.50	2.20	1.60	855.70
	± SD	1.18	2.21	0.79	2.35	6.59	3.51	1.21	4.00	3.89	1.14	1.17	59.42
G2 250	Mean	13.85	40.42	8.59	16.30	47.66	34.41	14.05	27.60	68.50	2.30	1.60	843.90
	± SD	0.96	2.24	0.88	2.27	6.89	3.64	0.97	3.84	3.31	1.16	0.97	56.15
G3 500	Mean	14.16	40.24	8.31	17.18	48.91	35.32	13.89	27.50	68.30	2.30	1.90	847.40
	± SD	1.01	2.60	0.73	2.17	6.57	3.28	1.31	4.53	4.67	1.16	1.10	61.78
G4 1000	Mean	14.13	40.34	8.38	16.96	48.57	35.09	14.13	26.90	68.90	2.40	1.80	841.40
	± SD	1.30	2.58	0.75	1.94	6.05	3.24	1.07	4.04	2.88	1.26	0.92	64.59
G5(R) 0	Mean	13.73	39.95	8.46	16.38	47.70	34.61	13.97	28.10	67.90	2.40	1.60	842.70
	± SD	1.26	2.40	0.82	2.34	6.12	4.87	1.13	3.87	3.60	1.26	0.97	62.75
G6(R) 1000	Mean	13.77	39.89	8.30	16.70	48.26	34.65	14.08	27.60	68.50	2.30	1.60	848.50
	± SD	1.02	2.28	0.67	1.93	3.65	3.69	1.13	4.25	4.14	1.16	1.17	65.99

Table 3: Effect Of Ariflex Tablet On Clinical Chemistry Parameters – Male Rats

Group & Dose (mg/kg)		Bili (mg/dl)	Albumin (g/dl)	TP (g/dl)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Gluc (mg/dl)	UN (mg/dl)	Na (mmol/L)	K (mmol/L)	Cholesterol (mg/dl)
G1	Mean	0.61	4.06	6.63	73.70	107.10	158.30	129.10	12.55	154.90	6.86	156.80
0	± SD	0.10	1.03	0.85	7.15	6.14	6.02	4.98	1.40	7.14	1.22	7.93
G2	Mean	0.58	3.78	6.45	71.50	110.00	157.40	128.70	13.89	156.50	7.47	155.40
250	± SD	0.08	0.99	0.72	9.18	6.67	8.73	4.06	1.47	7.17	1.41	7.78
G3	Mean	0.58	3.71	6.56	73.60	109.50	157.50	130.00	13.09	156.50	7.32	156.30
500	± SD	0.10	1.05	0.70	9.40	6.17	8.58	3.40	1.80	7.47	1.38	9.01
G4	Mean	0.57	3.92	6.35	73.10	109.40	156.50	128.10	12.18	155.00	7.22	156.80
1000	± SD	0.09	0.90	0.69	7.19	6.50	7.47	4.93	1.58	7.07	1.23	6.51
G5(R)	Mean	0.56	3.62	6.37	73.80	110.20	156.60	128.30	13.65	155.90	7.48	155.50
0	± SD	0.06	1.15	0.67	7.84	6.88	7.55	5.81	1.25	6.51	0.84	8.82
G6(R)	Mean	0.56	3.96	6.00	75.30	111.90	157.50	129.10	12.79	155.80	7.51	157.50
1000	± SD	0.10	1.21	1.19	9.49	6.01	9.77	2.77	1.46	5.98	0.91	8.02

Table 4: Effect Of Ariflex Tablet On Clinical Chemistry Parameters – Female Rats

Group & Dose (mg/kg)		Bili (mg/dl)	Albumin (g/dl)	TP (g/dl)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Gluc (mg/dl)	UN (mg/dl)	Na (mmol/L)	K (mmol/L)	Cholesterol (mg/dl)
G1	Mean	0.58	3.87	6.64	74.10	112.00	158.50	129.20	14.50	158.60	7.78	153.50
0	± SD	0.06	0.86	0.52	8.14	4.71	8.32	5.67	1.55	6.15	1.15	5.44
G2	Mean	0.56	3.62	6.35	70.80	108.50	156.80	127.90	11.95	155.60	7.26	154.50
250	± SD	0.08	0.98	0.65	9.07	5.99	7.70	4.43	1.27	6.17	1.33	6.85
G3	Mean	0.55	3.65	6.25	69.80	107.80	155.20	128.80	12.45	155.10	6.86	153.40
500	± SD	0.08	1.18	0.59	5.33	5.81	7.76	4.24	1.75	7.16	1.26	7.59
G4	Mean	0.55	3.60	6.46	68.90	106.10	154.50	128.30	11.73	155.40	6.90	153.90
1000	± SD	0.08	1.05	0.64	5.59	6.15	8.18	4.27	1.05	7.46	1.32	8.72
G5(R)	Mean	0.55	4.32	6.68	74.00	109.70	159.30	129.20	12.79	156.10	7.48	155.20
0	± SD	0.09	1.10	0.87	8.79	6.11	7.85	4.64	1.40	8.32	1.21	8.95
G6(R)	Mean	0.56	4.17	6.33	75.00	110.80	158.60	127.50	12.05	154.70	7.65	153.80
1000	± SD	0.07	1.13	0.78	7.51	5.53	9.64	5.80	1.59	7.30	1.17	8.83

Table 5: Effect Of Ariflex Tablet On Relative Organ Weights (%)- Male Rats

Group & Dose (mg/kg)		Adrenals	Testes	Brain	Kidney	Liver	Heart	Spleen	Epididymis	Thymus
G1	Mean	0.024	0.941	0.956	0.934	4.535	0.588	0.505	0.519	0.237
0	± SD	0.003	0.083	0.061	0.065	0.467	0.087	0.069	0.047	0.034
G2	Mean	0.023	0.914	0.927	0.920	4.652	0.575	0.507	0.508	0.228
250	± SD	0.003	0.076	0.078	0.063	0.345	0.111	0.085	0.064	0.027
G3	Mean	0.023	0.901	0.921	0.975	4.541	0.567	0.488	0.515	0.230
500	± SD	0.003	0.106	0.086	0.060	0.570	0.080	0.096	0.065	0.030
G4	Mean	0.023	0.932	0.910	0.928	4.722	0.572	0.473	0.485	0.235
1000	± SD	0.002	0.097	0.065	0.089	0.539	0.096	0.072	0.069	0.023
G5(R)	Mean	0.020	0.810	0.819	0.820	3.973	0.472	0.441	0.428	0.204
0	± SD	0.003	0.064	0.058	0.068	0.286	0.096	0.076	0.028	0.021
G6(R)	Mean	0.020	0.810	0.809	0.811	3.957	0.525	0.445	0.435	0.209
1000	± SD	0.002	0.081	0.056	0.059	0.307	0.056	0.069	0.034	0.024

TABLE 6: EFFECT OF ARIFLEX TABLET ON RELATIVE ORGAN WEIGHTS (%)- Female Rats

Group & Dose (mg/kg)		Adrenals	Ovaries	Brain	Kidney	Liver	Heart	Spleen	Uterus	Thymus
G1	Mean	0.024	0.032	0.978	0.956	4.679	0.563	0.508	0.248	0.241
0	± SD	0.003	0.009	0.110	0.107	0.331	0.127	0.058	0.023	0.022
G2	Mean	0.024	0.031	0.920	0.987	4.592	0.564	0.534	0.239	0.231
250	± SD	0.004	0.006	0.093	0.080	0.411	0.100	0.053	0.029	0.031
G3	Mean	0.025	0.031	0.944	0.955	4.715	0.544	0.503	0.241	0.252
500	± SD	0.003	0.005	0.071	0.095	0.418	0.115	0.083	0.043	0.027
G4	Mean	0.024	0.032	0.973	0.913	4.966	0.577	0.539	0.235	0.258
1000	± SD	0.003	0.009	0.084	0.156	0.574	0.111	0.069	0.032	0.027
G5(R)	Mean	0.021	0.029	0.840	0.842	4.301	0.547	0.472	0.222	0.219
0	± SD	0.004	0.005	0.086	0.057	0.363	0.050	0.047	0.020	0.021
G6(R)	Mean	0.022	0.028	0.825	0.857	4.374	0.527	0.476	0.226	0.214
1000	± SD	0.002	0.004	0.086	0.067	0.328	0.095	0.070	0.020	0.022

DISCUSSION

The interest in use of herbal preparations in different parts of the world has been growing considerably with corresponding developments in the phytomedicinal therapy. Herbal remedies positioned themselves in various forms such as dietary supplements, mono or polyherbal drugs, dietary ingredients etc. and have become famous and safe commercial commodities. However, the herbal preparations, irrespective of the popular belief that they are safe based on ancient literature, required to be confirmed for their non-toxic/relatively less toxic effects compared to the chemical therapeutic counterparts.[12] This critical prerequisite, especially in the form of acceptance by the western countries, provided the impetus to carry out scientific studies in accordance with the various established regulatory guidelines applicable to the geographic requirements.

Typically, safety studies on herbal compounds intended for oral use involve acute oral toxicity study in rodents which helps to determine the dose levels for short-term and long-term repeated dose toxicity studies. Despite the alternative views on use of LD_{50} data, acute studies still continue to be considered valuable in establishing target organ toxicity. In the present study, Ariflex tablet did not produce mortality/morbidity or adverse clinical signs up to the dose level of 2000 mg/kg which indicates the wide margin of safety level upon exposure of single large dose. It is to be noted that the test substances are generally labeled 'Unclassified (category 5)' and the cut off value is 5000 mg/kg body weight according to the Globally Harmonized System (GHS) for classification of chemicals when they have been comparatively safe at limit dose levels (i.e. 2000 mg/kg). Sub acute oral toxicity studies are carried out to evaluate the likely adverse effects upon prolonged exposure of a test substance to animals and to gather information about the deleterious health effects due to repeated exposures including target organ toxicity, cumulative effects, and to determine the dose level at which there is no observed adverse effect.[13] In the current study, treatment with Ariflex tablet up to 1000 mg/kg was well tolerated. Absence of treatment related deaths or toxic signs throughout the study are direct indication of relatively harmless nature of the test compound over prolonged exposure. The compounds with toxicity potentials are believed to impact on feed intake, metabolic processes, and consequently on the body weight gain. As is observed from scientific literature, a decrement of more than 10% in body weight gain is considered to be detrimental on long term administration of test materials. However, such a trend was not observed in the current study since the body weight gain of different groups remained comparable till the end of the study period with corresponding normal feed consumption

clearly demonstrated the normal metabolic process in rats administered with Ariflex tablet. The body weight gain of both sexes of different groups was found to be continuously increasing over treatment period including the recovery period. Functional observational tests (open field observations, locomotor activity, grasping strength) and Ophthalmoscopy examination of treated rats revealed no considerable alterations. These observations, in general, reveal that the herbal preparation did not interfere with neuromuscular physiology and autonomic activities of treated animals. No abnormal dose dependent changes have been observed in the blood parameters, the variations can be considered spontaneous, incidental and no treatment related in the tested rats. Repeated dose safety studies provide information on target organ toxicity upon continuous exposure of test substance intended for prolonged use in target species. The present investigation did not record any treatment related gross pathological lesions. No statistical significant changes were observed in the relative weight. No toxicologically significant or treatment related changes in urine analysis parameters (data not shown) were observed at all the dose levels. The normal values of majority of hematological and clinical chemistry end points revealed no organ damage related effects. No significant test item related microscopic observations were found in other organs.

CONCLUSION

Based on the results of the study, it can be concluded that **Ariflex tablet** is non toxic up to 2000 mg/ kg body weight, when administered as a single dose by oral gavage to Sprague Dawley rats and can be categorized ‘Unclassified (category 5)’ according to the Globally Harmonized System (GHS) for classification of chemicals and the cut off value is 5000 mg/kg body weight. The No Observed Adverse Effect Level (NOAEL) of Ariflex tablet can be considered as 1000 mg/kg in both male and female rats, under the experimental conditions and doses employed in Repeated Dose 90-day Oral Toxicity Study in Rat.

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