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Antiarthritic activity of ethanolic seed extracts of *Diplocyclos palmatus* (L) C. Jeffrey in experimental animals

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ABSTRACT

To evaluate the antiarthritic activity of ethanolic seed extract of Diplocyclos palmatus (L) C. Jeffrey in rodents. The antiarthritic activity of ethanolic extract of Diplocyclos palmatus (L) C. Jeffrey (EEDP) was studied against Freund's complete adjuvant induced arthritis in rats. The EEDP was administered at the doses of 100, 200 and 400 mg/kg body weight. The parameters including paw volume, joint diameter, thermal and mechanical hyperalgesia, biochemical parameters, haematological parameters and histopathology of synovial joint was observed. Diclofenac was taken as standard. EEDP showed significant (P<0.001) anti-inflammatory activity by reducing increased paw volume and joint diameter in arthritic rats. EEDP at 100, 200 and 400 mg/kg significantly (P<0.001) increased the animals paw mechanical and thermal hyperalgesia. EEDP (200 and 400 mg/kg) exhibits anti-arthritic activity by improving the altered haematological parameters (CRP, WBC, RBC, platelets and Hb) and biochemical parameters (AST, ALT and ALP). Histopathological examination showed reduced cell infiltration and erosion of joint cartilage in EEDP treated arthritic rats. This study validates the ethnomedicinal use of ethanolic extract of Diplocyclos palmatus (L) C. Jeffrey seeds in the treatment of pain, inflammation and arthritic conditions.

Key words: Rheumatoid arthritis, *Diplocyclos palmatus* (L) C. Jeffrey, Freund's complete adjuvant., Hyperalgesia, Synovial joint

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease affecting about 1% of the population in developed countries [1]. Although the disease can start at any age, the peak onset is between 25 and 55 years, women being affected about three times more frequently than men [2]. RA primarily affects the synovial joints of all extremities and less often the spinal column and is pathologically characterized by severe inflammation and progressive destruction of cartilage and subchondral bone. Recent data have shown that pathological changes occur early during the disease [3]. Rheumatoid arthritis is characterized by a series of pathological processes of the joints, such as leukocyte infiltration, pannus formation and extensive destruction of the articular cartilage and bone[4].

The most commonly prescribed medication for RA treatment is steroidal, non-steroidal anti-inflammatory drugs (NSAIDs), disease modifying antirheumatic drugs (DMARDs) and immunosuppressant drugs [5]. The goal of these drugs has been to relieve pain, decrease joint inflammation, and prevent joint destruction and to restore function of disabled joints [6]. However, the side effects of currently available drugs include gastric ulcer, renal damage, bronchospasm and cardiac abnormalities have limited their use [7]. Many researchers have focused in recent years on medicinal plants derived natural products such as flavonoids, steroids, polyphenols, coumarins, terpenes and

alkaloids due to their wide range of pharmacological significance including analgesics, anti-inflammatory and antiarthritic activities with lesser side effects [8,9].

Diplocyclos palmatus (L) C. Jeffrey syn *Bryonia lacinosa* (N.O. Cucurbitaceae) plant locally known as 'Shivlingi' is distributed throughout India, an annual climber with bright red fruit and is reported to be highly medicinal [10]. Locally in India its seeds are being used for promoting conception in women. Plant is used against snake-bite. Its leaves are used in inflammation [11]. Roots are used for treatment of asthma. The seeds are used for increasing sperm count also as an aphrodisiac [12]. The main active constituents of the plants are Bryonin, a bitter principle [13]; punicic acid, source of seed oil [14]; non ionic glucomannon [12]; and goniothalamin [15].

The objective of the study was to evaluate antiarthritic activity of ethanolic extract of the seeds of *Diplocyclos palmatus* (L) C. Jeffrey in rodents.

MATERIALS AND METHODS

2.1 Procurement of Plant material

Seeds of *Diplocyclos palmatus* (L) C. Jeffrey were obtained from a commercial supplier of Pune and then identified and authenticated by Department of Botany, Agharkar Research Institute, Pune, India and voucher specimen is deposited at that Institute.

2.2 Extraction Procedure

The seeds of the plants were dried in shade and powdered. Dried powder (300 g) was subjected to ethanol extraction by maceration for 48 h. The percentage yields of ethanol extract of *Diplocyclos palmatus* (L) C. Jeffrey (EEDP) was 4.5 g.

2.3 Drugs and Chemicals

Freund's Complete Adjuvant (FCA) was purchased from Sigma-Aldrich, St. Louis, MO, USA. Biochemical diagnostic kits of aspartate aminotransferase, alanine aminotransferase, total protein and alkaline phophatase (Accurex biomedical Pvt. Ltd) were purchased from authorized vendors.

2.4 Experimental animals

Female Wistar rats weighing 200-250 g and female Swiss albino mice weighing 20-25 g were used for the study. The animals were procured from National Toxicology Centre, Pune and housed in the animal house maintained under standard hygienic conditions, at 25 ± 2^{o}C, humidity (60 ± 10%) with 12 hour day and night cycle, with food and water *ad libitum*. The study was carried out as per CPCSEA norms after obtaining approval (CPCSEA/01/2011) from the Institutional Animal Ethical Committee of college.

2.5 Acute oral toxicity study

Healthy female Swiss albino mice were subjected to acute toxicity studies as per OECD guidelines-425. The animals were fasted overnight and divided into groups with 5 animals in each group. Extract (EEDP) were administered orally at one dose level of 2000 mg/kg body weight. The mice were observed continuously for behavioral and autonomic profiles for 2 h and for any sign of toxicity or mortality up to 48 h [16].

2.6 Freund's complete adjuvant induced arthritis

Arthritis was induced by the indradermal injection of 0.1 ml of Freund's complete adjuvant (FCA, Sigma) in the right hind paw. The animals were divided into six groups of six animals each as follows:

Group 1- Non-arthritic control, 2% Tween 80, p.o.
Group 2- Arthritic control, 2% Tween 80, p.o.
Group 3- Arthritic animals treated with standard, diclofenac 5 mg/kg, p.o.
Group 4- Arthritic animals treated with test drug, EEDP 100 mg/kg, p.o.
Group 5- Arthritic animals treated with test drug, EEDP 200 mg/kg, p.o.
Group 6- Arthritic animals treated with test drug, EEDP 400 mg/kg, p.o.
The dosing of all the groups was started from day 12, once day [17].

The following parameters were measured at regular intervals (Day 0, 1, 4, 8, 12, 16, 20, 24 and 28) body weight, paw volume, joint diameter, thermal hyperalgesia and mechanical hyperalgesia. On day 28, blood was withdrawn by retro-orbital puncture and used for biochemical assays. The animals were sacrificed on day 28 to study the synovial joint histology.

2.6.1 Quantification of paw edema

The severity of arthritis was quantified by measuring volume of hind paws using a plethysmometer. Paw volume (ml) was measured on days 0, 1, 4, 8, 12, 16, 20, 24 and 28 after arthritis induction. Data were expressed as the volume of increase with respect to day 0 volume [18].

2.6.2 Joint diameter

Before injection joint diameter were measured using a digital vernier caliper (Mitutoyo digimatic caliper, Japan) after which adjuvant was administered. The joint diameter was measured again on day 1, 4, 8, 12, 16, 20, 24 and 28 [19].

2.6.3 Thermal hyperalgesia

Thermal hyperalgesia was tested to evaluate the effect of EEDP and a noxious thermal stimulus was determined using a thermal planter tester (UGO Basile, Italy). Briefly, rats were acclimatized to the testing room for at least 10 min prior to start of behavioral testing. Following acclimation, radiant heat was applied to the planter surface of the hind paw until the rat lifted its paw. A photoelectric cell automatically tuned the heat surface off when the reflected light beam was interrupted (i.e. when the animal withdrew its paw) and the time at which this occurred was recorded as the paw withdrawal latency (PWL). The cut-off time was 15 s [20].

2.6.4 Mechanical hyperalgesia

Mechanical hyperalgesia of hind paws was evaluated by Von Frey Hairs (Almemo, Germany) of increasing gauge. The animals were allowed to acclimatize for 10 min in the Perspex box and Von Frey hairs (0.6 to 12.6g) were applied to planter surface to hind paws. A series of three stimuli were applied to each paw for each hair within a period 2-3 s. the lowest weight of Von Frey hair to evoke a withdrawal from the three consecutive applications was considered to indicate the threshold [21, 22].

2.6.5 Biochemical assays

The haematological parameters like haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), platelets and C-reactive protein (CRP) were determined by usual standardized laboratory method. The biochemical parameters like aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein (TP), and alkaline phophatase (ALP) were also determined [23].

2.6.6 Histological analysis

Rats were sacrificed on 28th day; hind limbs were removed and fixed in 10% buffered formalin. The limbs were decalcified in 5% formic acid, processed for paraffin embedding, sectioned at 5µm thickness, and subsequently stained with haematoxylin-eosin for examination under a light microscope with 10x magnifications. Sections were examined for the presence of hyperplasia of the synovium, pannus formation and destruction of the joint space [24-26].

2.7 Statistical analysis

The data was analyzed by one way ANOVA followed by Dunnett's test, two way ANOVA followed by Bonferroni's post hoc test. All statistical analyses were performed using Graph Pad Prism software (San Diego, CA). Data was considered statistical significant at $P<0.05$.

RESULTS

3.1 Effects of EEDP seeds on right hind paw edema of FCA-induced in rats:

One day after the FCA injection, primary arthritis of the right hind paw was induced, and inflammation was steady maintained for 28 days (Figure 1). Treatment with EEDP (100, 200 and 400 mg/kg) significantly reduced paw edema of right hind paw. A dose dependent effect was observed i.e. higher level of significance ($P<0.001$) with 200 and 400 mg/kg dose compared to $P<0.05$ at lower dose 100 mg/kg. Diclofenac 5 mg/kg showed significant ($P<0.001$) inhibition of right hind paw edema on days 20, 24 and 28.

Figure 1: Effect of oral administration of ethanol extract of *Diplocyclos palmatus* (L) C. Jeffrey seeds on right hind paw volume in arthritic rats. Data are expressed as mean ± S.E.M.; n=6 rats per group. Two way ANOVA followed by Bonferroni's post hoc test when compared with arthritic control group *P<0.05, **P<0.01, ***P<0.001.

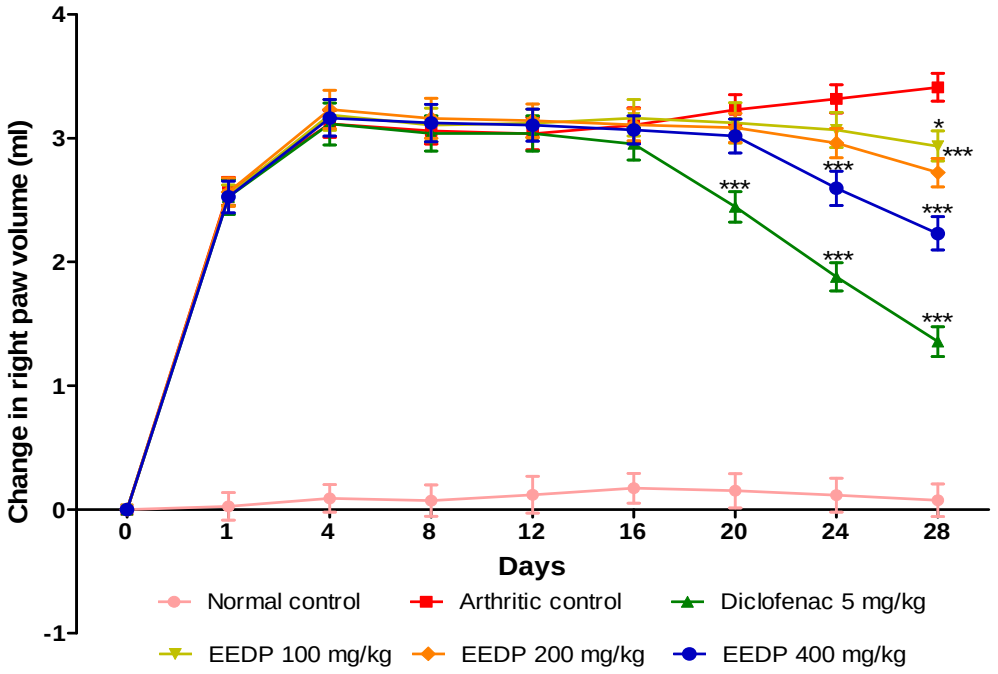
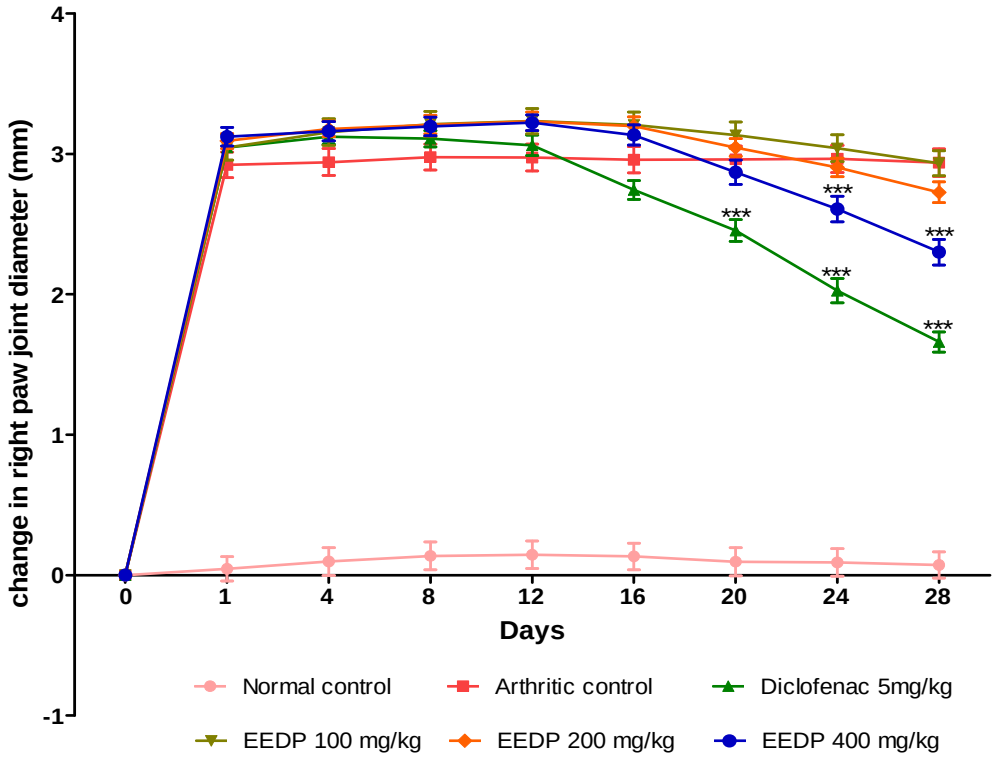


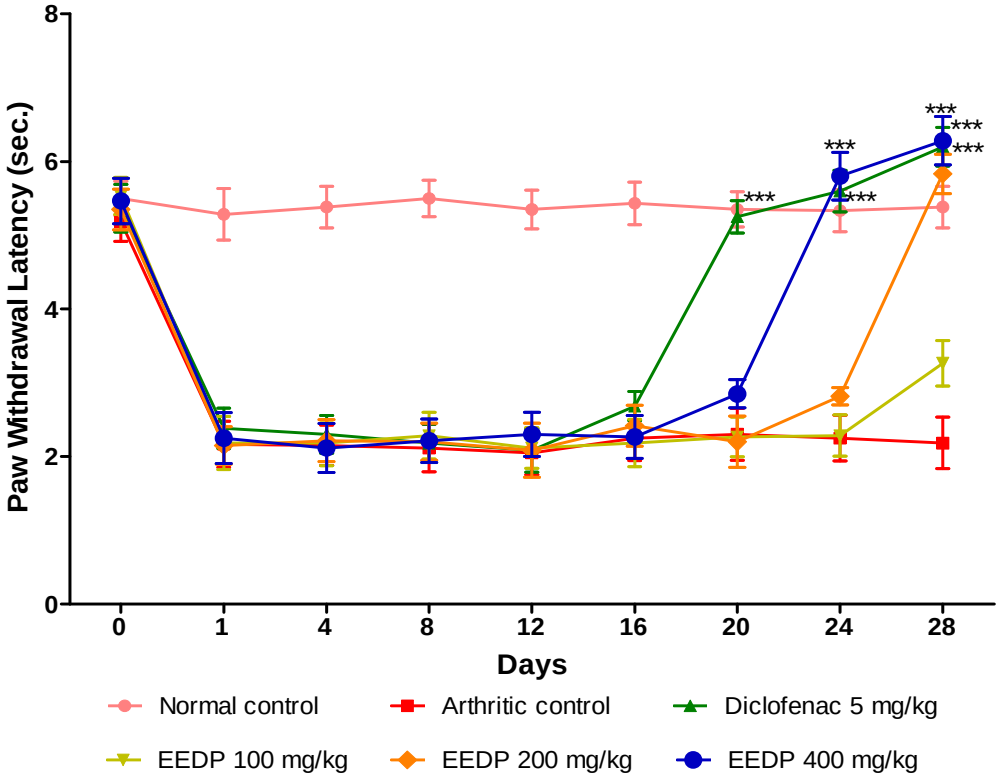
Figure 2: Effect of oral administration of ethanol extract of *Diplocyclos palmatus* (L) C. Jeffrey seeds on right hind paw joint diameter in arthritic rats. Data are expressed as mean ± S.E.M.; n=6 rats per group. Two way ANOVA followed by Bonferroni's post hoc test when compared with arthritic control group *P<0.05, **P<0.01, ***P<0.001.



3.2 Effects of EEDP seeds on right hind joint diameter of FCA-induced in rats:
There was a significant increase in the joint diameter in all the FCA induced arthritis groups when compared to the normal control (Figure 2). Administration of 5 mg/kg of diclofenac and 400 mg/kg of EEDP from day 12 onwards significantly (P<0.001) decreased the right paw joint diameter. This decrease in joint diameter was observed at days 20, 24 and 28.

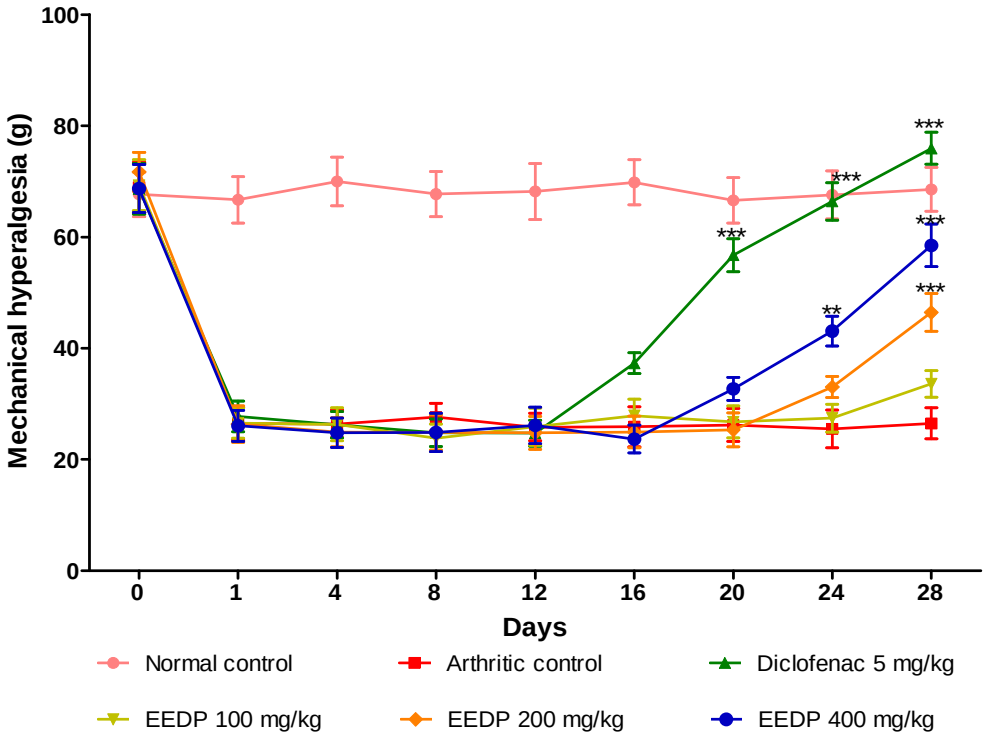
3.3 Effects of EEDP seeds on right hind paw thermal hyperalgesia of FCA-induced in rats:
The anti-nociceptive effect of EEDP seeds in chronic arthritis model was evaluated using plantar tests in FCA-induced arthritis rats (Figure 3). For 28 days, the paw withdrawal latency (PWL) of the right hind paw of arthritic rats was decreased compared to arthritic control. Oral administration diclofenac 5 mg/kg and EEDP 400 and 200 mg/kg showed significant (P<0.001) recovery from decreased right hind PWL on day 24 and 28.

Figure 3: Effect of oral administration of ethanol extract of *Diplocyclos palmatus* (L) C. Jeffrey seeds on right hind paw withdrawal latency in arthritic rats. Data are expressed as mean ± S.E.M.; n=6 rats per group. Two way ANOVA followed by Bonferroni's post hoc test when compared with arthritic control group *P<0.05, **P<0.01, ***P<0.001.



3.4 Effects of EEDP seeds on right hind paw mechanical hyperalgesia of FCA-induced in rats:
The mechanical hyperalgesia of the right hind paw was decreased compared to the basal level starting 1 day after the FCA injection (Figure 4). The oral treatment of 5 mg/kg of diclofenac significantly (P<0.001) suppressed the mechanical hyperalgesia at days 20, 24 and 28, while the treatment of 400 and 200 mg/kg of EEDP significantly (P<0.001) suppressed the mechanical hyperalgesia at days 24 and 28.

Figure 4: Effect of oral administration of ethanol extract of *Diplocyclos palmatus* (L) C. Jeffrey seeds on right hind paw mechanical hyperalgesia in arthritic rats. Data are expressed as mean ± S.E.M.; n=6 rats per group. Two way ANOVA followed by Bonferroni's post hoc test when compared with arthritic control group *P<0.05, **P<0.01, ***P<0.001.



3.5 Effects of EEDP seeds on biochemical parameters of FCA-induced in rats:

The challenge with FCA (0.1ml) showed significant (P<0.001) elevation of the serum AST, ALT, ALP and CRP level and decrease in the TP level, when compared to normal control. The treatment with EEDP 400 mg/kg showed significant (P<0.001) reduction of the serum AST, ALT, ALP and CRP as compared to arthritis control, whereas the effect on TP showed insignificant result. Diclofenac 5 mg/kg showed significant (P<0.05) reduction of the serum AST and ALT, where as ALP and TP showed insignificant results when compared to arthritis control (Table 1).

Table 1: Effect of oral administration of ethanol extract of *Diplocyclos palmatus* (L) C. Jeffrey seeds on biochemical parameters in arthritic rats.

Group	Normal control	Arthritic control	Diclofenac 5 mg/kg	EEDP 100 mg/kg	EEDP 200 mg/kg	EEDP 400 mg/kg
AST (U/L)	41 ± 3.2	129 ± 2.7	117 ± 3.1*	121 ± 2.7	118 ± 1.9*	88 ± 2.6***
ALT (U/L)	52 ± 1.7	186 ± 1.7	176 ± 3*	181 ± 1.9	173 ± 2.5**	125 ± 3.9***
ALP (U/L)	77 ± 2.1	451 ± 2.8	454 ± 2.5	438 ± 3.7*	434 ± 3.6**	396 ± 4.0***
Total protein (gm/dl)	6.9 ± 0.27	5.7 ± 0.17	5.7 ± 0.34	5.5 ± 0.17	5.8 ± 0.16	6.1 ± 0.15
CRP (mg/lit.)	1.6 ± 0.14	7.7 ± 0.30	2.5 ± 0.2***	7.3 ± 0.19	6.5 ± 0.28**	4 ± 0.26***

Data are expressed as mean ±S.E.M.; n=6 rats per g roup. One way ANOVA followed by Dunnett's test when compared with arthritic control group *P<0.05, **P<0.01, ***P<0.001.

3.6 Effects of EEDP seeds on haematological parameters of FCA-induced in rats:

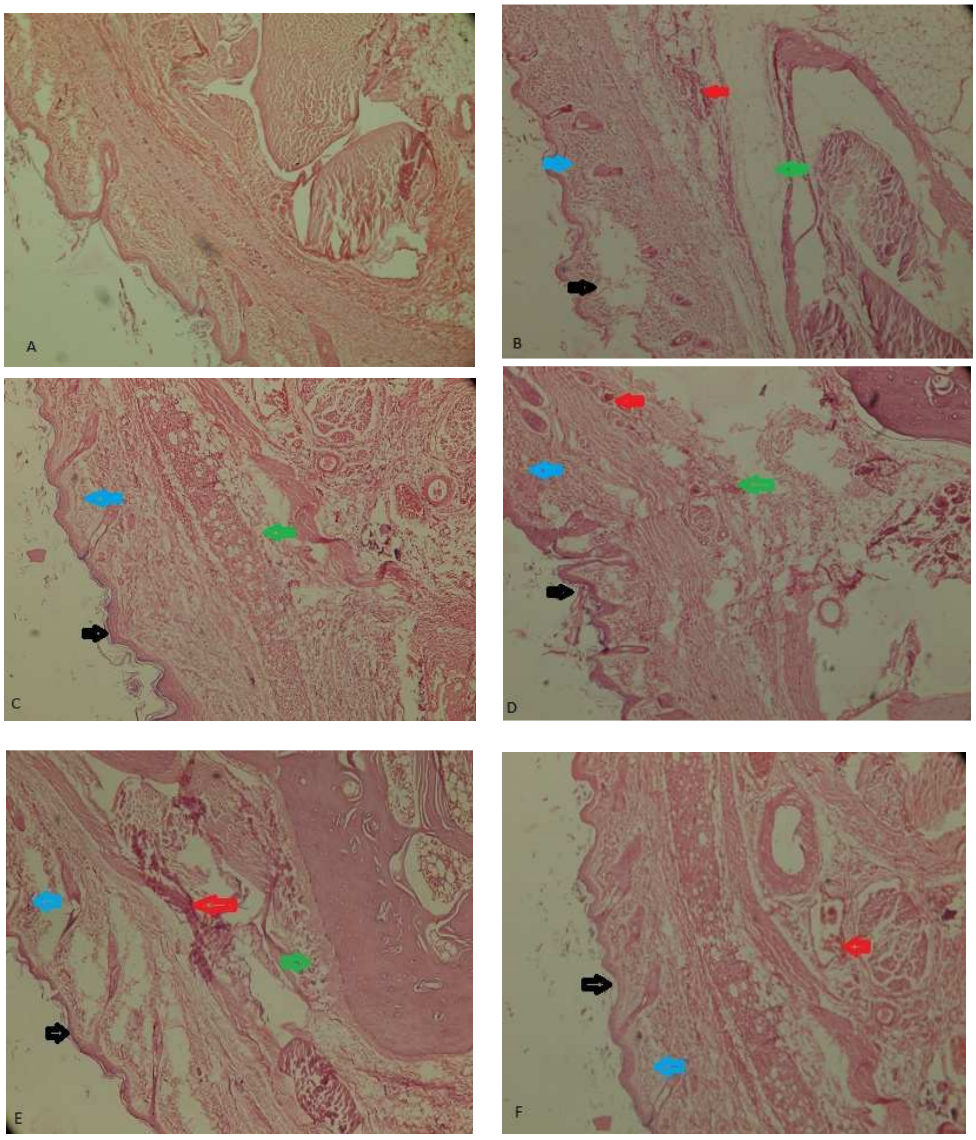
Levels of Hb, and RBC were decreased in arthritic rats with concomitant increases in WBC and platelet count. These changes were significantly (P<0.001) reverted to near normal levels in diclofenac 5 mg/kg and EEDP 400 mg/kg treated animals. Diclofenac showed a profound effect than EEDP. (Table 2)

Table 2: Effect of oral administration of ethanol extract of *Diplocyclos palmatus* (L) C. Jeffrey seeds on haematological parameters in arthritic rats.

Group	Normal control	Arthritic control	Diclofenac 5 mg/kg	EEDP 100 mg/kg	EEDP 200 mg/kg	EEDP 400 mg/kg
Hb (gm/100ml)	14 ± 0.21	9 ± 0.16	14 ± 0.08***	9.4 ± 0.15	9.6 ± 0.27	13 ± 0.11***
WBCs (thousands/µl)	7.5 ± 0.26	15 ± 0.26	9.1 ± 0.15***	15 ± 0.21	14 ± 0.11**	10 ± 0.15***
RBCs (million/µl)	7 ± 0.10	3.3 ± 0.12	6.3 ± 0.12***	3.5 ± 0.19	4.1 ± 0.22**	5.6 ± 0.13***
Platelets (lacks/µL)	9.6 ± 0.21	18 ± 0.17	12 ± 0.16***	18 ± 0.12	17 ± 0.25*	15 ± 0.27***

Data are expressed as mean ±S.E.M.; n=6 rats per group. One way ANOVA followed by Dunnett's test when compared with arthritic control group $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

Figure 5: Histopathology of synovial joint. (A) Normal non-arthritic (B) Arthritic control, (C) Diclofenac 5 mg/kg treated, (D) EEDP 100 mg/kg treated, (E) EEDP 200 mg/kg treated, (F) EEDP 400 mg/kg treated. (Black arrow – synovial lining, Blue arrow – influx of inflammatory cells, Red arrow – pannus formation, Green arrow – cartilage destruction)



3.7 Histopathology of Synovial joint:

Histopathology of synovial joint of normal rats showed intact morphology of synovium and synovial lining. No inflammation and influx of inflammatory cells was observed. FCA treated rats showed cartilage destruction, influx of inflammatory cells, pannus formation, disturbed synovial lining and chronic inflammation. Diclofenac treated rats

showed significant protection against cartilage destruction, vascular proliferation and synovial space thickening, low influx of inflammatory cells and no pannus formation. EEDP 400 mg/kg treated rats showed significant lesser cartilage destruction, synovial space thickening, vascular proliferation, low influx of inflammatory cells and no pannus formation. EEDP 200 mg/kg treated rats showed moderate cartilage destruction and synovial space thickening, influx of few inflammatory cells. EEDP 100 mg/kg treated rats showed minimal inflammation, influx of few inflammatory cells in synovium with evidence of disturbed synovial lining or pannus formation. (Figure 5)

DISCUSSION

The antiarthritic activity of ethanol extract of *Diplocyclos palmatus* (L) C. Jeffrey was investigated using the FCA induced arthritis model. The adjuvant containing 10 mg heat killed *Mycobacterium tuberculosis* in 1 ml paraffin oil. Rat adjuvant induced arthritis is a commonly used animal model for preclinical studies of non-steroidal anti-inflammatory drugs and disease-modifying anti-rheumatic drugs and it is suggested as the most convenient model for studying drugs affecting human arthritis [27] and has often been used to study the mechanisms of action and preventive effects of a number of disease-modifying anti-rheumatic drugs [28]. The phases of the adjuvant arthritis investigated in the present study were acute and polyarthritis/chronic phases corresponding to day 0-10 and day 10-28 post adjuvant inoculation, respectively [29]. The acute stage of arthritis is characterized by signs of hyperalgesia, lack of mobility and pause in body weight gain; during the acute period, hind paw and fore paw joint diameters increases [30]. In the later acute stages of disease (day 10+), rats with adjuvant arthritis are often relatively immobile due to the severity of paw swelling. It seems that bacterial peptidoglycan and muramyl dipeptide are responsible for its induction [31]. Since the composition of bacterial adjuvant is complex and the immune response is a multi-stage process of intercellular cooperation, the mechanism is unclear [32].

All arthritic control animals showed acute inflammatory edema at the ipsilateral (injected) paw around days 4-6 followed by subsequent chronic polyarthritis phase which begins around day 10-12. The progress of inflammatory edema in the contralateral (non-injected) paw was evident on day 12 indicative of systemic inflammation. Throughout the 28 day experiment, there was no significant change in the paw volume of the non-inflamed normal control group. During the development of adjuvant arthritis, paw volume and joint diameter was significantly increased while body weight gain was markedly retarded in arthritic rats. Our results showed that the EEDP significantly inhibited the development of chronic swelling induced by FCA. EEDP significantly reduced paw edema on 24^{th} and 28^{th} day, during the second and third phase of development. The percent inhibition of paw edema on 24^{th} and 28^{th} day was significantly higher, measured as paw volume and joint diameter, suggesting its inhibitory effect on the prostaglandin mediated pathway. Among the drugs studied only the EEDP 400 mg/kg and diclofenac 5 mg/kg partially restored body weight gain.

The model of adjuvant-induced arthritis in rats has been extensively used in the study of inflammatory processes and validated as a model of chronic pain [33]. This fact is corroborated by evidence of spontaneous pain behaviors in arthritic rats, such as reduced locomotor activity and increased itching and scratching behaviors in the affected paw [30] and an attempt at protection of the affected paw, as evidenced by curving and/or elevation, as well as avoidance to support its own weight [34]. Several studies using arthritic rats as a model of chronic pain evaluates hyperalgesia in different ways. Interlukin-1 β (IL-1β) is a potent mechanical and thermal hyperalgesic agent when injected into any number of peripheral tissues. Intraplantar injection of inflammatory agents, such as carrageenan, lipopolysaccharide (LPS), bacterial endotoxin or Freund's complete adjuvant (FCA) produce mechanical or thermal hyperalgesia associated with an upregulation of IL-1β and other inflammatory cytokines in the inflamed tissue and in the dorsal root ganglia (DRG) [35].

The use of adjuvant arthritis model offers an opportunity to study pathological changes in a variety of tissues other than joints. According to present study increase in the WBC and platelet counts, decrease in Hb and RBC level in arthritic rats was observed. The reduction in Hb and RBC levels in arthritic rats shows the presence of anemia in these rats [36]. Anemia is the most common extracellular manifestation in RA [37]. The most important cause might be the decreased level of plasma iron due to sequestering of iron in the reticuloendothelial system and synovial tissue that lead to failure of bone marrow to respond to anemia [38]. The decrease in plasma iron inturn was induced by IL-1 in association with the acute phase response [39]. Hence, it is provocative to speculate that the sequestration of less deformable erythrocytes by endothelial cells in the spleen plays a causative role in the shortened half life of erythrocytes and subsequently anemia resulting in adjuvant arthritis. The increase in both WBC and platelet counts might be due to the stimulation of immune system against the invading pathogenic microorganism [40]. This is

evident by the infiltration of inflammatory mononuclear cells in the joints of arthritic rats. In the present study, the level of Hb and RBC was significantly increased, while the level of WBC and platelets was significantly reduced by EEDP.

In present study, decrease in total serum protein and albumin levels observed in arthritic rats. In arthritis changes in plasma protein level with an increase in the globulin fraction and decrease in albumin fraction were well documented [41]. These biochemical abnormalities result from a more basic liver malfunction. General reduction of liver protein synthesis can be assessed by measuring albumin levels, because levels of this protein are lowered during inflammation and further, it was also reported that albumin synthesis was reduced by IL-1 [42]. Moreover the mediators released such as histamine, bradykinin and prostaglandins during inflammation increase the permeability of vascular tissues to albumin leading to reduction in its serum levels. Assessment of the levels of AST, ALT and ALP provides an excellent and simple tool to measure the antiarthritic activity of the target drug [43]. The activities of aminotransferases and ALP were significantly increased in arthritic rats, since these are good indices of liver impairment, which are also considered as the features of adjuvant arthritis [44]. Serum AST and ALT have been reported to play a vital role in the formation of biologically active chemical mediators such as bradykinins in inflammatory process confirmed a positive correlation between the increased activity of serum ALP and the disease activity in RA [45]. Elevated levels of serum ALP in arthritic rats can be due to increase in the liver and bone fraction or due to an increase of both isoenzymes [46, 47]. The decreased AST, ALT enzyme levels in EEDP treated rats indicate decreased bone loss and organ protective role of EEDP in arthritic rats. CRP is a marker for inflammation and its level rise dramatically during inflammatory processes [48]. Both EEDP (400 mg/kg) and diclofenac (5 mg/kg) significantly ($P<0.001$) reduced CRP in arthritic rat provide evidence for anti-inflammatory activity.

Phytochemical analysis of the EEDP seeds has mainly demonstrated the presence of saponins, flavonoids, terpenoids and steroids. Steroids can decrease inflammation and reduce the activity of the immune system, while triterpenoids impairs histamine release from mast cells and exerts anti-inflammatory effects. Flavonoids are often used for their antioxidant effect against free radicals. There are also strong indications that they have antiviral, anti-inflammatory and anti-hypertensive properties. We propose that the analgesic, anti-inflammatory and antiarthritic activity of the EEDP seeds could be due to combined effect of flavonoids, saponins, steroids and triterpenoids, which are the major components of the ethanol extract of this species.

CONCLUSION

It is concluded that the ethanol extract of *Diplocyclos palmatus* (L) C. Jeffrey seeds possess analgesic, anti-inflammatory and antiarthritic activities in animal model. The possible mechanisms involving inhibition of release and/or actions of vasoactive substances such as histamine, serotonin, kinins and prostaglandins. The results obtained justify the use of the plant extract in traditional medicine for the treatment of painful, inflammatory and arthritic conditions. Further work is ongoing to isolate, identify, characterize, and elucidate the structure of the phytoconstituents responsible for the observed pharmacological activities in this study.

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