



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

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ABSTRACT

Introduction: Despite 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 Amvatri 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 Amvatri capsule on arthritis. FCA induced arthritis animal model (*In-vivo*) was adopted to check therapeutic effectiveness of Amvatri 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. Amvatri capsule decreased significantly paw volume, Arthritic index, hematological parameters compared to disease control group. The significant increase in SOD and calalase level was found in test drug treated group. **Conclusion:** The No-Observed-Adverse-Effect-Level (NOAEL) of Amvatri capsule is 2000 mg/kg. The results suggest anti- inflammatory, anti-arthritic and antioxidant effect of Amvatri capsule.

KEYWORDS: Poly herbal formulation, Amvatri 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 Amvatri capsule is a poly herbal formulation and has been indicated for the treatment of rheumatoid arthritis, cervical spondylitis, joints pain etc. The ingredients of Amvatri capsule have proven anti arthritic properties.^{[7],[8],[9]}

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. Amvatri capsule.

AIM AND OBJECTIVES

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

MATERIALS AND METHODS

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

Table 1: Ingredients of Amvatri capsule (Each hard gelatine capsule contain).

Sl. No.	Name of Ingredient	Quantity
1	Ext. Withania somnifera	100 mg
2	Ext. Ricinus communis	100 mg
3	Colchicum luteum	100 mg
4	Smilax china	100 mg
5	Zingiber officinale	100 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^[10]: It was conducted according to OECD guideline AOT-425 to know single dose toxicity of Amvatri 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 2: Individual animal dosing record of Amvatri capsule.

Expt. Day	Animal No.	Gender	Test drug (mg)	Vehicle Distilled Water (ml)	Volume dose (ml)	Conc. (mg/ml)
1 st day	H	M	60	0.6	0.54	100
3 rd day	B	M	60	0.6	0.56	100
5 th day	T	F	50	0.6	0.58	83.33
7 th day	HT	F	60	0.6	0.54	100
9 th day	UM	F	50	0.6	0.58	83.33

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^[11]:** 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 Amvatri capsule in the current study.

Preparation of Reagents

- ✓ **Bovine Serum Albumin 0.5% (BSA):** Bovine Serum Albumin (500 mg) + H₂O (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 libitum*) 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	Amvatri Capsule (AC)	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 supernatant layer of solution was

collected after centrifugation for analysis of different assay i.e. total protein^[12], SOD activity^[13], Catalase activity^[14] and lipid peroxidation.^[15]

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 4: Showing individual animal observation & Mortality record.

Animal No.	Gender	Experiment Day, Unit : gm			Mortality
		1 st	7 th	14 th	
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

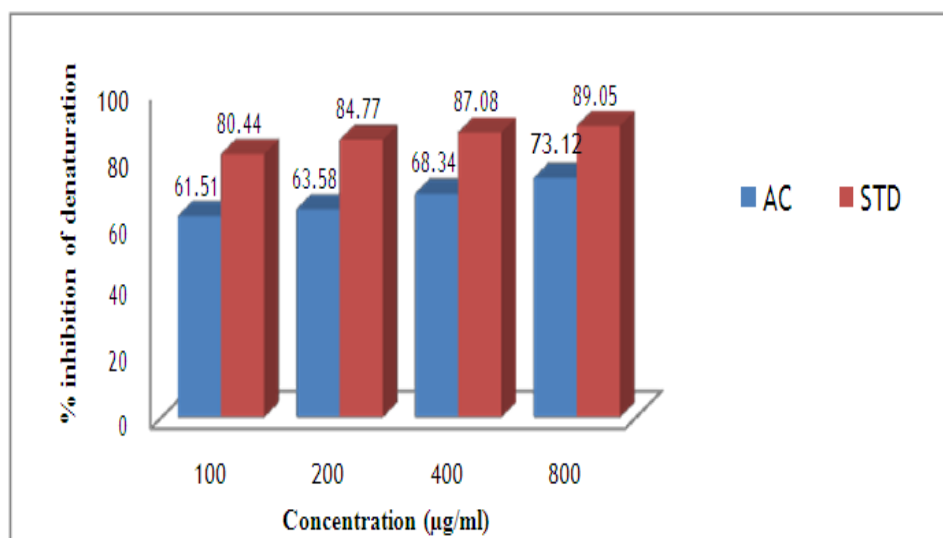
H: Head, B: Body, T: Tail, HT: Head & Tail, UM: Unmarked, M: Male, F: Female

(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 5: Details of Denaturation inhibition by Amvatri 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
Amvatri Capsule (AC)	100	0.330	0.127	38.48	61.51
	200	0.357	0.130	36.41	63.58
	400	0.417	0.132	31.65	68.34
	800	0.480	0.129	26.87	73.12



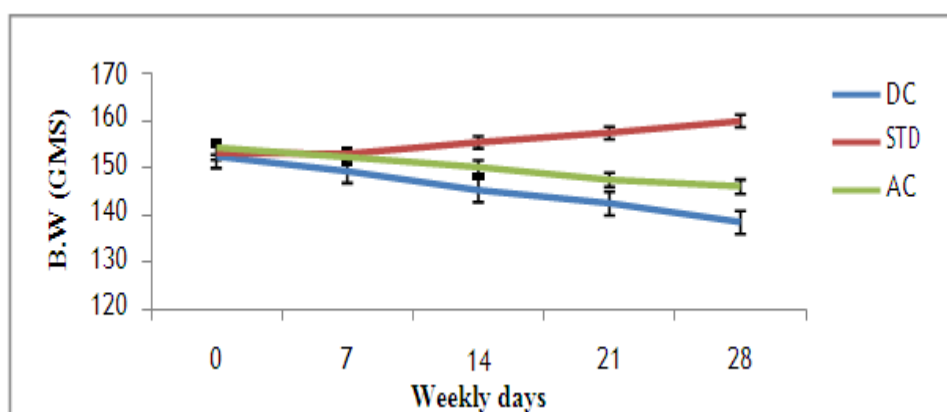
Graph 1: Showing % inhibition of denaturation at different concentrations.

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 comparison of test drug treated group.

Table 6: Weekly body weight record of all animals.

Group	0 day	7 th day	14 th day	21 st day	28 th day
I (DC)	152.5	149.3	145.3	142.5	138.5
II (Std.)	153.16	153	155.5	157.5	160
III (AC)	154.33	152.3	150.16	147.5	146.1



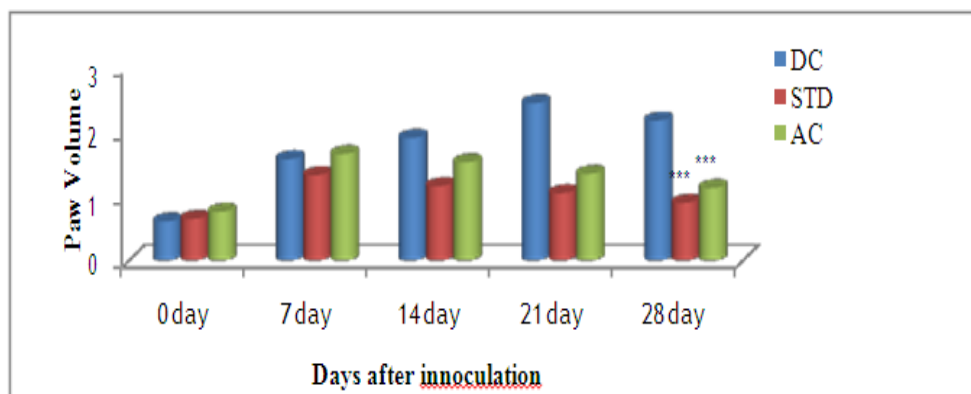
Graph 2: Showing Weekly body weights of all animals.

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 7: Weekly Paw volume record of all animals.

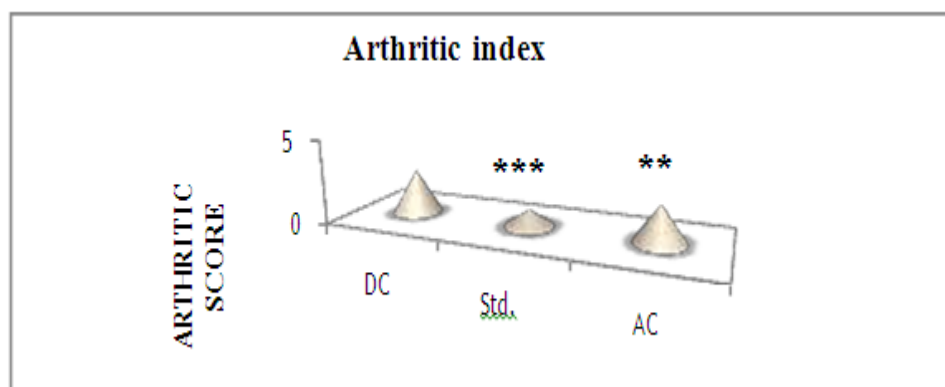
Group	0 day	7 th day	14 th day	21 st day	28 th day
I (DC)	0.61	0.61	0.61	0.61	0.61
II (Std.)	0.65	0.65	0.65	0.65	0.65
III (AC)	0.76	0.76	0.76	0.76	0.76

Graph 3 Showing Weekly Paw volume of all animals (All values represented as mean $\pm$ 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 (AC)
Arthritic Score	2.667	1	2.167

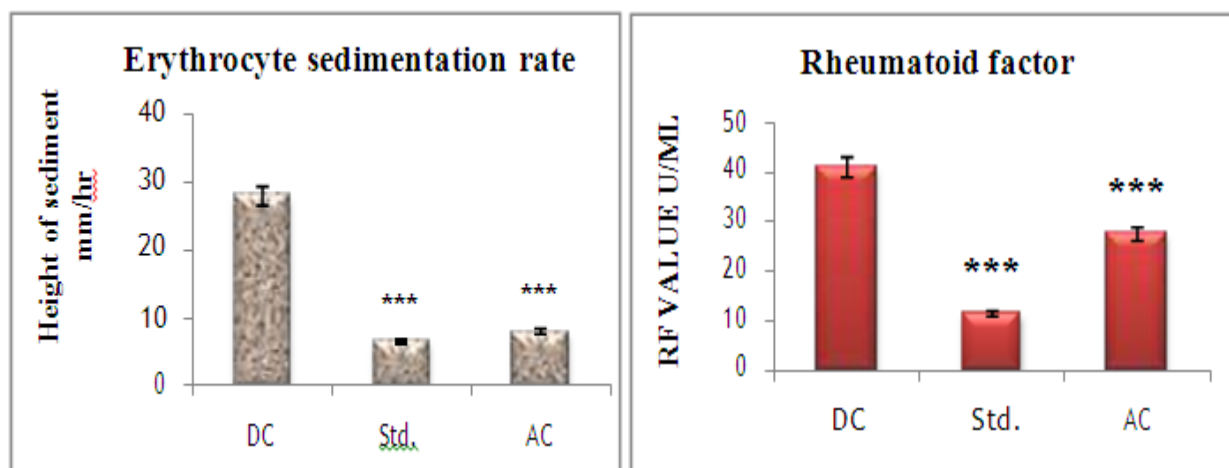
Graph 4: Showing Arthritic Index (All values represented as mean $\pm$ SEM of 6 animals.).

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

Table 9: Hematological Parameter in different groups.

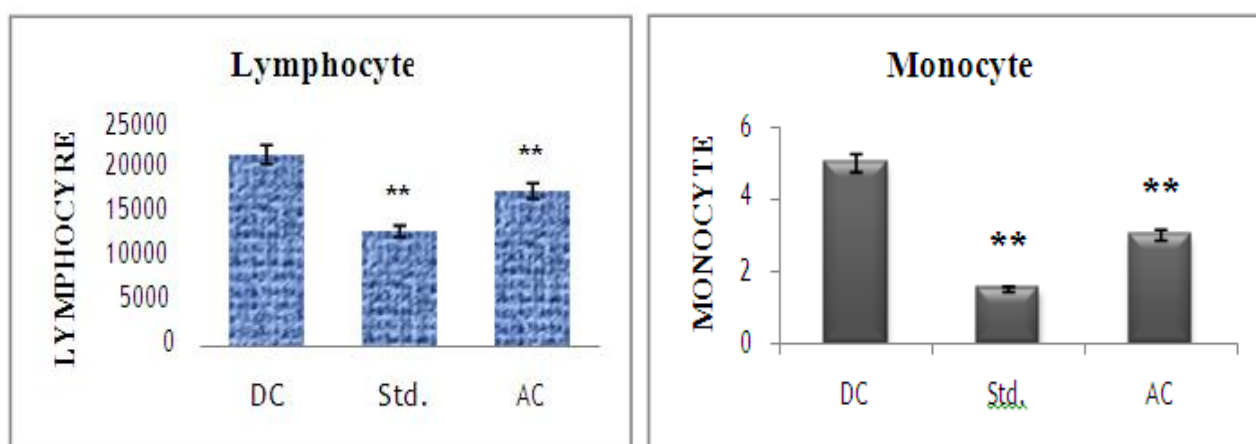
Group	RF	ESR	MONOCYTE	LYMPHOCYTE
I (DC)	41.00 $\pm$ 1.0	28.00 $\pm$ 4.0	5.0 $\pm$ 0.0	21650
II (Std.)	11.50 $\pm$ 1.5	6.5 $\pm$ 1.5	1.5 $\pm$ 0.5	13000
III (AC)	27.50 $\pm$ 0.5	8.0 $\pm$ 0.0	3.0 $\pm$ 0.0	17550

RF: Rheumatoid factor, ESR: Erythrocyte sedimentation rate.



Graph 5: Value of ESR & RF (All values represented as mean $\pm$ SEM of 2 animals).

*** $P \leq 0.001$ Vs. Disease control.

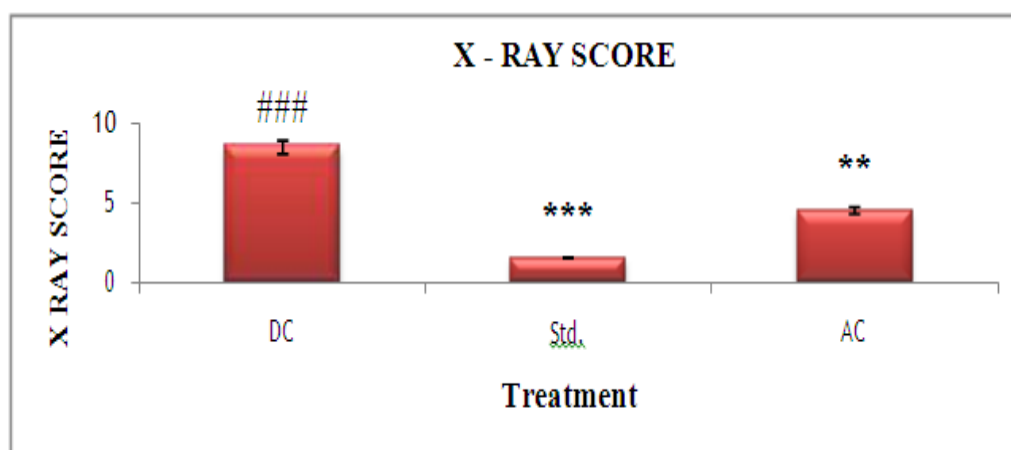


Graph 6: Value of Lymphocyte & Monocyte (All values represented as mean $\pm$ SEM of 2 animals).

** $P \leq 0.01$ Vs. Disease control, * $P \leq 0.05$. Vs. Disease control)

X ray analysis in adjuvant treated rats (Figure 2) Table 10 Radiological Analysis of Bone Destruction.

Group	I (DC)	II (Std.)	III (AC)
X-ray Score	8.5	1.5	4.5



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

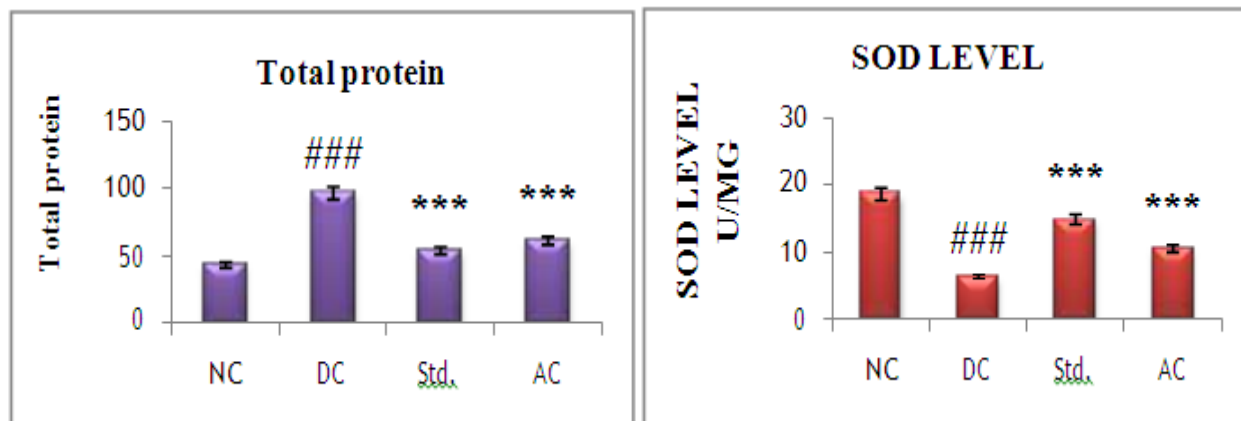
** $P \leq 0.01$ Vs. Disease control, *** $P \leq 0.001$ Vs. Disease control. ### $P \leq 0.001$ Significance difference Vs. Normal control.

(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.

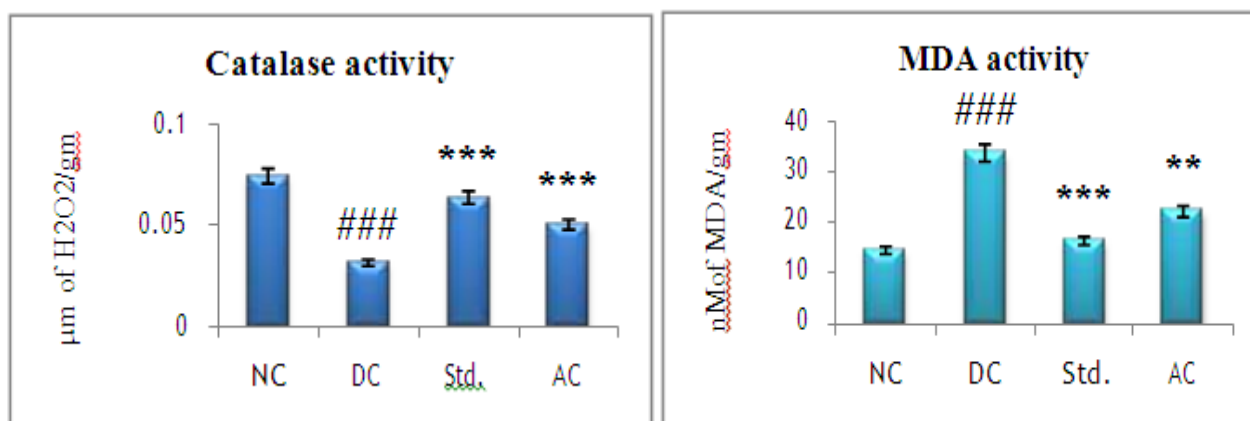
Table 11: Results of anti – oxidant study.

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 (AC)	61.24± 0.68	10.58± 0.55	0.05036±0.0021	22.26± 1.035
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. Amvatri capsule (200mg/kg)



C. Diclofenac (15mg/kg)

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

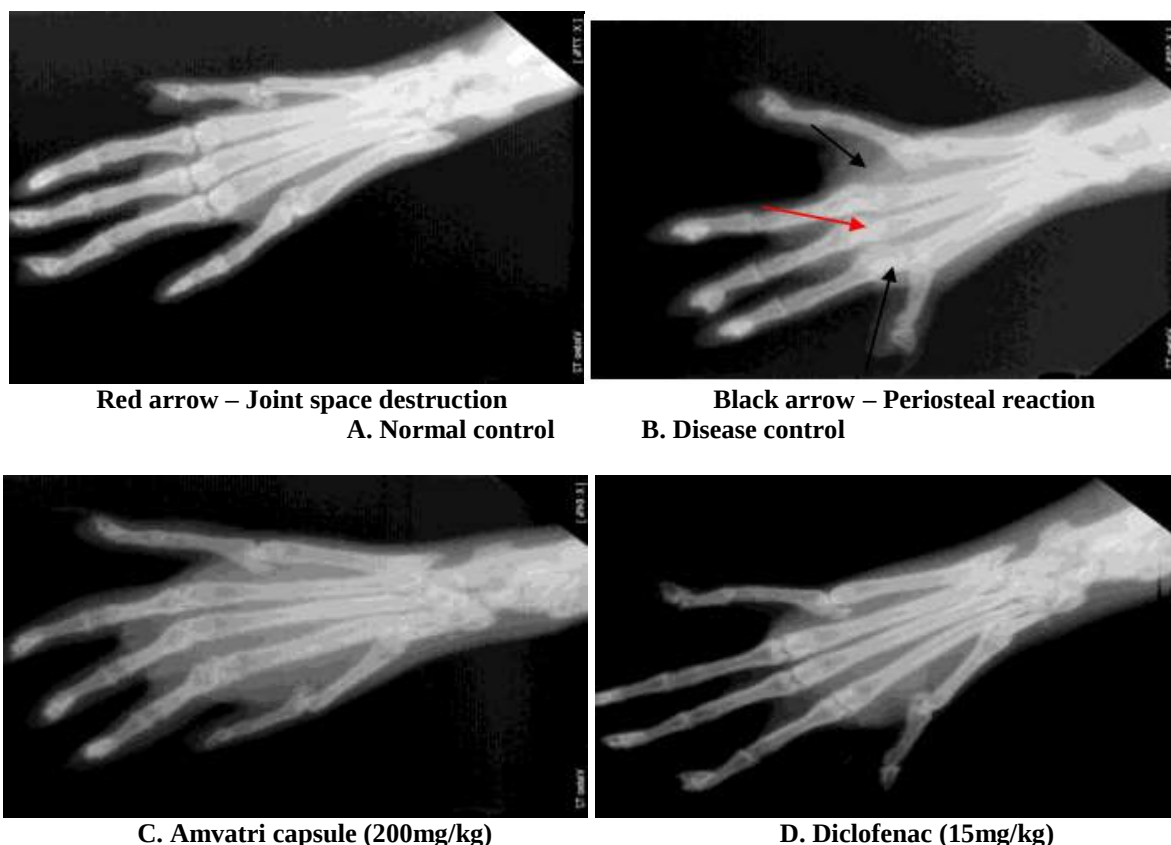


Figure 2: Radiological analysis of bone destruction.

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.^[16] This study can consider as a pioneer step for the establishment of safety profile and efficacy of Amvatri Capsule.

The study was done on Swiss Albino Mice for 14 days to rule out any toxic effect of Amvatri 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 4]. Animal daily observation was recorded and found to be 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 Amvatri Capsule which is indicated in RA has no oral toxicity effect. Hence, this can be used safely for therapeutic purposes.

The Amvatri capsule is a ploy herbal formulation containing various potent herbs [Table 1] having proven effect in arthritic conditions.^{[7],[8],[9]} 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 5]. 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 an important parameters for RA was found in AC treated group Standard drug treated group [Table 9]. 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 comparison 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, Amvatri 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 Amvatri 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 PHF with effective as anti oxidant in various arthritic conditions.

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