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ESTIMATION OF POTENTIAL ANTI-ARTHRITIC ACTIVITY OF *Trapa Bispinosa , Cassia Uniflora , Cissus Quadrangularis* AND *Bosevilla Serrata* PLANTS AGAINST FREUND'S COMPLETE ADJUVANT INDUCED ARTHRITIS BY USING ANIMAL MODELS

Amita B Dongare[1], Dr. Rakesh Kumar Jatav[2]

[1,2] School of Pharmacy, Dr. A. P. J. Abdul Kalam University, Indore (M. P).

ABSTRACT

Aim: Estimation of Anti-Arthritic Activity of sustained released tablet of *Trapa Bispinosa , Cassia Uniflora* , *Cissus Quadrangularis* and *Bosevilla Serrata* plants against Freund's complete adjuvant induced arthritis by using animal models.

Materials and Methods: *Trapa Bispinosa , Cassia Uniflora* , *Cissus Quadrangularis* and *Bosevilla Serrata* are well known plants available throughout India and they are commonly used for the treatment of various diseases including arthritis. The polyherbal sustained released tablet contains the ethanol extracts of *Trapa Bispinosa , Cassia Uniflora* , *Cissus Quadrangularis* and *Bosevilla Serrata* . The quality of the finished sustained released tablet was evaluated as per the World Health Organization's guidelines for the quality control of herbal materials. Arthritis was induced in female Wistar rats using Freund's complete adjuvant (FCA), and the antiarthritic effect of sustained released tablet was studied at doses of 200 and 400 mg/kg. The effects were compared with those of Diclofenac (20 mg/kg).

At the end of the study, blood samples were collected for biochemical and hematological analysis. The radiological examination was carried out before terminating the study.

Results: Sustained Released Tablet (TCBC) showed significant antiarthritic activity at 100 and 200 mg/kg, respectively, and this effect was comparable with that of Diclofenac (20 mg/kg).

The antiarthritic activity of Sustained Released Tablet is supported by hematological and histopathological analysis.

Conclusion: The sustained released tablet (TCBC 200 mg/kg) showed significant antiarthritic activity against Freund's complete adjuvant induced arthritis in Female Wistar rats.

Introduction

The oldest type of medical treatment that humans have ever used is herbal medicine. It is crucial to the advancement of modern society Civilization. In herbal medicine, formulations made from plants are used to treat illnesses. However, these formulations' lack of thorough evaluation is what poses the biggest problems for them. Thus, assessment is required to guarantee the quality and purity of the herbal product. Developing a system is extremely important. Due to the enormous potential for variation among different batches of medicine, of evaluation for every plant medicine available on the market[1] . A typical protective reaction to tissue injury is inflammation, which involves a variety of processes including enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown, and repair[2] . It is characterized by pain, stiffness in the joints, redness, swelling, and loss of joint function. Membrane changes, an increase in vascular permeability, and protein denaturation are all linked to inflammation [3] .

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A chronic, inflamed, and systemic autoimmune disorder, arthritis. Due to an immune-mediated response, the synovial joint is inflamed [4] . A fifth of all elderly people worldwide have arthritis [5]. The current approach to treating arthritis includes reducing this related Nonsteroidal anti-inflammatory drugs (NSAIDs) are used to treat pain and inflammation, and anti-rheumatic drugs are used to slow the progression of the disease [6]. Due to negative side effects from NSAIDs and disease-modifying antirheumatic medications, arthritis patients frequently look for alternative therapies that are both efficient and safe. Therefore, these patients frequently favor complementary and alternative therapies [7].

Single herb or more than one herb, or Polyherbal , are the two principles used in Ayurveda for drug formulation. This was emphasized in the "Sarangdhar Samhita," which dates back centuries to 1300 A.D. Additionally, PHF was used to record increased therapeutic effectiveness with fewer toxic side effects[8].

To achieve the greatest therapeutic efficacy, the traditional Indian medical system combined extracts from various plants rather than using just one type [9].

Ethanolic extracts of powder of *Trapa bispinosa ,* leaves of *Cassia Uniflora , Bosevilla Serrata leaves and Cissus Quadrangularis* were used to prepare Sustained released tablet and evaluate the anti arthritic activity . The preliminary acute toxicity of Sustained released tablet and individual plant extract was not showed any significant toxic effects upto 2000 mg/kg [10].

Experimental Animals:

In the present investigation Female Wistar rats were selected to induce arthritis because rats develop a chronic swelling in multiple joints under the influence of inflammatory cells, erosion of joint cartilage due to bone destruction [11].

Female Wistar rats weighing from 200 to 300 g were used. The rats were housed under standard conditions of temperature (23–25°C), and relative humidity (55%) with 12 h light and 12 h dark cycle. They were fed with a standard pellet diet and tap water ad libitum. The experiment was designed and carried out according to the norms of the ethical committee (CPSCEA) and approved by the institutional animal ethical committee (SGRS/IAEC/05/2020-21).

Plant materials

Taxonomically identified fruits of *Trapa Bispinosa ,leaves of Cassia Uniflora* , *Bosevilla Serrata leaves and Cissus Quadrangularis* was procured from crude drug supplier M/s. Gokuldas Goverdhandas (237, Budhawar peth ,Pune).The collected plants were identified and authentified at the **Sumatibhai Shah Ayurved Mahavidyalay.**

Extraction process of Cassia uniflora (CU) powder

1. Drug: Cassia uniflora (CU) powder
2. Solvent: Water
3. Extraction Method: Maceration

Procedure for Extraction:

The 100 gm of powder crude drug of Cassia uniflora were soaked in 600 ml of water for 48 hrs. Then filter it through the whatman filter paper. The filtrate is then concentrated under reduced pressure in vacuum at 40 °C for 25 min using a rotary evaporator[12].

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Extraction process of Boswellia serrata (BS) powder

1. Drug : Boswellia serrata (BS) powder
2. Solvent : Ethanol (95%)
3. Extraction Method : Soxhlet extraction(continuous extraction)

Procedure for Extraction:

The powder crude drug of Boswellia serrata is weigh & Pack in soxhlet apparatus, extract with ethanol (95%) at 10-20 C for 72 hrs. Ethanolic Extract is then filter & dry to collect solid form[13].

Extraction process of Cissus Condragularis (CS)

1. Drug : **Cissus Condragularis (CS)**powder
2. Solvent : Ethanol (95%)
3. Extraction Method : Soxhlet extraction(continuous extraction)

Procedure for Extraction:

The powder crude drug of **Cissus Condragularis** is weigh & Pack in soxhlet apparatus, extract with ethanol (95%) at 10-20 C for 72 hrs. Ethanolic Extract is then filter & dry to collect solid form[14].

Extraction process of *Trapa bispinosa Roxb (TB)*

1. Drug : ***Trapa bispinosa Roxb (TB)***powder
2. Solvent : 50% mixture of hydro ethanol
3. Extraction Method : Soxhlet extraction(continuous extraction)

Dried fruits of *Trapa bispinosa Roxb* was extracted with 50% mixture of hydro ethanol and concentrated. The concentrated material was washed several times with petroleum ether to remove the resinous matter. Then the mass was diluted with a mixture of water and ethanol (75:25) filtered and concentrated ,dried to get the powder from the extract[15].

Preliminary Phytochemical Studies[16]

Previously, various preliminary phytochemical tests were performed for the extracts used for sustained released tablet formulation using standard procedures, and the above formulations showed the presence of main carbohydrates, alkaloids, glycosides, phenols, tannins, flavonoids, and saponins which majorly responsible for the desired activity.

Preparation of Polyherbal Granules [17]

Polyherbal granules were prepared using by wet granulation method. The polyherbal extract was mixed well with lactose monohydrate, added the required quantity of starch to obtain a smooth mass, and then passed through # 100 to produce granules. Prepared granules were gently subjected to drying (< 0.05 was considered statistically significant, and $P < 0.001$ was considered statistically highly significant, as compared to control group.

Preparation of Sustained Released tablet[18,19,20] : Sustain release tablets were prepared by **Wet Granulation Method**.All the powders were passed through the 100 mesh.Required quantities of drugs (*Trapa Bispinosa , Cassia Uniflora* , *Cissus Quadrangularis* and *Bosevilla*

Serrata) and excipients were mixed thoroughly, and a sufficient volume of wetting agent (80% or 90% Ethanol) was added slowly.
Once the Sustain Release tablets were prepared, the characterization was done by determining following parameters:

Evaluation of granules:
1. Angle of repose
2. Bulk density
3. Tapped density
4. Hausner's ratio and Compressibility index

Evaluation of Tablet:
1. Hardness
2. Thickness
3. Friability
4. Weight variation
5. Drug release study (in-vitro)

Evaluation of granules:

i. Angle of repose

A simple method whereby the weighed quantity of granules was allowed to flow through an orifice (funnel) at a fixed height was used to determine flow rate. The time taken for the weighed granules to flow out completely from the orifice was recorded. This was performed in triplicate. The flow rate was obtained by the equation below:

$$\mathbf{Tan\theta} = \frac{\mathbf{h}}{\mathbf{r}}$$

Where,
h = Height of pile
r = Radius of the base of pile
θ = Angle of repose

ii. Bulk density

The volume of a known quantity of the granules from each batch was obtained before and after tapping. The volume before tapping was used to determine the bulk density. The bulk density was obtained by the equation below:

$$\mathbf{Bulk\ density} = \frac{\mathbf{Weight\ of\ sample\ in\ gram}}{\mathbf{Volume\ occupied\ by\ the\ sample}}$$

iii. Tapped density

The volume of a known quantity of the granules from each batch was obtained before and after tapping. The volume after tapping was employed to determine tapped density.
The tapped density was obtained by the equation below:

$$\mathbf{Tapped\ density} = \frac{\mathbf{Weight\ of\ sample\ in\ gram}}{\mathbf{Tapped\ volume}}$$

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iv. Hausner's ratio and Compressibility index

In recent years the compressibility index and the closely related Hausner's ratio have become the simple, fast and popular methods for predicting flow characteristics. Both the compressibility index and the Hausner's ratio were determined by using bulk density and the tapped density of granules.

$$\textbf{Hausner's ratio} = \frac{\textbf{Tapped density } (\textbf{gm/cm}^2)}{\textbf{Bulk density } (\textbf{gm/cm}^2)}$$

$$\%\ \textbf{Carr's compressibility index} = \frac{\textbf{Bulk density} - \textbf{Tapped density}}{\textbf{Tapped density}} \times \textbf{100}$$

Evaluation of Tablet:

1. Hardness

Hardness of the tablets was measure by LABINDIA, TH 1510 M hardness testing apparatus.

2. Thickness:

Thickness of the tablets was measure by Vernier calliper.

3. Friability

Ten tablets were randomly selected, dusted and weighed. The tablets were placed in a Roche friability tester and subjected to its tumbling actions at 25 revolutions per minute for four minutes. Afterwards, the tablets were once again dusted and reweighed to determine the percentage loss of weight.

$$\%\ \mathbf{F} = \frac{(\mathbf{W_0} - \mathbf{W})}{\mathbf{W_0}} \times \mathbf{100}$$

Where, F = friability

W_0 = initial weight of the ten tablets

W = final weight of the ten tablets

4. Weight variation

20 tablets were weighed individually using an electronic balance. The average weight was calculated and individual tablet weight was then compared with average value and the deviation was recorded .

5. Drug release study (in-vitro)

The in-vitro drug release study was carried out using the dissolution test apparatus. The 900 ml of dissolution media was kept at 37°C and the rotating speed was 100 rpm. The dissolution media used were 0.1 N HCL. Samples were withdrawn from the dissolution media at 30, 60, 90, 120, 150, 180, 210, 240, 270 and 300 min and assayed by UV method at 274 nm.

Table 1: Formulation table for Sustain Release Tablets

Name of the ingredient	Quantity in mg	
	100 mg tablet	200 mg tablet
Trapa bispinosa	25 mg	50 mg

Cassia Uniflora	25 mg	50 mg
Cissus Quadrangularis	25 mg	50 mg
Bosevilla Serrata	25 mg	50 mg
HPMC	60	60
Xanthan Gum	20	20
Lactose	200	200
Microcrystalline cellulose	120	120
Magnesium Sterate	10	10
Ethanol	Q.S.	Q.S.

Acute Toxicity Study [21,22].

For the acute toxicity study on mice, "Fixed-dose" method of the organization for economic cooperation and development guideline 420 was followed . The formulation was suspended in distilled water and administered by gavages (orally) at single doses of 2000 mg/kg. The animals had free access to water and food throughout the experiment, except for the fasting period before the oral administration of the single dose of the formulation. The general behavior of the rats was continuously monitored for 3 h and then every 30 min for the next 3 h till 24 h and then daily for a total of 14 days. Changes in the normal activity of rats, their body weights, sign and symptoms of toxicity, and mortality were monitored and recorded.

From the acute toxicity study, the LD50 cut-off dose for extracts was found to be 1000 mg/kg body weight. Hence, the therapeutic doses were taken as 100 mg/kg and 200 mg/kg body weight.

In-vivo Evaluation Selected Sustained Released Tablet Complete Freund's adjuvant Induced Arthritis in Female Wistar Rats[23,24]:

The protocol for conducting the in vivo study in female adult wistar rats was approved by the Institutional Ethical Committee (IEC) of SGRS/IAEC/05/2020-21.

The **Female Wistar Rats** were divided into five different groups of six animals, each as follows:

Group I: Normal control.
Group II: Arthritic control.
Group III: Sustained Released Tablet formulation (TCBC100).
Group IV: Sustained Released Tablet formulation (TCBC200).
Group V: Diclofenac sodium (20 mg/kg b.wt orally)

Induction of Arthritis:

Arthritis was induced by a single sub-planter injection of 0.1 ml of Complete Freund's adjuvant containing 1.0 mg dry heat-killed Mycobacterium tuberculosis per milliliter sterile paraffin oil into a foot pad of the left hind paw of **Female Wistar Rats** except vehicle control . The swelling in hind paws were periodically examined in each paw from the ankle using plethysmometer. Diclofenac sodium and Polyherbal capsules were administered orally in the form of freshly prepared Solution in distilled water.

Measurement of paw swelling:

On 0th day, the left hind paw swelling of al rats was measured using vernier caliper and on 1st day arthritis was induced in all rats using CFA. The mentioned drug treatment was started on 1st

day and continued for 14 days. The assessment of antiarthritic activity was carried out by measuring change in paw swelling and body weight on 0, 5th and 21st day after induction.

$$\% \text{ Inhibition} = \frac{(Vc - Vo)control - (Vt - Vo)Test}{(Vc - Vo)Control} \times 100$$

The percent inhibition of paw swelling as determined as: Where
VC is the paw swelling after induction,
V0 is the paw swelling before induction,
Vt is the paw swelling after treatment.

Estimation of Hematological parameters:
On the 22nd day , blood was withdrawn through retro-orbital vein puncture of all groups and the biochemical parameters such as Hemoglobin content, Total WBC count, ESR, RBC, PCV were analyzed.

Histopathological examination:
The animals were sacrificed by cervical dislocation and the ankle joints of hind limbs were removed along with this the kidney and liver were placed in 10% buffered formalin. The fixed tissues were then decalcified and the sections were stained with Hematoxylin and Eosin. Slides were reviewed for the evaluation of histopathological changes like soft tissue swelling, bone demineralization, pannus formation, cartilage erosion and joint space narrowing.

Results and discussion

Authentication: The raw materials used in the formulation were individually procured from crude drug supplier M/s. Gokuldas Goverdhandas (237, Budhawar peth ,Pune).The collected plants were identified and authentified at the **Sumatibhai Shah Ayurved Mahavidyalay.**

Phytochemical Analysis
The chemical tests for various Phyto constituents in the raw materials were carried out and the results were recorded and detailed in Table 2

Table 2: Phytochemical analysis of Plants

Sr. No.	Test	*Cassia uniflora*	Trapa bispinosa	Bosevilla serrata	Cissus quandragularis
1.	**Flavonoids:** Shinoda Test	+	+	+	+
2.	**Alkaloids:**				
	Mayer's Test	+	+	+	+
	Hager's Test	+	+	+	+
	Dragendorff's Test	+	+	+	+

3.	**Glycosides:** Legal's Test Keller Killani Test	 + +	 + +	 + +	 + +
4.	**Steroids:** Salkowski Test Liberman-Burchard's Test	 + +	 - -	 - -	 - -
5.	**Phenols:** $FeCl_3$ Test Lead Acetate Test $KMnO_4$ Test	 + + +	 + + +	 + + +	 + + +
6.	**Terpenoids:** Salkowski Test	 +	 +	 +	 +
7.	**Saponins:** Foam Test	 +	 +	 +	 +
8.	**Tannins:** $FeCl_3$ Test Lead Acetate Test $KmnO_4$ Test	 + + +	 + + +	 + + +	 + + +
9.	**Proteins:** Biuret Test	 +	 +	 +	 +
10.	**Carbohydrate:** Molisch's Test	 +	 +	 -	 -
11	**essential oil, gum and resin**	-	-	+	-

+ present , - absent

Evaluation of sustain release tablet

PREFORMULATION AND FORMULATION DEVELOPMENT STUDIES

A. Evaluation of granules:

The pre compression parameters of formulations blend were conducted for angle of repose, bulk density, tapped density, compressibility index, and hausners ratio. The two most important attributes for the direct compression formula are good flow and good compressibility. Inter particulate interactions that influence the bulking properties of a powder with powder flow. A comparison of bulk density and tapped density can give a measure of the relative importance of this interaction in given powder. The powder flow depends on three general areas. The physical property of particle (e.g. shape, size, compressability) , the bulk powder properties (e.g. size distribution, compaction), and the processing environment (storage, humidity). Pre compression parameter are evaluated, these are mentioned in following Table 3

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Table 3: Evaluation of granules

Sr. No.	Evaluation Parameters	Results	
		TCBC100	TCBC200
1	Angle of Repose (°)	35±0.72	33±0.48
2	Bulk Density (gm/ml)	0.37±0.020	0.38±0.32
3	Tapped Density (gm/ml)	0.45±0.43	0.46±0.39
4	Hausner's Ratio	1.18±0.82	1.16±0.8
5	Carr's Compressibility Index (%)	16±0.64	11±0.52

Values are expressed as Mean ± SD, (Where N = 3).

Fig 1: Evaluation of granules

Fig 1.1 : Angle of Repose

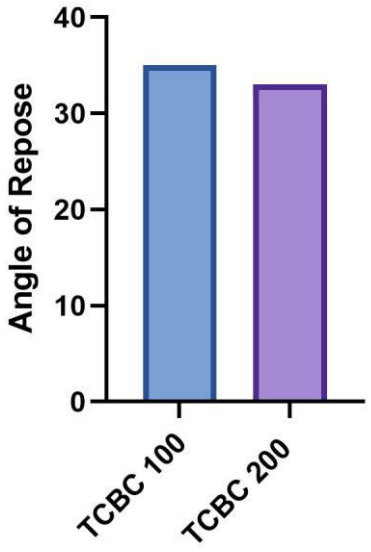

Fig 1. 2 : Bulk Density

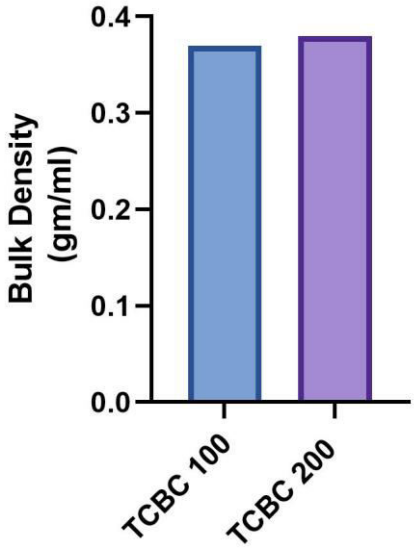

Fig 1. 3 : Tapped Density

Fig 1. 4 : Hausner's Ratio

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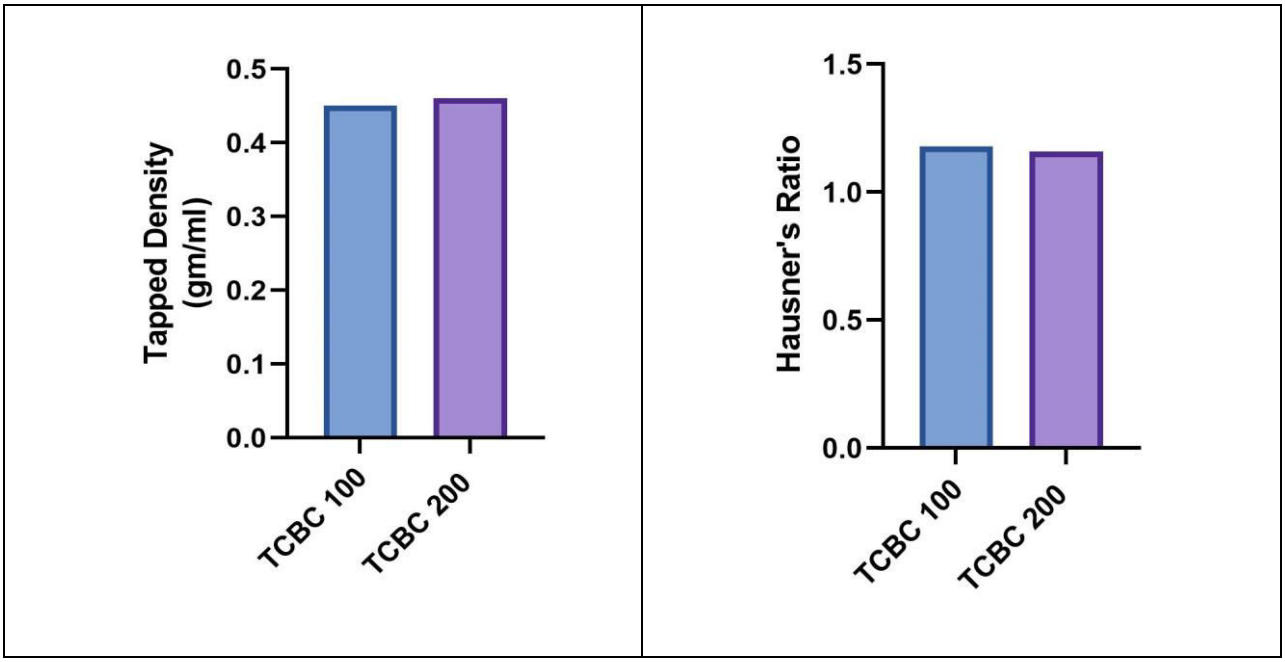
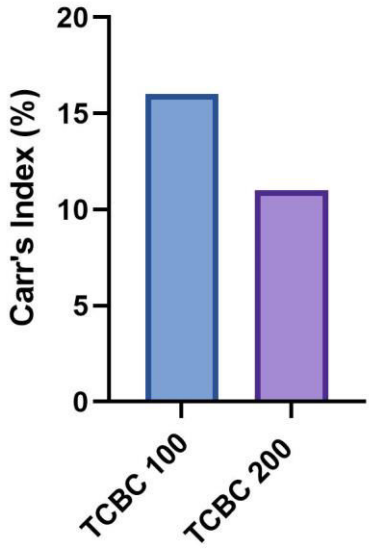


Fig 1. 5 : Carr's Compressibility Index



B. Evaluation of Sustain Release Tablet:

Evaluation of tablets was done by studying various parameters like weight variation, thickness, hardness, friability and % drug release. The % drug release was observed at 274 nm. The results were presented in Table 4 and all results were found to be within pharmacopeial standards.

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Table 4: Evaluation of sustain release tablet

Sr. No.	Evaluation Parameters	Results	
		TCBC100	TCBC200
1	Hardness (kg/cm^2)	5.3±0.10	5.5±0.13
2	Thickness (mm)	3.01± 0.044	3.08±0.052
3	Friability (% loss)	0.68± 0.11	0.53±0.11
4	Weight variation (mg)	520± 0.82	515±0.64

Values are expressed as Mean ± SD, (Where N = 3).

Fig 2: Evaluation of sustain release tablet

Fig 2.1 : Hardness

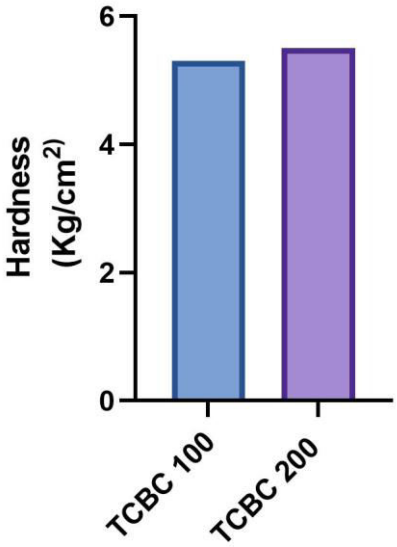

Fig 2.2 : Thickness

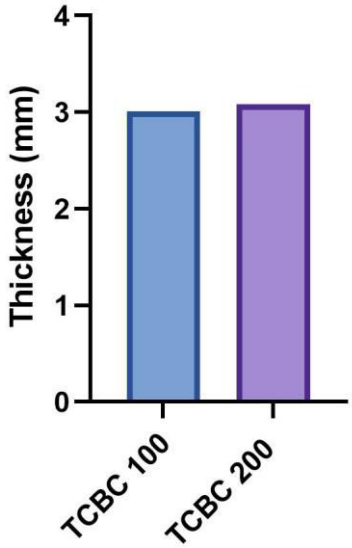

Fig 2.3: Friability

Fig 2.4 : Weight Variation

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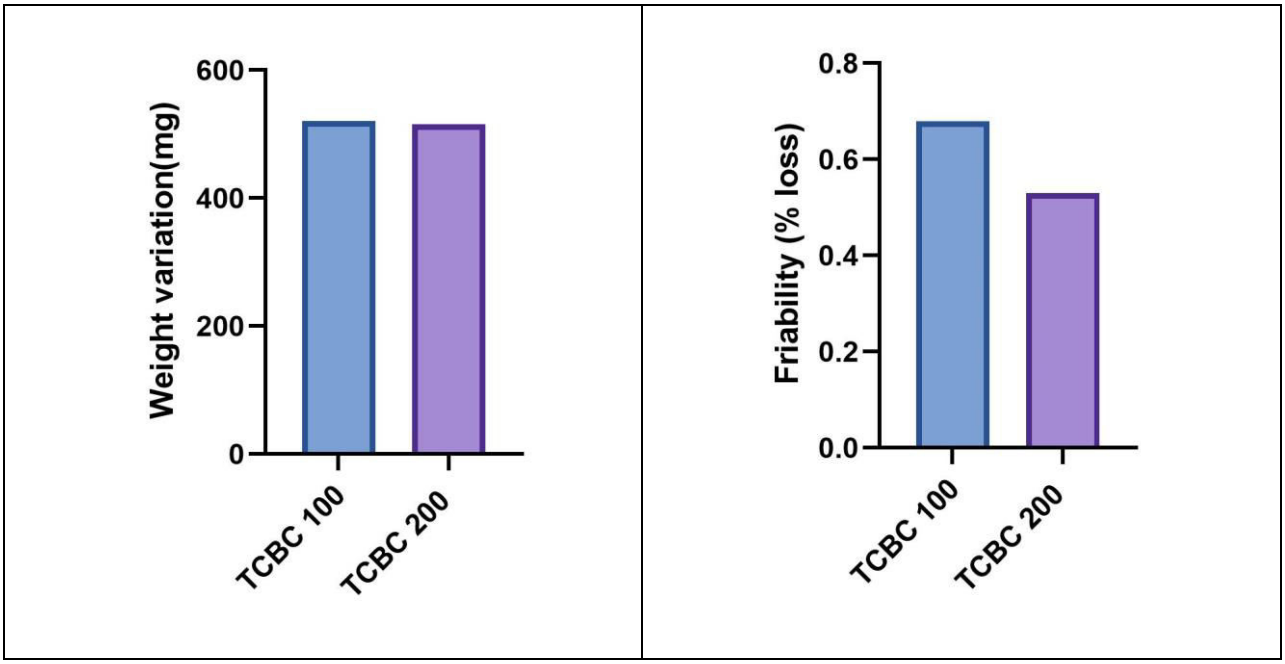

Table 5: In-vitro % Drug Release of Sustained released tablet.

Sr. No.	Time (hrs)	% Drug Release	
		TCBC100	TCBC200
1	0.5	03±0.63	04±0.10
2	1	06±0.52	07±0.17
3	1.5	08±0.04	12±0.06
4	2	13±0.17	18±0.25
5	3	15±0.26	21±0.24
6	4	26±0.41	37±0.34
7	5	35±0.66	46±0.31
8	6	45±0.36	52±0.36
9	7	53±0.12	66±0.65
10	8	68±0.25	75±0.14

Values are expressed as Mean ± SD, (Where N = 3).

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Fig 3 : In-vitro % Drug Release of TCBC 100

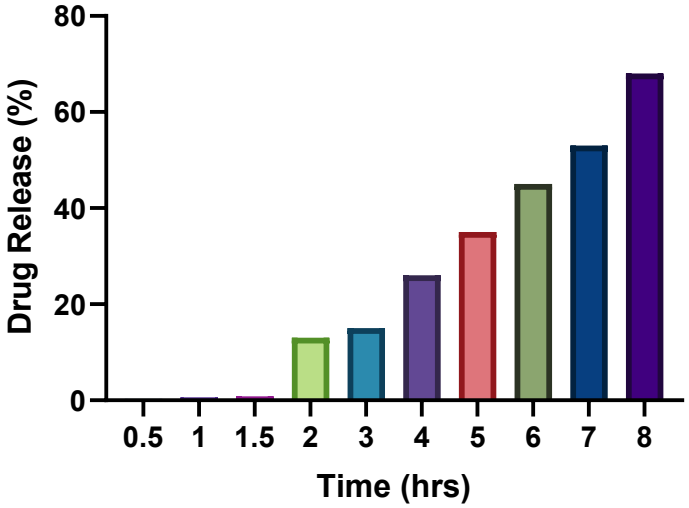
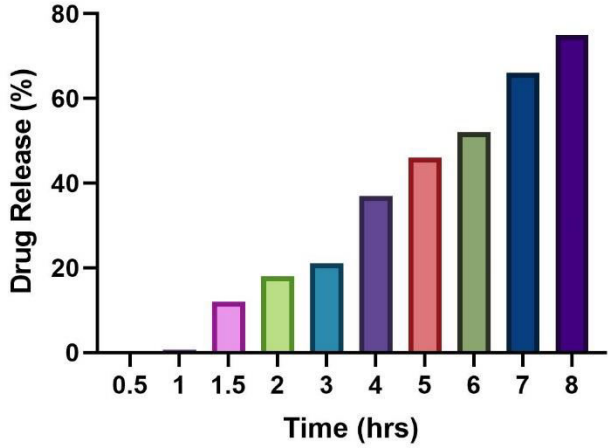


Fig 4 : In-Vitro % Drug Release of TCBC 200



TCBC 100 and TCBC 200 showed significant sustained drug release at various time intervals .

***In Vivo* Anti-Arthritic Activity of Sustain Release Tablet Formulation:**

Acute Oral Toxicity Study [19.20]

From the acute toxicity study, the LD50 cut-off dose for extracts was found to be 1000 mg/kg body weight. Hence, the therapeutic doses were taken as 100 mg/kg and 200 mg/kg body weight**.**

Effect on Paw Volume:

After the administrations of the herbal sustain release tablet formulation (F1 and F2) from day 1 to 12, change in the paw volume of rat was recorded on 0^{th}, 5^{th} and 21^{st} days (Table 6 and figure 5). The arthritic control group showed signs of arthritis development as seen by increase in the paw volume. Significant ($p<0.0001$) reduction in rat paw volume was observed in standard Diclofenac sodium group and sustain release tablet treated groups on 21^{st} day after CFA induction. When compared with standard Diclofenac sodium group sustain release tablet formulation with F2 (TCBC 200 mg/kg) showed most significant decrease in the paw volume results. While F1 (TCBC 100 mg/kg) formulation found to be less significant as compared to standard diclofenac sodium group.

CFA induced arthritis is the most widely used model in which the clinical and pathological changes are comparable with those seen in human rheumatoid arthritis.

Table 6 : Effect of sustain release tablet formulation on paw volume of CFA induced arthritic rats.

Group	Treatment (mg/kg)	Paw volume (ml)		
		Day 0	Day 5	Day 21
I	Normal Control	0.041±0.02	0.066±0.03	0.041±0.04
II	Arthritic Control	1.5±1.50****	2.7±2.70****	3.56±3.56****
III	Standard	1.64±1.64****	0.85±0.85****	0.17±0.17****
IV	F1 Formulation (TCBC 100mg)	1.92±1.92****	1.44±1.44****	0.81±0.81****
V	F2 Formulation (TCBC 200mg)	1.72±1.72****	1.36±1.36****	0.36±0.36****

Values are expressed as mean ± SEM (n = 6), **** $p < 0.0001$ compared with arthritic control. Data was analysed using one-way ANOVA followed by Dunnett's multiple comparison test.

Fig 5 : Effect of sustain release tablet formulation on paw volume of CFA induced arthritic rats.

Fig 5.1 Paw volume on day 0	Fig 5.2 Paw volume on day 5

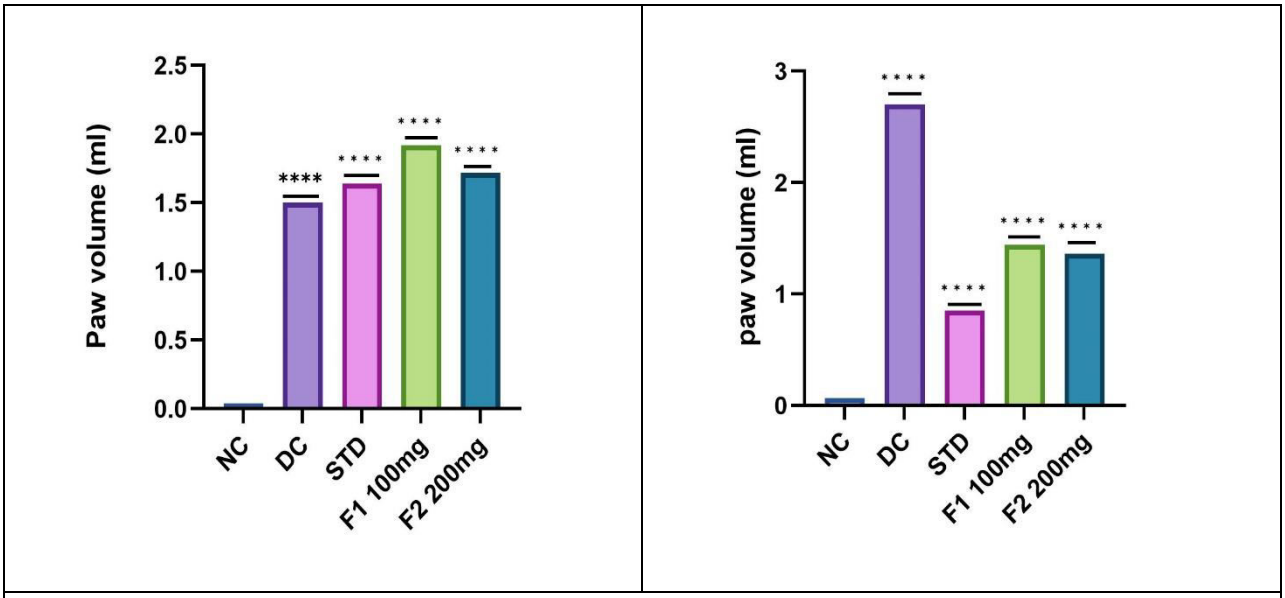
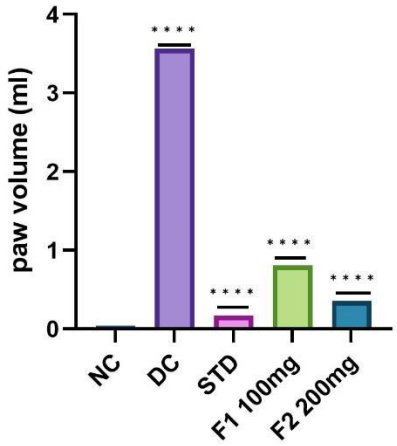


Fig 5.3 Paw volume day 21



Percentage Inhibition of Paw Volume:

The activity of Trapa Bispinosa , Cassia Uniflora ,Bosevilla Serrata , Cissus Quandragularis sustain release tablet formulation code with F1 and F2 and Diclofenac sodium to inhibit arthritic paw edema was examined against CFA-control group and found to be significant at $p < 0.0001$ respectively (Table 7 and Figure 6). Highest percentage of inhibition of paw volume was shown by F2 (TCBC200 mg/kg) as compared to F1 at a dose (TCBC 100 mg/kg).

Table 7: Effect of sustain release tablet formulation on % inhibition of paw volume in CFA induced arthritic rats.

Group	**Treatment (mg/kg)**	**Percentage Inhibition of Paw Volume (%)**	
		Day 5	**Day 21**

I	**Arthritic Control**	**0**	**0**
II	**Standard**	**38.32±2.46******	**51±0.53******
III	**F1 Formulation (TCBC100 mg/kg)**	**24.63±2.64******	**46±1.07******
IV	**F2 Formulation (TCBC200 mg/kg)**	**29.83±2.20******	**64.64±1.62******

Values are expressed as mean ± SEM (n = 6), **** p < 0.0001 compared with arthritic control. Data was analysed using one-way ANOVA followed by Dunnett's multiple comparison test

Fig 6: Effect of sustain release tablet formulation on % inhibition of paw volume in CFA induced arthritic rats.

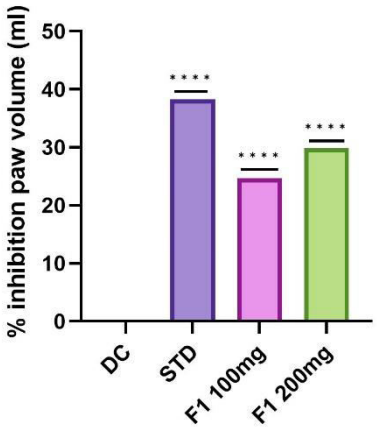
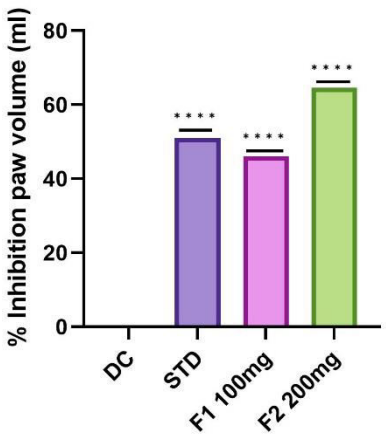
Fig 6.1 Percentage inhibition of paw volume day 5	Fig 6.2 Percentage inhibition of paw volume day 21
	

Table 8: Changes in body weight(g)

Group	Treatment	Day 0	Day 5	Day 21
I	**Arthritic Control**	**134.14±4.92**	**125.00±4.59**	**115.83±5.65**
II	**Standard**	**137.50±4.45**	**145.83±2.54**	**153.50±2.06**
III	**Formulation F1(TCBC100mg/Kg)**	**150.83±2.58**	**158.67±1.28**	**168.33±0.65**
IV	**Formulation F1(TCBC200mg/Kg)**	**121.67±6.68**	**124.33±4.65**	**137.00±3.63**

The values are expressed as mean ±SD, (n=6).

Body weight of rat increased in polyherbal formulation treated groups and also diclofenac in the diclofenac treated groups. There was a statistically significance increase the body

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weight, in the polyherbal formulation treated groups on comparing with arthritic control group.

Fig 7 : Change in body weight

Fig 7.1 Change in body weight day 0

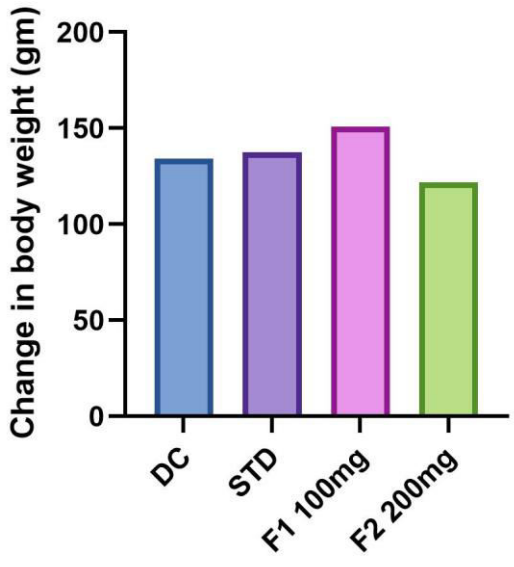

Fig 7.2 Change in body weight day 5

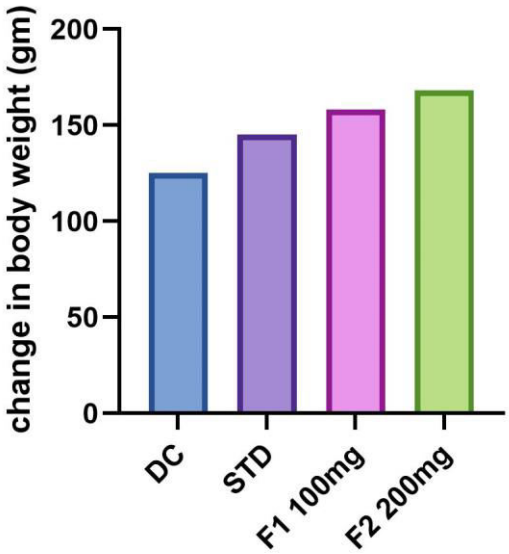

Fig 7.3 Change in body weight day 21

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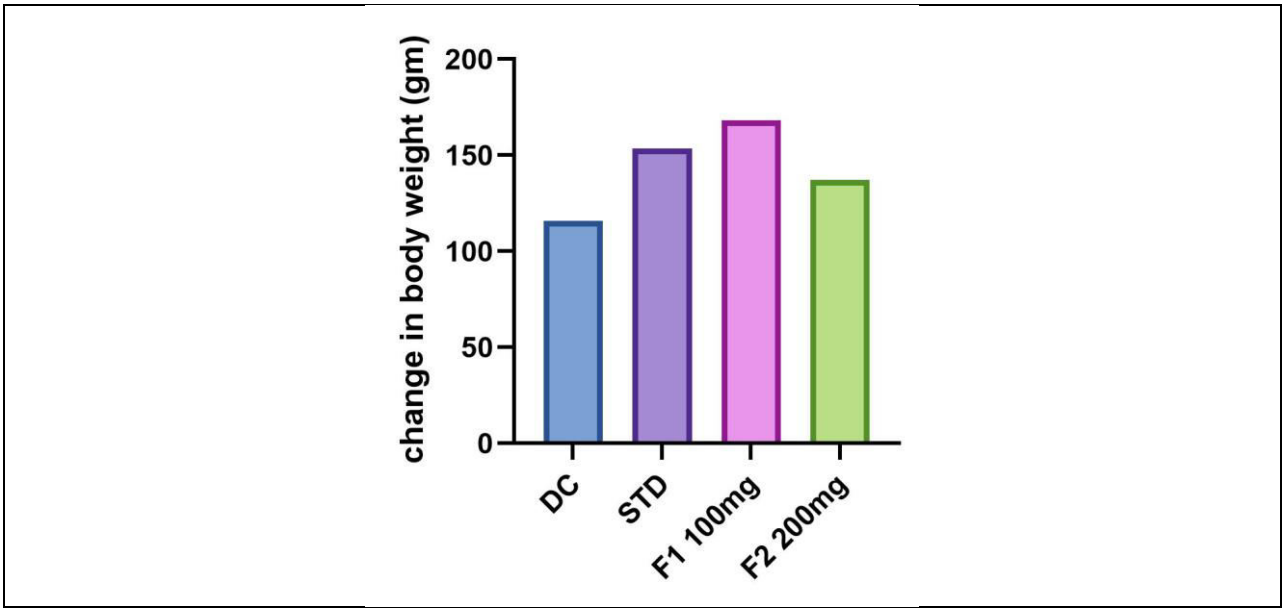

HEMATOLOGICAL PARAMETERS

Table 9: Hematological parameters

Parameters	Group I	Group II	Group III	Group IV	Group V
WBC(Cells/cumm)	6.5±0.3	7.4± 0.41	7.1±0.32	6.9±0.83	6.4±0.2
RBC(Cells/cumm)	6.48±0.21	5.83±0.13	6.14±0.22	6.03±0.63	6.76±0.02
DC-lymphocytes	24.8±0,42	50.2±0.42	36.9±0.38	35.0±0.45	30.49±0.05
DC- monocytes	4.12±0.14	3.26±0.32	5.32±0.87	3.65±0.39	4.83±0.01
DC- eosinophils	1.34±0.12	2.45±0.74	1.55±0.34	1.33±0.08	1.39±0.17
DC- neutrophils	71.34±0.14	46.12±0.43	57.32±12	61.34±0.41	65.21±0.18
PCV(%)	33.2±0.32	24.8±0.04	32.3±0.54	33.16±0.64	36.93±0.74
ESR(mm/hrs) 30 min	2.32±0.02	1.3±0.32	1.1±0.21	3.02±0.07	2.12±0.77
60 mins	5.12±0.28	21.9±1.21	6.42±0.42	4.5±0.35	5.76±0.09
Hb(g/dl)	11.3±0.45	9.4±0.61	10.35	10.0±0.07	11.9±0.57

The values are expressed as mean ±SD, (n=6).

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Fig 8: Hematological parameters

Fig 8 .1 : WBC

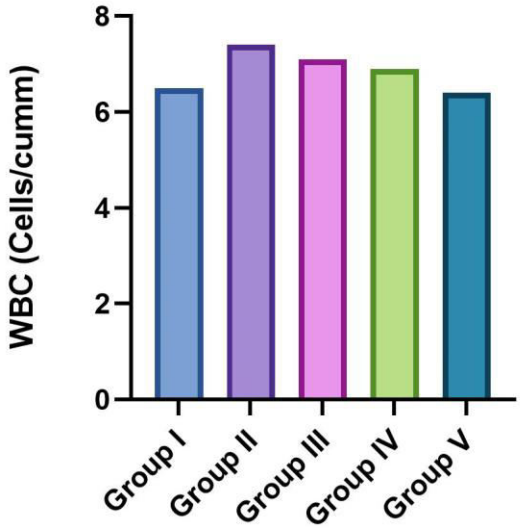

Fig 8 .2 : RBC

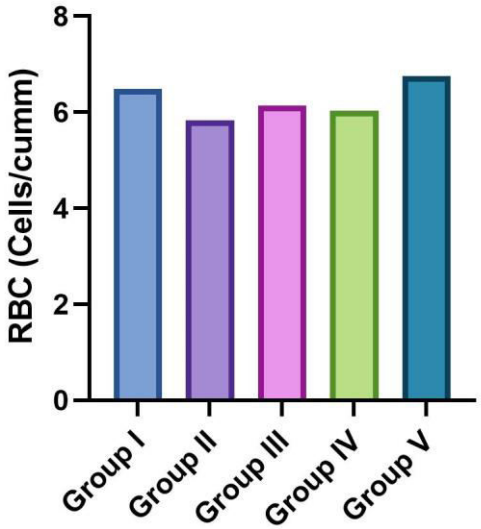

Fig 8 .3 : Lymphocytes

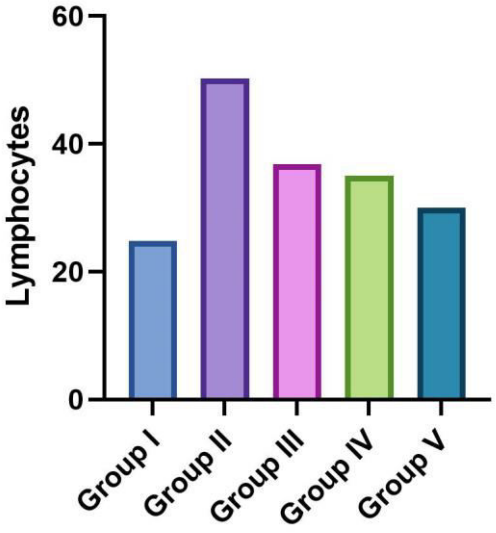

Fig 8 .4 : Monocytes

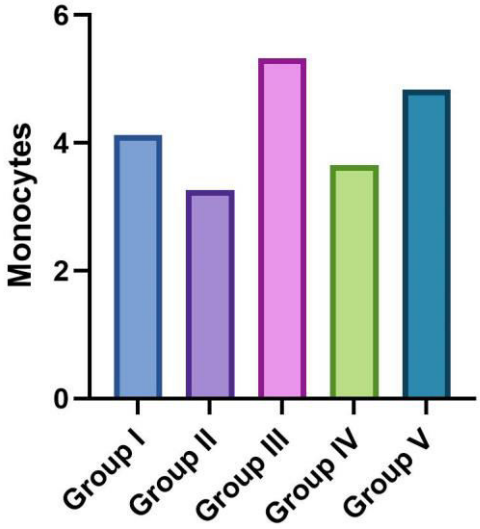

Fig 8 .5 : Eosinophils

Fig 8 .6 Neutrophils

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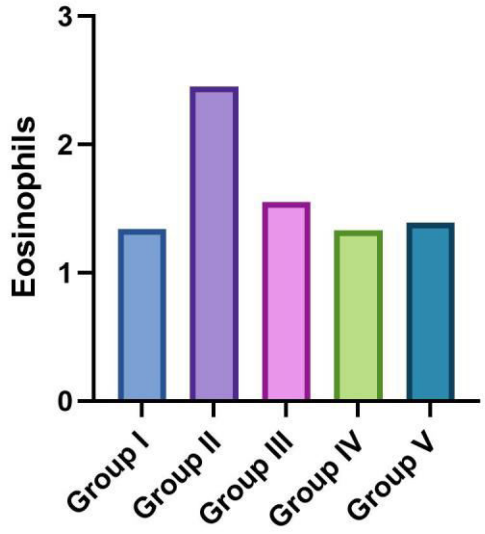


Fig 8 .7 : PVC%

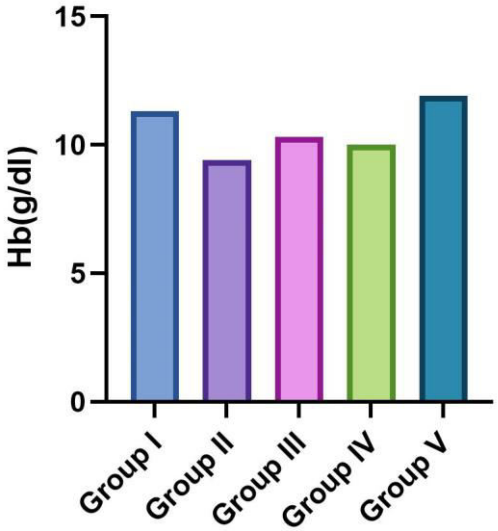
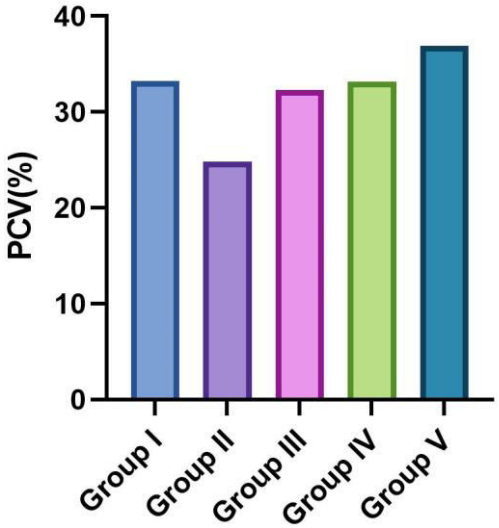


Fig 8 .8 : Neutrophils



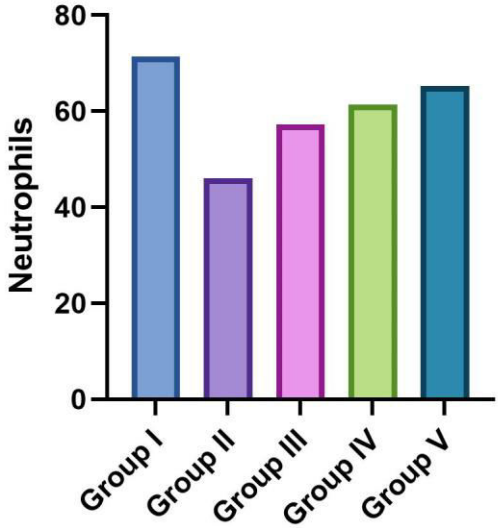


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Fig 8.9 : ESR (30 Minutes)

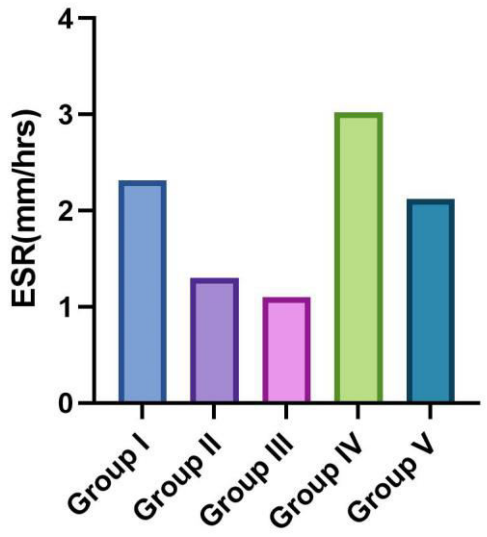


Fig 8.10 : ESR (60 Minutes)

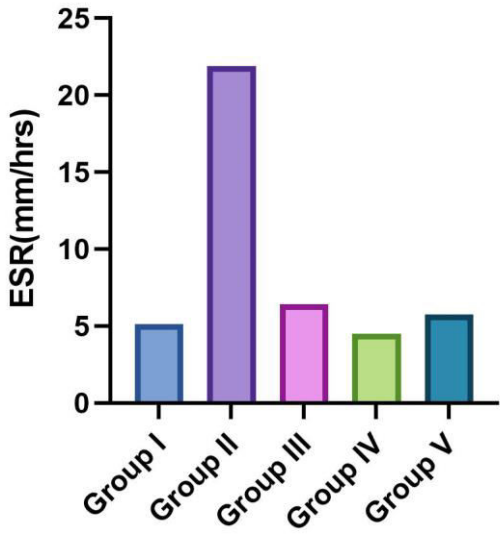
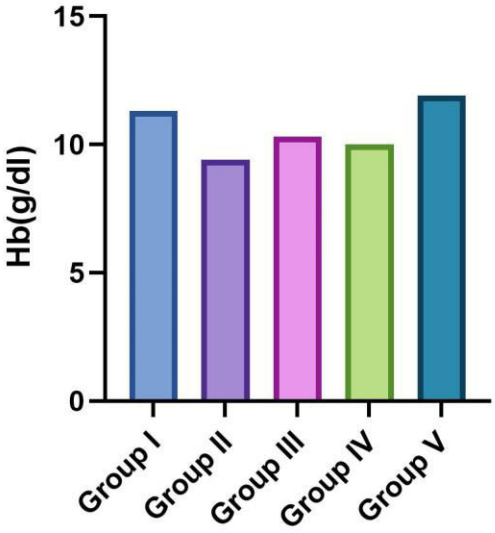


Fig 8.11 : Hemoglobin



Hematological parameters did not shows any difference between the sustained released tablet formulation treated groups, diclofenac sodium treated group and normal control. The arthritic control group there was elevated in Erythrocyte Sedimentation Rate and Reduce Haemoglobin, Red Blood cell, Differential Count on comparing with normal control. This shows that the polyherbal sustained released tablet does not affect the hematological parameters.

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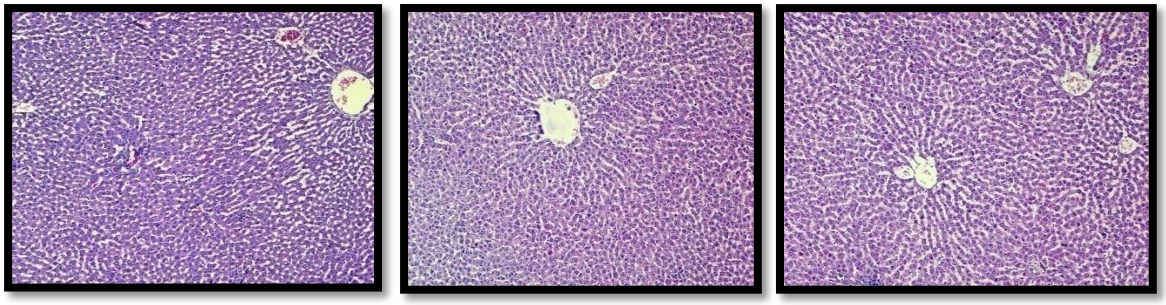
Histopathological Results

Liver:

Microscopic examination of liver of disease control animal (Group II) showed normal hepatic parenchyma with focal areas of cellular swelling of the hepatocytes and focal congested vascular tissue was noted. Focal areas in the hepatic parenchyma showed granular cytoplasmic changes. However other animals did not show any abnormality of pathological changes when compared with control group (Figure 9).

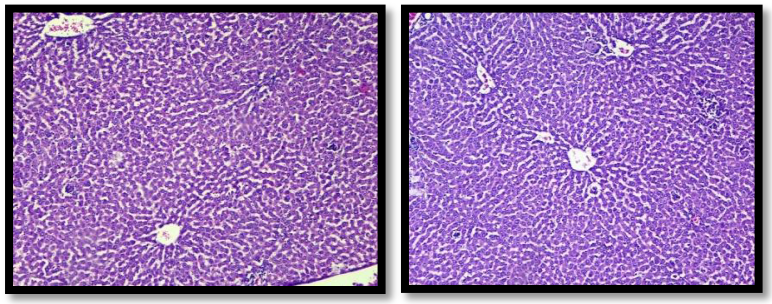
Figure 9 : Effect of Sustain release tablet formulation on histopathology of isolated liver of Arthritic rats in CFA induced rat model.



I) Normal Control Pathological grade 0

II) Disease Control Pathological grade ++

III) Standard Pathological grade 0



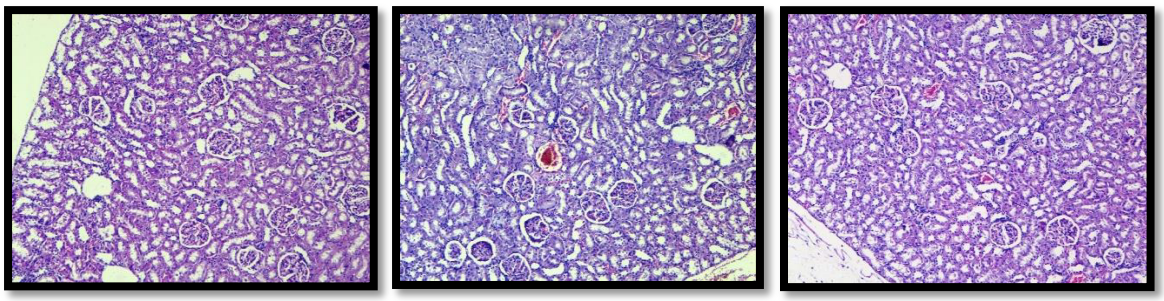
IV) Test 100 mg/kg Pathological grade 0

V) Test 200 mg/kg Pathological grade 0

Kidney:

Microscopic examination of the kidneys showed that there is no any abnormality of pathological change was observed in all group animals (Figure 10).

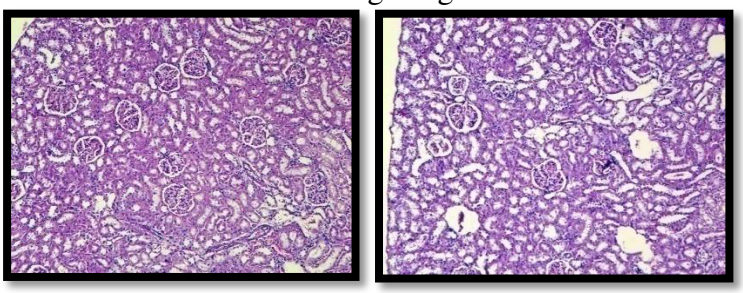
Figure 10 : Effect of Sustain release tablet formulation on histopathology of isolated Kidney of Arthritic rats in CFA induced rat model.



I)Normal Control Pathological grade 0

II) Disease Control Pathological grade 0

III) Standard Pathological grade 0



IV)Test 100 mg/kg Pathological grade 0

V) Test 200 mg/kg Pathological grade 0

Knee Joint:

The histopatholoical assessment of tissue section from rat knee joint was performed on day 21st of the treatment. The joint histological architecture of rats not treated with CFA was serves as a normal (Group I). The histopathology of paw tissue (Figure 11) revealed that adjuvant-induced arthritic rats (Group II) showed (+2) mild to moderate (+3) degenerative changes of the cartilage and bone tissue. The cartilage tissue showed focal areas with uneven surface suggestive of damage and erosion. Focal infiltration of inflammatory cells was also noted in the joint tissue. Overall, mild pathological changes were reported in the joint tissue with pathological changes associated with arthritis and degenerative features of bone and cartilage. Diclofenac treated group and sustain release tablet treated groups (Group III, IV and V) showed minimal (+1) degenerative changes of the cartilage and bone tissue. The cartilage tissue showed only one focus with degenerative features of bone (Figure 11). Overall, mild pathological changes were reported in the joint tissue with pathological changes associated with arthritis and degenerative features of bone and cartilage.

Figure 11 : Effect of Sustain release tablet formulation on histopathology of isolated Knee joint of Arthritic rats in CFA induced rat model.

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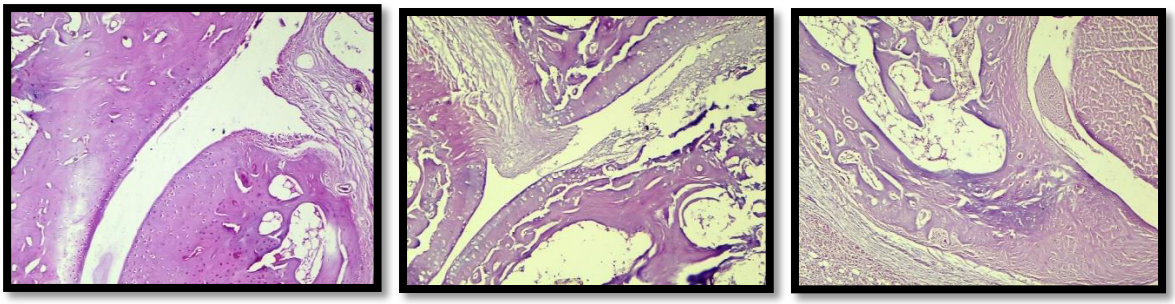
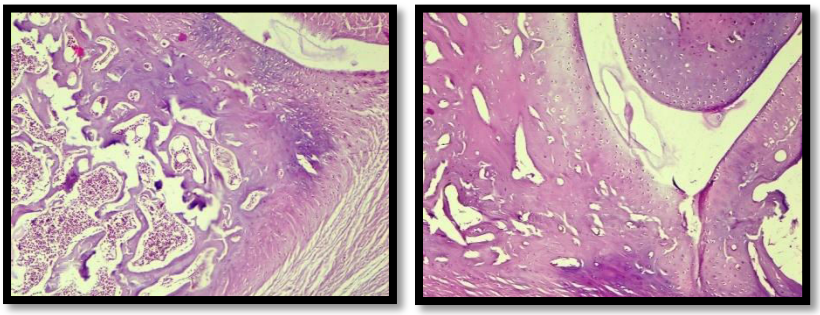


I)Normal Control Pathological grade 0

II) Disease Control Pathological grade +++

III) Standard Pathological grade ++



IV)Test 100 mg/kg Pathological grade ++

V)Test 200 mg/kg Pathological grade ++

Summary and conclusion

The present study was attempt for development of sustained released tablet of different herbal plants and evaluate its anti arthritic activity against Freunds adjuvant induced arthritis in female wistar rats .

Based on the extensive review of literature, four active ingredients were selected for the formulation of polyherbal sustained released tablet to treat rheumatoid arthritis.

The raw materials which are procured are subjected to various raw material analysis for their identy, quality and purity. The materials which complied with the specification were taken for further studies.

Preformulation studies such as bulk density, tapped density, compressibility index, housner's ratio and angle of repose were done for the all raw materials.

The formulated sustained released tablets were standardized as per WHO guidelines using various parameters and include quantification of phytoconstituent.

Pharmacological studies (in vivo) were carried out to study the efficacy of the sustained released tablets formulation in the treatment of rheumatoid arthritis.

Acute toxicity studies were performed according to OECD guidelines and for invivo studies was fixed. In vivo studies using complete freund's adjuvants induced model was done in rats. The test drug shows results are body weight, paw swelling, hematologic, histopathologic study was done.

The paw swelling showed a decrease in sustained released tablets polyherbal formulation treated groups and also diclofenac sodium treated group. There was a statistically significance decrease the paw swelling in the sustained released tablets polyherbal formulation treated groups on comparing with arthritic control group.

Body weight of rat increased in polyherbal formulation treated groups and also diclofenac in the diclofenac treated groups. There was a statistically significance increase the body weight, in the polyherbal formulation treated groups on comparing with arthritic control group.

Hematological parameters did not shows any difference between the sustained released tablets formulation treated groups, diclofenac sodium treated group and normal control. The arthritic control group there was elevated in RBC, DC, ESR and Hb on comparing with normal control. This shows that the sustained released tablets formulation does not affect the hematological parameters.

Histopathologic examination of the joint showed severe cartilage erosion in arthritic control group compared with sustained released tablets formulation treated group and diclofenac sodium treated group. This shows that sustained released tablets formulation suppressed the inflammation changes associated with arthritis which was equivalent to diclofenac sodium.

This shows that sustained released tablet formulation suppressed the inflammation changes associated with arthritis which was equivalent to diclofenac sodium. Future studies can be directed towards the exact mechanism of action responsible for this anti arthritic activity and further the study can be extended for clinical trials also.

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