

## Ethanol Extract of *Brassica Rapa* Attenuate the Complete Freund's Adjuvant-Induced Inflammation in Rats

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Turnip is an ancient edible plant belongs to family Braaicacaceae. Traditionally it is used for the treatment of various disorders such as diabetes, hepatotoxicity, ulcer, and anti-inflammatory activity. Therefore the current investigation was attempted to investigate the anti-inflammatory activity of *Brassica rapa* root extract against complete fluids adjuvant induced inflammation in experimental animals. The Ethanol extract of *Brassica rapa* was prepared by a hot extraction procedure and phytochemical analysis of different extract was done by different chemical tests. Daily oral treatment of indomethacin, and extract for twenty-one days after three days of CFA administration significantly decrease the paw volume, hepatic biomarkers as well as ameliorated the level of a hematological parameter such as hemoglobin, RBC, WBC, ESR and bodyweight of experimental animals. From the result of the current investigation, it can be concluded that the *Brassica rapa* extract possesses anti-inflammatory activity. Further study is required to explore the mechanism responsible for its anti-inflammatory activity.

**Keywords:** Arthritis; *Brassica rapa*; CFA; ESR; Hemoglobin.

Rheumatoid arthritis (RA) is a progressive inflammatory, an autoimmune disorder that affects worldwide approximately 1% adult population, <sup>1</sup> characterized by swelling, joint pain, pannus formation, destruction of joint, impaired functions, and increase morbidity and mortality<sup>2</sup>. Prevalence is found to be two to three times higher in women over the age of 40 years than the man <sup>3</sup>. The exact cause of this polyarticular disease is still unclear.

Disease-modifying anti-rheumatic drugs, glucocorticoid, and nonsteroidal anti-inflammatory drugs are used in the treatment of arthritis <sup>4</sup>. However, prolong use (more than six months) of these drugs produce various ill-effects such as Weight loss, nausea, Diarrhea, anxiety, and thinning of muscles<sup>5</sup>.

In the traditional system of medicine various, ayurvedic specialists utilize different indigenous plants for the treatment of joint disorders, and theses drugs can effectively decrease the inflammatory response and cure the disease. As per World Health Organization (WHO), nearby approximately 75% of the global occupants rely upon traditional medicines for their healthiness<sup>6</sup>. *Brassica rapa*, is a biennial herbaceous plant that has gained much more attention in traditional medicine, named as turnip, and belongs to the Braaicacaceae family <sup>7</sup>. It is a fast, growing, leafy vegetable, and the whole plant (root and leave) is used for edible purposes due to its high nutritional value. Traditionally, it is used to treat various types

of disease like sore throats, jaundice, and hepatitis. Besides, it also used in the prevention of heart attack and the boost of the cardiovascular system. Moreover, it reduces the risk of osteoporosis, joint damage, and help in the development of bone formation. Besides, it has also reported that various extract of turnip possesses, anti-inflammatory, and analgesic properties<sup>8-13</sup>. Therefore the current research has been designed to explore the anti-inflammatory activity of *Brassica rapa* root extract against the CFA induced inflammation in rats.

#### Drugs and Chemicals

CFA, Bovine serum albumin, egg albumin (Sigma-Aldrich, USA), AST, ALT, and total protein kit (Auto span Pvt. Ltd) and all other reagents used in the present study were freshly prepared and purchased from local suppliers.

### MATERIAL AND METHODS

*Brassica rapa* were procured from the local market of Agra and authenticated (NISCAIR/RHMD/Consult/2015/2929-122) by Dr. Sunita Garg, Chief Scientist, CSIR-NISCAIR, New Delhi. The dried roots of *Brassica rapa* were chopped and powdered in the grinder. The coarse powder was subjected to extraction in the soxhlet apparatus and successively extracted with pet ether, chloroform, and ethanol. Individual extract of *Brassica rapa* was concentrated in hicom rotary evaporator and excess of solvent was recovered. The ethanol extract was chosen for the investigation of anti-arthritis potential in wistar rats.

#### Preliminary phytochemical screening

The Preliminary phytochemical examination of various extracts of *Brassica rapa* was subjected to standard protocols of identification tests for alkaloids, tannins, glycosides, flavonoids, and phenols<sup>14</sup>.

#### Total phenol and flavonoid Content

The presence of total phenol content in EEBR was determined by using Folin ciocalteu reagent<sup>15</sup> with a slight change in the assay procedure. Gallic acid was used as a standard and absorbance of test solution was recorded in UV-Visible Spectrophotometer at 725 nm. The number of overall polyphenols was expressed as milligram of gallic acid equivalents (mg GAE/g). The flavonoid content in EEBR was determined in colorimetric by using aluminium chloride<sup>16</sup>.

Quercetin was used as a standard and absorbance of the test solution was recorded in UV-Visible Spectrophotometer at 420 nm. The quantity of flavonoids was expressed as quercetin equivalents (mg quercetin/g extract).

#### Bovine serum albumin

The inhibitory effect of *Brassica rapa* extract on protein denaturation was assessed according to the procedure illustrated by Mizushima & Kobayashi, 1968. The sample of the resultant solution has pH 6.3, adjusted with 1N HCl, and incubated at 37°C, heated at 57°C for 20 minutes. The resultant sample was then cooled, followed by addition of phosphate buffer and absorbance was measured using Spectrophotometer at 660 nm<sup>17</sup>. Percent inhibition of protein denaturation was calculated as follows:

$$\text{Percentage inhibition} = 100 - \left( \frac{\text{Abs test solution} - \text{Abs of control}}{\text{Abs test control}} \right) \times 100$$

#### Egg albumin

The inhibitory effect of *Brassica rapa* extract on protein denaturation was assessed according to the procedure illustrated by Hasan *et al.*, 2015. Briefly, 5 ml of sample solution contained egg albumin 0.2 ml, phosphate buffer 2.8 ml and 2ml of *Brassica rapa* extract and diclofenac sodium at different concentrations from (12.5 to 800 µg/ml) respectively. The test tubes of each sample incubated at 37°C for 15 min and then warmed at 70 °C for 5 min and the absorbance of test and standard were measured using a spectrophotometer at 660 nm<sup>18</sup>.

$$\text{Percentage inhibition} = \left( \frac{\text{Abs of control} - \text{Abs of test solution}}{\text{Abs of test control Absorbance} - (\text{Abs})} \right) \times 100$$

#### Animals and experimental design

Healthy Wistar rats of both sex weighing 180-200, housed in the polypropylene cages under standard condition 12 h light and 12 h dark cycle and allowed to free access to food (Aishrwad) and water. Prior approval was taken from the Institutional animal ethical committee (IAEC) to carried out the experimental work. The whole experimental protocol was designed for 21 days, and animals were divided into different groups containing six animals in each group, namely

control, CFA, CFA+Indomethacin (10 mg/kg)<sup>19</sup>, CFA+ EEBR 200 and CFA+ EEBR400.

### Induction of arthritis

The experimental protocol used in the current study by the method portrayed by Voon *et al.*<sup>20</sup>. Briefly, 0.1 mL of CFA was injected into the sub plantar region of the left hind paw of each animal under light anesthesia (Thiopental sodium). The time of adjuvant injection was referred to as day 0. Three days after induction of arthritis, daily oral treatments were started for 21 days and on the last day, one hour after the treatment the body weight and paw volume were measured, and then animals were sacrificed and blood samples were collected via cardiac puncture.

### Assessment of Arthritis

The paw volume of each animal was measured before the administration of CFA injection using a digital plethysmometer (Roxel India). Further, after administration of CFA body weight and paw volume of each animal were measured at different day's intervals 0, 7, 14, and 21. Besides the arthritic index was assessed using arthritic scoring techniques<sup>21</sup>.

### Biochemical and hematological analysis

On the last day of the experiment blood sample of each animal was collected in test tubes by cardiac puncture and allowed to stand for 30 min to separate the serum. The serum was then centrifuged at 4000 rpm, after which total RBC,

WBC was determined by hemocytometer, ESR was measured by Westergren and SGOT, SGPT and Total protein were examined by a commercially available kit (Span diagnostic Pvt. Ltd).

### Statistical Analysis

The results were expressed as mean  $\pm$  S.E.M. Statistical analysis was performed using Graph Pad Prism 5.2® software. Two-way analyses of variances (ANOVA) were performed on percentage inhibition of paw volume and effect on weight variation in arthritic rats followed by Turkey's post hoc test. All other data were analyzed by one-way ANOVA followed by Newman keuls test.

## RESULTS

Phytochemical investigation of various extract of *Brassica rapa* shows the presence of various phytoconstituent such as alkaloids, steroids, carbohydrates, glycosides, flavonoids, and anthocyanins. Further, the ethanol extract of *Brassica rapa* showed the (102.28  $\pm$  1.21 mg GAE/g) and (22.25  $\pm$  2.20 mg quercetin/g extract).

### Effect of *Brassica rapa* extracts on inhibition of protein denaturation using BSA and egg albumin

The inhibitory effect of *Brassica rapa* on denaturation of protein is depicted in fig-1 (BSA) and Fig-2 (Egg albumin). The outcome

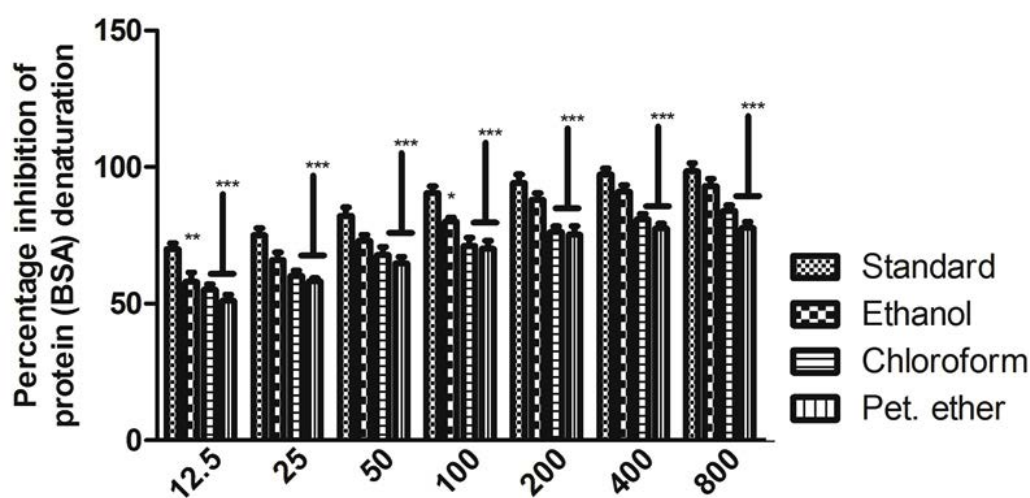


Fig. 1. Effect of different extracts of *Brassica rapa* against protein denaturation using bovine serum albumin

of the current investigation showed that ethanol extract of *Brassica rapa* exhibited the maximum 93.10% and 91.75% protection against the BSA and Egg albumin denaturation of protein at 800  $\mu\text{g/ml}$  compared to chloroform and petroleum ether extract, which is near to standard drug aspirin 98.20%.

The changes in body weight of control, CFA, indomethacin (10 mg/kg), and BREE (200 and 400mg/kg), treated arthritic rats are depicted in fig 3. There was a continuous loss of body weight was seen in all the CFA treated experimental rodents. Treatment of BREE (200 and 400mg/kg) showed a dose-dependent remarkable improvement on the loss of body weight against the CFA induced inflammation

### Effect on paw swelling

The effect of EEBR on CFA induced paw swelling is depicted in Fig.4 Sub plantar injection of CFA significantly ( $p < 0.001$ ) increased the swelling of the paw in experimental animals. Daily treatment of EEBR (200 and 400mg/kg) and indomethacin(10mg/kg) for twenty-one days after three-day of CFA injection, attenuated the CFA induced paw volume ( $p < 0.001$ ) in Wistar rats compared to CFA treated group.

### Estimation of Biochemical parameters

#### Effect on AST and ALT

Fig.5 and 6 illustrate the effect of EEBR on CFA induced changes in the level of serum AST and ALT in Wistar rats. Sub plantar injection of

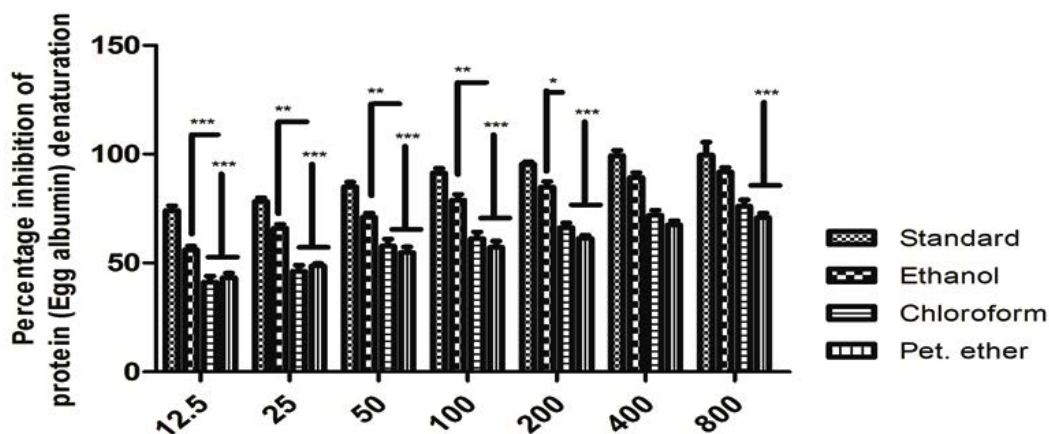


Fig. 2. Effect of different extracts of *Brassica rapa* against protein denaturation using egg albumin

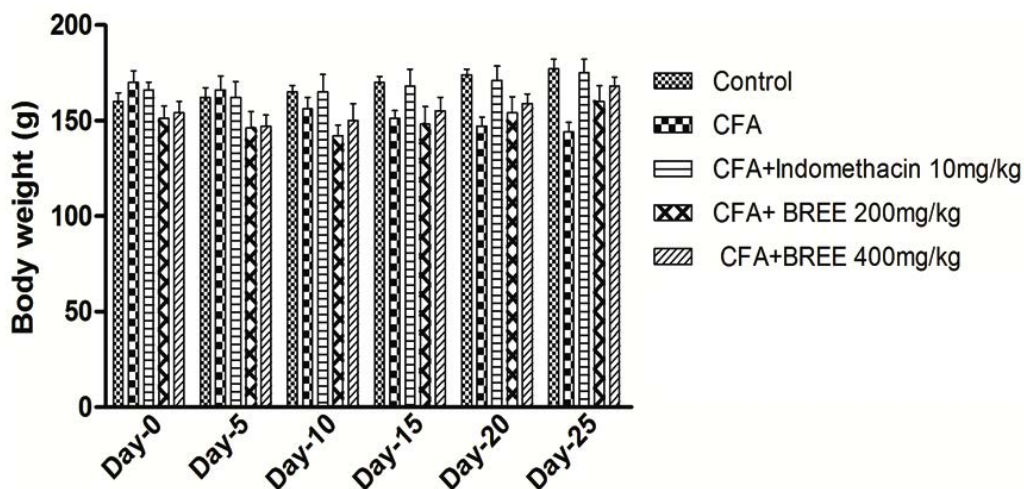


Fig. 3. Effect of *Brassica rapa* ethanol extract on CFA-induced changes in body weight

CFA significantly ( $p < 0.001$ ) increased the serum AST and ALT levels in all the groups of animals compared to control. Repeated treatment of EEBR showed a remarkable ( $p < 0.05$ ) curtailment in elevated levels of blood AST and ALT against the CFA induced arthritic group. Moreover, the treatment of a higher dose of 400mg/kg of EEBR treatment showed a remarkable reduction in hepatic biomarker compared to indomethacin treated group.

**Effect on Serum total Protein**

Fig-7 demonstrates the effect of EEBR on CFA induced change in serum total protein concentration in experimental animals. There was

a substantial reduction in the level of serum total protein that was observed in all the experimental animals compared to the control group of rodents. Statistical analysis revealed that repeated treatment of EEBR and indomethacin treated rats showed a remarkable ( $p < 0.05$ ) increment in their serum total protein compared to the CFA group.

**Hematological parameters**

The effect of EEBR on RBC, WBC, Hb, and ESR of adjuvant induced inflammation in experimental animals are depicted in Table-1. Statistically analysis revealed that sub plantar injection of CFA significantly changed the level of hematological parameters such as RBC wbc

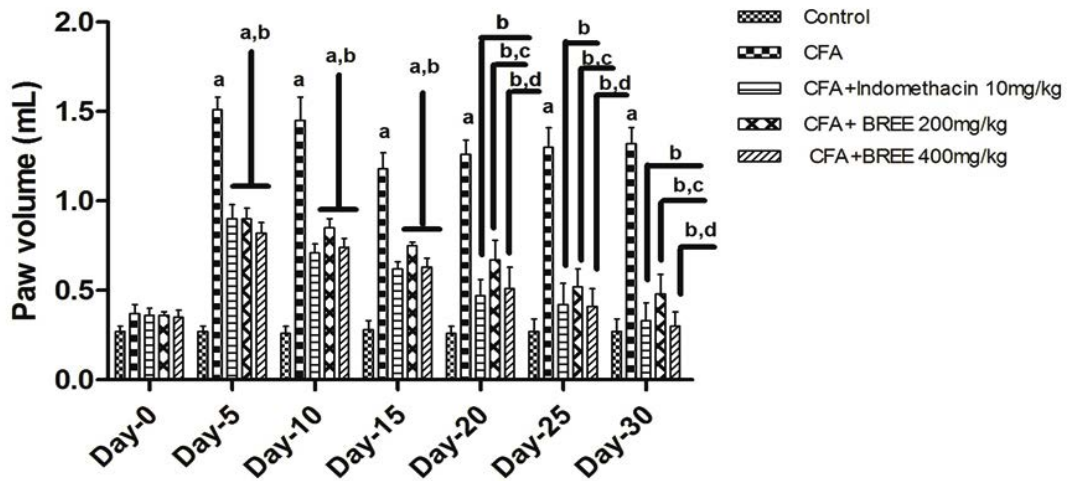


Fig. 4. Effect of *Brassica rapa* ethanol extract on CFA-induced changes in paw volume

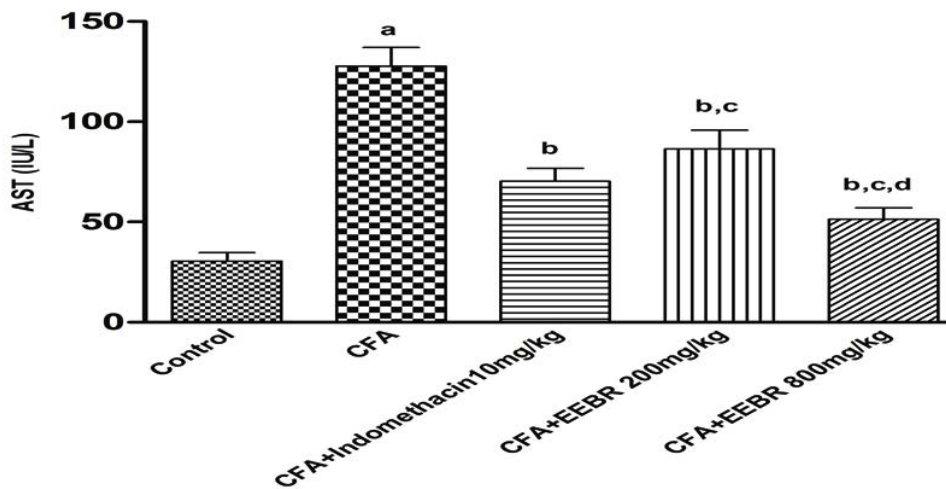


Fig. 5. Effect of *Brassica rapa* ethanol extract on CFA-induced changes in AST level

and ESR as well as body weight of experimental animals rodents compared to control group of animals. There were significant decrease in RBC from  $7.94 \pm 0.33$  to  $6.24 \pm 0.33 \text{ mm}^3$ , Hb from  $14.35 \pm 0.31$  to  $9.6 \pm 0.86 \text{ g/dL}$  and increase in WBC from  $7022 \pm 4.2$  to  $8963 \pm 5.26$  and ESR from  $0.62 \pm 0.07$  to  $2.89 \pm 0.08 \text{ mm/h}$ . Repeated treatment of EEBR reverse the elevated count of WBC, ESR

level, and boost up the Hb and RBC count all most up to normal range in all the experimental animals. Moreover, the treatment of indomethacin attenuated the elevated level of ESR and WBC count but did not show any much more significant effect on RBC count and hemoglobin level compare to EEBR.

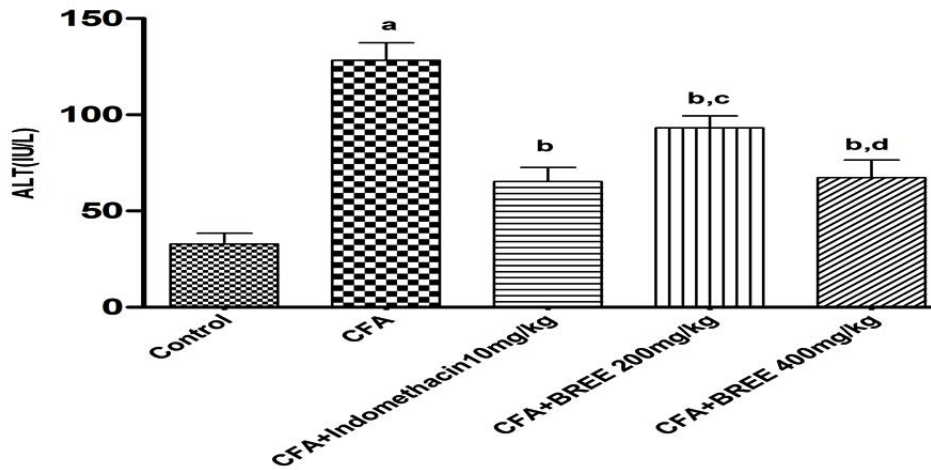


Fig. 6. Effect of *Brassica rapa* ethanol extract on CFA-induced changes in ALT level

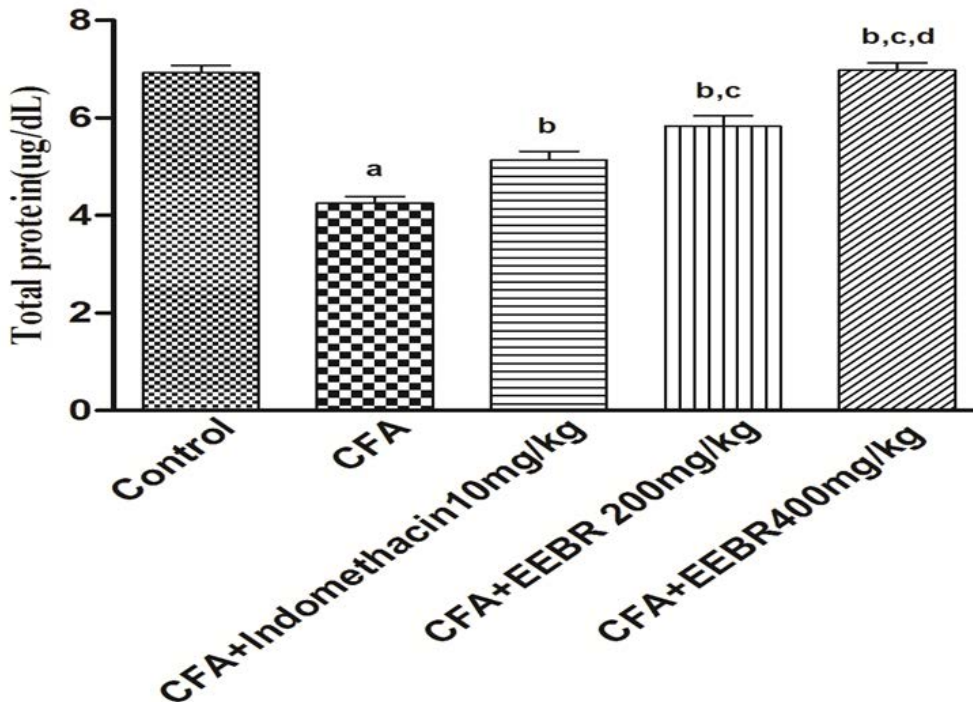


Fig. 7. Effect of *Brassica rapa* ethanol extract on CFA-induced changes in total protein level

**Table 1.**

Groups	RBC mm <sup>3</sup>	Hb% g/dl	WBC mm <sup>3</sup>	ESR
Control	7.94±0.33	14.35±0.31	7022±4.2	1.12±0.07
CFA	6.24±0.33 <sup>a</sup>	9.6±0.86 <sup>a</sup>	8963±5.26 <sup>a</sup>	2.89±0.08 <sup>a</sup>
CFA+Indomethacin(10mg/kg)	7.35±0.55 <sup>b</sup>	12.51±0.67 <sup>b</sup>	7031±6.7 <sup>b</sup>	0.64±0.04 <sup>b</sup>
CFA+EEBR (200mg/kg)	7.1±0.32 <sup>a</sup>	11.87±0.71 <sup>a</sup>	8085±8.17 <sup>c</sup>	1.16±0.05 <sup>b,c</sup>
CFA+EEBR (400mg/kg)	7.86±0.44 <sup>b,c</sup>	12.86±0.8 <sup>b,d</sup>	7070±7.49 <sup>b,d</sup>	0.97±0.10 <sup>a,b,d</sup>

## DISCUSSION

Medicines have a pivotal role in the treatment of various diseases of human beings since ancient times and herbal drugs are frequently used as a traditional medicine for the relief of pain and inflammation. These drugs can not only prevent the inflamed joint from pain and bone destruction but in comparison to allopathic medicine, these are safe, free from side effects, and suitable for patients<sup>22</sup>. The present investigations demonstrate that ethanol extract of *Brassica rapa* exhibited the inhibition of protein denaturation activity as well as attenuated the CFA induced inflammation in experimental animals. Therefore ethanol extract of *Brassica rapa* could be an probable option in the treatment of RA.

In the current investigation, the inflammation lowering potential of *Brassica rapa* extracts was investigated, using BSA and egg albumin method. Protein denaturation is one of the reasons for joint inflammation, which may occur due to loss of secondary and tertiary structure of proteins<sup>23</sup>. In the present study, the ethanol extract of *Brassica rapa* extracts successfully inhibited the denaturation of protein (albumin). Moreover, the ethanol extract showed the highest inhibitory percentage of protein denaturation, close to standard drug. The augmentation in absorbance of test samples concerning control showed that *Brassica rapa* can decrease the protein denaturation by heat.

Adjuvant induced inflammation model is one of the most accepted screening methods for the assessment of anti-inflammatory drugs because it elicits the similar symptoms of RA as occurs in humans such as pain, redness, and swelling in joints<sup>24</sup>. Therefore in the current study adjuvant-induced arthritis model was selected for the demonstration

of the anti-arthritis activity of *Brassica rapa* root. Subcutaneous injection of CFA induce the inflammation in a two different phase (Acute and chronic)<sup>25</sup> and increases the paw volume in experimental animals similar to our previous finding under publication. In the present investigation oral administration of BREE attenuated the CFA induced paw volume in experimental animals that shows that deterioration in the progression of the disease. This might be because of secondary metabolites, potent antioxidant enzymes that decrease tissue injury and promote health<sup>26</sup>. Loss of body weight most frequently occurs in RA patients which maybe occur due to loss of appetite and inadequate absorption of nutrients from the gastrointestinal intestine<sup>27</sup>. The outcomes of the current investigation demonstrated that there may be a close connection between the loss of body weight and inflammation of joints. Treatment of BREE attenuated the loss of body weight in adjuvant induced inflammation in experimental animals, which could be due to presence of vitamin B and omega 3 fatty acids<sup>28</sup>. Our findings were in agreement with the result of<sup>29</sup> which shows that supplementation of vitamin B improves the health status of the patient.

Various researchers have proposed that the estimation of liver biomarkers such as SGOT, SGPT, and total protein is an excellent approach to measure the anti-arthritis potential of test drugs<sup>30-32</sup>. In the current investigation, CFA caused a significant elevation in the level of SGOT and SGPT and reduction in total protein concentration similar to that of earlier findings which indicate that impairment in hepatic tissue<sup>33</sup>. Treatment of BREE significantly diminished the activity of SGOT and SGPT and reverses the diminished concentration of protein to normal level in CFA induced arthritic rats and thus demonstrating its ability in protecting

the liver against the CFA induced arthritis in experimental animals.

In our study, CFA caused a reduction in the level of hemoglobin and red blood cell count. A low level of hemoglobin represents the anemic condition that may be due to the inadequate absorption of iron or maybe the destruction of premature RBC and decreased the response of erythropoietin towards the bone<sup>34</sup>. Ethanol extract of *Brassica rapa* attenuated the elevated count of white blood cells to normal levels in arthritic rats and restored the percentage of hemoglobin, RBC count, and elevated ESR in diseased animals.

### CONCLUSION

The present study revealed that repeated treatment of BREE attenuated the CFA induced symptoms of arthritis such as paw volume in bodyweight and hepatic markers in the experimental rodents. The symptomatic relief in the present study might be due to the presence of flavonoid or any other phytoconstituents present in the extract. Further study is required to confirm the anti-arthritic potential of the extract.

#### Source of Funding

Nil.

#### Conflict of Interest

The authors declare that they have no conflict of interests and thankful to GLA University, Mathura, Uttar Pradesh, India for the facility provided for the work. Bhupesh Chandra semwal developed the concept, designed experiment and prepared the manuscript. Neha singh performed the experiment and Shilpi pathak help in the experiment.

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