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ACUTE ORAL TOXICITY OF RHUMAVIN CAPSULE (POLY HERBAL FORMULATION) WITH ITS EFFECT AGAINST RHEUMATOID ARTHRITIS

Nilesh Patel[1], Dr. Janmejay Patel[2], Achal Patel[3] and Prof. Dr. Upendra U. Zala[4]*

[1]Associate Professor & Head, Department of Pharmacology, Shree S K Patel College of P'ceutical Education & Research, Ganpat University, At. Kherva – 382711, Dist. Mehsana Gujarat, India.
[2]CEO, Petlad Mahal Arogya Mandal Pharmacy, At. Piplata -387355, Dist. Kheda, Gujarat, India.
[3]MBBS Student, Pramukh Swami Medical College, Karamsad -388325, Dist. Anand, Gujarat, India.
[4]Professor& Head, Post Graduate Department of Rasashastra evam Bhaishajya Kalpana, J. S. Ayurved Mahavidyalaya, Nadiad - 387001, Gujarat, India.

***Corresponding Author: Prof. Dr. Upendra U. Zala**
Professor& Head, Post Graduate Department of Rasashastra evam Bhaishajya Kalpana, J. S. Ayurved Mahavidyalaya, Nadiad - 387001, Gujarat, India.

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ABSTRACT

Introduction: In Contempt of the availability of advanced medicinal systems, approximately 80% of the world population still depends on herbs and herbal formulations for the treatment of arthritic conditions. Though it is in use for wide variety of clinical applications, toxicological evaluation of herbal ingredients or combinations is still in infancy. **Aim:** To evaluate acute oral toxicity on Swiss albino mice and efficacy with anti-oxidant properties on arthritic rats of Rhumavin capsule. **Method:** The protocol of present study was certified by IAEC (SKPCPER/IAEC/2016-02/05) as per the CPCSEA. The acute oral toxicity was assessed according to OECD guideline AOT-425 to know single dose (2000 mg/kg) toxicity of test drug on swiss albino mice. Animals were periodically observed individually for 14 days for any clinical signs of toxicity or mortality after administration of test drug. Bovine serum denaturation assay (*In-vitro*) was done to investigate effectiveness of Rhumavin capsule on arthritis. FCA induced arthritis animal model (*In-vivo*) was adopted to check therapeutic effectiveness of Rhumavin capsule against arthritis. FCA was challenged to sub plantar region after treatment in Rats and various parameter were measured i.e. paw volume, arthritic index, hematological and radiological parameters. Different assay analysis (total protein, SOD activity, Catalase activity, Lipid peroxidation) were done to establish its anti-oxidant properties. **Results:** There were no physical changes, behavioral changes and mortality observed in Swiss albino mice during 14 days. Body weight of all animals did not reveal any significant change as compared to vehicle control group. Rhumavin capsule decreased significantly paw volume, Arthritic index, hematological parameters compared to disease control group. The significant increase in SOD and catalase level was found in test drug treated group. **Conclusion:** The No-Observed-Adverse-Effect-Level (NOAEL) of Rhumavin capsule is 2000 mg/kg. The results suggest anti- inflammatory, anti-arthritic and antioxidant effect of Rhumavin capsule.

KEYWORDS*:* Poly herbal formulation (PHF), Rhumavin capsule, NOAEL, Mortality, OECD Guideline, Rheumatoid Arthritis, Anti-oxidant.

INTRODUCTION

The medicinal plants have been enormously benefitting sources of wide therapeutic applications along with their relatively low toxic and less side effects. Despite the availability of advanced medicinal systems, approximately 80% of the world population still depends on herbs and herbal formulations for the treatment of various disease conditions.[1] Although the herbal formulations are in use for wide variety of clinical applications, the toxicological evaluation of herbal ingredients or combinations is still in infancy. The toxicity characteristics of the test materials need to be confirmed prior to human clinical trials, though herbal entities are believed to be relatively safe. Generally this is accomplished by conducting general preclinical safety studies to uncover potential toxic effects of drug in question.[2]

Rheumatoid arthritis (RA) is a severely disabling chronic autoimmune disorder that leads to progressive inflammation of the joints and surrounding tissues.[3] It affects approximately 1-2% of the population worldwide and the prevalence of RA was reported to be around 0.75% in the adult population in India.[4] The treatment of RA is done with various disease modifying agents such as NSAIDs and DMARDs which have limited efficiency and immunosuppressive properties with risk of adverse effect and other complications.[5]

Due to this, moreover RA patients (60 % - 90%) are

prefer complementary and alternative medicine instead of conventional therapy.[6] The Rhumavin capsule is a poly herbal formulation(PHF) and has been indicated for the treatment of rheumatoid arthritis, cervical spondylitis, joints pain etc. The ingredients of Rhumavin capsule have proven anti arthritic properties.[7], [8]

With the above considerations, the present study was aimed to assess the acute oral toxicity as well as efficacy on Rheumatoid arthritis of newly developed poly herbal formulation i.e. Rhumavin capsule.

Aim and objectives

1. To evaluate acute oral toxicity of Rhumavin capsule on Swiss albino mice.
2. To evaluate efficacy with anti-oxidant properties of Rhumavin capsule on arthritic rats.

MATERIALS AND METHODS

Material: The test drug (Rhumavin capsule) was manufactured by following all the GMP standards. The detail of Rhumavin capsule is mentioned below;

Table: 01. Ingredients of Rhumavin capsule (Each hard gelatine capsule contain).

Sl. No.	Name of Ingredient	Quantity
1	Ext. Ricinus communis	50 mg
2	Ext. Pluchea lanceolate	50 mg
3	Ext. Tinospora cordifolia	50 mg
4	Ext. Boerhavia diffusa	50 mg
5	Ext. Zingiber officinale	50 mg
6	Ext. Trachyspermum ammi	50 mg
7	Ext. Smilax Glabra	50 mg
8	Commiphora wightii	150 mg

Method: The present study was performed after obtained permission from IAEC (SKPCPER/IAEC/2016-02/05) as per the CPCSEA, Ministry of Environment, Forest and Climate Change (MoFCC), Government of India.

(A) Acute oral toxicity:[9] It was conducted according to OECD guideline AOT-425 to know single dose toxicity of Rhumavin capsule on swiss albino mice. All the Animals were kept in proper condition and acclimatized prior to dosing. They were divided in different groups. Each mouse was treated with a limit single oral dose of 2000 mg/kg of extract in sequence at 48 h intervals. Animals were observed individually for any clinical signs of toxicity or mortality at least once during the first 30 min after dosing periodically during the first 24 h, and daily thereafter for 14 days. Body weight of all animals was recorded once in a week. The detail of dosing record is as follow;

Table: 02. Individual animal dosing record of Rhumavin capsule.

Expt. Day	Animal No.	Gender	Test drug (mg)	Vehicle Distilled Water (ml)	Volume dose (ml)	Conc. (mg/ml)
1st day	H	M	50	0.6	0.55	83.33
3rd day	B	M	55	0.6	0.57	91.67
5th day	T	F	50	0.6	0.58	83.33
7th day	HT	F	52	0.6	0.58	86.66
9th day	UM	F	52	0.6	0.58	86.66

Expt.: Experiment, Conc.: Concentration, H: Head, B: Body, T: Tail, HT: Head & Tail, UM: Unmarked, M: Male, F: Female.

(B) Effect on Arthritis: This effect was evaluated by *In Vitro* and *In Vivo* assay.

***In Vitro* assay:**[10] It was done by Bovine serum Denaturation method. Bovine serum albumin (BSA) is one type of protein derived from the cows which is standard protein for the experiment in lab. The production of auto antigen may be due to denaturation of the protein in certain arthritic condition. So, inhibition of denaturation of protein was evaluated at different concentration of Rhumavin capsule in the current study.

Preparation of Reagents:

- ✓ **Bovine Serum Albumin 0.5% (BSA):** Bovine Serum Albumin (500 mg) + H20 (100ml)
- ✓ **Phosphate buffer saline:** NaCl (8 gm) + KCl (0.2 gm) + Na2HPO4 (1.44gm) + KH2PO4 (0.24 gm) + Distilled water (800ml)

The pH of solution was adjusted 6.3 by using 1N HCl and made up to volume 1000 ml with addition of distilled water (D.W.).

Preparation of various solutions (1 ml)

- ✓ **Test solution:** BSA (0.9 ml) + 0.1 ml A.C. (100, 200, 400, 800 µg)BSA (0.9 ml) + 0.1 ml STD (100, 200, 400, 800 µg)
- ✓ **Test control:** BSA (0.9 ml) + D.W.(0.1 ml)
- ✓ **Product control:** D.W. (0.9 ml) + Test solution (0.1 ml)
- ✓ **Standard solution:** BSA (0.9 ml) + 0.1 ml Diclofenac potassium (100, 200 400 800 µg)

Procedure: 1 ml (100, 200, 400, 800 µg/ml) Test drugs solution (AC), Standard drug solution (100, 200, 400, 800µg/ml) and Product control solutions were taken. The samples were incubated at 35°C for 25 min. and kept the

samples at 57°C for further 3 min. After cooling, 2.5 ml of P.B. was added into it. The Absorbance was measured using UV-visible spectrophotometer at 250 nm.

***In-vivo* assay:** It was done in Freund's adjuvant (FCA) arthritis model after experimental protocol approved by CPCSEA. The female Albino wistar Rats (n = 18) with age of 8-12 weeks and having weight between 150 to 200 gm were taken from animal room of the institute. They were maintained in controlled temperature as well as humidity and standard diet and water (ad libitium) were provided.

Table: 03. Grouping of Animals.

Group No.	Group Name	No. of animals
I	Disease control (DC)	6
II	Standard drug (Diclofenac potassium) treated (Std.)	6
III	Rhumavin Capsule (RC)	6

Procedure: On first day, the normal paw volume of Female albino wistar rats (n = 18) was measured by plethysmometer. After that, 0.1 ml CFA (Complete Freund's Adjuvant) was injected in to sub plantar region on the left hind paw (mycobacterium butyricum 6 mg being suspended in heavy paraffin oil by thoroughly grinding with mortar pestle to give final concentration of 6 mg/ml). Administration of test compound (RC) and Standard drug was started on the next day and continued for consecutive 28 days. The right paw was considered as reference for comparison. The paw volumes of both legs were noted every week. **(pearson CM & Wood FD)**The various parameters for arthritis were analyzed i.e. body weight, inflammation, arthritic index, ESR, RA factor and radiological analysis of bone destruction.

(C) Anti-oxidant study: The joints of the hind paw were removed and washed with water. After that, it crushed finely in to mortar pestle by using few drops of phosphate buffer and homogenized in to homogenizer. This homogenized solution was collected in eppendorf and put in centrifugation machine for centrifugation at 3500 RPM for 15 min. The separated supernant layer of solution was collected after centrifugation for analysis of different assay i.e. total protein,[11] SOD activity,[12] Catalase activity[13] and lipid peroxidation.[14]

Statistical Analysis: Arithmetic mean and standard error of mean are calculated from the individual observations. The data are expressed as mean ± S.E.M. Statistical difference between the mean are calculated using One way analysis of variance (ANOVA) followed by Dunett's post hoc test using graph pad prism 5. $P < 0.05$ was considered as significant.

OBSERVATIONS AND RESULT

(A) Acute oral toxicity: The animals were observed continuously for behavioural changes, autonomic profiles and other signs of toxicity or mortality up to a period of 14 days. The body weight, food intake and water intake were also observed on 1st, 7th and 14th day. There were no physical and behavioural changes observed in Swiss albino mice during 14 days. Body weight of all animals did not reveal any significant change as compared to vehicle control group. Mortality was not observed in any animal of a group.

Table: 04. Showing individual animal observation & Mortality record.

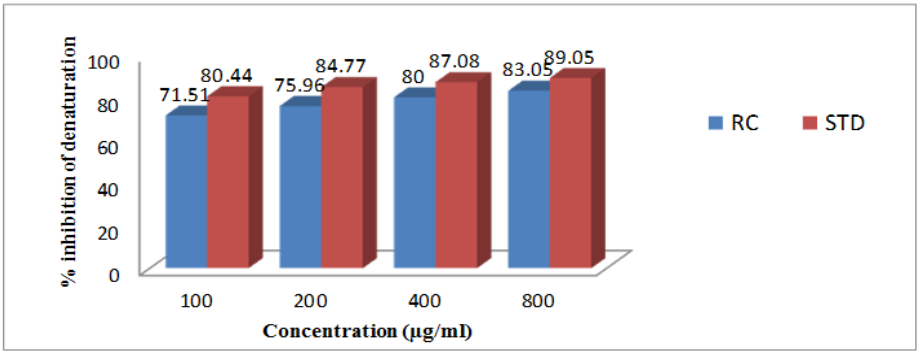
Animal No.	Gender	Experiment Day, Unit : gm			Mortality
		1st	7th	14th	
H	M	27	28	29	NIL
B	M	28	28	29	NIL
T	F	24	25	26	NIL
HT	F	27	28	28	NIL
UM	F	24	25	26	NIL

H: Head , B: Body , T: Tail , HT: Head & Tail, UM: Unmarked, M: Male , F: Female

(B) Effect on Arthritis: The results of *In-Vitro* evaluation of anti arthritic activity by Bovine serum denaturation method (BSA) at different concentrations are as follow;

Table: 05. Details of Denaturation inhabitation by Rhumavin Capsule.

Drug	Concentration (µg/ml)	Test absorbance	Product control	% denaturation	% inhibition of denaturation
Diclofenac potassium (STD)	100	0.491	0.096	19.55	80.44
	200	0.670	0.102	15.23	84.77
	400	0.836	0.108	12.92	87.08
	800	0.987	0.108	10.94	89.05
Rhumavin. Capsule (RC)	100	0.337	0.096	28.48	71.51
	200	0.397	0.096	24.30	75.96
	400	0.520	0.104	19.30	80
	800	0.602	0.102	16.94	83.05



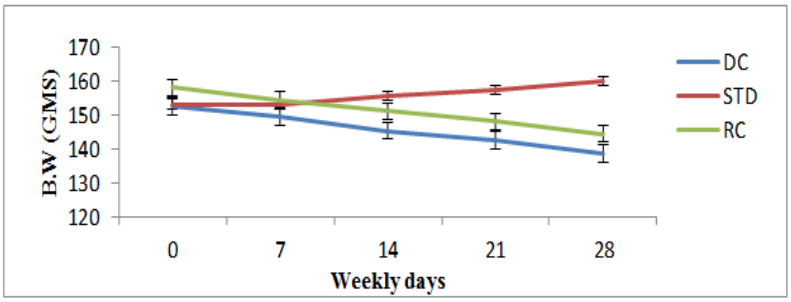
Graph: 1. Showing % inhibition of denaturation at different concentrations.

The results of *In-vivo* anti arthritic activity by Freund's Adjuvant Induced Arthritis in Rats model is as follow;

Body Weight: DC group showed significant decrease in body weight in compression of test drug treated group.

Table: 06. Weekly body weight record of all animals.

Group	0 day	7th day	14th day	21st day	28th day
I (DC)	152.5	149.3	145.3	142.5	138.5
II (Std.)	153.16	153	155.5	157.5	160
III (RC)	158	154.33	151	148	144.3

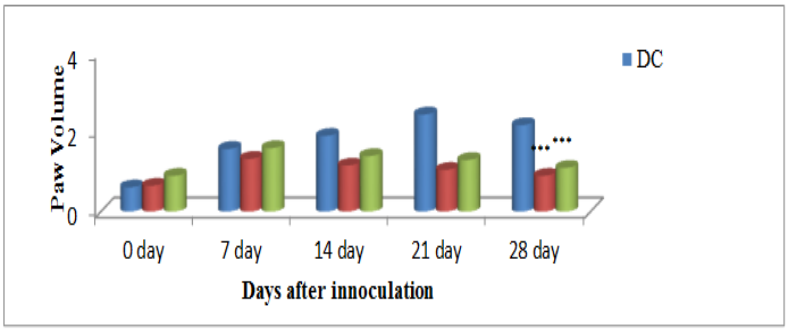


Graph: 2. Showing Weekly body weights of all animals.

Paw Volume: During 28 day treatment, paw volume in DC group is found increased in time dependent manner **(Figure 1)**. The significant difference observed in paw volume of all groups is as follow;

Table: 07. Weekly Paw volume record of all animals.

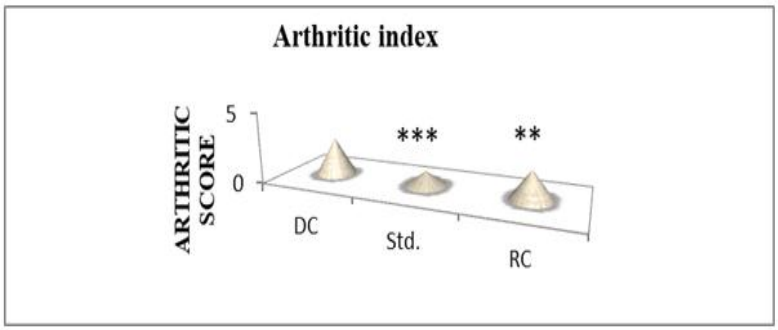
Group	0 day	7th day	14th day	21st day	28th day
I (DC)	0.61	0.61	0.61	0.61	0.61
II (Std.)	0.65	0.65	0.65	0.65	0.65
III (RC)	0.9	1.6	1.4	1.3	1.1



Graph: 3. Showing Weekly Paw volume of all animals. (All values represented as mean ± SEM of 6 animals**);**
*** P ≤ 0.001 Vs. disease control, ** P ≤ 0.01 Vs. Disease control,

Table: 08. Arthritic Score of different groups.

Group	I (DC)	II (Std.)	III (RC)
Arthritic Score	2.667	1	2

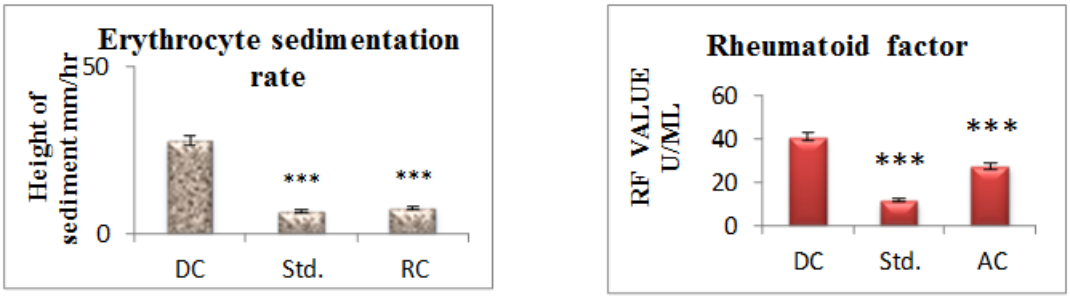


Graph: 4. Showing Arthritic Index (All values represented as mean ± SEM of 6 animals.**);**
*** P ≤ 0.001 Vs. disease control, ** P ≤ 0.01 Vs. Disease control,

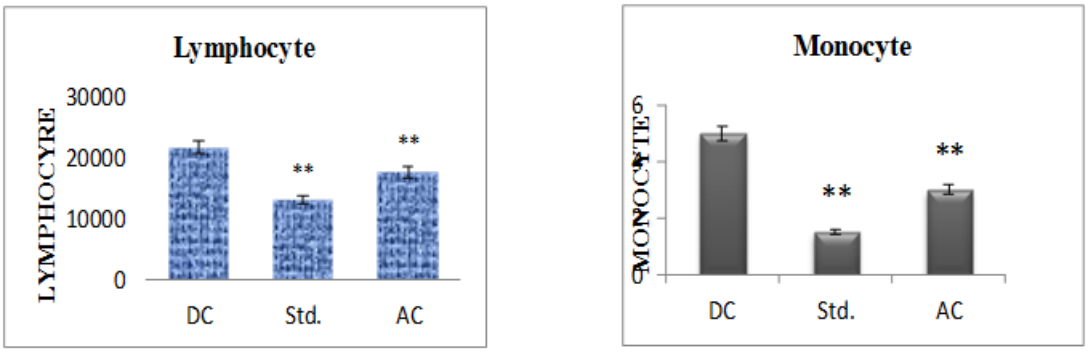
Table: 09. Hematological Parameter in different groups.

Group	RF	ESR	MONOCYTE	LYMPHOCYTE
I (DC)	41.00 ± 1.0	28.00 ± 4.0	5.0 ± 0.0	21650
II (Std.)	11.50 ± 1.5	6.5 ±1.5	1.5 ± 0.5	13000
III (RC)	23.00 ± 1.0	7.5 ± 2.5	2 ± 0.0	15650

RF: Rheumatoid factor, ESR: Erythrocyte sedimentation rate



Graph: 5. Value of ESR & RF. (All values represented as mean ± SEM of 2 animals**);**
***P ≤0.001 Vs. Disease control.



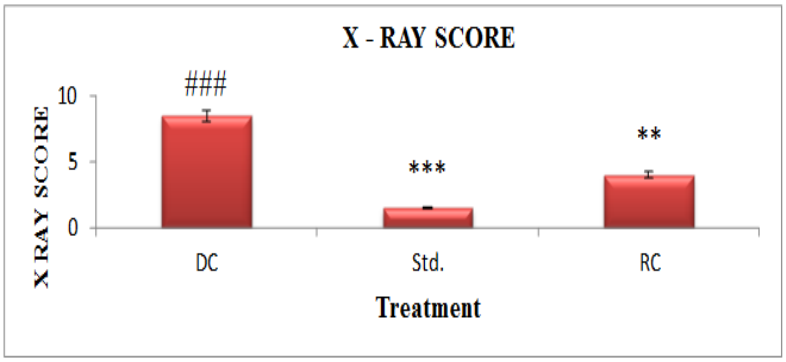
Graph: 6. Value of Lymphocyte & Monocyte (All values represented as mean ± SEM of 2 animals**);**

Table: 10. Radiological Analysis of Bone Destruction.

Group	I (DC)	II (Std.)	III (RC)
X-ray Score	8.5	1.5	4

** P ≤ 0.01 Vs. Disease control, * P ≤ 0.05. Vs. Disease control**)**

X ray analysis in adjuvant treated rats. (Figure 2)

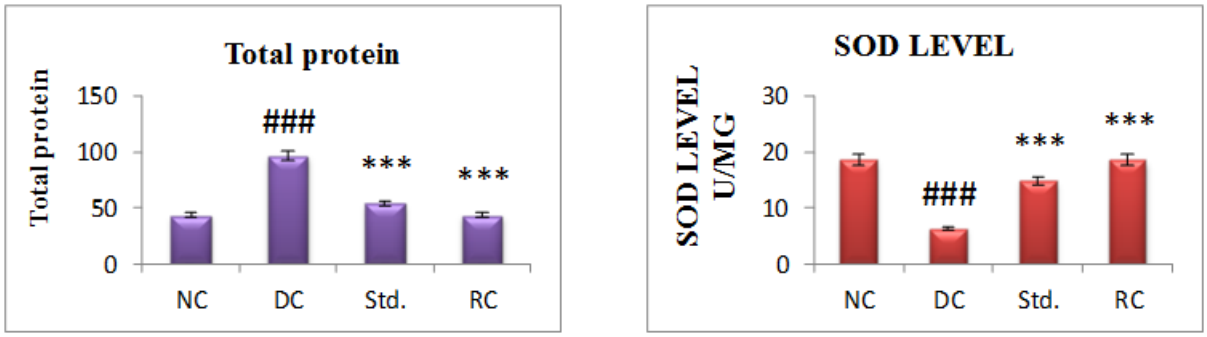


Graph: 7. X-ray score (All values represented as mean ± SEM of 2 animals**);**
** P ≤0.01 Vs. Disease control, *** P ≤0.001 Vs. Disease control. ###P ≤ 0.001 Significance difference Vs. Normal control.

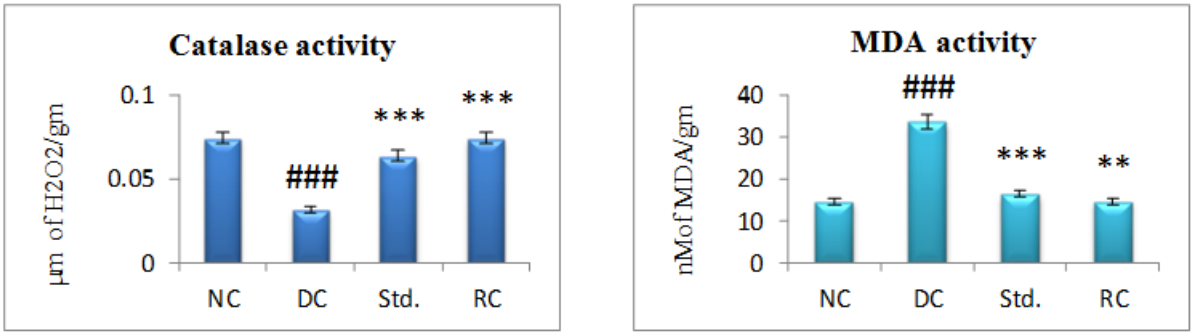
(C) Anti-oxidant study: It showed protective effect on oxidative stress in terms of significant reduction of MDA level and increased SOD & Catalase level compared with non treated group.

Table: 11. Results of anti – oxidant study.

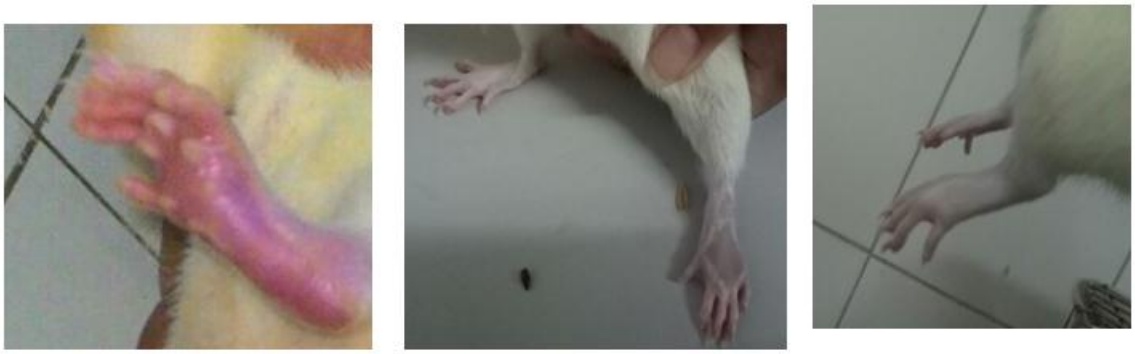
Group	Total protein	SOD	Catalase	LOP - MDA
I (DC)	96.55 ± 0.99	6.383± 0.51	0.03151 ± 0.0029	33.78±3.22
II (Std.)	53.90±1.66	14.90± 0.29	0.06376±0.00054	16.44± 0.40
III (RC)	56.54 ±1.98	12.19 ± 0.034	0.05835±0.00083	19.61 ± 0.43
IV (NC)	43.45 ± 0.99	18.64± 0.14	0.07433 ± 0.0030	14.57± 0.144



Graph: 8. Values of Total protein & SOD activity. (mean ± SEM of 2 animals**);**
*** P≤ 0.001 Vs. Disease control, ###P ≤ 0.001 significance difference Vs. normal control.



Graph: 9. Values of Catalase activity & LOP-MDA level. (mean ± SEM of 2 animals**);**
*** P≤ 0.001 Vs. Disease control, ** P ≤ 0.01 Vs. Disease control, ### P ≤ 0.001 significance difference Vs. normal control.



A. Disease control **B. Rhumavin capsule (200mg/kg)** **C. Diclofenac (15mg/kg)**

Figure: 1. Photographs of the arthritic rats were taken after 28 day.

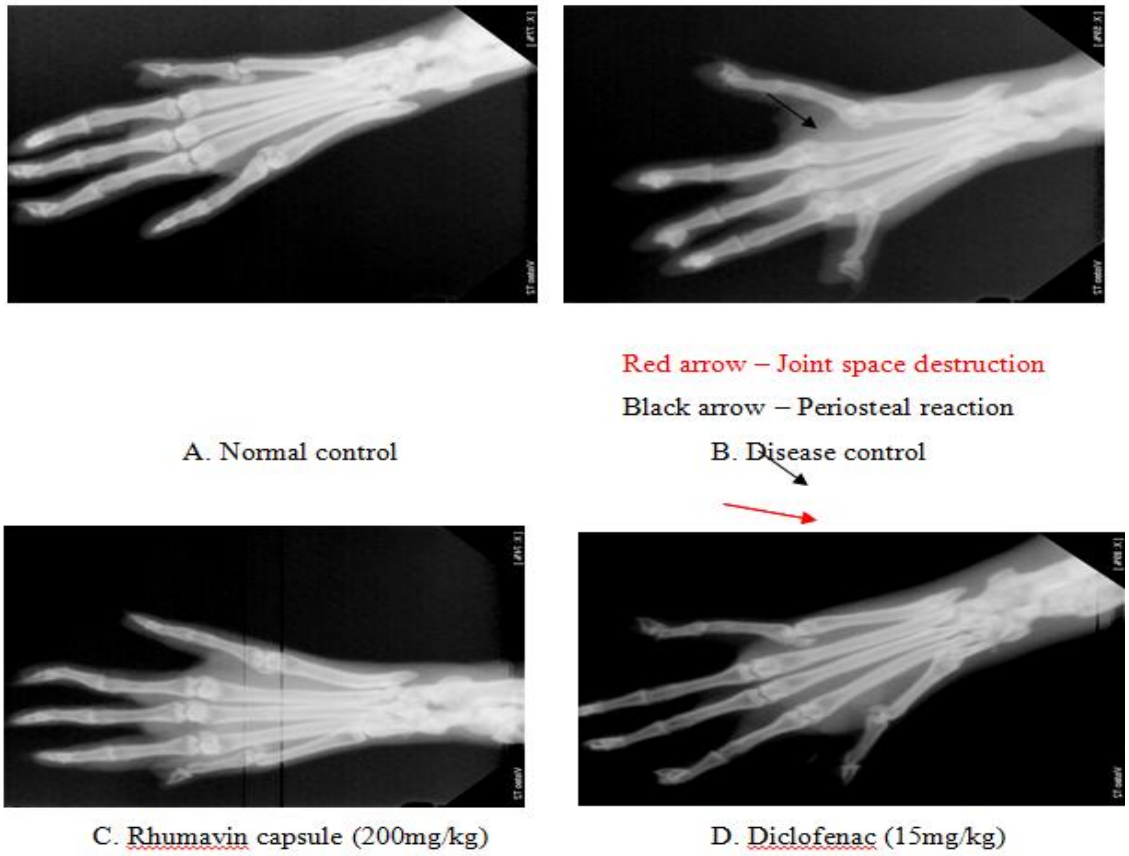


Figure: 2. Radiological analysis of bone destruction.

DISCUSSION

The interest in use of herbal preparations in different parts of the world has been growing considerably. However, popular belief that herbal preparations are safe based on ancient literature, required to be confirmed for their non-toxic/relatively less toxic effects compared to the chemical therapeutic counterparts.[15] This study can consider as a pioneer step for the establishment of safety profile and efficacy of Rhumavin Capsule.

The study was done on Swiss Albino Mice for 14 days to rule out any toxic effect of Rhumavin Capsule at the dose of 2000 mg/kg. Individual animal weekly body weight was recorded and found to be increasing during the observation period (Table 04). Animal daily observation was recorded and found to be same and mortality rate was Nil (Table 04). There were no physical and behavioral changes observed in animals during the observation period. This study reveals that Rhumavin Capsule which is indicated in RA has no oral toxicity effect. Hence, this can be used safely for therapeutic purposes**.**

The Rhumavin capsule is a ploy herbal formulation (PHF) containing various potent herbs (Table 01) having proven effect in arthritic conditions. The *In-Vitro* evaluation of anti arthritic activity of test drug by Bovine serum denaturation method (BSA) at different concentrations shows decrease in % inhibition of denaturation as compare to Standard drug treated group

(Table 05). The *In-Vivo* assay done in FCA arthritis model by measuring effect of test drug on various parameters. The AC treated group shows significant reduction in inflammation as compare to DC and Std groups. The significant reduction in ESR and RF important parameters for RA was found in AC treated group Standard drug treated group (Table 09). All these findings are suggestive of its potent anti arthritic and anti inflammatory activities. The radiography score of AC treated group was found nearly normal in compression with DC group. The anti-oxidant study showed protective effect on oxidative stress in terms of significant reduction of LOP-MDA level and increased SOD & Catalase level compared with non treated group. Thus, Rhumavin capsule can be safely use in various conditions like, rheumatoid arthritis, cervical spondylitis, joints pain, backache etc.

CONCLUSION

The No-Observed-Adverse-Effect-Level (NOAEL) of Rhumavin capsule is 2000 mg/kg as it did not have any toxic effect at that dose. It is found potent anti inflammatory and anti arthritic poly herbal formulation(PHF) with effective as anti oxidant in various arthritic conditions.

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1. Petalad Mahal Arogya Mandal Pharmacy (PMAMP), At. Po. Pipalata - , Dist. Kheda, Gujarat, India.
2. Shree S.K. Patel College of Pharmaceutical Education and Research, Ganpat University, Ganpat Vidyanagar-384012, Gujarat, India.
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