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Research Article

STUDIES ON POLYHERBAL DRUG (JPR-1) USED FOR ARTHRITIS

Mansoor Ahmad[1*], Farah-Saeed[2], Mehjabeen[3], Noor Jahan[4]

[1]Research Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Karachi, Pakistan
[2]Department of Pharmacognosy, Dow College of Pharmacy, Dow University of Health Sciences, Ojha Campus, Karachi, Pakistan
[3]Department of Pharmacology, Federal Urdu University, Karachi, Pakistan
[4]Department of Pharmacology, Dow College of Pharmacy, Dow University of Health Sciences, Ojha Campus, Karachi, Pakistan

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*Corresponding Author: **Prof. Dr.Mansoor Ahmad**
Research Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Karachi, Pakistan
Email: herbalist53@yahoo.com, +92300-210-2275

ABSTRACT

The term arthritis refers to more than 200 kinds of diseases causing pain, swelling, and damage to joint and connective tissue. Musculoskeletal conditions are a major burden on individuals, health systems, and social care systems. The aim of this study was to carry out preliminary, pharmacognostical, phytochemical analysis along with clinical studies on poly-herbal drug, JPR-1, prepared in our laboratory in 1997, for the treatment of different types of arthritis. Following pharmacognostic parameters were evaluated by chemical colour reaction tests, fluorescence analysis, chemical testing for powdered drug, powder microscopy. The clinical studies were carried out by dividing the patients into four groups; and then determining the presence of different diseases, prevalence of different types of arthritis and determination of different parameters used for the diagnosis of arthritis in these four age groups. JPR-1 exhibited significant improvement in following arthritis conditions in descending series gouty arthritis> back pain> rheumatoid arthritis> osteoporosis> osteoarthritis> fibromyalgia> psoriatic arthritis. The drug also showed significant alleviation in side effects associated with allopathic arthritis medicines intake.

Keywords: Musculo-Skeletal Conditions, Inflammation, Uric Acid, Erythrocyte Sedimentation Rate, Arthritis.

INTRODUCTION

Arthritis is a world-wide problem not only of old age people but also of young people[1]. In advance countries like USA, European countries the percentage of arthritis victims is very high and mostly they rely on NSAIDs (synthetic drugs)[2-6]. In India and Pakistan nosignificant data is available but it is a serious problem of the people. In Karachi, Pakistan amongst the urban population; the incidence of rheumatoid arthritis were stated to be 0.142% [7]. However, in northern region of Pakistan the assessed occurrence was found to be 0.55% [8]. The occurrence of rheumatoid arthritis was almost 2.5 times higher in females than males[9].The 80% population of Pakistan is utilizing herbal medicine. Though it does not cure the problem but provides relief comfort with no/less side-effects[10-15]. An attempt has been made to develop a polyherbal drugs with having known curing features of arthritis. The polyherbal (JPR-1) drug was prepared as per WHO recommendations.The drug was prepared in the laboratory of Pharmacognosy department and analyzed by phytochemical reactions, pharmacology, toxicology studies and clinical data.

MATERIALS AND METHOD

Polyherbal drug (JPR-1) which is mixture of five common herbs (some are from cooking spices), was registered in Intellectual Property Organization of Pakistan (Patent office Karachi; Pakistan) with patent no. 971/2012 dated 23/05/20012.

Extraction procedure

JPR-1 was formulated by Prof. Dr. Mansoor Ahmad, Research Institute of Pharmaceutical Sciences, Karachi. The drug was crushed into fine powder and then macerated with methanol and kept for 15 days at room temperature for percolation. The methanol extract was then filtered.

This procedure was repeated thrice. Methanol from extract was evaporated under reduced pressure in a rotary evaporator to obtain a dark yellow residue and later dried in a lyophilizer (Freeze dryer model FD1, Eyela, Tokyo Rikakikai Co. Ltd., Japan).

Chemicals

All chemicals used were purchased from Merck, Germany; BDH Chemicals England and Sigma-Aldrich, USA.

Phytochemical Analysis

The presence of alkaloids, tannins, saponins, carbohydrates, sterols, proteins, triterpenes and steroids, were determined by methods described byJanarthanan et al. 2012; Sofowara 1993; Trease & Evans 1989, Harborne 1973[16-19].

Fluorescence Analysis

The air dried plant material was subjected to fluorescence analysis under ultra violet light and day light after giving treatment with various chemical and organic solvents like 1N Sodium hydroxide in methanol, 1N Sodium hydroxide in distilled water, 50% Nitric acid, 50% Sulphuric acid, 1N Hydrochloric acid[20].

Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared Spectrometry (FTIR) of JPR-1 was carried out on FT-IR model Nicolet Avator 330-FT-IR (USA). The spectrum of JPR-1 was determined as described by Griffiths & de-Hasseth (2007)[21].

Agglutination Activity

Hemagglutination activity was determined on different dilution of JPR-1 samples in 8 blood groups (A^+,A^-,B^+,B^-,O^+,O^-,AB^+,AB^-) with the method described by Alam & Usmanghani (1994) and Mosby's Medical, Nursing and Allied Health Dictionary (1994)[22-23].

Thin Layer Chromatography

Extract of JPR-1 in small quantity was dissolved in ethanol (analytical grade) for Thin Layer Chromatography TLC. Ready-made TLC plates (Silica gel 254 fluorescent, Merck, Germany) were used. Sample of JPR-1 was applied on TLC plates as described by Stahl [24]. A solvent system that is $CHCl_3$-MeOH (90:10) used to develop TLC plates and separate UV-active chemical compounds on TLC plates and observed under UV-lights of 254nm and 366nm. After marking, spots were detected by spraying the reagents (Vanillin-Sulphuric acid; 1:100). The R_f value of each spot was determined (Table 8 and Fig.2).

Protocol for clinical studies

The clinical studies on JPR-1were carried out as per WHO protocol [25-27].The case histories of 6,929 patients suffering from different forms of arthritis were collected. From which 5,103 patient were treated completely; while 1,826 were under treatment at the time of data collection. The patients were divided into four age groups for the proper compilation of the data: Group I: 7-15 years, Group II: 16-40 years, Group III: 41-60 years and Group IV: 61 or above (see tables 1, 2 and graphs 1, 2). During treatment with JPR-1, the following diagnostic parameters were closely monitored to assess the effect produced by the drug inside the patients, to assess the therapeutic efficacy, the dose and the time period required for the drug to be continued (table 3 and graph 3).

RESULTS AND DISCUSSION

The presence of different chemical constituents present in JPR-1 is shown in table 4.Chemical reaction tests of powdered JPR-1 sample were carried out as shown in table 5.Fluorescence analysis of JPR-1 sample was carried as such and by treatment with chemical reagents and observed under ordinary, short and long wavelength UV light (table 6).FT-IR of JPR-1 showed important peaks were obtained at 3300(acid OH), 2920(alkyl Sp3 C-H),2850(aldehyde C-H), 1740(anhydride, 3-4 membered ring C=O), 1700(C=O aldehyde, ketone, ester, acid), 1650(Amide C=O), 1450(Aromatic C=C), 1150(C-O), and 1030cm^1(C-O stretch). (figure 1). JPR-1 revealed significant agglutination activity at 5g/ml dose in all the blood groups. Moderate agglutination activity was found in 2.5g/ml dose. At 1.25g/ml, weak agglutination activity was observed. Whereas, no agglutination activity was observed at dose levels 0.625g/ml and 0.3125g/ml (table 7).

Thin-layer chromatography of JPR-1 sample was carried out using a solvent system: Chloroform-methanol (90:10) (table 8 and figure 2).

Out of 6,929 patients using JPR-1; 5,103 users have been cured. The above clinical studies revealed that in comparison with other diseases musculo-skeletal conditions are a major burden on individuals in all age groups and among the most stressful musculoskeletal conditions are: Osteoarthritis, Rheumatoid arthritis, Osteoporosis, Gout, Fibromyalgia, Psoriatic arthritis and Low back pain. The different types of arthritis are more prevalent in the age group 41-60 years.

The use of JPR-1 demonstrated remarkable changes in diagnostic parameters. JPR-1 decreased the swelling and pain associated with different types of arthritis. Reduction in uric acid level and erythrocyte sedimentation rate is observed. The use of JPR-1 also produced changes in blood parameters which include increase in hemoglobin. Liver function test remains within limits.

This research work shows that JPR-1 is effective in all the cases of arthritis except for the cases in which some deformity has appeared or the patient is diabetic.

The different types of arthritis have become the major cause of disability in the population world-wide and it has been estimated that by 2030, in US alone 67 million people of age 18 onwards will be diagnosed with any one type of arthritis[28].

The above clinical studies revealed that in comparison with other diseases musculo-skeletal conditions are a major burden on individuals in all age groups; and among the most stressful musculoskeletal conditions are: osteoarthritis, rheumatoid arthritis, osteoporosis and low back pain. JPR-1, prepared in our lab from 5 herbs of medicinal importance, possesses anti-oxidant, anti-tumor, fungistatic, anti-bacterial, anti-inflammatory, anti-mutagenic, gastro-protective and hepato-protective properties[29-31]. It is used for the treatment of arthritis due to its potential of reducing pain, improving mobility of joints, increasing collagen synthesis, reducing risk of total joint replacement by reducing disease progression due to the presence of flavonoids, various volatile oils, carbohydrates, resins and proteins.

NSAIDs act through the inhibition of cyclo-oxygenase and modification of prostaglandin synthesis. While JPR-1 have multiple mechanisms of actions working concomitantly. It inhibits the activity and synthesis of the various enzymes associated with inflammation, such as COX-2, 5-lipo-oxygenase, glutathione-S-transferase, pro-inflammatory leukotrienes (IL-1, IL-2, IL-6, IL-8, IL-12), prostaglandins, arachidonic acid, inflammatory cytokines, TNF-α, monocyte chemo-attractant protein, protein kinase, migratory inhibitory protein[32-35]. JPR-1 mechanism of action mimics that of aspirin

without causing vascular thrombosis. The effectiveness of JPR-1 for treating different types of arthritis is matched with the steroidal and non-steroidal anti-inflammatory drugs but with no adverse effects[36-37].JPR-1 additionally inhibits oxidative DNA damage and relieves oxidative stress that enables it to regulate the formation of nitric oxide which plays a pivotal role in inflammation. JPR-1 several modes of action also involve blocking TRPV1 activation and thereby impede TRPV1-mediated pain hyper-sensitivity[38]. Inhibition of platelet aggregation through potentiation of prostacyclin synthesis and inhibition of thromboxane synthesis is responsible for the cardio-protective effect of JPR-1. It exhibits gastro-protective effects by inhibition of intestinal spasms and increased gastrin, secretin, bicarbonate and pancreatic enzyme secretion. It also inhibits ulcer formation.

JPR-1 involves the increase in the level of Glucosamine sulphate. Glucosamine is a basic component of articular cartilage glycosaminoglycans and acts as a substrate in the synthesis of proteoglycans. With aging, the body decreases the synthesis of glucosamine, and the synovial fluid becomes thin and is no longer effective in lubricating the joint. Glucosamine is used to provide the building block for cartilage regeneration. Therefore, JPR-1 helps in inhibiting or reversing joint degeneration[39-43].

The onset of action of JPR-1 is slower in comparison to NSAIDs but has longer half-life. JPR-1 has no serious side effects. While the use of NSAIDs is often accompanied by serious gastro-intestinal complications, which can also be fatal. NSAIDs are truly a silent epidemic that has caused a tremendous amount of pain and death.

Homeopathic drugs have slower onset of action in comparison to JPR-1 and comparatively less efficacy. Unani drugs have more or less the same onset of action as JPR-1; but have been found to be less effective in comparison.

JPR-1 exhibited its efficacy in following series: GI disturbances associated with arthritis (96.64%) > allergic reactions due to arthritis medicines intake (96.19%) > side-effects of corticosteroids (93.85%) > liver problems due to arthritis medicines intake (91.21%) > cardiovascular problems due to arthritis medicines intake (86.27%) > kidney problems due to arthritis medicines intake (85.33%) > gouty arthritis (78.26%) > back pain (67.56%) > rheumatoid arthritis (64.11%) > osteoporosis (61.87%) > osteoarthritis (61.5%) > fibromyalgia (44.44%) > psoriatic arthritis (29.41%) (Tables 9a and 9b; Graphs 3a and 3b).

The recommended diet while undergoing treatment with JPR-1 include turnip, pumpkin, radish, carrot, potato, rice, chicken, fish, pulses, milk, yogurt and all juicy fruits except apple, banana and mango.

JPR-1 has been successful in providing complete cure as well as in alleviating the symptoms of many arthritis patients; except for the cases in which some form of deformity has appeared or patient is diabetic due to dysfunctioning of different organs in both instances. The therapeutic potential of chemical constituents present in JPR-1 are well-supported by the pharmacognostic evaluation, phytochemical screening and thinlayer chromatographic techniques.

CONCLUSION

The polyherbal drug, JPR-1 is thus an effective and safe remedy for the treatment of different types of arthritis without any hazardous side effects. This has been established after carrying out pharmacognostic, chemical, pharmacological, toxicological evaluation and clinical studies on JPR-1. Therefore, JPR-1 can be prescribed alone or in combination for providing relief and cure to the ailing arthritis patients.

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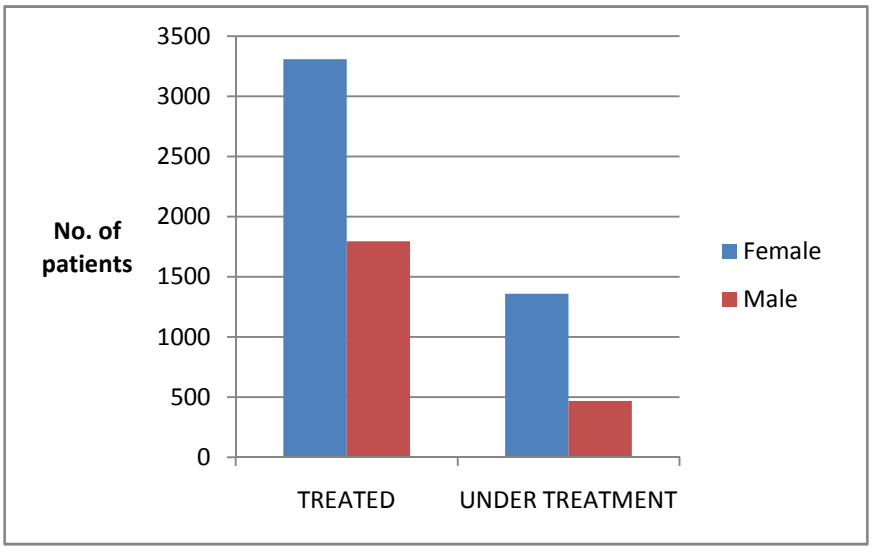
Table 1: The patients treated and under-treatment with JPR-1

PATIENTS	NO. OF PATIENTS	TREATED	UNDER TREATMENT
Female	4667	3309	1358
Male	2262	1794	468
Total	6929	5103	1826



Graph 1: The patients treated and under-treatment with JPR-1 (according to gender)

Table 2: The total no. of patients included in study and cured according to age-groups

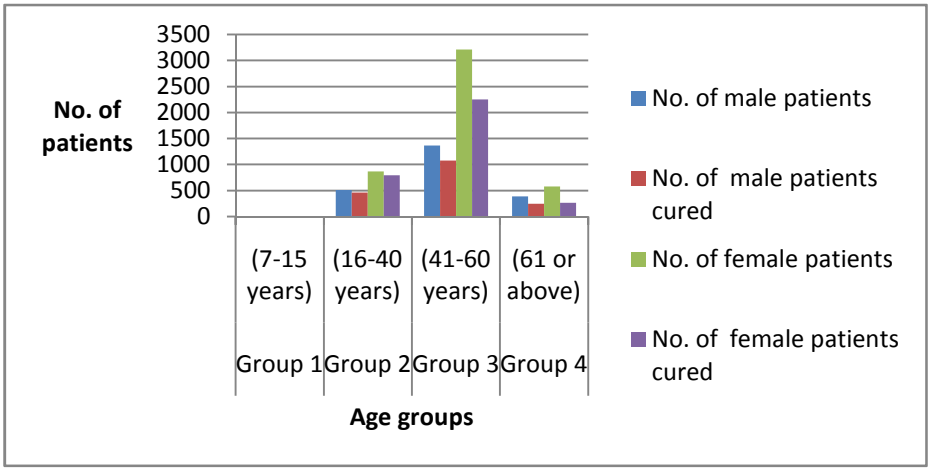
Patients	Group 1 (7-15 years)	Group 2 (16-40 years)	Group 3 (41-60 years)	Group 4 (61 or above)
No. of male patients included in study	01	509	1364	388
No. of male patients cured	01	464	1080	249
No. of female patients included in study	02	871	3213	581
No. of female patients cured	01	792	2253	263

Table 3: Presence of diagnostic parameters in arthritis patients according to gender and different age groups

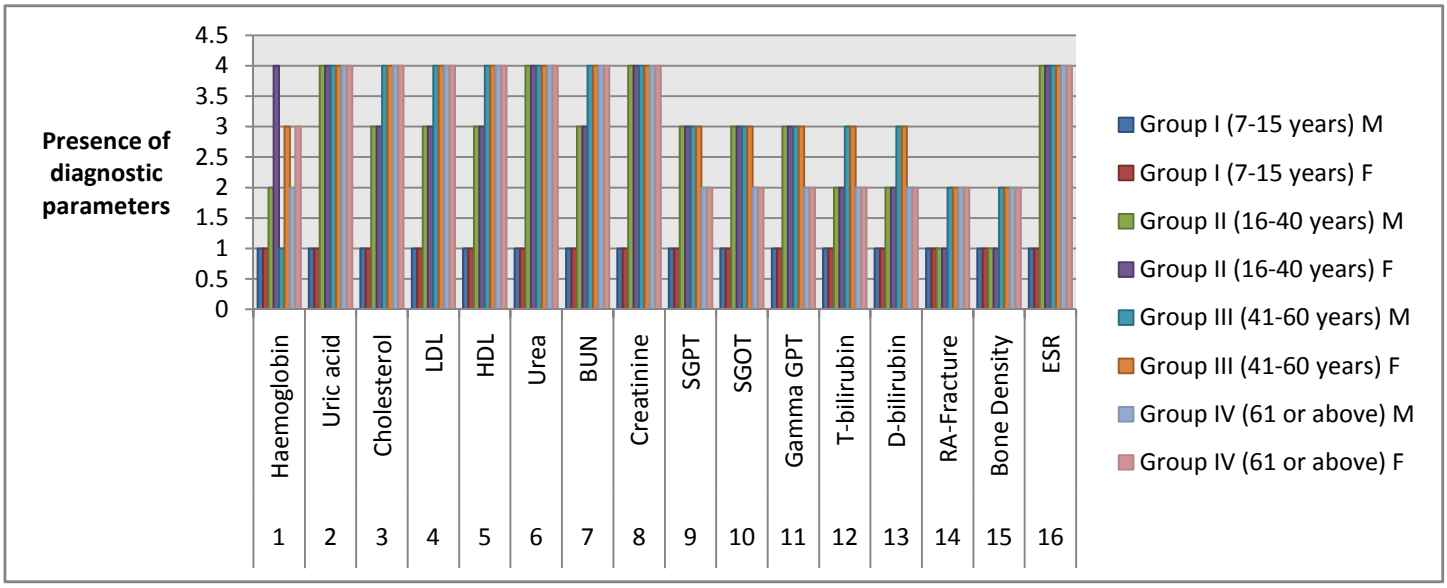
Sl. No.	Parameters	Group I (7-15 years)		Group II (16-40 years)		Group III (41-60 years)		Group IV (61 or above)	
		M	F	M	F	M	F	M	F
1	Haemoglobin	1	1	2	4	1	3	2	3
2	Uric acid	1	1	4	4	4	4	4	4
3	Cholesterol	1	1	3	3	4	4	4	4
4	LDL	1	1	3	3	4	4	4	4
5	HDL	1	1	3	3	4	4	4	4
6	Urea	1	1	4	4	4	4	4	4
7	BUN	1	1	3	3	4	4	4	4
8	Creatinine	1	1	4	4	4	4	4	4
9	SGPT	1	1	3	3	3	3	2	2
10	SGOT	1	1	3	3	3	3	2	2
11	Gamma GPT	1	1	3	3	3	3	2	2
12	T-bilirubin	1	1	2	2	3	3	2	2
13	D-bilirubin	1	1	2	2	3	3	2	2
14	RA-Fracture	1	1	1	1	2	2	2	2
15	Bone Density	1	1	1	1	2	2	2	2
16	ESR	1	1	4	4	4	4	4	4

M=stands for male; F = stands for female

Rare = 1; slightly positive = 2; moderately positive = 3; highly positive = 4



Graph 2: The total no. of patients included in study and cured according to age-groups



Graph 3: Presence of diagnostic parameters in arthritis patients according to gender and different age groups
M = stands for male; F = stands for female

Table 4 Identification of chemical constituents of JPR-1 by specific chemical tests

Chemical Constituents	Chemical Tests	Chemical Reaction
Tannins	Lead acetate test	+
Tannins	Phenazone test	+
Saponins	Froth test	+
Saponins	Ether test	–
Alkaloids	Dragendorff test	+
Carbohydrates	Molisch test	+
Triterpenes	Libermann- Burchard Test	+
Proteins	Ethanol test	+
Proteins	Picric and tannic acid test	+
Sterols	Salwaski test	+
Steroids	Antimony pentachloride test	++
Steroids	Napthol-sulphuric acid test	++
Steroids	Sulphuric acid test	++

Slightly positive = +; positive = ++; negative = –

Table 5: Chemical reaction test of powdered JPR-1 sample

Chemical reaction test	Observations
Powder triturated with water	Insoluble
Powder shaked with water	Yellow precipitates in yellow solution
Powder treated with 5% NaOH and heated	Froth formation on heating. The colour changes to reddish-orange.
Powder treated with 5% $FeCl_3$	Precipitates and froth formation. The colour changes to brown.
Powder treated with 66% H_2SO_4	Colour changes to reddish-brown with dense orange precipitates.
Powder pressed between two filter papers	Greasy and leaves yellow spot

Table 6: Fluorescence Analysis of JPR-1

Treatment	Source of light	Observation
Dry powder as such	Ordinary light	Green with brown spots
	254 nm UV	Yellow
	365 nm UV	Yellowish-brown
Powder treated with 1N NaOH in methanol	Ordinary light	Brown with purple ting
	254 nm UV	Brown with yellow ting
	365 nm UV	Reddish-brown with yellow ting
Powder treated with 1NHCl	Ordinary light	Yellow with brown ting
	254 nm UV	Mustard with yellow ting
	365 nm UV	Yellow
Powder treated with 1N NaOH in water	Ordinary light	Brown
	254 nm UV	Brown
	365 nm UV	Orange
Powder treated with 50% HNO_3	Ordinary light	Green with brown precipitates
	254 nm UV	Brown with yellow precipitates
	365 nm UV	Yellowish with brown precipitates
Powder treated with 50% H_2SO_4	Ordinary light	Dark green colour
	254 nm UV	Dark green colour
	365 nm UV	Reddish-brown

Table 7: Agglutination activity of JPR-1

JPR-1	A^+	B^+	O^+	AB^+	A^-	B^-	O^-	AB^-
5	+3	+4	+3	+3	+4	+3	+3	+3
2.5	+2	+2	+2	+2	+2	+2	+2	+2
1.25	+1	+1	Fine Traces	-	+2	Fine traces	Fine traces	Fine traces
0.625	-	-	-	-	-	-	-	-
0.3125	-	-	-	-	-	-	-	-

Table 8: Thin-layer Chromatography of JPR-1

Drug	Spray reagent	Physical method	Rf value ($CHCl_3$-MeOH) (90:10)
JPR-1	Vanillin-Sulphuric acid	Naked eye UV (254nm) UV (366nm)	0.02
			0.07
			0.11
			0.22
			0.30
			0.50
			0.57
			0.64
			0.71
			0.75
			0.82

Table 9 (A): Percentage of arthritis patients cured with JPR-1

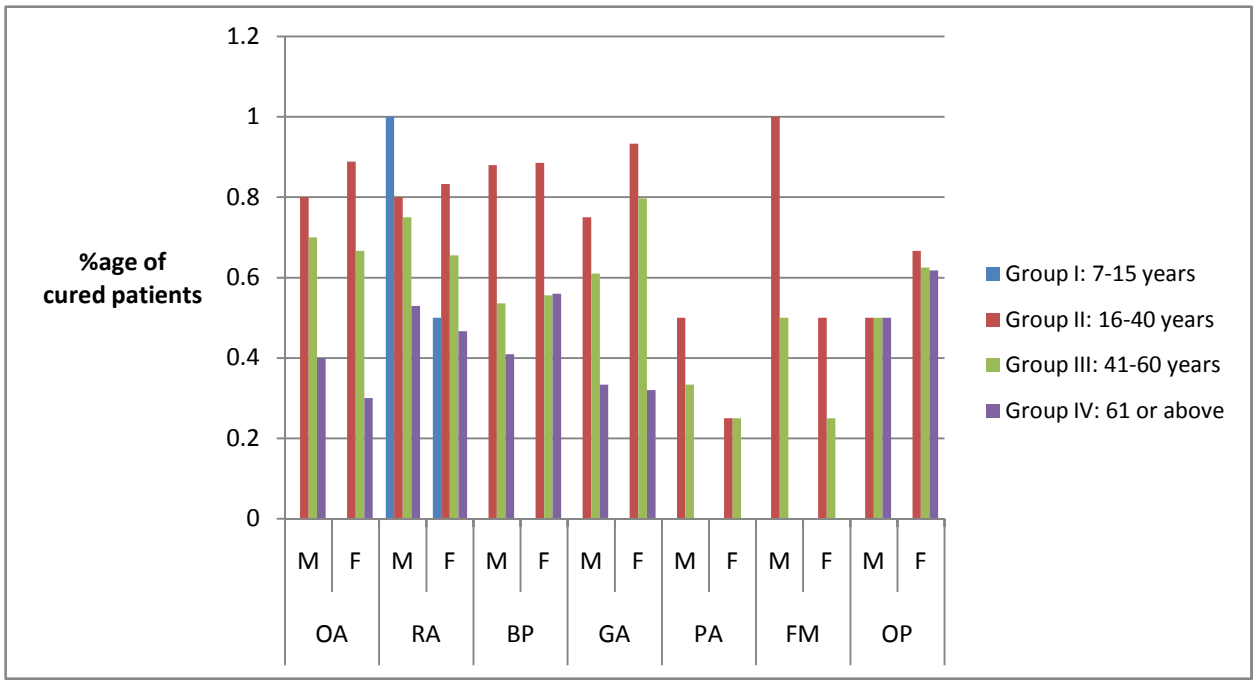
Age groups of patients		Osteoarthritis (n=1200)		Rheumatoid arthritis (n=928)		Back pain (n=1557)		Gouty arthritis (n=483)		Psoriatic arthritis (n=17)		Fibromyalgia (n=09)		Osteoporosis (n=800)	
		M	F	M	F	M	F	M	F	M	F	M	F	M	F
Group I: 7-15 years	Treated	Nil	Nil	01	02	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	Cured	Nil	Nil	01 (100%)	01 (50%)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Group II: 16-40 years	Treated	10	90	10	30	250	350	20	150	02	04	01	02	02	18
	Cured	08 (80%)	80 (88.89%)	08 (80%)	25 (83.33%)	220 (88%)	310 (88.57%)	15 (75%)	140 (93.33%)	01 (50%)	01 (25%)	01 (100%)	01 (50%)	01 (50%)	12 (66.67%)
Group III: 41-60 years	Treated	100	750	200	450	345	540	41	232	03	08	02	04	30	640
	Cured	70 (70%)	500 (66.67%)	150 (75%)	295 (65.56%)	185 (53.62%)	300 (55.56%)	25 (60.97%)	185 (79.74%)	01 (33.33%)	02 (25%)	01 (50%)	01 (25%)	15 (50%)	400 (62.5%)
Group IV: 61 or above	Treated	50	200	85	150	22	50	15	25	Nil	Nil	Nil	Nil	08	102
	Cured	20 (40%)	60 (30%)	45 (52.94%)	70 (46.67%)	09 (40.91%)	28 (56%)	05 (33.33%)	08 (32%)	Nil	Nil	Nil	Nil	04 (50%)	63 (61.76%)
M/F cured patients		98/160 (61.25%)	640/1040 (61.53%)	204/296 (96.91%)	391/632 (61.86%)	414/617 (67.09%)	638/940 (67.87%)	45/76 (59.21%)	333/407 (81.81%)	02/05 (40%)	03/12 (25%)	02/03 (66.67%)	02/06 (33.33%)	20/40 (50%)	475/760 (62.5%)
Total cured patients		738/1200 (61.5%)		595/928 (64.11%)		1052/1557 (67.56%)		378/483 (78.26%)		05/17 (29.41%)		04/09 (44.44%)		495/800 (61.87%)	

M = stands for male; F = stands for female

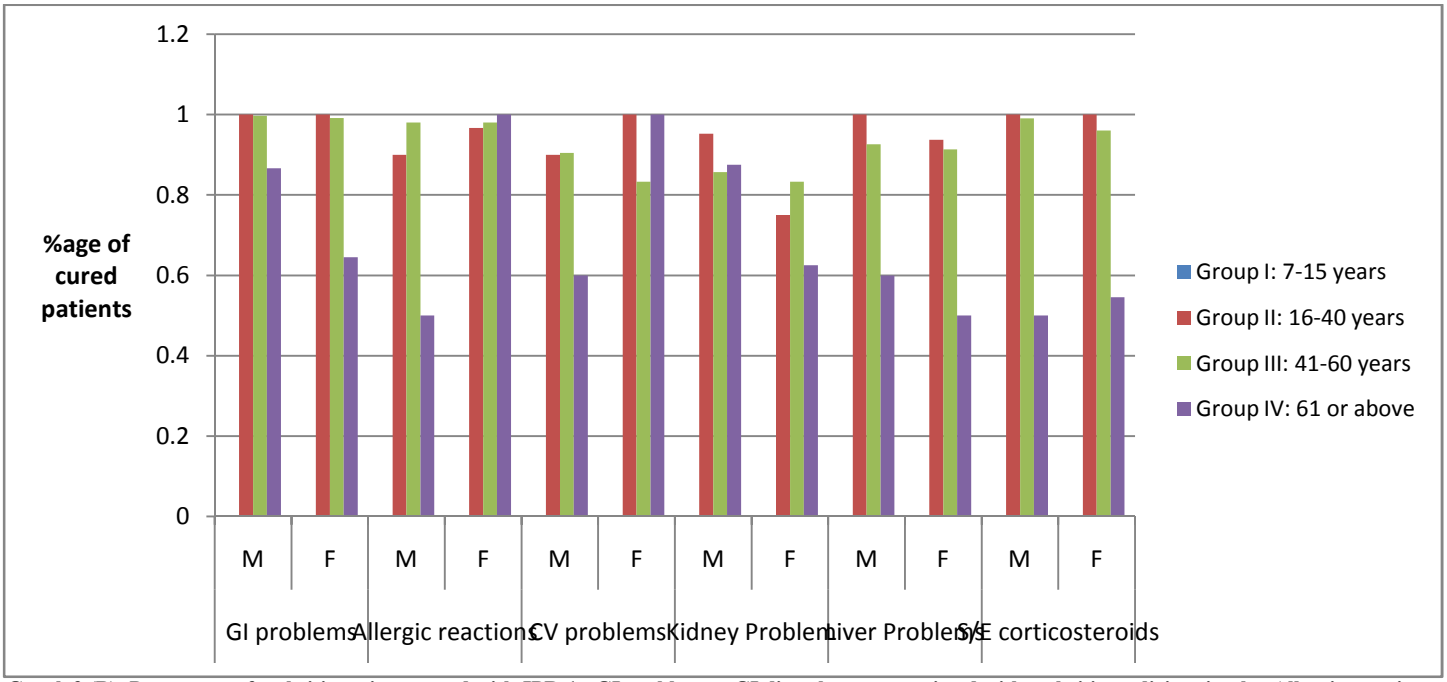
Table 9 (B): Percentage of arthritis patients cured with JPR-1

Age groups of patients		GI disturbances associated with arthritis medicines intake (n=1013)		Allergic reactions due to arthritis medicine intake (n=184)		Cardio-vascular problems due to arthritis medicines intake (n=51)		Kidney Problem due to arthritis medicines intake (n=75)		Liver Problems due to arthritis medicines intake (n=91)		Side effects of corticosteroids (n=521)	
		M	F	M	F	M	F	M	F	M	F	M	F
Group I: 7-15 yrs	Treated	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	Cured	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Group II: 16-40 yrs	Treated	140	130	20	60	10	02	21	04	18	16	05	15
	Cured	140 (100%)	130 (100%)	18 (90%)	58 (96.67%)	09 (90%)	02 (100%)	20 (95.23%)	03 (75%)	18 (100%)	15 (93.75%)	05 (100%)	15 (100%)
Group III: 41-60yrs	Treated	320	242	51	50	21	12	14	12	27	23	210	250
	Cured	319 (99.68%)	240 (99.17%)	50 (98.03%)	49 (98%)	19 (90.47%)	10 (83.33%)	12 (85.71%)	10 (83.33%)	25 (92.59%)	21 (1.3%)	208 (99.04%)	240 (96%)
Group IV: 61 or above	Treated	150	31	02	01	05	01	16	08	05	02	30	11
	Cured	130 (86.67%)	20 (64.51%)	01 (50%)	01 (100%)	03 (60%)	01 (100%)	14 (87.5%)	05 (62.5%)	03 (60%)	01 (50%)	15 (50%)	06 (54.54%)
M/F cured patients		589/610 (96.55%)	390/403 (96.77%)	69/73 (94.52%)	108/111 (97.29%)	31/36 (86.11%)	13/15 (86.67%)	46/51 (90.19%)	18/24 (75%)	46/50 (92%)	37/41 (90.24%)	228/245 (93.06%)	261/276 (94.56%)
Total cured patients		979/1013 (96.64%)		177/184 (96.19%)		44/51 (86.27%)		64/75 (85.33%)		83/91 (91.21%)		489/521 (93.85%)	

M = stands for male; F = stands for female



Graph 3 (A): Percentage of arthritis patients cured with JPR-1; OA = osteoarthritis, RA = rheumatoid arthritis, BP = back pain, GA = gouty arthritis, PA = psoriatic arthritis, FM = fibromyalgia, OP = osteoporosis



Graph 3 (B): Percentage of arthritis patients cured with JPR-1; GI problems = GI disturbances associated with arthritis medicines intake, Allergic reactions = Allergic reactions due to arthritis medicines intake, CV problems = cardiovascular problems due to arthritis medicines intake, Kidney Problems = Kidney problems due to arthritis medicines intake, Liver Problems = Liver problems due to arthritis medicines intake, S/E corticosteroids = side-effects of corticosteroids

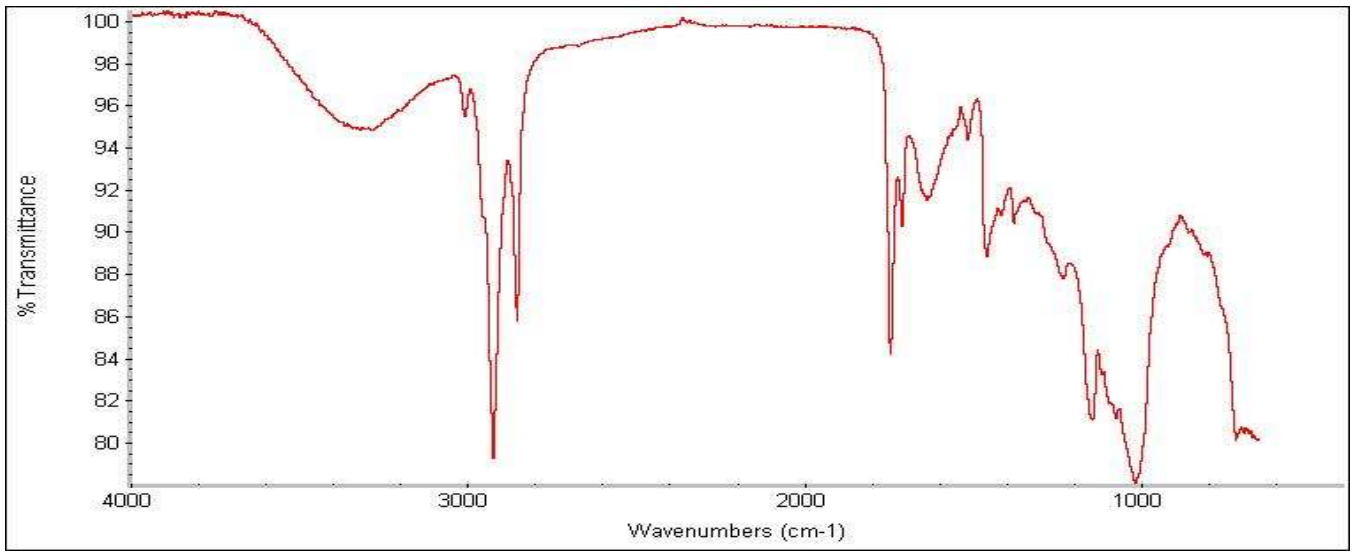
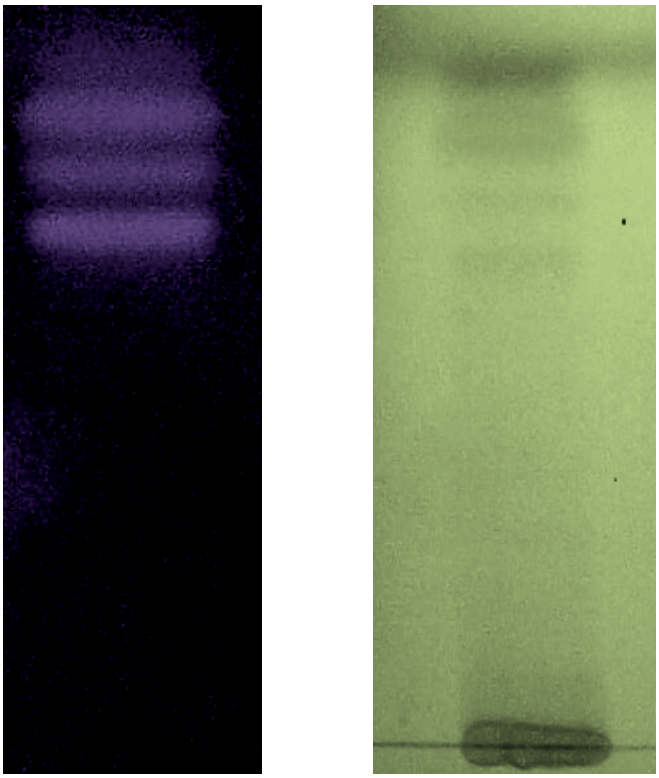


Figure 1: FT-IR spectra of JPR-1; Significant peaks observed at 3300cm-1 (acid OH); 2920cm-1(alkyl Sp3 C-H); 2850cm-1 (aldehyde C-H); 1740cm-1 (anhydride, 3-4 membered ring C=O); 1700cm-1 (C=O aldehyde, ketone, ester, acid); 1650cm-1 (Amide C=O); 1450 (Aromatic C=C); 1150cm-1 (C-O); 1030cm-1 (C-O stretch)



At long wavelength (366nm) At short wavelength (254nm)

Figure 2: Thin-layer chromatography of JPR-1

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