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Original Article

Safety evaluation of a proprietary ayruveda-based polyherbal preparation (arthralgex) used for arthritis

Avaliação de segurança de uma preparação polierbal proprietária à base de ayruveda (arthralgex) usada para artrite

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Abstract

Arthralgex is a proprietary polyherbal preparation used in clinics to treat rheumatoid arthritis for decades. Its safety evaluation has not been reported. The study is aimed at evaluating the safety of arthralgex using Wistar rats, as per OECD guidelines. According to OECD 407, rats of either gender were separated into six groups (n= 6 each). The dose of arthralgex was decided based on an acute toxicity study. Under the treatment group, separate set of rats received arthralgex in three dose levels like - low, medium, and high (200, 400 & 800 mg/kg/day; p.o for 28 days). Satellite groups received high dose (800 mg/kg/day, p.o for 28 days), and control group received equal volume of vehicle. On day 28, blood samples were collected to estimate hematology and biochemistry parameters. Subsequently, rats were euthanized to collect organs for weighing and histopathology. Satellite groups were maintained for an additional 14 days post-treatment to assess toxicity reversibility and euthanized on day 43. Arthralgex did not show any signs of toxicity or major change in body weight in the acute toxicity study. Arthralgex has no significant adverse effect on general health status as confirmed by body weight, feed intake, hematology, biochemistry, urine analysis, internal organs, relative organ weight, and histopathological evaluation after 28 day treatment. Arthralgex could be considered safe for short-term treatment. Present findings may help researchers in dose fixing for sub-chronic and chronic toxicity studies, which is essential for safety evaluation for long-term use.

Keywords: ayurveda, arthralgex, polyherbal formulation, sub-acute toxicity.

Resumo

Arthralgex é uma preparação poli-herbal patenteada usada em clínicas para tratar a artrite reumatoide há décadas. Sua avaliação de segurança não foi relatada. O estudo tem como objetivo avaliar a segurança do arthralgex em ratos Wistar, conforme diretrizes da OCDE. De acordo com a OCDE, 407 ratos de ambos os sexos foram separados em seis grupos (n = 6 cada). A dose de arthralgex foi definida com base num estudo de toxicidade aguda. No grupo de tratamento, um conjunto separado de ratos recebeu arthralgex em três níveis de dose: baixo, médio e alto (200, 400 e 800 mg/kg/dia, respectivamente, por via oral, por 28 dias). Os grupos satélites receberam altas doses (800 mg/kg/dia, por via oral, por 28 dias), e o grupo controle recebeu igual volume. No dia 28, foram coletadas amostras de sangue para estimar parâmetros hematológicos e bioquímicos. Posteriormente, os ratos foram eutanasiados para coletar órgãos para pesagem e histopatologia. Os grupos satélites foram mantidos por mais 14 dias após o tratamento para avaliar a reversibilidade da toxicidade e sacrificados no dia 43. Arthralgex não mostrou quaisquer sinais de toxicidade ou alteração importante no peso corporal no estudo de toxicidade aguda. Arthralgex não tem efeito adverso significativo no estado geral de saúde, conforme confirmado pelo peso corporal, ingestão de ração, hematologia, bioquímica, análise de urina, órgãos internos, peso relativo dos órgãos e avaliação histopatológica após 28 dias de tratamento. Arthralgex pode ser considerado seguro para tratamento de curto prazo. As descobertas atuais podem ajudar os pesquisadores na fixação de doses para estudos de toxicidade subcrônica e crônica, o que é essencial para avaliação de segurança para uso a longo prazo.

Palavras-chave: ayurveda, arthralgex, formulação poli-herbal, toxicidade subaguda.

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1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by inflammation, nonspecific swelling in peripheral joints with damaged cartilage, leading to joint deformities (Firestein, 2003; Jeon et al., 2019). The epidemiological data indicates that about 0.1% of the world's population suffers from RA. The incidence of RA is more in females and it peaks after 50 (Kvien et al., 2006). Diverse anti-RA drugs are available in the market, which includes but is not limited to NSAIDs, selective COX-2 inhibitors, and disease-modifying anti-rheumatic drugs. Both NSAIDs and corticosteroids are commonly used as adjuvants, because they relieve pain and inflammation in RA (Verhoeven et al., 2019).

Ayurveda is a way of life which uses plant-based products to treat ailments in both developed and developing countries, chiefly due to affordability, availability, and safety (Pandey et al., 2013; Jakaria et al., 2018). Currently, the population affected with chronic diseases is increasing, and drugs derived from plants are proven effective treatments (Margină et al., 2015; Margină et al., 2020). Plant extracts are commonly used in Europe and Asia as a polyherbal formulations for treating various diseases and disorders (Islam et al., 2014). In India, over 80% of the population take Ayurvedic medicines (Dargan et al., 2008), because of ethnic acceptability and compatibility with the body with minimal side effects.

Arthralgex administered continuously for a long-duration to treat RA. Therefore, evaluation of product safety is essential before prescribing to patients. In addition, recent publications indicate toxic effects of plant extracts / phytochemicals at higher doses in preclinical animal models in great detail (Guldiken et al., 2018). Case studies reported possible risk of hepatic injuries in certain individuals due to herbal and dietary supplements usage that accounts for 20% of the cases of hepatotoxicity in the United States (Navarro et al., 2017). Such studies cautions researchers to assess phytochemicals safety in long-term use. Toxicity studies improve product knowledge by augmenting its utilization and preventing undesirable effects, a major obstacle for long-term use of modern medication (Tabasum et al., 2019). As per Organisation for Economic Co-operation and Development (OECD) TG 407 (OECD, 2008b), sub-acute toxicity study is conducted only after knowing snapshot details from acute toxicity studies. It improves understanding about the probable toxic effect on health likely to occur from continuous use of product over a short period (Prabu et al., 2013; Patel et al., 2022). Therefore, acute toxicity study became a foundation for conducting subsequent toxicity studies. In addition, sub-acute toxicity reports in rats are considered essential to support clinical studies and marketing (Greaves, 2007). To our knowledge, both acute and sub-acute toxicity study reports of arthrralgex are not available so far. Hence, the objective was to find out the toxicological profile of arthralgex in vivo. This will also help researchers in dose fixing for conducting long-term studies.

2. Material and Methods

2.1. Animals

Both healthy Wistar albino rats (male & female) (180 ± 20 g) and female Swiss mice (28 ± 2 g) were selected and housed at NUCARE (Nitte University Centre for Animal Research and Experimentation) facility. Standard laboratory environment (12-hour light/dark cycle; relative humidity 60 ± 5%; temperature 22 ± 2 °C) were maintained with free access to commercial pellet diet and purified drinking water. Protocol was approved by the institutional animal ethics committee (NGSM Institute of Pharmaceutical Sciences) in accordance with Committee for the Control and Supervision of Experiments on Animals (CCSEA, approval no: NGSMIPS/JUNE-2021/256).

2.2. Composition of arthralgex

Each 1 mL of Arthralgex contains 55.55 mg of each 18 ingredients (Table 1).

2.3. Limit test/dose selection assay/acute oral toxicity

The study was conducted as per OECD guidelines for testing chemicals (OECD, 2008a). Here, the mortality-inducing dose is considered a toxic dose, which serves as a base for dose selection for conducting a sub-acute toxicity study. Female Swiss albino mice and female Wistar rats (n=3) were selected and fasted overnight with access to water. Arthralgex was administered (2000 mg/kg) orally and monitored over 14 days for a change in general behaviors, if any (Mathiasen and Moser, 2018; Wu et al., 2018).

Table 1. Ingredients of arthralgex.

S. No.	Plant name
1	Kadamba (Anthocephaluscadamba)
2	BilvaMula (Aeglemarmelos)
3	Agnimantha (Clerodendrumphlomidis)
4	Shalaparni (Desmodiumgangeticum)
5	Prishniparni (Urariapicta)
6	Gokshura (Tribulusterrestris)
7	Kantakari (Solamumxanthocarpum)
8	Brihati (Solanumindicum)
9	Shyonaka (Oroxylumindicum)
10	Kashmari (Gmelinaarborea)
11	Patala (Trichosanthesdioica)
12	Devadaru (Cedrus deodar)
13	Rasna (Pluchealanceolata)
14	Shunti (Zingiberoffcinale)
15	Punarnava (Boerhaaviadiffusa)
16	Aragvadha (Cassia fistula)
17	Guduchi (Tinosporacordifolia)
18	Yastimadhu (Glycyrrhiza glabra)

2.4. Sub-acute oral toxicity

As per OECD Test No. 407 (OECD, 2008b), rats of either sex were randomized into 6 groups (n=6 each). Rats were marked for individual recognition and kept in separate numbered cages as below:

I) Vehicle control [1A (Male) & 1B (Female)] - Distilled water for 28 days.
II) Low dose [2A (Male) & 2B (Female)] - Arthralgex (200 mg/kg/day, oral) for 28 days.
III) Medium dose [3A (Male) & 3B (Female)] - Arthralgex (400 mg/kg/day, oral) for 28 days.
IV) High dose [4A (Male) & 4B (Female)] - Arthralgex (800 mg/kg/day, oral) for 28 days.
V) Satellite vehicle control [5A (Male) & 5B (Female)] - Distilled water for 28 days, and kept under observation for additional 14 days post-treatment (day 42).
VI) Satellite high dose [6A (Male) & 6B (Female)] - Arthralgex (800 mg/kg/day, oral) for 28 days, and kept under observation for additional 14 days post-treatment (day 42).

Daily experimental rats were observed for mortality, if any. Any changes in the clinical signs, feed intake, and body weight were documented every week. Both vehicle control and treatment groups (Group I – Group IV) were euthanized using isoflurane inhalation anesthesia on 28th day to collect about 1.5 mL of blood by cardiac puncture, and tissue samples for investigation. Both satellite groups (Group V – Groups VI) were observed for additional 14 days post-treatment to access reversibility or recovery if any. During these period also, experimental rats were observed for mortality, if any. Any changes in the clinical signs, feed intake, and body weight were documented weekly. On 43rd day rats were euthanized using isoflurane inhalation anesthesia to collect blood and tissue samples for investigation (Thanabhorn et al., 2006; Sireeratawong et al., 2012). The schematic sub-acute toxicity study design is shown in Table 2.

2.4.1. Clinical observations

For assessing treatment effects on experimental rats, they were observed for mortality, if any. Any change in clinical signs, including Irwin Test or Functional Observational Battery (FOB), were carried out and recorded on the 28th day before blood sampling and euthanizing for organ collection for histopathology. Similarly, on the 43rd day, satellite group were analyzed (Mathiasen and Moser, 2018; Wu et al., 2018).

2.4.2. Urine analysis

Small quantity of urine was collected on 28th and 43rd day before sacrifice for qualitative analysis using urinalysis reagent strips (ACON Biotech (Hangzhou) Co., Ltd. China.

2.5. Statistical analysis

Data were analyzed using GraphPad Prism (version 8.4.3, San Diego, CA, USA). Each value represents mean ± SEM of the independent experiment (n=6). Data were analyzed by ANOVA followed by student's T-test. *P < 0.05, **P< 0.01, ***P< 0.001

Table 2. The schematic sub-acute toxicity study design.

<table>
<tr><td colspan="4">Wistar rats (n=6, Both Male & Female)</td></tr>
<tr><td colspan="4">Treatment groups (mg/kg)</td></tr>
<tr><td colspan="2">Test groups</td><td rowspan="2"></td><td colspan="2">High dose reversal / Satellite groups</td></tr>
<tr><td>• Vehicle control</td><td>• Low dose - 200
• Medium dose - 400
• High dose - 800</td><td>• Vehicle control</td><td>• High dose - 800</td></tr>
<tr><td colspan="2">Weakly parameters:
• Body weight
• Feed intake</td><td>Treatment for 28 days</td><td colspan="2">Weakly parameters:
• Body weight
• Feed intake</td></tr>
<tr><td colspan="2" rowspan="2">End point evaluation on day 29:
• Haematology
• Clinical biochemistry
• Relative organ weights
• Gross pathology & Histopathlogical examination*</td><td rowspan="2">No treatment for 14 days (Satellite groups)</td><td colspan="2">Weakly parameters:
• Body weight
• Feed intake</td></tr>
<tr><td colspan="2">End point evaluation on day 43:
• Haematology
• Clinical biochemistry
• Relative organ weights
• Gross pathology & Histopathlogical examination*</td></tr>
<tr><td colspan="4">Analysis, Data compilation & Interpretation</td></tr>
<tr><td colspan="4">Results & Conclusions</td></tr>
</table>

*Histopathology of all 28 organs (Male) and 29 organs (Female) mentioned in the guideline OECD 407 (OECD, 2008b).

3. Results

3.1. Acute toxicity study

Arthralgex at the highest dose (2000 mg/kg, oral) didn't show any signs of toxicity. All the three mice and rats were survived until study period of day 14. Necropsy examination of the carcasses on day 14 did not show any treatment related gross pathological changes. Based upon these observations, we have selected three doses: low, medium and high dose (200, 400, and 800 mg/kg, respectively) for sub-acute toxicity study (OECD, 2008a).

3.2. Sub-acute toxicity study

During the study period, either 28 days treatment groups or the satellite reversal groups did not show any toxic signs such as altered locomotor activity, piloerection, or any major changes in feed and water intake (data not shown for simplicity). There was no mortality in the treatment groups. Both feed intake and body weights (Table 3) did not show any significant changes (P<0.05) between the treatment and control groups.

3.2.1. Hematology & biochemical parameters

No significant changes in hematological (Table 4) and bone marrow (Table 5) parameters at tested dose levels of arthralgex.

Treatment with arthralgex did influence biochemical parameters such as AST, ALT, total cholesterol, BUN, and total bilirubin levels (Table 6). These effects are not dose-dependent and reversible by day 43.

Table 3. Effect of oral administration of arthralgex over the body weight and feed intake in rats.

Day	Dose (mg/kg)				Reversal group (mg/kg)	
	0	200	400	800	0	800
Body weight						
Male						
0	150.83 ± 3.52	160.83 ± 3.96	167.50 ± 3.82	161.67 ± 4.94	146.67 ± 3.07	152.50 ± 4.23
7	169.17 ± 4.55	170.83 ± 5.97	184.17 ± 4.90	183.33 ± 7.60	160.00 ± 4.65	176.67 ± 6.01
14	183.33 ± 7.26	191.67 ± 4.77	204.17 ± 5.39	200.83 ± 10.91	171.67 ± 10.06	194.17 ± 8.00
21	194.17 ± 7.35	200.00 ± 5.77	209.17 ± 5.54	205.83 ± 11.50	185.00 ± 11.76	211.67 ± 7.60
28	191.67 ± 6.54	204.00 ± 3.96	225.83 ± 4.17	209.17 ± 14.52	209.17 ± 13.44	225.83 ± 9.61
35	--	--	--	--	207.50 ± 13.83	243.33 ± 10.93
42	--	--	--	--	224.17 ± 10.68	253.33 ± 10.14
Female						
0	155.00 ± 4.28	151.67 ± 4.41	160.00 ± 7.19	163.33 ± 3.33	160.00 ± 3.42	149.17 ± 1.54
7	150.00 ± 6.19	159.17 ± 4.17	162.50 ± 7.04	163.33 ± 3.80	158.33 ± 4.77	158 ± 4.22
14	164.17 ± 5.07	167.50 ± 5.12	170.00 ± 8.16	170.00 ± 4.08	165.00 ± 5.48	162.50 ± 7.93
21	177.50 ± 7.93	171.67 ± 7.38	172.50 ± 8.04	170.00 ± 5.00	162.50 ± 6.16	154.00 ± 7.42
28	175.83 ± 6.11	172.50 ± 5.44	180.00 ± 8.47	180.83 ± 6.64	172.50 ± 7.93	162.00 ± 4.64
35	--	--	--	--	175.00 ± 8.76	175.00 ± 5.00
42	--	--	--	--	194.17 ± 8.98	172.00 ± 5.61
Feed intake (g)						
Male						
7	7.50 ± 0.37	11.67 ± 0.15	11.67 ± 0.15	14.17 ± 0.37	11.67 ± 0.75	14.17 ± 0.37
14	14.16 ± 0.52	11.66 ± 0.22	11.67 ± 0.30	12.50 ± 0.22	10.0 ± 0.29	12.5 ± 0.37
21	12.50 ± 0.07	13.33 ± 0.45	13.3 ± 0.30	12.50 ± 0.37	16.67 ± 0.60	13.3 ± 0.30
28	11.6 ± 0.15	14.16 ± 0.22	12.5 ± 0.37	13.33 ± 0.45	15.65 ± 0.30	16.67 ± 0.29
35	--	--	--	--	12.50 ± 0.22	15.83 ± 0.37
42	--	--	--	--	13.33 ± 0.45	14.16 ± 0.07
Female						
7	13.33 ± 0.45	10.0 ± 0.15	12.50 ± 0.07	9.17 ± 0.37	10.0 ± 0.30	9.17 ± 0.37
14	8.33 ± 0.37	11.67 ± 0.30	9.16 ± 0.07	8.33 ± 0.30	10.0 ± 0.15	11.67 ± 0.15
21	13.33 ± 0.15	12.5 ± 0.22	10.0 ± 0.30	10.0 ± 0.29	12.5 ± 0.52	8.33 ± 0.15
28	8.33 ± 0.15	9.16 ± 0.22	12.50 ± 0.22	12.5 ± 0.07	12.5 ± 0.37	10.0 ± 0.17
35	--	--	--	--	12.5 ± 0.07	12.0 ± 0.82
42	--	--	--	--	11.67 ± 0.15	11.0 ± 0.41

Means bearing * vary significantly between groups (*P < 0.05, **P< 0.01, ***P< 0.001).

Table 4. Effect of oral administration of arthralgex over the hematological parameters in rats.

Parameters	Dose (mg/kg)				Reversal group (mg/kg)	
	0	200	400	800	0	800
			Male			
RBC (mill/c.mm)	8.14 ± 0.26	8.08 ± 0.16	8.34 ± 0.28	8.04 ± 0.19	8.54 ± 0.26	8.69 ± 0.13
Hb (g/dl)	13.02 ± 0.30	13.85 ± 0.23	14.07 ± 0.25	13.73 ± 0.26	13.80 ± 0.29	14.27 ± 0.20
PCV (%)	42.73 ± 1.00	41.13 ± 1.00	43.17 ± 1.42	41.62 ± 1.14	43.35 ± 1.26	45.82 ± 0.61
Retic. (%)	1.53 ± 0.07	1.42 ± 0.06	1.27 ± 0.09	1.37 ± 0.11	1.53 ± 0.07	1.37 ± 0.07
WBC (mill/c.mm)	8,833.33 ± 1,221.66	13,983.33 ± 1,596.96	13,033.33 ± 1,597.43	14,550.00 ± 2,674.29	9,233.33 ± 1,596.59	8,433.33 ± 765.80
Neutro. (%)	18.17 ± 1.11	17.67 ± 3.05	15.67 ± 1.52	14.17 ± 1.22	14.83 ± 3.03	16.83 ± 1.85
Eosin. (%)	2.00 ± 0.45	2.67 ± 0.67	2.50 ± 0.34	2.00 ± 0.00	1.83 ± 0.17	2.00 ± 0.37
Baso. (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lympho.(%)	75.67 ± 3.23	78.17 ± 4.11	80.83 ± 1.51	82.83 ± 1.22	82.33 ± 3.14	80.17 ± 1.64
Mono. (%)	1.00 ± 0.00	1.50 ± 0.50	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
PLT (lac/c.mm)	7.65 ± 0.34	7.43 ± 0.24	8.23 ± 0.32	8.67 ± 0.50	7.16 ± 0.34	8.52 ± 0.38
			Female			
RBC (mill/c.mm)	6.81 ± 0.50	7.00 ± 0.69	7.83 ± 0.16	7.54 ± 0.44	6.18 ± 0.26	7.14 ± 0.28
Hb (g/dl)	13.83 ± 0.22	13.77 ± 0.23	13.92 ± 0.18	13. 37 ± 0.37	13.23 ± 0.25	13. 58 ± 0.25
PCV (%)	40.83 ± 3.38	36.97 ± 1.92	40.38 ± 0.71	38.07 ± 1.96	43.47 ± 0.94	40.54 ± 1.09
Retic. (%)	1.37 ± 0.08	1.33 ± 0.10	1.50 ± 0.07	1.42 ± 0.05	1.60 ± 0.09	1.24 ± 0.10
WBC (mill/c.mm)	9,266.67 ± 1,438.67	11,183.33 ± 1,067.53	10,850.00 ± 851.96	12,033.33 ± 1,341.06	10,966.67 ± 1,409.18	12,560.00 ± 2,038.04
Neutro. (%)	20.17 ± 6.06	14.33 ± 1.33	13.67 ± 1.52	19.00 ± 4.52	13.67 ± 2.25	18.00 ± 1.48
Eosin. (%)	2.50 ± 0.50	1.67 ± 0.33	1.50 ± 0.22	2.67 ± 0.49	1.83 ± 0.17	2.00 ± 0.00
Baso. (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lympho. (%)	76.33 ± 6.54	7.50 ± 1.48	84.17 ± 1.45	77.33 ± 5.00	83.50 ± 2.20	79.00 ± 1.48
Mono. (%)	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
PLT (lac/c.mm)	7.66 ± 0.33	7.15 ± 0.91	8.10 ± 0.42	7.65 ± 0.26	6.52 ± 0.41	7.25 ± 0.26

RBC: Red Blood Corpuscles; Hb: Hemoglobin; PCV: Packed Cell Volume; Retic.: Reticulocytes; WBC: White Blood Corpuscles; Neutro.: Neutrophils; Eosin.: Eosinophils; Baso.: Basophils; Lympho.: Lymphocytes; Mono.: Monocytes; PLT: Platelet. Means bearing * vary significantly between groups (*P < 0.05, **P< 0.01, ***P< 0.001).

Table 5. Effect of oral administration of arthralgex over the bone marrow in male and female rats.

Groups and dose(mg/kg)	Observations
Vehicle control	All the six bone marrow smears from control group (male & female) bone marrow showing features suggestive of normal haematopoiesis.
Low dose (200 mg/kg)	All the six bone marrow smears in the low dose group (male & female) show features suggestive of normal haematopoeisis.
Medium dose(400 mg/kg)	All the six bone marrow smears in the medium dose group (male & female) show normocellular smears with features suggestive of normal haematopoeisis.
High dose (800 mg/kg)	All the six bone marrow smears in the high dose group (male) show normocellular smears with features suggestive of mild reactive plasmacytosis.No pathological changes seen. Four bone marrow smears in the high dose group (female) show features suggestive of reactive marrow with mild reactive plasmacytosis. The other two bone marrow smears in the high dose group (female) show features suggestive of normal haematopoeisis. No pathological changes seen.
Vehicle control	All the six bone marrow smears in the control reversal group (male & female) show features of predominantly normal haematopoiesis. No pathological changes seen.
High dose reversal (800 mg/kg)	All the six bone marrow smears in the high dose reversal group (male & female) show features suggestive mild reactive plasmacytosis. No other haematopoeitic abnormalities seen in any of these smears in the reversal groups.

3.2.2. Gross pathology

Both the organ weights (data not shown) and relative organ weights (Table 7) did not show any significant changes in most organs in both male and female rats. However, the mild changes in the spleen, adrenal gland, liver, and kidney are not dose-dependent and completely reversible by day 43. Similarly, the gross pathological examination did not show any treatment-related abnormality in any dose levels.

3.2.3. Histopathology

Microscopic examination also did not show any significant changes in any of the organs studied in all the dose levels. However, few dilated sinusoids are evident but not significant in the liver. Other organs were unremarkable in all cases. Representative photographs of the histological sections of vital organs are shown in Figure 1 for female and male rats treated with vehicle and arthralgex respectively.

3.2.4. Urine analysis

The details of urine analysis by qualitative method in experimental rats were presented in Table 8. Arthralgex did not show any treatment-related abnormalities in any dose levels.

Table 6. Effect of oral administration of arthralgex over the biochemical parameters in rats.

Parameters	Dose (mg/kg)				Reversal group (mg/kg)	
	0	200	400	800	0	800
			Male			
Glucose (mg/dl)	84.81 ± 5.74	89.12 ± 6.60	97.02 ± 5.62	94.89 ± 11.98	96.90 ± 3.20	92.91 ± 14.74
AST (U/L)	145.62 ± 10.56	189.90 ± 10.53*	176.08 ± 9.57	196.67 ± 10.26*	211.98 ± 14.30	177.60 ± 13.73
ALT (U/L)	60.86 ± 5.18	56.89 ± 5.39	77.23 ± 3.62	68.11 ± 6.12	70.42 ± 4.04	68.07 ± 4.28
ALP (U/L)	298.75 ± 36.21	302.53 ± 48.08	343.38 ± 17.38	270.13 ± 34.06	261.98 ± 39.90	201.08 ± 23.07
TP (g/dL)	7.41 ± 0.19	7.25 ± 0.63	7.80 ± 0.74	6.68 ± 0.27	7.31 ± 0.33	6.96 ± 0.73
Albumin (G/dL)	2.42 ± 0.15	3.16 ± 0.30	3.13 ± 0.17	2.66 ± 0.20	2.92 ± 0.12	2.27 ± 0.17
T. Chol. ((mg/dL)	51.06 ± 4.12	68.24 ± 5.72*	64.08 ± 2.11	55.60 ± 1.10	60.39 ± 5.03	59.06 ± 4.72
TG (mg/dL)	122.52 ± 15.67	87.88 ± 15.58	108.52 ± 13.14	91.14 ± 11.03	124.80 ± 15.04	127.07 ± 14.38
BUN (mg/dL)	38.30 ± 3.42	49.34 ± 2.47*	46.58 ± 2.71	50.69 ± 1.91*	45.46 ± 2.84	39.33 ± 3.85
Creat. (mg/dL)	0.58 ± 0.03	0.59 ± 0.03	0.58 ± 0.02	0.65 ± 0.05	0.54 ± 0.06	0.56 ± 0.03
T. Bil. (mg/dL)	0.33 ± 0.03	0.62 ± 0.05***	0.36 ± 0.04	0.57 ± 0.04	0.45 ± 0.08	0.40 ± 0.04
Ca (mg/dL)	11.06 ± 0.54	12.35 ± 0.60	10.09 ± 0.29	10.74 ± 0.63	10.85 ± 1.14	12.25 ± 1.38
Phos. (mg/dL)	7.52 ± 0.97	9.07 ± 0.58	8.10 ± 0.44	8.94 ± 0.80	8.81 ± 0.63	6.89 ± 0.14**
Na^+ (meq/L)	138.33 ± 0.33	138.17 ± 0.31	138.00 ± 0.37	138.00 ± 0.37	138.17 ± 1.08	138.33 ± 0.42
K^+ (meq/L)	4.30 ± 0.09	4.27 ± 0.08	4.23 ± 0.08	4.28 ± 0.09	3.92 ± 0.12	4.17 ± 0.10
			Female			
Glucose (mg/dl)	97.92 ± 5.60	89.56 ± 10.28	90.03 ± 7.07	83.69 ± 6.63	135.38 ± 11.31	127.27 ± 31.66
AST (U/L)	171.95 ± 14.37	126.10 ± 6.14*	152.62 ± 4.47	193.32 ± 10.60***	173.10 ± 7.14	163.58 ± 12.07
ALT (U/L)	68.13 ± 3.01	65.36 ± 4.01*	51.62 ± 4.71	72.13 ± 3.43**	64.55 ± 2.92	55.21 ± 2.82
ALP (U/L)	191.48 ± 20.59	143.62 ± 9.60	179.33 ± 32.57	244.93 ± 36.00	194.70 ± 25.68	181.26 ± 34.02
TP (g/dL)	7.19 ± 0.26	6.97 ± 0.25	8.19 ± 0.63	6.94 ± 0.12	6.43 ± 0.57	6.64 ± 0.26
Albumin (G/dL)	2.50 ± 0.08	2.93 ± 0.13	2.67 ± 0.38	2.70 ± 0.20	2.93 ± 0.12	2.39 ± 0.19
T. Chol. ((mg/dL)	56.60 ± 7.06	68.69 ± 4.13	71.52 ± 6.81	66.79 ± 3.45	68.05 ± 4.93	51.93 ± 2.12*
TG (mg/dL)	72.65 ± 4.55	78.66 ± 7.40	79.06 ± 8.80	102.82 ± 5.48	109.11 ± 8.77	86.80 ± 13.75
BUN (mg/dL)	64.18 ± 30.36	43.01 ± 1.65	48.19 ± 2.77	46.97 ± 1.51	50.37 ± 6.98	33.48 ± 2.88
Creat. (mg/dL)	0.63 ± 0.04	0.62 ± 0.02	0.59 ± 0.01	0.65 ± 0.02	0.63 ± 0.04	0.55 ± 0.02
T. Bil. (mg/dL)	0.39 ± 0.01	0.55 ± 0.03*	0.35 ± 0.02	0.65 ± 0.07*	0.42 ± 0.07	0.39 ± 0.10
Ca (mg/dL)	11.49 ± 0.49	11.80 ± 0.92	9.97 ± 0.38	11.26 ± 0.74	12.14 ± 0.87	11.84 ± 0.76
Phos. (mg/dL)	7.98 ± 1.04	8.11 ± 0.63	7.81 ± 0.58	9.01 ± 0.47	8.19 ± 0.55	5.63 ± 0.32**
Na^+ (meq/L)	137.83 ± 0.48	138.17 ± 0.48	137.83 ± 0.31	138.17 ± 0.31	138.83 ± 0.31	140.40 ± 1.08
K^+ (meq/L)	3.93 ± 0.08	4.17 ± 0.06	4.13 ± 0.08	4.32 ± 0.11	4.03 ± 0.06	4.30 ± 0.11

AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; ALP: Alkaline Phosphatase; TP: Total protein; T. Chol.: Total Cholesterol; TG: Triglycerides; BUN: Blood urea nitrogen; Creat.: Creatinine; T. Bil.: Total bilirubin; Ca: Calcium; Phos.: Phosphate; Na^+: Sodium; K^+: Potassium. Means bearing * vary significantly between groups (*P < 0.05, **P< 0.01, ***P< 0.001).

Table 7. Effect of oral administration of arthralgex over the relative organ weights in rats.

Parameters	Dose (mg/kg)				Reversal group (mg/kg)	
	0	200	400	800	0	800
			Male			
Heart	0.375 ± 0.013	0.345 ± 0.019	0.350 ± 0.016	0.345 ± 0.016	0.329 ± 0.007	0.321 ± 0.008
Adrenal	0.025 ± 0.003	0.028 ± 0.003	0.026 ± 0.003	0.027 ± 0.003	0.031 ± 0.002	0.026 ± 0.003
Brain	0.924 ± 0.029	0.773 ± 0.032	0.803 ± 0.030	0.841 ± 0.059	0.785 ± 0.036	0.708 ± 0.039
Liver	4.507 ± 0.153	4.480 ± 0.077	4.361 ± 0.260	4.207 ± 0.275	4.149 ± 0.209	4.027 ± 0.141
Testis	1.184 ± 0.109	1.139 ± 0.035	1.112 ± 0.039	1.126 ± 0.111	1.059 ± 0.040	1.038 ± 0.039
Kidney	0.769 ± 0.015	0.748 ± 0.014	0.720 ± 0.020	0.758 ± 0.037	0.708 ± 0.028	0.702 ± 0.019
Spleen	0.404 ± 0.027	0.370 ± 0.015	0.305 ± 0.022*	0.329 ± 0.024	0.408 ± 0.029	0.391 ± 0.018
Lung	1.116 ± 0.061	1.060 ± 0.151	1.183 ± 0.100	0.937 ± 0.066	0.915 ± 0.045	0.877 ± 0.067
			Female			
Heart	0.335 ± 0.11	0.383 ± 0.012	0.360 ± 0.009	0.359 ± 0.028	0.358 ± .017	0.373 ± 0.011
Adrenal	0.042 ± 0.002	0.048 ± 0.002	0.035 ± 0.002*	0.036 ± .002*	0.038 ± .001	0.040 ± 0.003
Brain	0.935 ± 0.040	1.006 ± 0.029	0.961 ± 0.036	1.025 ± 0.034	0.905 ± .032	0.961 ± 0.058
Liver	3.499 ± 0.191	4.218 ± 0.172*	3.682 ± 0.154	3.870 ± 0.201	4.057 ± 0.213	3.855 ± 0.187
Uterus	0.108 ± 0.016	0.097 ± 0.010	0.097 ± 0.010	0.108 ± 0.006	0.110 ± 0.018	0.094 ± 0.007
Kidney	0.658 ± 0.014	0.774 ± 0.012*	0.728 ± 0.022	0.712 ± 0.029	0.662 ± 0.029	0.712 ± 0.036
Spleen	0.409 ± 0.032	0.438 ± 0.037	0.372 ± 0.030	0.376 ± 0.030	0.388 ± 0.039	0.396 ± 0.044
Lung	0.900 ± 0.132	1.020 ± 0.106	1.157 ± 0.170	1.209 ± 0.284	1.046 ± 0.049	1.115 ± 0.058

Means bearing * vary significantly between groups (*P < 0.05, **P< 0.01, ***P< 0.001). The relative organ weights of the adrenal glands, kidneys, and testes mentioned are the combined weights of both the right and left organ.

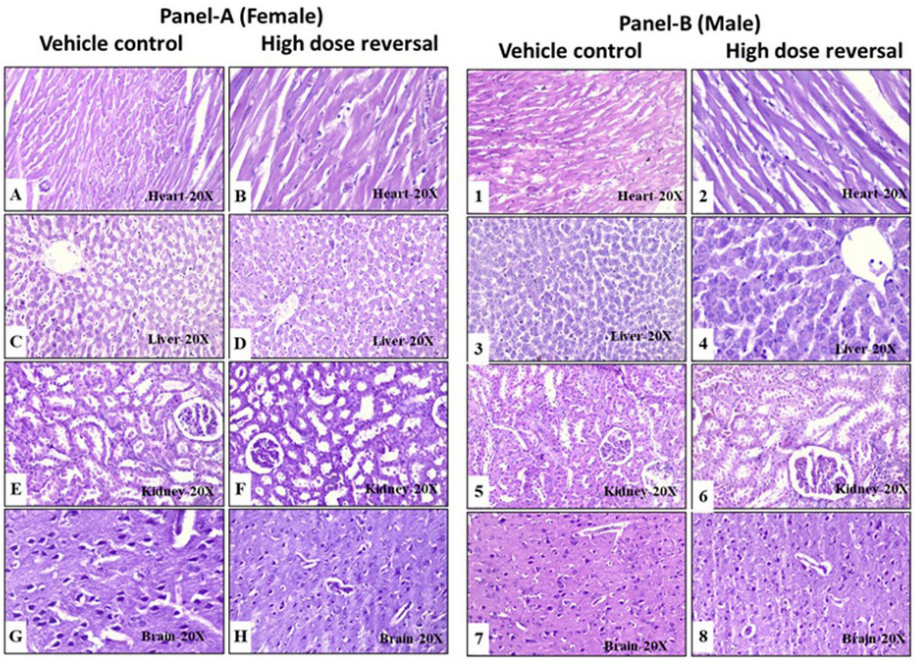

Figure 1. Panel-A (Female): H & E sections of vital organs of vehicle control and high dose reversal arthralgex (800 mg/kg) treated female rats. (A, B) Heart sections of rat treated with vehicle (A) and arthralgex (B) at 800 mg/kg showing normal architecture 20X (C, D) Liver sections of rat treated with vehicle (C) and arthralgex (D) at 800 mg/kg with normal features, 20X (E, F) Kidney sections of rat treated with vehicle (E)the arthralgex (F) at 800 mg/kg showing glomeruli, tubules with no changes,20X (G, H) Lung sections of the rat treated with vehicle (G) and arthralgex (H) at 800 mg/kg showing normal structures, 20X; **Panel-B (Male)**: H & E sections of vital organs of vehicle control and high dose reversal arthralgex (800 mg/kg) treated female rats. (1-2) Heart sections of rat treated with vehicle (1) and arthralgex (2) at 800 mg/kg showing normal architecture 20X (3, 4) Liver sections of rat treated with vehicle (3) and arthralgex (4) at 800 mg/kg with normal features, 20X (5, 6) Kidney sections of rat treated with vehicle (5) the arthralgex (6) at 800 mg/kg showing glomeruli, tubules with no changes, 20X (7, 8) Lung sections of the rat treated with vehicle (7) and arthralgex (8) at 800 mg/kg showing normal structures, 20X. Note: Histopathology of all other organs (male and female) are comparable and unremarkable in all doses with respective controls. Hence, for simplicity and convenience their histopathology is not included.

Table 8. Effect of oral administration of arthralgex over the urine in male and female rats.

Parameters	Dose (mg/kg)				Reversal group (mg/kg)	
	0	200	400	800	0	800
Male						
Sp. Gr.	1.02 ± 0.00	1.02 ± 0.00	1.02 ± 0.00	1.02 ± 0.00	1.02 ± 0.00	1.02 ± 0.00
pH	7.58 ± 1.11	6.83 ± 0.75	7.75 ± 0.76	7.08 ± 0.58	6.50 ± 0.63	7.0 ± 0.45
Glucose	0	0	0	0	0	0
Bilirubin	0	0	0	0	0	0
Ketones	±	±	±	±	±	±
Occult Blood	0	0	0	0	0	0
Urobilinogen	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
Nitrite	0	0	0	0	0	0
Female						
Sp. Gr.	1.02 ± 0.01	1.02 ± 0.00	1.02 ± 0.01	1.02 ± 0.00	1.02 ± 0.01	1.02 ± 0.00
pH	6.0 ± 0.00	6.17 ± 0.68	6.42 ± 0.66	6.67 ± 0.61	7.17 ± 0.52	6.20 ± 0.45
Glucose	0	0	0	0	0	0
Bilirubin	±	0	0	0	0	0
Ketones	±	±	±	0	±	±
Occult Blood	0	0	0	0	0	0
Urobilinogen	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
Nitrite	0	0	0	0	0	0

Qualitative: 0 = Absent; ± = Difficult to judge; + = Trace; ++ = Small amount of analyte; +++ = Moderate amount of analyte; ++++ = Large amount of analyte.

4. Discussion

Arthralgex is a proprietary polyherbal formulation prepared based on ancient literature and is used extensively to treat rheumatoid arthritis in clinics along the borders of Karnataka- Kerala. It is prepared by Sahasraksha Vaidya Shala, Ukkinadka, Badiyadka, Kerala, India. Despite extensive use over many years, with clinical benefits, there have been no efforts to study and document arthralgex's toxicity if any. We have evaluated this product for its toxicity as per OECD guidelines.

Present study evaluates Arthralgex toxicity profile in rats by sub-acute toxicity study. Generally significant changes in the body weight of test subject are used as an indicator in toxicity evaluation. Single dose of Arthralgex did not alter body weight significantly indicating the safety of the preparation. Further, repeated oral dosing to rats for 28 days evaluated toxicity, if any, by arthralgex. It provides evidence of treatment-induced alteration in hematology, biochemical, and reflect organ morphology and histopathology. Generally this kind of the study helps for assessing the toxicity profile of a test substance (Loha et al., 2019; Lin et al., 2022).

Ayurvedic preparations usage in managing lifestyle disorders is unquestionable (Chandola, 2012). Contribution of World Health Organization in promoting the global acceptance of ayurveda deserves appreciation (Chaudhary and Singh, 2011). In general, effective polyherblal preparations need not be free from toxicity but awareness about toxicity is generally poor. The general perception is that all ayurvedic preparations are safe because they are prepared by following ancient literature. People believe that preparations used so for such long periods would not have any toxic effects (Atsamo et al., 2011). However, literature indicates that acute or sub-acute toxicity studies in animals suggest that many currently used plants are highly toxic (Prabu et al., 2013; Raman and Lau, 1996; Basch et al., 2003; Farzaei et al., 2020). An acute oral toxicity study, which was conducted before efficacy study in our lab (communicated) (Ukkinadka and Badanthadka, 2023) did not cause any toxicity to rats and was tolerated up to the upper limit (2000 mg/kg, oral). For the sub-acute study, we therefore employed $1/10^{th}$ dose as the low dose (200 mg/kg), twice the low dose as medium dose (400 mg/mg/kg) and twice the medium dose as high dose (800 mg/kg) (OECD, 2008b). Sub-acute arthralgex treatment didn't make significant alteration in animal behavior or body weight at all three doses. Earlier studies have considered change in body weight as an indicator of toxic effects for test products (Tofovic and Jackson, 1999; Raza et al., 2002; Teo et al., 2002). Both hematology and biochemistry suggests that arthralgex drug exposure did not adversely affect the overall health of the rats including behavior, metabolic, and untoward effects on body organs (Prabu et al., 2013; Patel et al., 2022; Olayode et al., 2019). Thus, indicating non-toxic nature of arthralgex on blood parameters, correlating the metabolism with vital organs function.

Any changes in organ weight after treatment are sensitive markers of toxicity in preclinical study under controlled environment. However, treatment did not make significant differences in organ weights or relative organ weight, suggesting arthralgex is non-toxic. Evaluation of pathological changes mediated by test product in experimental animals makes the foundation for product's safety before Phase-I studies. Histopathological study further supports this findings (Greaves, 2007). Both gross and microscopic evaluation of target organs of the rats treated with arthralgex did not show any significant pathological changes attributed to the non-toxic nature of arthralgex in experimental rats.

5. Conclusion

The present research demonstrated that arthralgex is safe without significant toxicity with 'no observed adverse effect level (NOAEL)' of 800 mg/kg/day oral dose in Wistar rats gavaged for 28 days for either sex under the experimental conditions. This dose did not cause any mortality or significant hematological and biochemical changes. Further, both gross and histopathological observations support these findings. However, further studies are essential to assess long-term toxicity for investigating reproductive toxicity, teratogenicity, and carcinogenicity if any.

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