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*calliobotrys*

## Evaluation of *in vitro* and *in vivo* anti-arthritic potential of *Berberis calliobotrys*

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### Abstract

The present study was commenced to evaluate the anti-arthritic effect of 70% methanol extract and *n*-butanol and aqueous fractions of *Berberis calliobotrys* using both *in vitro* and *in vivo* arthritis models. Extract and fractions were investigated *in vitro* for inhibition of protein (bovine serum and egg albumin) denaturation and human red blood cell membrane stabilization. *In vivo* anti-arthritic activity of extract and fractions at 50, 100 and 200 mg/kg was assessed using turpentine oil and formaldehyde-induced arthritis, while, 200 mg/kg dose was evaluated against complete Freund's adjuvant-induced arthritis. *B. calliobotrys* produced significant ( $p < 0.001$ ) dose dependent inhibition of protein denaturation and human red blood cell membrane stabilization. In turpentine oil, formaldehyde and complete Freund's adjuvant-induced arthritis models, *B. calliobotrys* significantly ( $p < 0.001$ ) reduced joint and paw swelling. *B. calliobotrys* markedly improved body weight, hematology profile, radiological and histopathological parameters in complete Freund's adjuvant model. It could be concluded that *B. calliobotrys* holds anti-arthritic potential, supporting its traditional use in treatment of rheumatoid arthritis.

### Introduction

Rheumatoid arthritis is a chronic inflammatory, auto-immune disorder that influence multiple joints (Afuwape et al., 2002). Moreover, this disease is accompanied by synovial inflammation and hyperplasia, autoantibody production, cartilage and bone deformation with functional impairment and systemic complications e.g., cardiovascular, pulmonary, psychological, and skeletal disorders (McInnes and Schett, 2011). Therapy includes disease modifying anti-rheumatic drugs, non-steroidal anti-inflammatory drugs, biologic response modifiers and corticosteroids. Although, these drugs are currently being used but their toxic profile prevail over beneficial effects, thus, decreasing patient adherence to therapy.

Traditional herbal remedies are more acceptable in most of ethnic societies as compared to allopathic medicines because they are considered to be safest approach

of treating diseases with least side effects on human health (Ahmad et al., 2012).

Various plants of family *Berberidaceae* are used as traditional herbal medicines in Gilgit and other Northern Areas of Pakistan (Mokhber-Dezfuli et al., 2014). Roots and stem bark of these plants are used for treatment of internal and external wounds, infections, piles, jaundice, liver problems, kidney stone, diabetes, sore throat, leucorrhoea, bleeding, uterine tumors, swellings and its related problems (Khan and Khatoon, 2007). *Berberis calliobotrys* Bienert ex Aitch, commonly known as Chowenj and Zarch, is a medicinally imperative plant having folkloric use in arthritis by aboriginal people.

The present study was designed to evaluate the anti-arthritic activity of 70% methanol extract and some fractions of *B. calliobotrys* in both *in vitro* and *in vivo* systems thus, supporting its traditional use in arthritis.



## Materials and Methods

### Plant material

Stem of *B. calliobotrys* was collected from hilly areas of Quetta, Balochistan. Plant was identified and authenticated by Dr. Rasool Buksh, Taxonomist, Department of Botany, University of Balochistan.

### Preparation of extract/fractions

Crude extract of *B. calliobotrys* was prepared using cold maceration technique. The finally grounded stem powder (1.3 kg) was soaked in 4 L of water-methanol mixture (70:30) for three days at room temperature with intermittent shaking afterward filtration. The soaking and filtration process was repeated 2-fold more. Finally, all the filtrates were combined and concentrated under reduced pressure using rotary evaporator. The crude extract was then air-dried to obtain a thick pasty mass with a yield of 6.3% (Yuchi et al., 2012). Furthermore, the crude extract was sequentially fractionated with *n*-butanol for three times in a separating funnel.

### Experimental animals

Sprague-Dawley rats of either sex weighing from 200-300 g were used. The rats were housed under standard conditions of temperature (23 to 25°C), relative humidity (55%) with 12 hours light and 12 hours dark cycle. They were fed with standard pellet diet and tap water *ad libitum*.

### Drugs and chemicals

Diclofenac sodium (Sigma-Aldrich, USA), aspirin (Uni-Chem, Germany), bovine serum albumin (Sigma-Aldrich, USA), *n*-butanol (Sigma-Aldrich, USA), complete Freund's adjuvant (Sigma-Aldrich, USA), turpentine oil (Uni-Chem, Germany), egg albumin from fresh hen's egg, formaldehyde (VWR, International Ltd, England), sodium chloride (Sigma-Aldrich, USA), disodium hydrogen phosphate (Merck, Germany), DMSO (Riedel-de-Haen, USA), potassium dihydrogen phosphate (Riedel-de-Haen, USA), sodium hydroxide (Sigma-Aldrich, USA), hydrochloric acid (Riedel-de-Haen, USA) and methanol (Riedel-de-Haen, USA) were used.

Assessment of *in vitro* anti-arthritis activity

### Inhibition of protein denaturation method using bovine serum albumin

The anti-arthritis activity of *B. calliobotrys* was evaluated using method of bovine serum albumin denaturation. The reaction mixture (0.5 mL) containing 0.45 mL bovine serum albumin (5% aqueous solution) and 0.05 mL of varying concentrations (12.5, 25, 50, 100, 200, 400, 800 µg/mL) of aqueous methanolic extract, *n*-butanol, aqueous fraction of *B. calliobotrys* and aspirin were prepared. pH of reaction mixture was adjusted at 6.3 using a small amount of 0.1 N HCl. Then, samples were

incubated at 37°C for 20 min and afterward heated at 57°C for 30 min. Phosphate buffer saline (2.5 mL; pH 6.3) was added to each tube after cooling the samples. Absorbance was measured through spectrophotometer at 660 nm. Test control consisting of 0.05 mL distilled water in case of aspirin, *n*-butanol and aqueous fractions while, DMSO for aqueous methanolic extract were prepared. Whereas, product control did not contain bovine serum albumin. The percentage inhibition of protein denaturation was computed as follows (Rahman et al., 2012):

$$\% \text{Inhibition} = \{[100 - (A - B)]/C\} \times 100$$

Where 'A' is the absorbance of test solution, 'B' is the absorbance of product control and 'C' is the absorbance of test control

### Inhibition of protein denaturation method using egg albumin

*In vitro* anti-arthritis activity of *B. calliobotrys* was scrutinized against protein denaturation method using fresh hen's egg albumin. The reaction mixture (5 mL) consisting of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (pH 6.4) and 2 mL of different concentrations (12.5, 25, 50, 100, 200, 400 and 800 µg/mL) of hydroalcoholic extract, butanolic and aqueous fractions of *B. calliobotrys* and diclofenac sodium were prepared. Similar volume of doubled distilled water for diclofenac sodium, *n*-butanol fraction and aqueous fraction while, DMSO for aqueous methanolic extract served as a control. The mixtures were incubated at 37 ± 2°C in incubator for 15 min and then heated at 70°C for 5 min. After cooling their absorbance were measured at 660 nm by using vehicle as a blank. The percentage inhibition of protein denaturation was calculated by using the following formula (Prakash et al., 2013):

$$\text{Percentage inhibition} = 100 \times [\text{Absorbance of test sample} / \text{Absorbance of control} - 1]$$

### HRBC membrane stabilization method

Anti-arthritis activity of *B. calliobotrys* was also determined using human red blood cell membrane stabilization method. The blood was collected from healthy human volunteer who had not taken any NSAIDs for 2 weeks prior to the experiment and was mixed with equal volume of sterilized alsevers solution. The blood solution was centrifuged in a centrifugation machine at 3,000 rpm for 15 min and the upper layer was carefully removed with a syringe or sterile pipette. The packed cells remained at the bottom were separated and washed with isosaline solution and a 10% v/v suspension was made with isosaline. Human red blood cells suspension was used for the study.

The assay mixtures contained phosphate buffer (1 mL), hypotonic saline (2 mL), red blood cell suspension (0.5 mL), and 0.5 mL of different concentrations of extract, reference sample and control were mixed in a discrete

manner. The test solution comprising of 1 mL of phosphate buffer, 2 mL of hypotonic saline, 0.5 mL of various concentrations (12.5, 25, 50, 100, 200, 400 and 800 µg/mL) of diclofenac sodium and aqueous methanolic extract, *n*-butanol and aqueous fractions of *B. calliobotrys* and 0.5 mL of 10% w/v human red blood cells were formulated. Likewise, 1 mL of phosphate buffer and 2 mL of distilled water and 0.5 mL of 10% w/v human red blood cells in isotonic saline served as test control in case of aqueous fraction, *n*-butanol fraction and diclofenac sodium. Alternatively, DMSO was incorporated in the test control solutions for hydroalcoholic extract. Then, all the assay mixtures were incubated at 37°C for 30 min and centrifuged at the rate of 3,000 rpm. The supernatant liquid was poured out and the hemoglobin content was estimated by UV spectrophotometer at 560 nm. The percentage of human red blood cell membrane stabilization or protection against hypotonicity induced hemolysis was calculated by using the following formula (Gautam et al., 2013):

Percentage protection =  $100 - [(optical\ density\ sample / optical\ density\ control) \times 100]$

#### *Assessment of in vivo anti-arthritis activity*

##### **Turpentine oil induced joint edema in rats**

To inspect the anti-arthritis effect of *B. calliobotrys* against turpentine oil induced joint edema method described by Kaithwas et al., 2012 was employed. Sprague-Dawley rats of either sex were fasted for a period of 24 hours before the commencement of experiment however; the animals were allowed free access to water. Animals were randomly divided into 11 groups of 5 rats each. Group I served as control and received distilled water (3 mL/kg/p.o.) and Group II served as standard drug treated group and received the standard drug, aspirin (100 mg/kg; p.o.). While, Group III, IV, V received aqueous methanolic extract of *B. calliobotrys* (50, 100 and 200 mg/kg; p.o.), respectively, and Group VI, VII, VIII were treated with aqueous fraction of *B. calliobotrys* at 50, 100 and 200 mg/kg orally in that order. Likewise, Group IX, X, XI were administered same doses of *n*-butanol fraction as mentioned above through oral route. Hydroalcoholic extract and its fractions were dissolved or suspended in distilled water. Acute non-immunological inflammatory joint edema was induced by injecting 0.02 mL turpentine oil in synovial cavity of right knee joint 30 min after drug administration. Joint diameter was measured at hourly interval for 6 hours using digital vernier calliper. The percentage inhibition of edema was calculated using following formula (Dhage et al., 2013):

Percentage inhibition of edema =  $(1 - V_t) / V_c \times 100$

Where,  $V_t$  and  $V_c$  are the joint diameter of treated and control rats

##### **Formaldehyde induced arthritis in rats**

The aspire of this experiment was to seek out the anti-arthritis effect of *B. calliobotrys* against formaldehyde-induced chronic non-immunological arthritis in rats as illustrated by Kaithwas et al. (2012). Sprague-Dawley rats of either sex were distributed into 11 groups of 5 rats each at random. Group I served as control and received distilled water (3 mL/kg; p.o.), Group II was administered with standard drug aspirin at dose of 100 mg/kg body weight orally for the duration 10 days. Similarly, Group III, IV, V were treated with aqueous methanolic extract of *B. calliobotrys* with 50, 100, 200 mg/kg p.o. and Group V, VII, VIII were received *n*-butanol fraction at 50, 100, 200 mg/kg body weight orally for 10 days, respectively. Likewise, Group IX, X, XI were treated with aqueous fraction of *B. calliobotrys* at 50, 100 and 200 mg/kg body weight, correspondingly, for a period 10 days. On day 1st 30 min after drug administration arthritis was induced by subplanter injection of 0.1 mL of 2% formaldehyde solution and repeated on day 3<sup>rd</sup>. Arthritis was assessed by measuring mean increase in paw diameter for a period of 10 days using digital vernier calliper. The percentage inhibition of edema was calculated using aforementioned formula.

##### **Complete Freund's adjuvant-induced arthritis in rats**

Experimental chronic immunological arthritis was induced in rats according to the method of Newbould (1963). The left footpad of each rat was injected subcutaneously with 0.05 mL (0.5% w/v) of complete Freund's adjuvant. Sprague-Dawley rats of either sex were separated into 6 Groups of 5 rats each. Group 1 was not administered complete Freund's adjuvant and served as normal control. Group II as arthritic control received control vehicle (distilled water, 3 mL/kg; p.o.), Group III was treated with standard drug (aspirin, 100 mg/kg, p.o.), Group IV, V and VI were treated with aqueous methanolic extract, *n*-butanol fraction and aqueous fraction at the dose level of 200 mg/kg body weight orally 1 day before the complete Freund's adjuvant injection. Treatment was continued for 14 days. The magnitude of inflammatory response was evaluated by measuring the increase in left hind paw thickness after complete Freund's adjuvant injection using digital vernier caliper (Winter et al., 1962). However, the body weight changes were observed every day and the percentage inhibition of paw edema was calculated as stated above. After 14th days, animals were killed with chloroform, blood samples were withdrawn by intracardiac puncture for hematological analysis and their legs were amputated at knee joints and immediately fixed on cardboard for radiographical studies. Amputated legs were fixed with 10% neutral buffered formalin and after 48 hours specimens were placed into 5% formic acid for decalcification for 7 days. The ankle joints were cut into approximately equal

halves in the frontal plane using the collateral ligament as a guide. When the specimens were grossed, they were placed into fresh decalcifying solution for the night and processed for paraffin embedding. The tissue was segmented at 5 micron and stained with hematoxylin and eosin for histopathological assessment (Durie et al., 1993).

#### Statistical analysis

All the data concerning anti-arthritis study are expressed as mean  $\pm$  SEM. Statistical analysis was carried out by two-way ANOVA followed by Bonferroni multiple comparison *Post hoc* test and one-way ANOVA followed by Dunnett's using the "GraphPad-Prism" statistic computer program. A difference in the mean values of  $p < 0.05$  was considered statistically significant.

## Results

The crude extract of *B. calliobotrys* exhibited significant ( $p < 0.001$ ) inhibition of bovine serum albumin denaturation as 89.8% at 800  $\mu\text{g/mL}$ . These results were comparable to that of standard drug aspirin that is 97.6% at 800  $\mu\text{g/mL}$ . While, butanolic and aqueous fractions showed 86.5% and 83.3% inhibition of bovine serum albumin denaturation, correspondingly, at 800  $\mu\text{g/mL}$  as compared to aspirin. Likewise, crude extract, butanol fraction as well as aqueous fraction produced 93.7%, 88.0% and 79.8% protection against denaturation of egg albumin at 800  $\mu\text{g/mL}$  concentration, respectively. Even as, diclofenac sodium demonstrated 99.2% inhibition of egg albumin denaturation at 800  $\mu\text{g/mL}$  in a concentration-dependent manner. On the other hand, hydroalcoholic extract of *B. calliobotrys* provided 48.8% protection against hypotonic saline induced erythrocyte lysis at 800  $\mu\text{g/mL}$ . Whereas, *n*-butanol and aqueous fraction produced 45.7% and 41.8% stabilization of HRBC membrane at 800  $\mu\text{g/mL}$ , correspondingly, as compared to standard. Nonetheless, diclofenac sodium exhibited 74.8% at 800  $\mu\text{g/mL}$  membrane stabilization (Table I).

Oral dosage of 50 mg/kg of aqueous methanolic extract, *n*-butanol and aqueous fraction of *B. calliobotrys* exhibited 64.4, 59.0 and 56.1% inhibition of joint inflammation in turpentine oil-induced joint edema in rats, respectively. At the dose of 100 mg/kg, the reduction in joint edema was 66.7, 62.2 and 60.0% in hydroalcoholic extract, *n*-butanol and aqueous fraction of *B. calliobotrys* treated rats in that order. While, maximum dose of 200 mg/kg of hydroalcoholic extract, *n*-butanol and aqueous portion of *B. calliobotrys* crude extract showed highly significant ( $p < 0.001$ ) decrease in knee joint swelling with 70.1, 65.8 and 63.3% inhibition, correspondingly. The positive control aspirin (100 mg/kg) also produced significant ( $p < 0.001$ ) inhibition

(69.3%) in the turpentine oil mediated joint edema in rats (Table II).

Subplanter injection of formaldehyde (0.1 mL) on day 1 and 3 to the rat hind paw led to increase in paw diameter. Maximum paw swelling was noted at day 3rd with the progressive decrease in paw diameter throughout the surveillance time of 10 days. In control group paw diameter was continually increased up to 17.3 mm during this assessment span. The aqueous methanol extract at the test doses of 50 mg/kg, 100 mg/kg and 200 mg/kg significantly ( $p < 0.001$ ) reduced the paw edema by 73.4%, 78.2%, 82.6% respectively at day 10 whereas, *n*-butanol fraction at 50 mg/kg, 100 mg/kg and 200 mg/kg showed 68.7, 72.1 and 76.6%, accordingly. On the other hand, aqueous fraction demonstrated 56.2%, 63.1% and 70.4% inhibition of paw swelling at 50 mg/kg, 100 mg/kg and 200 mg/kg in that order. Whereas, aspirin at 100 mg/kg manifested 76.1% decrease in paw engorgement during 10 days study duration (Table II).

The paw diameter of CFA control rats increased strikingly whereas, significant ( $p < 0.001$ ) decrease in paw diameter was observed in treatment groups as compared to CFA control group (Table III; Figure 1). Oral administration of aqueous methanolic extract, *n*-butanol fraction and aqueous fraction of *B. calliobotrys* at dose of 200 mg/kg to arthritic rats reduced paw diameter significantly ( $p < 0.001$ ) and exhibited 80.6%, 77.5% and 70.1% inhibition, respectively, as compared to CFA control group at the end of study period of 15 days. Arthritic rats showed a considerable reduction in hemoglobin and RBC, while, WBC, platelets count, RF, ESR were significantly increased compared to normal rats. Administration of crude extract, *n*-butanol and aqueous fraction (200 mg/kg) of *B. calliobotrys* to arthritic rats enhanced hemoglobin and RBC ( $p < 0.001$ ) to near normal levels pertaining to standard (aspirin) at 100 mg/kg. The increase in WBC count, platelet, RF and ESR were significantly ( $p < 0.001$ ) in the extract-administered arthritic group (Table IV). Body weight of CFA treated rats was extensively decreased in comparison with normal control group whereas, body weight of crude extract (200 mg/kg) treated rats was substantially increased during the study period. Likewise, body weight of group treated with *n*-butanol fraction was decreased initially but later on a significant improvement was seen in their body weight. Alternatively, treatment with aqueous fraction exhibited a slight decrease in body weight during trial period. In general, aforementioned results verify the appreciably significant ( $p < 0.001$ ) protective effect of *B. calliobotrys* against loss of body mass in rheumatoid arthritis in comparison with aspirin which also exhibit significant ( $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$ ) inhibitory effect on cachexia in arthritic rats (Table V). From the results it was found

Table I

*In vitro* anti-arthritis activity of *Berberis calliobotrys*

Treatment groups	% Inhibition of protein (BSA) denaturation (concentration µg/mL)					% Inhibition of protein (egg albumin) denaturation (concentration µg/mL)					% HRBC membrane stabilization (concentration µg/mL)											
	12.5	25	50	100	200	400	800	12.5	25	50	100	200	400	800	12.5	25	50	100	200	400	800	
Aqueous methanol extract	67.2 (0.3) <sup>a</sup>	72.9 (0.1) <sup>a</sup>	78.6 (0.7) <sup>a</sup>	81.7 (0.8) <sup>a</sup>	85.8 (0.3) <sup>a</sup>	87.9 (0.2) <sup>a</sup>	89.7 (0.3) <sup>a</sup>	50.4 (1.4) <sup>a</sup>	61.5 (0.6) <sup>a</sup>	70.7 (1.9) <sup>a</sup>	78.3 (0.4) <sup>a</sup>	83.8 (0.3) <sup>a</sup>	87.7 (0.1) <sup>a</sup>	93.7 (0.0) <sup>a</sup>	27.7 (0.3) <sup>a</sup>	29.9 (2.8) <sup>a</sup>	34.9 (4.4) <sup>a</sup>	37.6 (5.9) <sup>a</sup>	40.7 (0.1) <sup>a</sup>	43.3 (0.9) <sup>a</sup>	48.7 (0.6) <sup>a</sup>	
Butanolic fraction	65.6 (0.1) <sup>a</sup>	68.1 (0.9) <sup>a</sup>	72.5 (0.3) <sup>a</sup>	75.3 (3.8) <sup>a</sup>	81.6 (0.3) <sup>a</sup>	85.3 (0.4) <sup>a</sup>	86.4 (2.9) <sup>a</sup>	52.5 (0.0) <sup>a</sup>	58.6 (0.4) <sup>a</sup>	69.4 (0.6) <sup>a</sup>	75.2 (0.0) <sup>a</sup>	79.5 (0.0) <sup>a</sup>	83.3 (0.0) <sup>a</sup>	86.0 (0.1) <sup>a</sup>	18.5 (0.2) <sup>a</sup>	23.6 (0.3) <sup>a</sup>	27.4 (0.1) <sup>a</sup>	31.5 (0.3) <sup>a</sup>	36.4 (0.1) <sup>a</sup>	41.8 (0.5) <sup>a</sup>	45.7 (0.3) <sup>a</sup>	
Aqueous fraction	55.1 (0.7) <sup>a</sup>	61.5 (0.1) <sup>a</sup>	64.6 (0.7) <sup>a</sup>	69.1 (0.8) <sup>a</sup>	74.2 (1.5) <sup>a</sup>	78.3 (0.0) <sup>a</sup>	83.2 (0.6) <sup>a</sup>	47.5 (0.0) <sup>a</sup>	50.8 (0.0) <sup>a</sup>	58.3 (0.0) <sup>a</sup>	61.2 (0.2) <sup>a</sup>	69.2 (0.0) <sup>a</sup>	73.9 (0.0) <sup>a</sup>	79.8 (0.0) <sup>a</sup>	14.6 (0.4) <sup>a</sup>	17.6 (0.6) <sup>a</sup>	22.1 (0.3) <sup>a</sup>	27.9 (0.4) <sup>a</sup>	33.5 (1.0) <sup>a</sup>	37.2 (0.3) <sup>a</sup>	41.7 (0.3) <sup>a</sup>	
Aspirin	75.5 (1.2)	80.8 (0.1)	89.6 (0.7)	90.4 (1.0)	92.7 (0.2)	95.6 (0.5)	97.5 (0.5)															
Diclofenac sodium								73.0 (0.0)	77.8 (0.0)	82.6 (0.0)	88.5 (0.0)	94.6 (0.0)	97.4 (0.0)	99.1 (0.0)	45.5 (0.0)	46.9 (2.4)	55.3 (0.0)	60.3 (0.0)	64.8 (0.0)	67.6 (1.3)	74.7 (0.01)	

Values are expressed as mean ± SEM; n=3; Two-way ANOVA followed by Bonferroni post test used and p<0.05 was considered as significant as compared to standard control where \*p<0.001

**Table II**  
**Effect of *Berberis calliobotrys* on joint edema and paw swelling in rats**

Treatment groups	Turpentine oil-induced joint edema										Formaldehyde induced arthritis				
	Increase in joint diameter (mm)										Increase in paw diameter (mm)				
	1 <sup>st</sup> hour	2 <sup>nd</sup> hour	3 <sup>rd</sup> hour	4 <sup>th</sup> hour	5 <sup>th</sup> hour	6 <sup>th</sup> hour	2 <sup>nd</sup> day	4 <sup>th</sup> day	6 <sup>th</sup> day	8 <sup>th</sup> day	10 <sup>th</sup> day				
Arthritic control (3 mL/kg)	8.9 ± 0.0	10.0 ± 0.0	13.2 ± 0.0	15.3 ± 0.0	18.7 ± 0.0	21.4 ± 0.0	8.7 ± 0.0	10.8 ± 0.0	12.2 ± 0.0	15.0 ± 0.0	17.2 ± 0.0				
Standard aspirin (100 mg/kg)	7.1 ± 0.0 <sup>a</sup> (19.8%)	6.8 ± 0.0 <sup>a</sup> (31.9%)	7.5 ± 0.0 <sup>a</sup> (42.9%)	7.3 ± 0.0 <sup>a</sup> (53.3%)	7.2 ± 0.0 <sup>a</sup> (61.5%)	6.5 ± 0.0 <sup>a</sup> (69.3%)	5.0 ± 0.0 <sup>a</sup> (42.9%)	5.5 ± 0.0 <sup>a</sup> (49.0%)	5.1 ± 0.0 <sup>a</sup> (57.8%)	4.8 ± 0.0 <sup>a</sup> (68.0%)	4.1 ± 0.0 <sup>a</sup> (76.1%)				
Aqueous methanol extract (50 mg/kg)	7.9 ± 0.0 <sup>a</sup> (11.6%)	7.8 ± 0.0 <sup>a</sup> (21.6%)	8.1 ± 0.0 <sup>a</sup> (38.8%)	7.9 ± 0.0 <sup>a</sup> (50.9%)	7.8 ± 0.0 <sup>a</sup> (58.3%)	7.6 ± 0.0 <sup>a</sup> (64.4%)	5.7 ± 0.0 <sup>a</sup> (33.8%)	6.1 ± 0.0 <sup>a</sup> (43.9%)	5.7 ± 0.0 <sup>a</sup> (53.1%)	5.3 ± 0.0 <sup>a</sup> (64.3%)	4.5 ± 0.0 <sup>a</sup> (73.4%)				
Aqueous methanol extract (100 mg/kg)	7.4 ± 0.0 <sup>a</sup> (17.2%)	7.3 ± 0.0 <sup>a</sup> (26.5%)	7.6 ± 0.0 <sup>a</sup> (42.7%)	7.7 ± 0.0 <sup>a</sup> (49.9%)	7.5 ± 0.0 <sup>a</sup> (60.0%)	7.1 ± 0.0 <sup>a</sup> (66.7%)	4.9 ± 0.0 <sup>a</sup> (43.0%)	5.4 ± 0.0 <sup>a</sup> (49.6%)	5.0 ± 0.0 <sup>a</sup> (58.9%)	4.6 ± 0.0 <sup>a</sup> (69.4%)	3.7 ± 0.0 <sup>a</sup> (78.2%)				
Aqueous methanol extract (200 mg/kg)	6.8 ± 0.0 <sup>a</sup> (23.1%)	6.7 ± 0.0 <sup>a</sup> (33.0%)	6.6 ± 0.0 <sup>a</sup> (50.1%)	6.5 ± 0.0 <sup>a</sup> (57.2%)	6.4 ± 0.0 <sup>a</sup> (65.5%)	6.3 ± 0.1 <sup>a</sup> (70.1%)	4.6 ± 0.0 <sup>a</sup> (46.8%)	5.0 ± 0.2 <sup>a</sup> (54.0%)	4.7 ± 0.1 <sup>a</sup> (61.5%)	3.7 ± 0.0 <sup>a</sup> (75.3%)	3.0 ± 0.0 <sup>a</sup> (82.6%)				
n-Butanol fraction (50 mg/kg)	8.1 ± 0.0 <sup>a</sup> (9.3%)	7.8 ± 0.0 <sup>a</sup> (21.5%)	9.5 ± 0.0 <sup>a</sup> (27.9%)	9.2 ± 0.0 <sup>a</sup> (40.0%)	9.0 ± 0.0 <sup>a</sup> (52.0%)	8.7 ± 0.0 <sup>a</sup> (59.0%)	6.0 ± 0.0 <sup>a</sup> (31.4%)	6.6 ± 0.0 <sup>a</sup> (39.6%)	6.2 ± 0.0 <sup>a</sup> (48.8%)	5.7 ± 0.0 <sup>a</sup> (61.3%)	5.4 ± 0.0 <sup>a</sup> (68.7%)				
n-Butanol fraction (100 mg/kg)	7.8 ± 0.0 <sup>a</sup> (11.8%)	7.7 ± 0.0 <sup>a</sup> (22.9%)	8.7 ± 0.0 <sup>a</sup> (34.5%)	8.6 ± 0.0 <sup>a</sup> (43.8%)	8.5 ± 0.0 <sup>a</sup> (54.4%)	8.1 ± 0.0 <sup>a</sup> (62.2%)	5.3 ± 0.0 <sup>a</sup> (38.6%)	5.8 ± 0.0 <sup>a</sup> (46.7%)	5.4 ± 0.0 <sup>a</sup> (56.0%)	5.0 ± 0.0 <sup>a</sup> (66.8%)	4.7 ± 0.0 <sup>a</sup> (72.1%)				
n-Butanol fraction (200 mg/kg)	7.6 ± 0.0 <sup>a</sup> (14.4%)	7.4 ± 0.0 <sup>a</sup> (26.1%)	7.5 ± 0.0 <sup>a</sup> (43.0%)	7.5 ± 0.0 <sup>a</sup> (59.9%)	7.4 ± 0.0 <sup>a</sup> (60.5%)	7.3 ± 0.0 <sup>a</sup> (65.8%)	4.9 ± 0.0 <sup>a</sup> (44.0%)	5.3 ± 0.0 <sup>a</sup> (50.8%)	5.0 ± 0.0 <sup>a</sup> (59.3%)	4.7 ± 0.0 <sup>a</sup> (68.4%)	4.0 ± 0.0 <sup>a</sup> (76.6%)				
Aqueous fraction (50 mg/kg)	8.4 ± 0.0 <sup>a</sup> (5.9%)	8.7 ± 0.0 <sup>a</sup> (12.3%)	9.7 ± 0.0 <sup>a</sup> (26.64%)	9.6 ± 0.0 <sup>a</sup> (37.43%)	9.4 ± 0.0 <sup>a</sup> (49.36%)	9.4 ± 0.0 <sup>a</sup> (56.07%)	6.5 ± 0.0 <sup>a</sup> (24.91%)	7.5 ± 0.0 <sup>a</sup> (30.70%)	7.3 ± 0.0 <sup>a</sup> (39.85%)	7.2 ± 0.0 <sup>a</sup> (52%)	7.1 ± 0.0 <sup>a</sup> (56.24%)				
Aqueous fraction (100 mg/kg)	8.2 ± 0.0 <sup>a</sup> (7.6%)	8.6 ± 0.0 <sup>a</sup> (14.2%)	8.9 ± 0.1 <sup>a</sup> (32.6%)	8.8 ± 0.0 <sup>a</sup> (42.5%)	8.6 ± 0.0 <sup>a</sup> (54.0%)	8.5 ± 0.0 <sup>a</sup> (60.0%)	6.3 ± 0.0 <sup>a</sup> (28.0%)	6.5 ± 0.0 <sup>a</sup> (39.4%)	6.3 ± 0.0 <sup>a</sup> (48.3%)	6.2 ± 0.0 <sup>a</sup> (58.8%)	6.0 ± 0.0 <sup>a</sup> (63.1%)				
Aqueous fraction (200 mg/kg)	8.1 ± 0.0 <sup>a</sup> (9.4%)	8.2 ± 0.0 <sup>a</sup> (17.4%)	8.3 ± 0.0 <sup>a</sup> (37.3%)	8.2 ± 0.0 <sup>a</sup> (46.4%)	8.1 ± 0.0 <sup>a</sup> (56.6%)	7.8 ± 0.0 <sup>a</sup> (63.3%)	6.1 ± 0.1 <sup>a</sup> (30.3%)	6.3 ± 0.1 <sup>a</sup> (42.0%)	6.1 ± 0.1 <sup>a</sup> (50.0%)	5.8 ± 0.1 <sup>a</sup> (61.3%)	4.8 ± 0.0 <sup>a</sup> (70.4%)				

Values are expressed as mean ± SEM; n=5, done by Two-way ANOVA followed by Bonferroni post test used and p<0.05 was considered as significant as compared to arthritic control where <sup>a</sup> = p<0.001

Table III				
Effect of <i>Berberis calliobotrys</i> on complete Freund's adjuvant-induced arthritis in rats				
Treatment groups	Increase in paw diameter (mm)			
	Day 1	Day 5	Day 10	Day 15
Arthritic control (3 mL/kg)	7.2 ± 0.0	16.2 ± 0.0	20.5 ± 0.0	23.7 ± 0.0
Normal control	4.1 ± 0.1 <sup>a</sup>	4.2 ± 0.2 <sup>a</sup>	4.2 ± 0.1 <sup>a</sup>	4.2 ± 0.1 <sup>a</sup>
Standard aspirin (100 mg/kg)	5.9 ± 0.0 <sup>a</sup> (18.2%)	7.2 ± 0.0 <sup>a</sup> (55.1%)	6.3 ± 0.0 <sup>a</sup> (69.1%)	5.8 ± 0.0 <sup>a</sup> (75.4%)
Aqueous methanol extract (200 mg/kg)	5.5 ± 0.0 <sup>a</sup> (23.6%)	6.7 ± 0.0 <sup>a</sup> (58.5%)	5.7 ± 0.0 <sup>a</sup> (72.1%)	4.6 ± 0.0 <sup>a</sup> (80.6%)
n-Butanol fraction (200 mg/kg)	5.6 ± 0.0 <sup>a</sup> (21.8%)	7.1 ± 0.0 <sup>a</sup> (55.9%)	6.3 ± 0.0 <sup>a</sup> (69.0%)	5.3 ± 0.0 <sup>a</sup> (77.5%)
Aqueous fraction (200 mg/kg)	6.2 ± 0.0 <sup>a</sup> (13.7%)	9.0 ± 0.0 <sup>a</sup> (44.5%)	7.8 ± 0.0 <sup>a</sup> (61.8%)	7.1 ± 0.0 <sup>a</sup> (70.1%)

Values are expressed as mean ± SEM; n=5; Two-way ANOVA followed by Bonferroni post test used and p<0.05 was considered as significant as compared to arthritic control; Where <sup>a</sup> = p<0.001

Table IV						
Effect of <i>Berberis calliobotrys</i> on hematological parameters in arthritic rats						
Treatment groups	Hb (g/dL)	RBCs 10 <sup>6</sup> /μL	WBCs 10 <sup>3</sup> /μL	Platelets 10 <sup>3</sup> /μL	ESR mm/1 <sup>st</sup> hr	RF IU/mL
Arthritic control (3 mL/kg)	9.3 ± 0.1	4.9 ± 0.0	9.4 ± 0.2	1225 ± 105.3	20.3 ± 0.8	48.3 ± 2.0
Normal control	14.2 ± 0.2 <sup>a</sup>	7.4 ± 0.2 <sup>a</sup>	5.2 ± 0.1 <sup>a</sup>	311 ± 3.2 <sup>a</sup>	3.0 ± 0.5 <sup>a</sup>	14 ± 0.0 <sup>a</sup>
Aspirin (100 mg/kg)	12.6 ± 0.1 <sup>a</sup>	6.7 ± 0.1 <sup>a</sup>	7.7 ± 0.2 <sup>a</sup>	732.3 ± 4.6 <sup>a</sup>	12.3 ± 1.2 <sup>a</sup>	25.3 ± 2.3 <sup>a</sup>
Aqueous methanol extract (200 mg/kg)	13.3 ± 0.1 <sup>a</sup>	6.8 ± 0.0 <sup>a</sup>	6.4 ± 0.2 <sup>a</sup>	404.3 ± 4.1 <sup>a</sup>	9.6 ± 0.8 <sup>a</sup>	21.3 ± 1.7 <sup>a</sup>
n-Butanol fraction (200 mg/kg)	12.4 ± 0.1 <sup>a</sup>	6.5 ± 0.1 <sup>a</sup>	7.5 ± 0.2 <sup>a</sup>	454.3 ± 18.7 <sup>a</sup>	11.3 ± 0.8 <sup>a</sup>	24.0 ± 1.1 <sup>a</sup>
Aqueous fraction (200 mg/kg)	11.6 ± 0.1 <sup>a</sup>	6.3 ± 0.0 <sup>a</sup>	8.1 ± 0.18 <sup>b</sup>	561.3 ± 11.5 <sup>a</sup>	14.0 ± 1.1 <sup>b</sup>	32.6 ± 1.7 <sup>a</sup>

Values are expressed as mean ± S.E.M; n=3; One-way ANOVA followed by Dunnett's test used and p<0.05 was considered as significant when compared with arthritic control group where <sup>b</sup> = p<0.01, <sup>a</sup> = p<0.001

Table V				
Effect of <i>Berberis calliobotrys</i> on weight of CFA-induced arthritic rats				
Treatment groups	1 <sup>st</sup> day	5 <sup>th</sup> day	10 <sup>th</sup> day	15 <sup>th</sup> day
Arthritic control (3 mL/kg; distilled water)	262.0 ± 3.6	223.6 ± 3.9	218.2 ± 3.5	208.4 ± 4.5
Normal control	269.4 ± 6.0 <sup>ns</sup>	270.2 ± 5.5 <sup>a</sup>	270.6 ± 5.9 <sup>a</sup>	274.4 ± 6.1 <sup>a</sup>
Aspirin (100 mg/kg)	243.8 ± 3.0 <sup>ns</sup>	246.2 ± 3.5 <sup>c</sup>	243.6 ± 2.9 <sup>b</sup>	245.4 ± 3.7 <sup>a</sup>
Aqueous methanol extract (200 mg/kg)	264.4 ± 8.5 <sup>ns</sup>	267.0 ± 10.0 <sup>a</sup>	265.6 ± 9.2 <sup>a</sup>	269.2 ± 8.5 <sup>a</sup>
n-Butanol fraction (200 mg/kg)	271.0 ± 4.1 <sup>ns</sup>	268.0 ± 5.1 <sup>a</sup>	269.0 ± 4.0 <sup>a</sup>	273.6 ± 2.6 <sup>a</sup>
Aqueous fraction (200 mg/kg)	272.6 ± 2.5 <sup>ns</sup>	264.0 ± 3.3 <sup>a</sup>	264.6 ± 2.2 <sup>a</sup>	270.8 ± 1.8 <sup>a</sup>

Values are expressed as mean ± SEM; n=5; done by Two-way ANOVA followed by Bonferroni post test and p<0.05 was considered as significant as compared to arthritic control where ns= non-significant, <sup>c</sup> = p<0.05, <sup>b</sup> = p<0.01 and <sup>a</sup> = p<0.001.





Figure 1: Effect of aspirin and *Berberis calliobotrys* on paw diameter of rats in CFA-induced arthritis

that the degree of periarticular bone resorption, bony erosion, joint space narrowing, tissue puffiness was markedly reduced with complete protection against joint destruction in rats treated with extract of *B. calliobotrys* (200 mg/kg). In the same manner, butanol fraction

showed minor swelling, periarticular bone resorption & bony erosion, no joint space narrowing and visibly no joint damage. Whereas, aqueous fraction showed acute soft tissue swelling, periarticular bone resorption & bony erosions, distinct joint space diminution, and

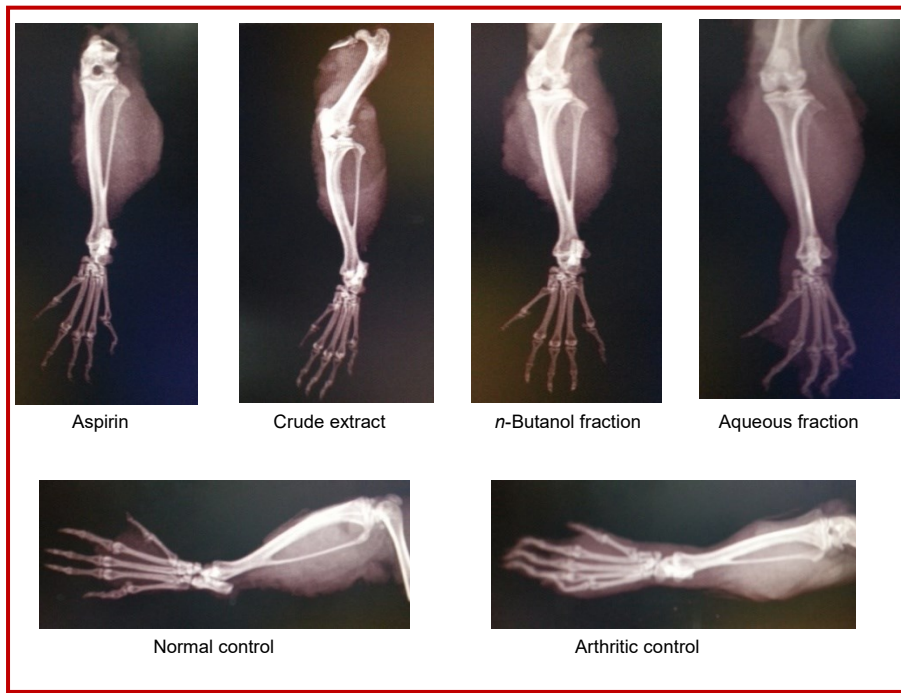


Figure 2: Radiographic analysis of hind limbs of Sprague Dawley rats in CFA-induced arthritis



Figure 3: Effects of different treatments on histopathological changes in ankle joints of Sprague Dawley rats

noticeable joint deformity. Likewise, mild changes in soft tissue inflammation, periarticular bone resorption & bony erosions with definite joint space reduction and fewer joint changes were evident in aspirin treated rats. While, X-ray examination of normal healthy rats verified no joint tissue swelling, normal bone and cartilage morphology as depicted in (Figure 2). Histopathological examination of ankle joint interpret that normal

rats had intact morphology of synovium with no signs of inflammation. Arthritic rats showed perceptible cartilage destruction, influx of inflammatory cells, pronounced pannus formation, disturbed synovial lining, fibrin deposition, and chronic inflammation and erosive changes in cartilage and bone. However, aspirin (100 mg/kg) treated rats revealed mild cartilage destruction, modest influx of inflammatory cells and

synovial space thickening and restricted pannus formation. Similarly, crude extract of *B. calliobotrys* (200 mg/kg) treated rats corroborated substantial protection against cartilage destruction, synovial space thickening, vascular proliferation, very low influx of inflammatory cells and no pannus formation nearly normal joint architecture of injected paw. Likewise, butanolic fraction (200 mg/kg) certified trivial cartilage destruction and synovial space thickening, influx of few inflammatory cells and no pannus formation. On the other hand, aqueous fraction (200 mg/kg) illustrated moderate cartilage destruction, influx of inflammatory cells in synovium, synovial space thickening with evidence of disturbed synovial lining or pannus formation (Figure 3).

## Discussion

Several classes of drugs such as non-steroidal anti-inflammatory drugs, corticosteroids, DMARDs and biologics are currently being used in the treatment of RA but their side effects and toxicities leave a necessity for new and more powerful natural agents. Since, natural products or bioactive compounds derived from them represent immense structural diversity, which is not commonly seen in synthetic compounds. Owing to these reasons, the present investigation was carried out to scrutinize the anti-arthritic effect of traditionally used plant *B. calliobotrys*. The findings of current study have revealed the dose-dependent antiarthritic effect of aqueous methanolic extract, *n*-butanol and aqueous fraction of *B. calliobotrys* in both *in vitro* methods as well as *in vivo* animal models. As previous researches have reported that denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases and production of autoantigens in certain rheumatic diseases may be due to *in vivo* protein denaturation. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding (Gupta et al., 2013). It has been avowed that several non-steroidal anti-inflammatory drugs for instance indomethacin, flufenamic acid, diclofenac sodium, salicylic acid avert the denaturation of BSA at pathological pH (6.2-6.5) (Williams et al., 2008). In current investigation aqueous methanol extract of *B. calliobotrys* showed maximum inhibition of protein denaturation (bovine serum albumin, egg albumin), whereas, among fractions butanolic fraction has been found to produce comparable anti-denaturation effect with standard drug (Table I). Thus, it can be assumed on the basis of present findings that the ability of preventing protein denaturation of *B. calliobotrys* could be responsible for its antiarthritic effect.

As erythrocyte membrane is akin to that of lysosomal membrane and inhibition of RBC hemolysis in hypo-

tonic milieu offer an additional mechanism of anti-arthritic effect. Because hypotonic solution causes the massive accretion of fluid within the cell resulting in rupturing of its membrane. Since, damage to the lysosomal membrane instigate the release of phospholipase A2 that brings about the hydrolysis of phospholipids to generate the inflammatory intermediaries (Umukoro and Ashorobi, 2006; Aitadafoun et al., 1996). Hence, stabilization of membrane prevents the cell rupture and successive release of the cellular contents, which consequently alleviate the tissue damage and aggravation of the inflammatory response (Okoli et al., 2008). The results imply that aqueous methanol extract, butanol and aqueous fractions of *B. calliobotrys* offer concentration dependent protection of HRBC membrane against hypotonicity induced lysis compared with standard diclofenac sodium. Thus, the data obtained has demonstrated the adequate membrane stabilizing property of *B. calliobotrys*. Direct interaction of phytochemical constituents with membrane components such as proteins seems nearly all plausible, even though, the exact mechanism of membrane stabilization is not recognized (Umukoro and Ashorobi, 2006). Turpentine oil-induced joint edema is characterized by increased vascular permeability and marked vasodilatation. The pathogenesis of turpentine oil induced arthritis entail a chronological release of the inflammatory mediators such as histamine and serotonin in early phase, kinin like substances in intermediate phase and prostaglandins in late phase. The acute inflammatory response in the knee joint of rat stimulated by turpentine oil was reduced in a dose-dependent approach by oral administration of *B. calliobotrys* at 50, 100 and 200 mg/kg (Table II). The doable inhibitory effect of *B. calliobotrys* on different phases of inflammation elicited in turpentine oil-induced joint edema might be due to inhibition of either lipoxygenase or cyclooxygenase enzymes (Riaz et al., 2007; Gautam et al., 2011). Formaldehyde induced arthritis is one of most commonly used acute non-immunological arthritis model for investigating the anti-arthritic potential and anti-proliferative activity of a plant extract (Greenwald, 1991). Studies have been reported that swelling around the ankle joint and paw of arthritic rats following the injection of formaldehyde may be owing to edema of particular tissues such as ligament and capsule (Rakesh and Prashant, 2012). Furthermore, formaldehyde produce localized inflammation and distinct biphasic pain that is to say an early neurogenic pain afterward tissue mediated response (Owoyele et al., 2011). In the present study hydroalcoholic extract, *n*-butanol and aqueous fractions of *B. calliobotrys* at the selected dose levels corroborated perceptible antiarthritic activity by reducing the paw swelling and soft tissue thickening at the depth side throughout the observation period (Table II). Results of this trial have also depicted that *B. calliobotrys* has the capability of acting both centrally as well as

peripherally in abating the biphasic pain possibly by inhibiting the arachidonic acid pathway in comparison with aspirin.

Complete Freund's adjuvant induced arthritis is a well established animal model in which clinical and pathological alterations are akin to those seen in human rheumatoid arthritis. Complete Freund's adjuvant is a mixture of heat killed *Mycobacterium tuberculosis* with liquid paraffin which stimulates cell-mediated immunity thus, potentiating the production of certain immunoglobulins in body (Newbould, 1963). Adjuvant induced arthritis in the rat can be alienated into three distinctive phases firstly the induction phase without the manifestation of synovitis, followed by early synovitis, and finally late synovitis accompanied by unremitting cartilage and joint tissue destruction (Woode et al., 2009). In the current study, CFA injected into the left hind paw of rat initiated an inflammatory response as primary lesions within 3-5 days subsequent to injection followed by secondary lesions which appeared after a delay of 11-12 days, typified as inflammation of hind legs (non-injected sites) (Vogel, 2002). Since, determination of rat paw swelling is an easy, sensitive and one of the quick procedures for assessing the extent of inflammation and the curative efficacy of drugs (Eric et al., 1996). Therefore, the inhibition of rat paw swelling by methanolic extract, *n*-butanol and aqueous fractions of *B. calliobotrys* (80.6%, 77.5% and 70.1%), correspondingly, might be due to its inhibitory effect on neutrophil infiltration, a critical event in inflammation as confronted by aspirin and other NSAIDs, pointing towards its anti-inflammatory potential (Vogel, 2002).

On the other hand, expression of secondary lesions in non-injected sites is due to the activation of proliferative T cells by Freund's adjuvant, which are the core reservoir of pro-inflammatory mediators. Immunosuppressant alleviate arthritis by offsetting such immunological reaction, which is not inhibited by anti-inflammatory agents as supported by the results of this test model where *B. calliobotrys* appreciably diminished or prevented the swelling of non-injected sites as compared to aspirin (Vogel, 2002). Adjuvant induced arthritis model offers an opportunity to examine the pathological changes in a variety of tissues other than joints. Anemia is the most common extracellular manifestation in RA (Hochberg et al., 1988) and may be caused by the decreased level of plasma iron due to sequestration of iron in the reticuloendothelial system and synovial tissue ultimately failure of bone marrow to counter anemia (Mowat, 1971). IL-1 in association with the acute phase response also decrease plasma iron content (Connolly et al., 1988) and it is challenging to speculate that the sequestration of less deformable erythrocytes by endothelial cells in the spleen also plays a causative role in shortened half life of erythrocytes thus, resulting in anemia. Alternatively, a rise in both

WBC and platelet counts might be due to the stimulation of immune system against the invading pathogenic microorganism and it is evident by the influx of inflammatory mononuclear cells in the joints of arthritic rats. In the present experiment *B. calliobotrys* and aspirin treated groups had considerably increased level of hemoglobin and RBC while, the level of WBC and platelets was significantly reduced in contrast to arthritic control group but comparable to normal control group (Table IV). Similarly, erythrocyte sedimentation rate is an imperative hematological index for the diagnosis as well as prognosis of infectious and inflammatory diseases.

With reference to aspirin *B. calliobotrys* extract together its fractions remarkably decreased ESR count in arthritic rats, thus justifying its significant role in arthritic conditions. RF, a key serologic marker, is an auto-antibody directed against the Fc portion of IgG and form immune complexes that contribute towards the succession of rheumatoid arthritis (Arnett et al., 1988; Scott et al., 2010). A note-worthy decrease in RF level in the serum of arthritic rats treated with aqueous methanolic extract, *n*-butanol and aqueous fraction unveil the protective effect of *B. calliobotrys* against rheumatoid arthritis (Table IV). From these hematological findings, it can be proposed that *B. calliobotrys* shifts the alterations in blood parameters towards normal by inhibiting the inflammatory response which might be due to its blocking action on pro-inflammatory cytokines and cyclo-oxygenase enzyme as well as suppressing the immune response as supported by previous studies (Ivanovska et al., 1999; Chatterjee and Pal, 1984). Rheumatoid cachexia refers to weight loss or muscle wasting, a distressing complication of rheumatoid arthritis. The rationale for this comorbidity may be an accelerated protease mediated degradation of muscle protein devoid of any modification in protein synthesis in investigational arthritis (Fagan et al., 1987).

The data obtained from the *in vitro* antiarthritic membrane stabilization method may be extrapolated to the effect of *B. calliobotrys* against muscle wasting. Similarly, rheumatoid cachexia is thought to be the outcome of cytokine mediated hypermetabolism (Roubenoff et al., 1997). Earlier reports have also recommended that absorption of <sup>14</sup>C-glucose and <sup>14</sup>C-leucine in rat's intestine become reduced during inflammatory condition (Somasundaran et al., 1983a) and anti-inflammatory drugs rectify the impaired absorption capacity of intestine (Somasundaran et al., 1983b). Results of present study have shown that aqueous methanolic extract, *n*-butanol fraction and aqueous fraction of *B. calliobotrys* possess significant protective effect on body weight of arthritic rats. Moreover, histopathological slides of ankle joints interpreted that the treatment with *B. calliobotrys* (200 mg/kg)

decreased vascularity, lymphocytic infiltration with less rheumatoid inflammation and angiogenesis, with no thickening of synovial membrane as compared to standard drug aspirin 100 mg/kg treated group. Degeneration of the ankle joint was not observed in any of the drug treated groups when compared with the negative control (Figure 2 and 3). Thus, histopathological findings have revealed the antiarthritic effect of *B. calliobotrys* by inhibiting the inflammatory response which might be due to its blocking action on pro-inflammatory cytokines and cyclooxygenase enzyme (Ivanovska et al., 1999; Chatterjee and Pal, 1984). Berberine (an isoquinoline alkaloid) is a pharmacologically potent constituent found in numerous plants of the genus *Berberis* and *Coptis* (Ye et al., 2009). Berberine has been shown to exert immunosuppressive and anti-inflammatory effects by suppressing Th17 and dendritic cell responses in several autoimmune diseases (Yang et al., 2013). Moreover, berberine is also involved in alleviating the joint inflammation and intense pain by inhibiting NF- $\kappa$ B, COX-2, TNF- $\alpha$ , IL-1 $\beta$ , IL-6 most prevailing factors in arthritis (Ivanovska et al., 1999; Chatterjee and Pal, 1984).

## Conclusion

*B. calliobotrys* is a medicinally valuable plant and its antiarthritic effect might be due to its anti-inflammatory, antioxidant and immunosuppressant actions, although, actual mechanism is not known

## Financial Issue

Self-funded

## Ethical Issue

All animals were treated in accordance with the guidelines of the NIH and approved by the Institutional Animal ethical committee at the Faculty of Pharmacy University of Sargodha.

## Conflict of Interest

Authors declare no conflict of interest

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