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

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ABSTRACT

Introduction: World Health Organization estimated that 80% of the world's population still depend on traditional medicines for their health care despite the availability of advanced medicinal systems. The toxicological evaluation of herbal formulation is necessary as it is in use for wide variety of clinical applications. **Aim:** To evaluate Rhumavin Tablet for its acute oral toxicity on Swiss albino mice and efficacy with anti-oxidant properties on arthritic rats. **Method:** The study protocol was approved by IAEC (SKPCPER/IAEC/2016-02/05) as per the CPCSEA. The acute oral toxicity was assessed by following OECD guideline AOT-425 to know single dose (2000 mg/kg) toxicity of test drug. 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 examine effectiveness of test drug on arthritis. FCA induced arthritis animal model (*In-vivo*) was adopted to assess effectiveness of test drugin arthritis. FCA was challenged to sub plantar region after treatment in Rats and various parameter such as paw volume, arthritic index, hematological and radiological parameterswere measured. Different assay analysis (total protein, SOD activity, Catalase activity, Lipid peroxidation) were done to establish its anti-oxidant properties. **Results:** There were no any physical as well as behavioral changes and mortality observed in any animal during study period. Body weight of all animals did not reveal any significant change as compared to vehicle control group. Rhumavin Tablet has significantly decreased paw volume, arthritic index, hematological parameters compared to disease control group. The significant increase in SOD and calalase level was observed in test drug treated group. **Conclusion:** The No-Observed-Adverse-Effect-Level (NOAEL) of Rhumavin Tablet is 2000 mg/kg. The obtained results advocate anti- inflammatory, anti-arthritic and antioxidant effect of Rhumavin Tablet.

KEYWORDS*:* Poly herbal formulation, Rhumavin Tablet, NOAEL, Mortality, OECD Guideline, Rheumatoid Arthritis, Anti-oxidant.

INTRODUCTION

Traditional medicines is sum total of the practices based on the theories, beliefs and experiences of different cultures and times which often inexplicable while used in the maintenance of health as well as prevention, diagnosis, improvement and treatment of diseases.[1] World Health Organization define the Traditional herbal medicines as naturally occurring, plant-derived substances with minimal or no industrial processing that have been used to treat illness within local or regional healing practices.[2] Despite the availability of advanced medicinal systems of treatment, approximately 80% of the world population still depends onalternative systems of medicine for the treatment of various illness.[3] The toxicity of poly herbal formulations is essential to be ruled out before used in human being, though it believed to be relatively safe.

Rheumatoid arthritis (RA) is a chronic autoimmune disorder and loss of possible joint function. Rheumatoid pannus formation leads to destruction of joints and surrounding tissues with inflammation, pain, swelling and stiffness.[4] The treatment of RA is done with various disease modifying synthetic agents such as NSAIDs and DMARDs. The main disadvantage with these potent drugs is their toxicity and reappearance of symptoms after discontinuation.[5] Research indicates 60–90% of people suffering from chronic pain as in RA and those

dissatisfied with current treatment are very likely to seek alternative treatments and use Complementary and Alternative medicine (CAM).[6] The Rhumavin Tablet is a poly herbal formulation indicated for the treatment of rheumatoid arthritis, cervical spondylitis, joints pain etc.

The present study was aimed to determine the acute oral toxicity as well as efficacy of Rhumavin Tablet in Rheumatoid arthritis.

AIM AND OBJECTIVES

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

MATERIALS AND METHODS

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

Table 1: Ingredients of Rhumavin Tablet (Each tablet contain).

Sl. No.	Name of Ingredient	Quantity
1	Ext. Ricinus communis	30 mg
2	Ext. Pluchea lanceolate	30 mg
3	Ext. Tinospora cordifolia	30 mg
4	Ext. Boerhavia diffusa	30 mg
5	Ext. Zingiber officinale	30 mg
6	Ext. Trachyspermum ammi	30 mg
7	Aconitum heterophyllum	20 mg
8	Smilax Glabra	20 mg
9	Shuddha Shilajit	20 mg
10	Strychnosnux-vomica	10 mg
11	MahaYograj Guggulu	50 mg
12	Vitex negundo	QS
13	Moringa oleeifera	QS
14	Dashmul Kwatha	QS

Method: The present study was conducted after got 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[7] It was performed by following OECD guideline AOT-425 to know single dose toxicity of Rhumavin Tablet on swiss albino mice. All the Animals were maintained in standard condition and acclimatized prior to dosing. They were randomly divided in different groups. Each animal was treated with a limit single oral dose of 2000 mg/kg of extract in sequence at 48 h intervals. They were observed individually for any clinical sign of toxicity or mortalityonce during first 30 min after dosing and periodically during 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 2: Individual animal dosing record of Rhumavin Tablet.

Expt. Day	Animal No.	Gender	Test drug (mg)	Vehicle Distilled Water (ml)	Volume dose (ml)	Conc. (mg/ml)
1st day	H	M	52	0.6	0.58	86.66
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	60	0.6	0.54	100

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** [8]**:** It was done with 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 Tablet in the present 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 (*adlibitium*) were provided.

Table 3: 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 Tablet(RT)	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 (AC) 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 volume 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,[9] SOD activity,[10] Catalase activity[11] and lipid peroxidation.[12]

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: All animals were continuously observed 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 neither any physical and behavioural changes nor 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.

Table 4: Showing individual animal observation & Mortality record.

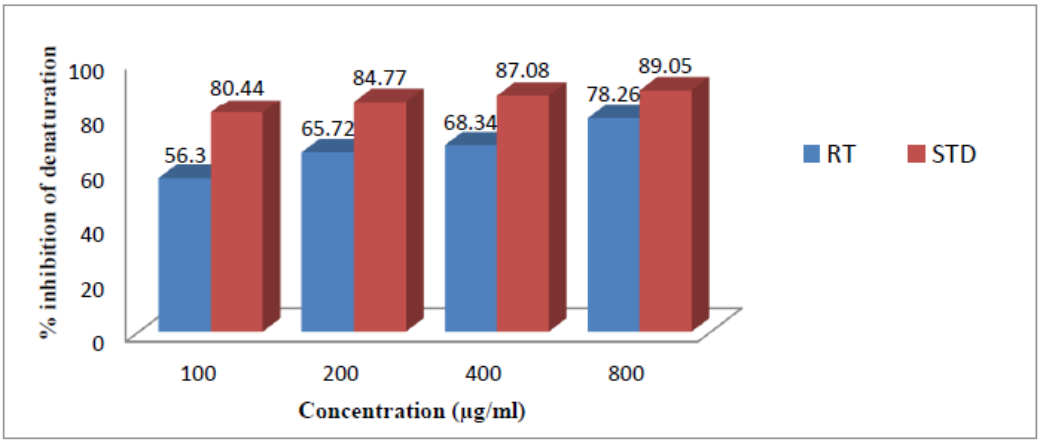
Animal No.	Gender	Experiment Day, Unit : gm			Mortality
		1st	7th	14th	
H	M	25	26	27	NIL
B	M	26	27	28	NIL
T	F	24	25	26	NIL
HT	F	25	26	27	NIL
UM	F	27	28	29	NIL

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 5: Details of Denaturation inhabitation by Rhumavin Tablet.

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 Tablet (RT)	100	0.358	0.157	43.97	56.30
	200	0.318	0.109	34.37	65.72
	400	0.417	0.132	31.65	68.34
	800	0.690	0.150	21.73	78.26



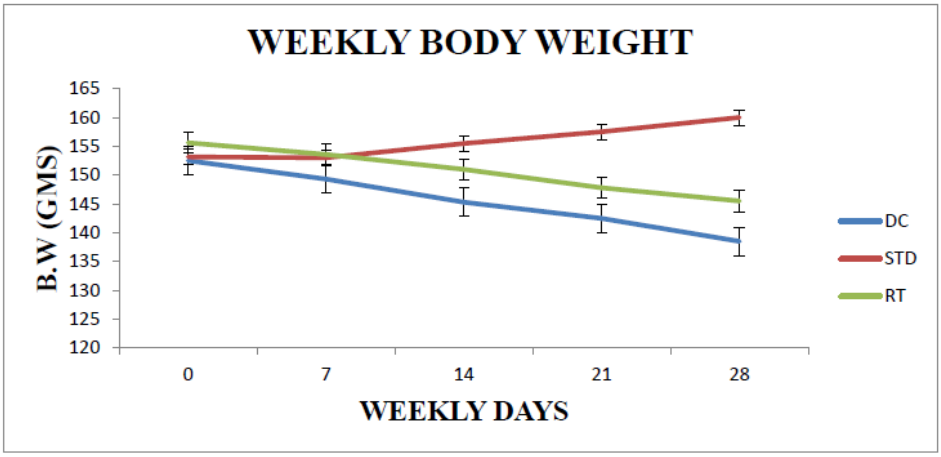
Graph 1 Showing % inhibition of denaturation at different concentrations.

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 6: Weekly body weight record of all animals.

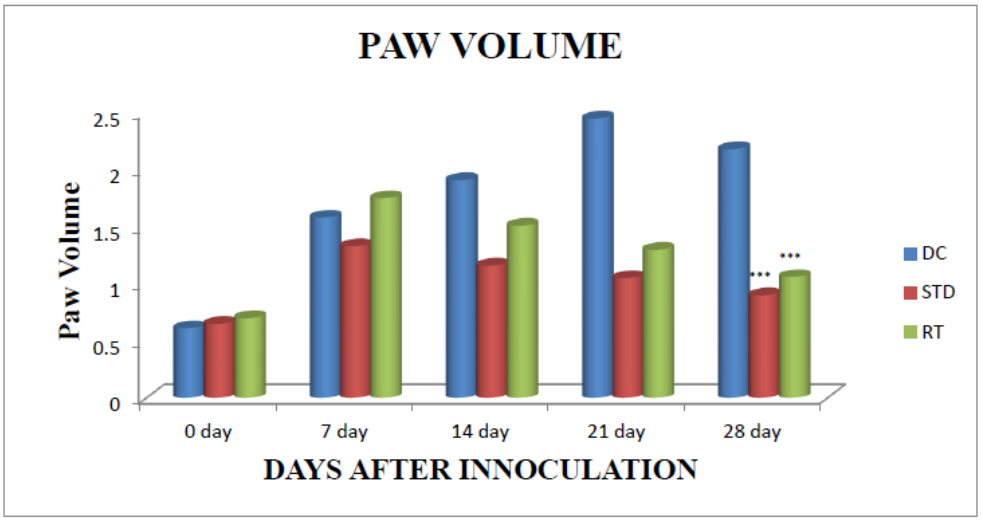
Group	0 day	7th day	14th day	21st day	28th day
I (DC)	152.5	149.3	145.3	142.5	138.5
II (Std.)	153.16	153	155.5	157.5	160
III (RT)	155.6	153.6	151	147.8	145.5



Graph 2: Showing Weekly body weights of all animals.

Table 7: 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 (RT)	0.7	1.75	1.51	1.3	1.06

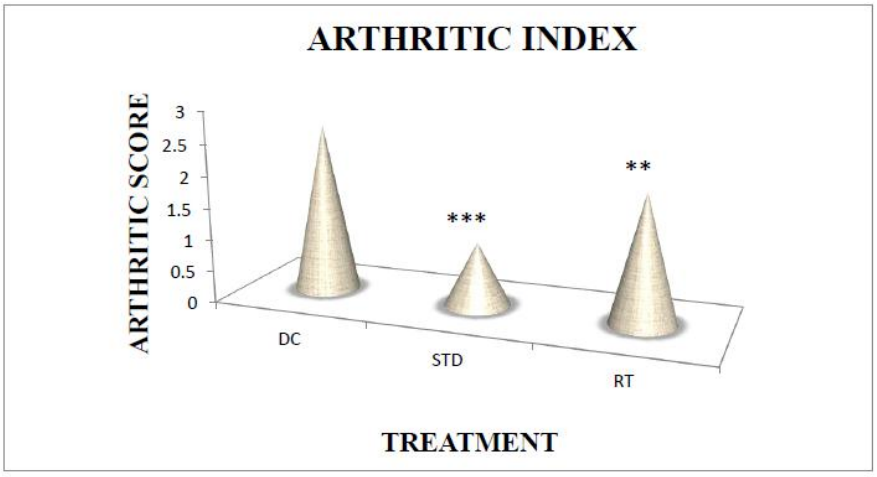


Graph 3: Showing Weekly Paw volume of all animals (All values represented as mean ± SEM of 6 animals).

$P \leq 0.001$ Vs. disease control, ** $P \leq 0.01$ Vs. Disease control,

Table 8: Arthritic Score of different groups.

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



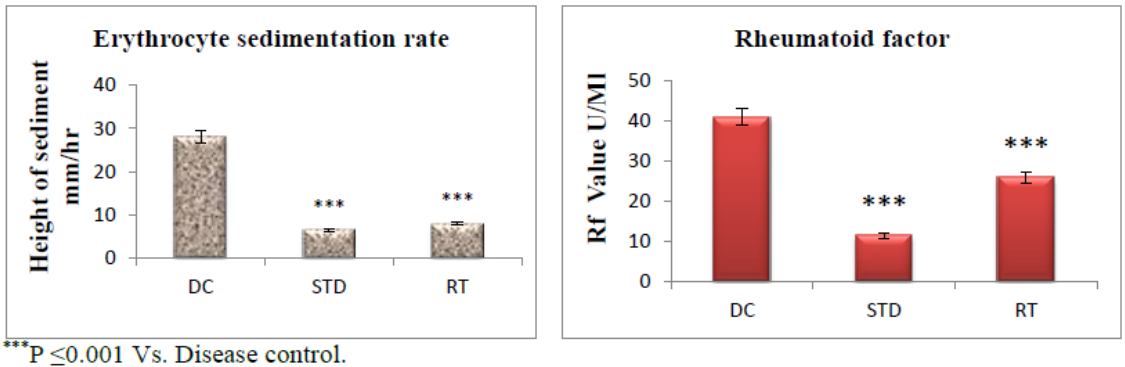
Graph 4: Showing Arthritic Index (All values represented as mean ± SEM of 6 animals.);

Table 9: Hematological Parameter in different groups.

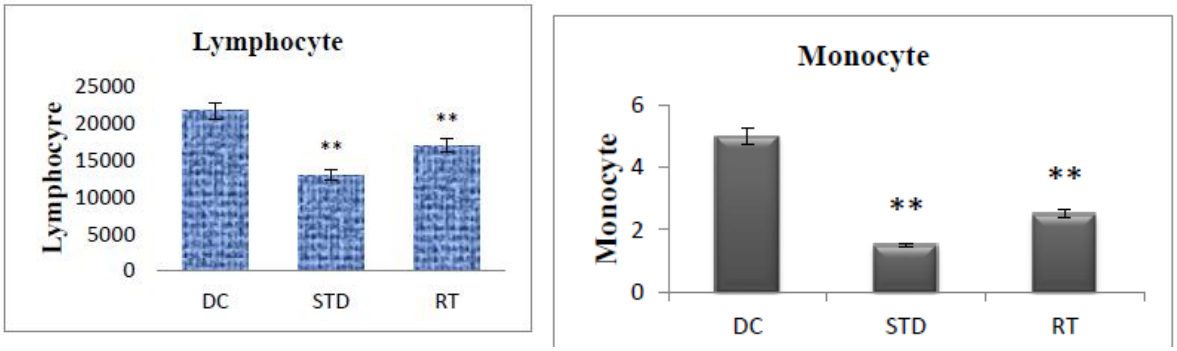
Group	RF	ESR	MONOCYTE	LYMPHOCYTE
I (DC)	41.00 ± 1.0	28.00 ± 4.0	5.0 ± 0.0	21650
II (Std.)	11.50 ± 1.5	6.5 ±1.5	1.5 ± 0.5	13000
III (RT)	26.00 ± 0.0	8.0 ± 2.0	2.5 ± 0.5	17000

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.

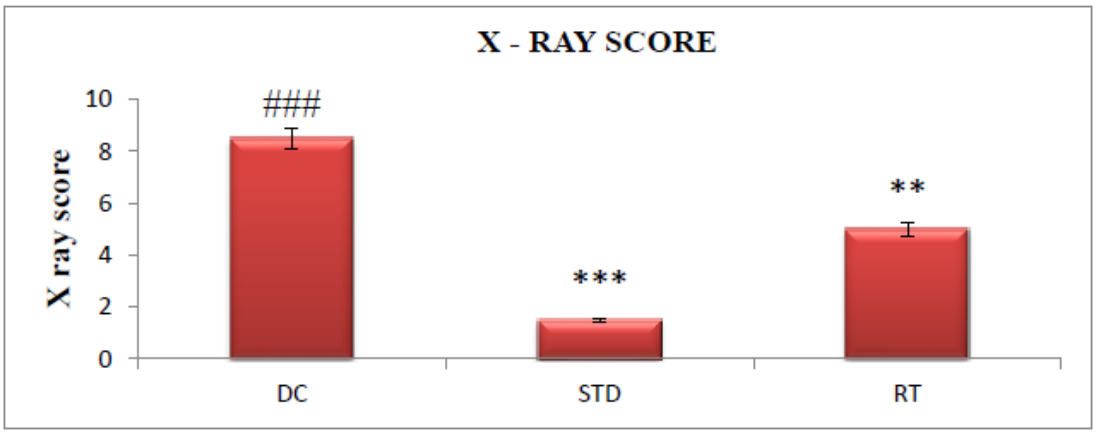


Graph 6: Value of Lymphocyte& Monocyte (All values represented as mean ± SEM of 2 animals).

Table 10 Radiological Analysis of Bone.

Group	I (DC)	II (Std.)	III (RT)
X-ray Score	8.5	1.5	5

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



Graph 7: X-ray score (All values represented as mean ± SEM of 2 animals).

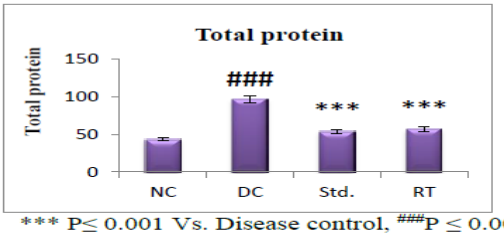
** P ≤0.01 Vs. Disease control, *** P ≤0.001 Vs. Disease control. ###P ≤ 0.001 Significance difference Vs. Normal control.

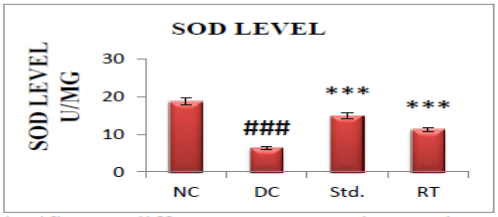
(C) Anti-oxidant study: It showed protective effect on oxidative stress in terms of significant reduction of MDA level and increased SOD & Catalase level compared with non treated group.

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 (RT)	56.61± 1.26	11.26± 0.16	0.05708±0.0028	21.43± 0.57
IV (NC)	43.45 ± 0.99	18.64± 0.14	0.07433 ± 0.0030	14.57± 0.144

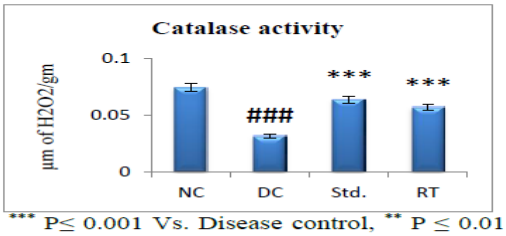
Graph 8 Values of Total protein& SOD activity(mean ± SEM of 2 animals);

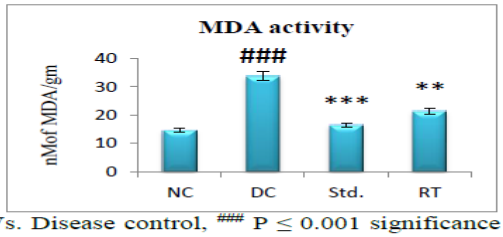




*** P≤ 0.001 Vs. Disease control, ###P ≤ 0.001 significance difference Vs. normal control.

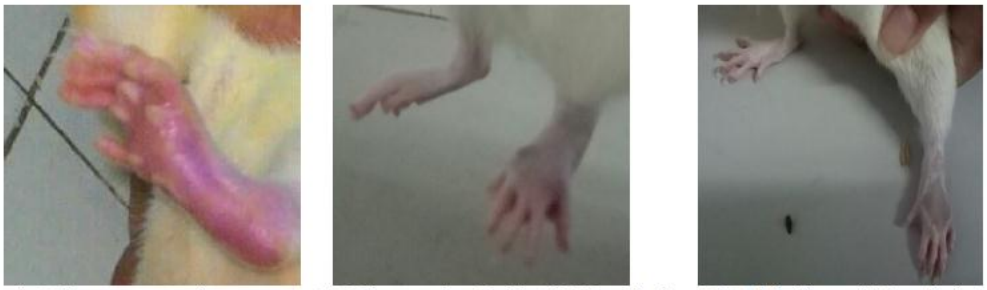
Graph 9 Values of Catalase activity& LOP-MDA level (mean ± SEM of 2 animals);





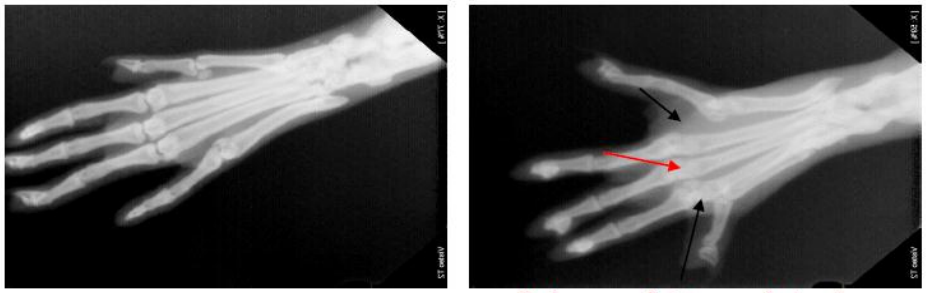
*** P≤ 0.001 Vs. Disease control, ** P ≤ 0.01 Vs. Disease control, ### P ≤ 0.001 significance difference Vs. normal control.

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



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

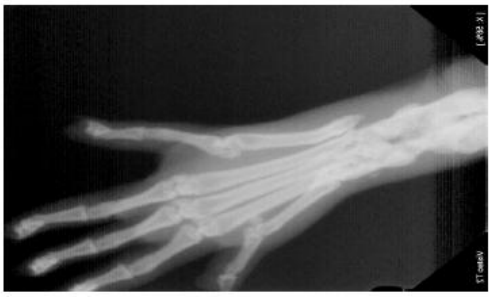
Figure 2 radiological analysis of bone destruction



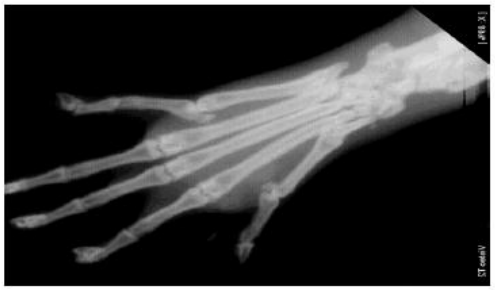
Red arrow – Joint space destruction

Black arrow – Periosteal reaction

A. Normal control B. Disease control



C. Rhumavin Tablet (200mg/kg)



D. Diclofenac (15mg/kg)

DISCUSSION

The present study can consider as a pioneer step for the establishment of safety profile and efficacy of Rhumavin Tablet as it does not reported till date.

The acute oral toxicity of Rhumavin Tablet was assessed on Swiss Albino Mice for 14 days of study period at the single dose of 2000 mg/kg. Body weight of individual animal was recorded weekly and found increased during the observation period **[Table 4]**. Animal daily observation was recorded and found same and mortality rate was Nil **[Table 4]**. There were no physical and behavioral changes observed in animals during the observation period. This study reveals that Rhumavin Tablet has no oral toxicity effect. Hence, it can be used safely for therapeutic purposes**.**

The Rhumavin Tablet is a ploy herbal formulation containing various potent herbs **[Table 1]** having proven effect in arthritic conditions.[13,14,15] The *In-Vitro* evaluation for 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 5].** The *In-Vivo* assay done in FCA arthritis model by measuring effect of test drug on various parameters. The RT treated group shows significant reduction in inflammation as compare to DC and Std groups. The significant reduction in ESR and RF an important parameters for RA was found in RT treated group as compare to Standard drug treated group **[Table 9]**. All these findings are suggestive of potent anti arthritic and anti inflammatory activities of test drug. The radiography score of RT 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 Tablet 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 Tablet is 2000 mg/kg as it did not have any toxic effect at that dose. It is found potent anti inflammatory and anti arthritic PHF with effective as anti oxidant in various arthritic conditions.

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REFERENCES

1. https://www.researchgate.net/publication/5623790_Herbal_Medicine_Today_Clinical_and_Research_Issues, 21/03/2020, 10:04 am.
2. Qazi Majaz A, Molvi Khurshid I, Herbal Medicine: A Comprehensive Review, International Journal Of Pharmaceutical Research, 2016; 8(2): 1-5.
3. Who Media Centre: Traditional Medicine. Fact Sheet N°134 2008. [Last Accessed On 2012 June]. AvailablFrom: Http://Www.Who.Int/Mediacentre/Factsheets/Fs134/En.
4. D.Yen, J.Cheung, H.Scheerens, F.Poulet, T.Mcclanahan, B.Mckenzie, M.A.Kleinschek, J.Owyan, W.Blumenschein, E.Murphy, M.Sathe, D.J.Cua, R.A.Kastelein, D.Rennick, Il-23isessentialfor T Cell–Mediated Colitisandpromotes Inflammation Via Il-17and Il 6, J. Clin.I nvestig, 2006; 116: 1310–1316.
5. Bi Bi Sara, A Krishna Shailja, A Review On Polyherbal Formulations Used In The Treatment Of Autoimmune Disease Like Rheumatiod Arthritis, Research & Reviews: Journal Of Herbal Science, 2019; 8(1): 11-16.
6. OECD Guideline For Testing Of Chemicals, Acute Oral Toxicity Up And Down Procedure, OECD – 425, Adopted On 17th December, 2001; 1-26. (Brown, J.H., H.K. Mackey1995).
7. Lowry Oh, Rosebrough Nj, Farr Al, Randall Rj, Protein Measurement With The Folin Phenol Reagent. J Biol Chem., 1951; 193(1): 265-75.

8. Misra HP, FridovichI, The Role Of Superoxide Anion In The Autoxidation Of Epinephrine And A Simple Assay For Superoxide Dismutase. J Biol Chem., 1972; 25; 247(10): 3170-5.
9. Tadayuki lwase *et al*, A Simple Assay for Measuring Catalase Activity: A Visual Approach, Sci Rep., 2013; 3: 3081.
10. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal Biochem., 1979; 95(2): 351-8.
11. Oreagba IA, Oshikoya KA, Amachree M, Herbal medicine use among urban residents in Lagos, Nigeria. BMC Complement Altern Med., 2011; 11: 117.
12. Khan MA *et al*, Effect of *Withania somnifera* (Ashwagandha) root extract on amelioration of oxidative stress and autoantibodies production in collagen-induced arthritic rats. J Complement Integr Med., 2015; 12(2): 117-125.
13. Funk J L *et al*, (2009), Comparative Effects of Two Gingerol-Containing *Zingiber officinale* Extracts on Experimental Rheumatoid Arthritis, J Nat Prod., Vol. Mar 27, 72(3), pp 403-407.
14. Abdullah Al-Nahain *et al*, Zingiber officinale: A Potential Plant against Rheumatoid Arthritis. http://dx.doi.org/10.1155/2014/159089, Vol. 2014, Article ID 159089, 2014; 8.