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# IMMUNOMODULATORY EFFECT OF YASADA BHASMA ON EXPERIMENTAL ANIMALS I.E IN ALBINO RATS

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**ABSTRACT**
*The main aim of the study was to scientifically validate the immuno modulatory effect of Yashada bhasma. Yashada bhasma, prepared as per Rasatarangini, was subjected to screening of immuno modulatory effect using five experimentl parameters. The parameters used were carrageenan induced paw edema , forced swimming induced stress, Cyclophosphamide induced neutropenia & leucopenia, Milk induced leucocytosis & Estimation of free radical generation showed significant results in all the parameters.*
**KEYWORDS:** *Yashada bhasma, Immuno modulators, Ayurveda*

## INTRODUCTION

In olden days all the leading authorities of Ayurveda have stressed the importance of four main Pramanas to obtain the real knowledge of any drug or formulations. The present era is based on scientific evidence. The entire hypothesis has to be proved by the available, affordable parameters or experiments to establish the facts. Hence it is essential to establish the drug efficacy an advocated in scientifically. Animal studies provide analogies and serve as useful model for the administration of a biological active agent to human beings. The pharmacological effect of a drug is evaluated by administering the same drug in experimental animals extrapolating the human dose. The result of the study contributes for the effective therapeutic use in humans. The experimental models are decided according to the drug, dose schedule & the drug delivery. Wister albino rats, mice & rabbits are usually used as animal species for evaluation of the pharmacokinetic & dynamic effect of a drug sample. The pharmacological effect of any compound are usually evaluated through Experiment on animal models. It is sometimes impossible to produce same etiopathological events as they occur in human-being on Dosa-Dusya parlance in the animals, but some pathological conditions like inflammation, neutropenia,leucopenia etc. in the experimental animal model could be deduced and test the drugs for their efficacy and adverse effects. However information on mode of action of drugs, it's effect on various body system and probable adverse effect is importantfor rational treatment of diseases.Hence pharmacology which deals with study of drugs particularly their action on living animals organs, tissue cells etc provides scientific data and forms a basis of rational therapeutic measure. The living body is a biological unit endowed with many capabilities, which are available for modulations with therapeutic manoeuvres.

## EXPERIMENTAL PARAMETERS

The following studies will be conducted to evaluate the immuno modulatory effect of Yasada bhasma in albino rats.

1. Study of effect of Yasada bhasma in cell medited immunity (CMI)
   (carrageenan induced paw edema in albino rates)
2. Evaluation of the effect of Yasada bhasma on forced swimming induced stress.
3. Estimation of hematological parameters (CBC)
   (Cyclophosphamide induced neutropenia & leucopenia)
4. Anti-stress and adaptogenic effects (Milk induced leukocytosis)
5. Estimation of free radical generation Anti-oxidant activity :-
   - SOD (superoxide dismutase)
   - MDA(malon-di-aldehyde)

## MATERIALS & METHODS OF PHARMACOPIAL STANDARD-

- Raw Yasada was procurred from open market at Bhubaneswar.
- Yasada bhasma sample was prepared in pharmacy of post graduate department of RS and BK, GAM Puri.Raw yasada was converted to bhasma adopting the classical methods
- Experimental study was carried out at Department of Pharmaceutics, Siksha ‘O’ Anusandhann, Bhubaneswar using the animals, equipment,and required chemicals as necessary.

## SELECTION OF EXPERIMENTAL MODELS

- All the experiments were carried out after obtaining necessary permission from the Institutional Animal Ethical committee (IAEC) constituted by the Department of Pharmaceutics, Siksha ‘O’ Anusandhann, Bhubaneswar .
- Immuno modulatory activity of the trial drug i.e. Yasada bhasma was carried out on albino rats of both sexes.
- All the rats were acclimatized for 7 days under standard husbandry condition i.e room temp (22°C-24°C), relative humidity 45%-55% and the light and dark cycle of 12:12 hours in the Animal House.
- The rats received rat pellets and clean water ad - libitum.

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- **Inclusion Criteria**

Healthy albino rats weighing about 180-250gm of either sex will be included in the study.

- **Exclusion Criteria**
  - ✓ Rats less than 180 gram-more than 250 gram
  - ✓ Pregnant and diseased rats
  - ✓ Rats which are under trial of other experiment

## EXPERIMENTAL DESIGN

- Rats were divided into three groups comprising six rats in each group.

| GROUP | DRUG ADMINISTERED | CLINICAL FINDINGS TO BE OBSERVED |
|---|---|---|
| Control | Normal Saline Water | Normal |
| Toxic control | Toxicity inducing chemical like | |
| | • Carrageenan | Paw edema |
| | • Cyclophosphamide | Neutropenia and leukopenia |
| | • Buffalo milk injection | Leucocytosis with high Eosinophillic Count |
| | • Forced swimming | Oxidative Stress |
| Treatment group | Inducing chemical+yasada bhasma | Changes in Signs and Symotoms on treated group |

## DOSE DETERMINATION

- As per the classical text , the daily adult human dose of Yasada bhasma is approximately 62.5 to 125 milligram (1/2 ratti to 1 ratti).
- In Classical text the daily human dose administration of Yasads Bhasma 62.5mg to 125 mg approximately. Passing in the human dose, the dose experimental animal was calculated by extrapolating the human dose to animal I.e extrapolating the human dose to animal as 125mg/kg based on the body surface area ratio.
- AED (mg/kg)=HED (mg/kg) x conversion factor for rat (0.018)
- Animal dose= (125mg/60) × (37/6) = 12.84mg (equivalent dose=13mg)
- Human dose=125mg
- Adult human body weight=60 KG
- Human KM=37
- Rat KM=6

## MODE OF ADMINISTRATION

Oral route was selected for administration of drug to different group of animals by using syringe with an attached gastric tube.

## METHODOLOGY

- **Carrageenan-induced paw edema in albino rats**:

The experiment involved three group of rats, with six animals in each group. The rats under control group received oral saline administration whereas the treatment group were given Yasad Bhasma orally at a dosage of 13mg/10ml/kg body weight. Thirty minutes later, 0.1 ml of 1% (w/v) carrageenan was injected into the sub-plantar region of the hind paw in all groups. A Plethysmometer was used to measure paw volume at an intervals of 30 minutes, followed by 60 minutes,120 minutes, 120 minutes,180 minutes,240 minutes after the carrageenan injection. (Taur DJ et al.,2017)

- **Evaluation of the effect of yasad bhasma on forced swimming induced stress**:

Three groups, each consisting of six rats, were formed. The control group received saline solution, while the test group was given an oral dose of 13mg/10ml/kg Body Weight Yasad Bhasma.Rats swam to exhaustion (via 30-minute sessions) in a 37×37×30 cm polypropylene tank full of water for 30 consecutive days. On 31st day, the rats underwent anesthesia with ketamine followed by spinal dislocations. The blood sample was collected through cardiac puncture, allowed to clot for 30 minutes on the working bench for serum separation, and then stored at -80°C for the assay. The brain, heart, kidney, and liver were immediately removed and preserved in 10% formalin for histopathological analysis. (Prusty SK et al.,2017)

- **Cyclophosphamide induced neutropenia & leucopenia**:

The cyclophosphamide induced toxic control group consisting of six rats received cyclophosphamide at a dose of 200 mg/kg body weightorally dissolved in distilled water.The control group animals received saline water orally while the treatment group were administerd cyclophosphamide in prescribed dose along with Yasad Bhasma at a dose of 13mg/10 ml/kg body weight orally for 14 days daily.on 15th day all the animals were sacrificed and blood samples were drawn for hematological parameters through cardiac puncture (Shenoy BK et al., 2022)

- **Buffalo milk induced leukocytosis**

It is reported that Buffalo milk induced leukocytosis in experimental animals which has been considered as an model to study immunomodulatory effect. In this study three groups have been taken consisting of 6 rats in each group i.e control, toxic control and treatment group. These Control was given a low dose of oral saline and the buffalo milk induced toxic control group received boiled and cooled buffalo milk at a dose of 4ml / kg B.weight. (Taur DJ et al., 2011)

## RESULTS

- **Carrageenan-induced paw edema in albino rats**:

The control group showed minimal variation in paw volume, ranging from 4.31 ± 0.012 ml at the start to 4.29 ± 0.1992 ml at the end, indicating no significant inflammation. In contrast, the carrageenan toxic group demonstrated a steady increase in paw volume from 4.36 ± 0.1122 ml initially to 4.49 ± 0.1213 ml at 240 minutes, signifying the development of edema on post-carrageenan injection. The Yasad Bhasma-treated group exhibited a moderated increase in paw volume, suggesting an

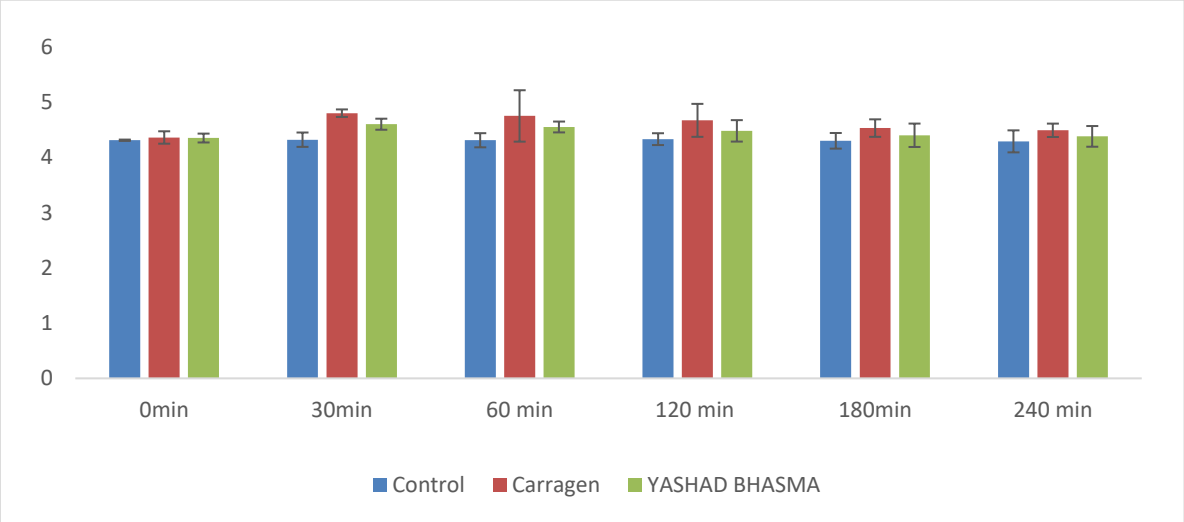
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attenuating effect on edema. Starting at 4.35 ± 0.0801ml, the paw volume in this group reached 4.6 ± 0.0999 ml at 30 minutes, 4.55 ± 0.0983 ml at 60 minutes, and 4.38 ± 0.1873 ml at 240 minutes. Compared to the carrageenan group, this controlled increase indicates that Yasad Bhasma effectively reduces the inflammatory response($p<0.05$). Notably, the treated group showed better control of paw volume than the toxic group at later time points (180 and 240 minutes), underscoring the anti-inflammatory potential of Yasad Bhasma, as shown in fig 1. The findings suggest that Yasad Bhasma protects against carrageenan-induced edema, highlighting its potential as an anti-inflammatory therapeutic agent.

**Table No.1 Effect of YASAD BHASMA on carrageenan-induced paw edema in rats.**

| Group | Paw volume (in min.) | | | | | |
|---|---|---|---|---|---|---|
| | 0min | 30min | 60min | 120min | 180min | 240min |
| Control | 4.31±0.012 | 4.32±0.13 | 4.31±0.1281 | 4.33±0.1071 | 4.3±0.1415 | 4.29±0.1992 |
| Carragenan Toxic control | 4.36±0.1122 | 4.8±0.0679 | 4.75±0.465 | 4.67±0.2984 | 4.53±0.1583 | 4.49±0.1213 |
| Yasad Bhasma treatment | 4.35±0.0801 | 4.6±0.0999 | 4.55±0.0983 | 4.48±0.1941 | 4.4±0.2119 | 4.38±0.1873 |

Fig 1. Effect of YASAD BHASMA on carrageenan-induced paw edema in rats. The statistical analysis was done using One-way ANOVA followed by Turkey's HSD test. All values are expressed as mean ± SD (n=3). *p<0.01 Control Vs Carrageenan ,*p<0.05




Control Vs Yasad Bhasma and #p<0.01 Carrageenan Vs Yasad Bhasma.

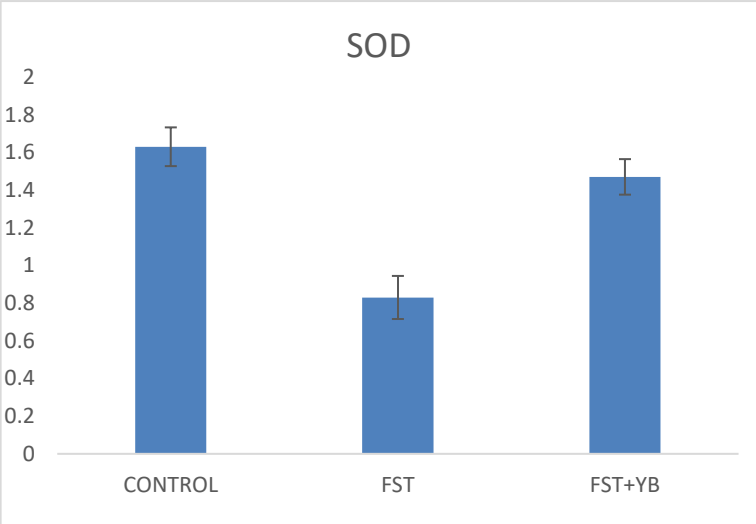
- **Evaluation of the effect of Yasad Bhasma on forced swimming induced stress**:

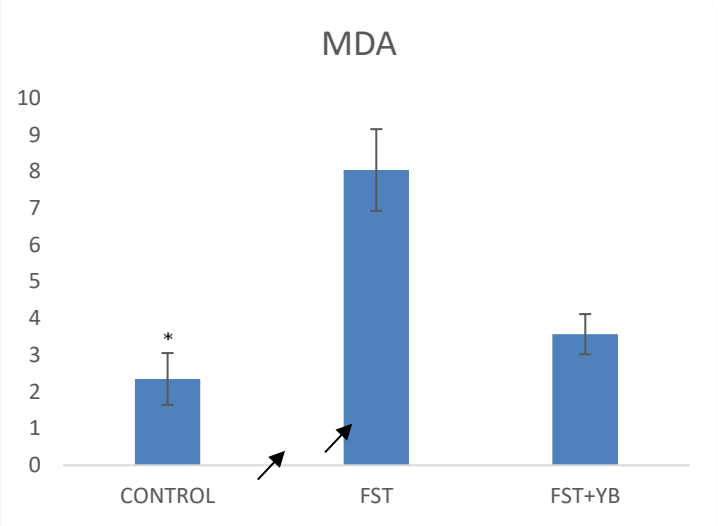
After 30 days the results indicate that forced swimming stress significantly impacted the oxidative status of the experimental groups. In Forced swimming Test group, the Superoxide dismutase(SOD) level decreased to 0.83±0.1143 compared to control group animals.However, administration of Yasad Bhasma (13mg/10ml/kg, p.o.) improved SOD levels to 1.47±0.0943, bringing them closer to the control group ($p<0.05$) suggesting enhanced antioxidant capacity. Regarding Malon dialdehyde(MDA) levels, the FST group exhibited a sharp increase to 8.04±1.1119, indicating high oxidative stress. In contrast, the Yasad Bhasma treated group reduced MDA levels to 3.57±0.05469, significantly lower than the FST group animals ($p<0.01$), though still above the control group. This result suggests that the Yasad Bhasma mitigated oxidative damage as shown in Figure 2.

Table 2. Antioxidant study (SOD and MDA Level) Fig 2: The statistical analysis was done using One-way ANOVA followed by Turkey's HSD test. All the values are expressed as mean ± SD (n=3). SOD *p<0.05 Control Vs FST+YB and #p<0.01 FST Vs FST+YB, MDA *p<0.005 Control Vs FST+YB and #p<0.01 FST Vs FST+YB.

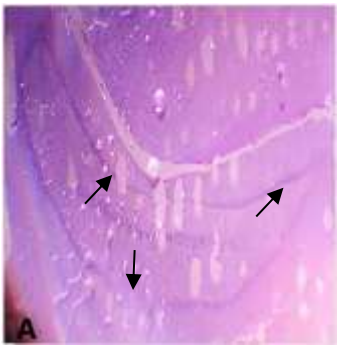
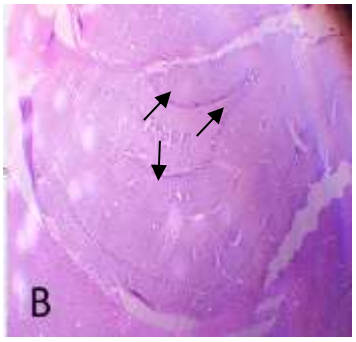
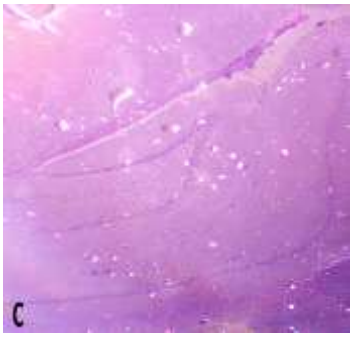
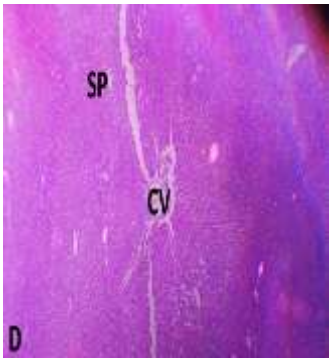
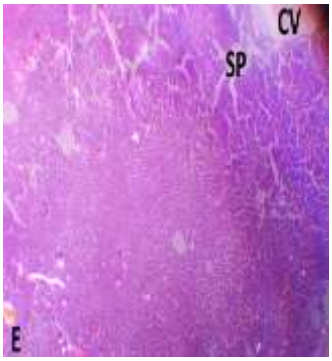
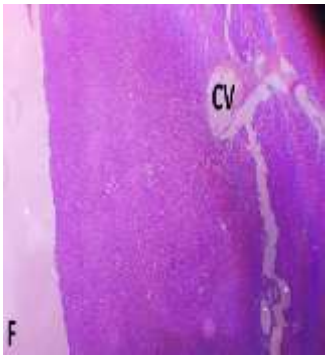
| Group | SOD level | MDA level |
|---|---|---|
| Control | 1.63±0.1028 | 2.35±0.7057 |
| FST | 0.83±0.1143 | 8.04±1.1119 |
| Yasad Bhasma | 1.47±0.0943 | 3.57±0.5469 |

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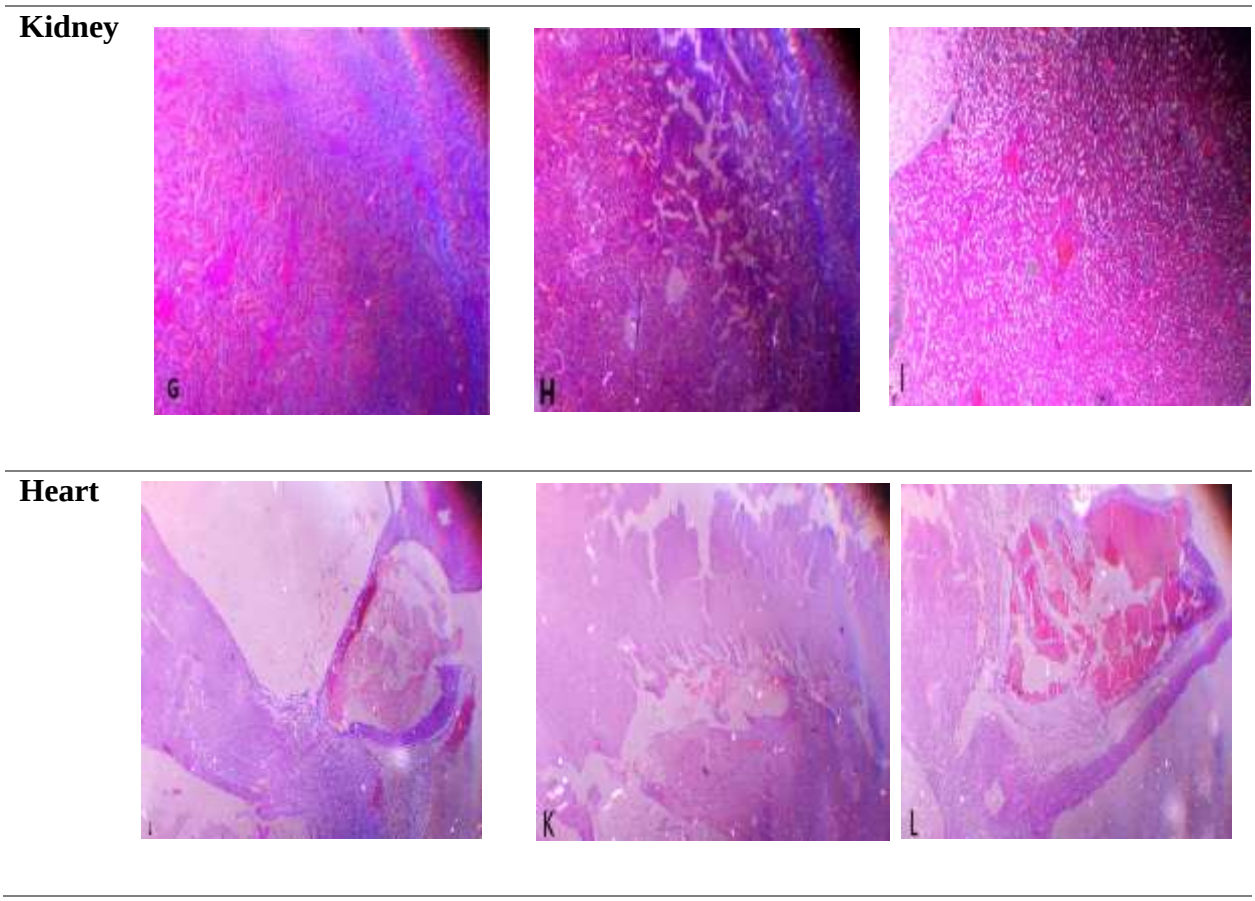



**Histological evaluation of different tissues**

| Organ | Control group | FST group | Yasad Bhasma group |
|---|---|---|---|
| Brain |  |  |  |
| Liver |  |  |  |

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**HISTOPATHOLOGICAL ANALYSIS**

Fig 3. (A) The control group's hippocampus exhibit typical structure. (B) The FST group shows reduced cell density and the presence of deteriorating dark neurons. (C) The Yasad Bhasma group displays a seemingly normal hippocampal structure with a few degenerating cells. (D) The control group's liver demonstrates normal architecture, including hepatocytes extending from the central vein, sinusoidal space, and portal triad. (E) The FST group's liver shows decreased cellular hypertrophy, sinusoidal space, and inflammatory cell infiltration. (F) The Yasad Bhasma group's liver exhibits moderate parenchymal cell hypertrophy, expanded sinusoidal space, and mild inflammatory cell infiltration. (G) The control group's kidney reveals normal renal tissue structure, including healthy parenchyma and blood vessels. (H) The FST group's kidney shows collapsed glomeruli and degeneration. (I) The Yasad Bhasma group's rat kidney displays normal glomeruli and regenerated tubules. (J) The control group's rat heart shows normally arranged myocardial muscle fibers. (K) The FST group's rat heart exhibits early infarction, significantly dilated and engorged blood vessels, and inflammatory cell infiltration. (L) The heart of the rats under Yasad Bhasma group's demonstrates normal myocardial muscle fibers, dilated blood vessels, and minimal inflammatory infiltration.

- **Cyclophosphamide induced neutropenia and leucopenia**:

Yasad Bhasma administration resulted in enhanced WBC counts, while RBC and platelet counts remained relatively stable compared to the control group. In contrast, cyclophosphamide (200mg/10ml/kg, p.o.) administration to rats led to a notable decrease in WBC, showing a significant difference from the control group. Oral administration of Yasad Bhasma (13mg/10ml/kg, p.o.) demonstrated a significant ($p<0.01$) increase in all parameters above compared to the cyclophosphamide-treated group. Figure 4 illustrates the immunostimulatory effect of Yasad Bhasma on cyclophosphamide-induced neutropenia and leucopenia.

**Table 3. Cyclophosphamide-induced neutropenia and leucopenia**

| Group | Leukocytes ($\times 10^3/\mu L$) | Neutrophils (%) |
|---|---|---|
| Control | 9.25±0.935 | 41.66±5.3166 |
| Cyclophosphamide | 4.9±0.801 | 18.5±2.429 |
| Yasad Bhasma | 8±0.811 | 29±2.6394 |

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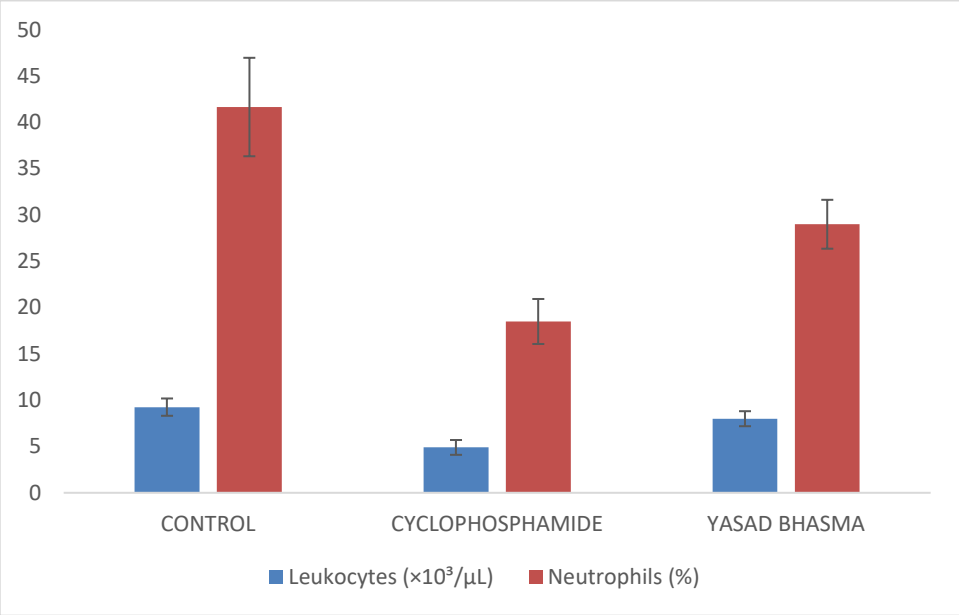



Fig 4. The statistical analysis was done using One-way ANOVA followed by Turkey's HSD test. All the values are expressed as mean ± SD (n=3). Leukocytes *p<0.01 Cyclophosphamide Vs Yasad Bhasma, Neutrophils #p<0.01 Cyclophosphamide Vs Yasad Bhasma.

- **Buffalo milk-induced leukocytosis**

The investigation examined the effects of Yasada Bhasma (13 mg/kg, P.O.) on eosinophil and total leukocyte counts. Control subjects displayed a total leukocyte count of 9±0.5354 and an eosinophil percentage of 6.01±1.1479. Buffalo milk administration led to a substantial increase in both parameters, with leukocyte counts elevating to 13.5±1.291 and eosinophil percentages surging to 16.9±1.96, indicating a robust inflammatory response and leukocytosis. Yasada Bhasma treatment (13 mg/kg, P.O.) resulted in a leukocyte count of 10±0.273, which was lower than the buffalo milk group, suggesting its potential to counteract buffalo milk-induced leukocytosis (p<0.01). Additionally, the eosinophil percentage in Yasad Bhasma-treated subjects was 9.3±1.223, significantly (p<0.01) reduced compared to the buffalo milk group, implying that Yasad Bhasma effectively inhibits eosinophilia as shown in Fig 5.

**Table 4. Buffalo *milk* induced Leukocytosis & eosinophilia**

| Group | Leukocytes (×10³/µL) | Eosinophil % |
|---|---|---|
| Control | 9±0.5354 | 6.01±1.1479 |
| Buffalo milk | 13.5±1.291 | 16.9±1.96 |
| Yasad Bhasma | 10±0.2733 | 9.3±1.2231 |

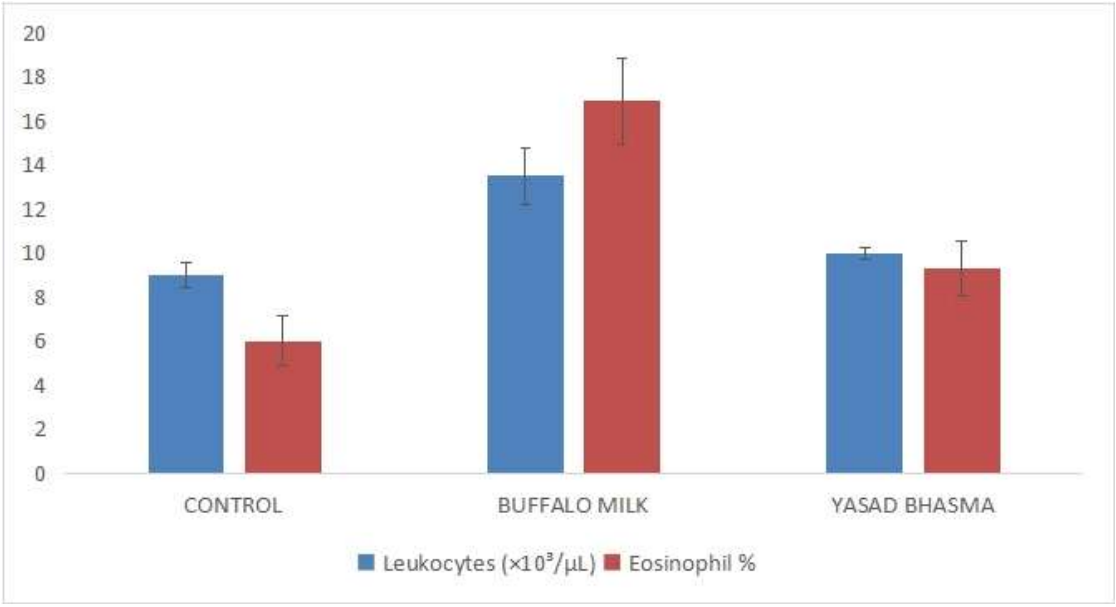



Fig 5. The statistical analysis was done using One-way ANOVA followed by Turkey's HSD test. All the values are expressed as mean ± SD( n=3). Total Leukocytes Count *p<0.01 BuffaloMilk Vs Yasad Bhasma, Eosinophil #p<0.01 01 BuffaloMilk Vs Yasad Bhasma.

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## EXTERIMENTAL STUDY



ANIMAL HOUSING AND FEEDING











VISCERAL TISSUE EXTRACTED