Original Article

Comparative Evaluation of Anti-Arthritic Activity of Pongamia Pinnata, Bryophyllum Pinnata and Their Combined Formulation in Fca Induced Arthritis Rat Model

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Abstract

The present study deals that the in-vivo comparative evaluation of anti-arthritic activity of *Pongamia pinnata* seeds, *Bryophyllum pinnata* leaves and their combined formulation using Dexamethasone as a standard. Rheumatoid arthritis (RA) is the most common inflammatory arthritis and affects the connective tissues throughout the body, but especially around the joints. *Pongamia pinnata and Bryophyllum pinnatum* have several uses which ascribed in traditional medicine. *Pongamia pinnata* used in diabetes, Epilepsy, hypertension, skin ailments and rheumatic arthritis. *Bryophyllum pinnatum* used in inflammatory disease, analgesic, ulcer, kidney and gall bladder stones. In vivo method, the antiarthritic effect of *Pongamia pinnata* seed *and Bryophyllum pinnata* leave combined extract (PBCE) was confirmed by Freund's complete adjuvant (FCA) arthritis induced rat. FCA consists of inactivated dried mycobacterium which stimulates effectively cell mediated immunity and produce immunoglobulin. In the present study, the results show that the *Pongamia pinnata* ethanolic extract (PPEE), *Bryophyllum Pinnatum* ethanolic extract (BPEE) and *Pongamia Bryophyllum* combined extract (PBCE) had significantly anti-arthritic effect on behaviors, hematological and biochemical parameters. Both plants are rich in antioxidant, flavonoids and polyphenols which are inhibit lipid. Treatment with PBCE reduces the all biochemical serum parameters similar as dexamethsone. The present investigation showed that PBCE (500mg/kg) was observed highly significant reduces paw swelling, hematological parameters and biochemical parameters in the FCA induced arthritis rats.

Keywords: Pongamia pinnatum, Bryophyllum pinnatum, Rheumatoid arthritis, flavonoids

INTRODUCTION

Arthritis is the inflammations of joints which can be further differentiate by the name Rheumatoid arthritis, psoriatic arthritis and gouty arthritis etc. Rheumatoid arthritis is a systemic autoimmune disease with chronic inflammation characterized by hyperplasia of synovial cells and angiogenesis in affected joints, which ultimately leads to the destruction of cartilage and bone.[1] Rheumatoid arthritis is inflammatory condition of connective tissue throughout the body but specially affects the joints. Rheumatoid arthritis is the most common arthritis which affects almost 1% population, in population women are 3 times more affected than men.[2] Rheumatoid arthritis symptoms mostly in knee become more painful and inflamed. Generalized symptoms of rheumatoid arthritis are fatigue, low blood cell count, dry eyes and dryness of the mouth. Environmental risk factors are diet, bronchiectasis, respiratory, oral, intestinal and genital tract mucosal site infection.[3] There are currently used for treatment have five main drug classes include analgesics, disease-modifying antirheumatic drugs (DMARDS), nonsteroidal anti-inflammatory drugs (NSAIDS), anticytokine therapies, glucocorticoids.[4] Traditional herbal medicine has fewer side effects. Natural products isolated and detailed investigation of their phytoconstituents and pharmacological action. Many chemical substances are extracted from herbal plant and development of newer drug compounds or natural medicine.

Many drugs approved by FDA in last decade for drug development has been from natural product in various diseases.[5] *Pongamia pinnata and Bryophyllum pinnatum* have several uses which ascribed in traditional medicine. The ethanolic extract of *B. pinnatum and P.pinnata* leaves have reported to be anti-inflammatory, analgesic, and immunosuppressant activity.[6,7] The ethanolic extract of *P.pinnata* seeds have reported to be anti-inflammatory activity. The crushed seeds paste are used for leporus sores, skin disease and painful rheumatic joints.[8,9] Arthritis is one of the most common autoimmune inflammatory conditions of unknown etiology.

MATERIALS AND METHODS

Collection and authentication of plant materials

The seeds of *Pongamia pinnata and Bryophyllum Pinnatum* leaves were collected from local vendors of Jaipur. The *Pongamia pinnata* seeds and *Bryophyllum Pinnatum* leave both plant materials were authenticated by herbarium of the

Department of Botany, University of Rajasthan, and Jaipur. The both plant materials powdered to a fine grade by using a laboratory scale mill. These shade dried parts of the plant was powdered and kept in air tight glass container until use.

Preparation of extracts

Dried Seeds and leaves were powdered mechanically through mesh sieve. The powdered plant materials were extracted with ethanol solvent using soxhlet apparatus for 48 hrs. Both plant materials extracted separately using ethanol solvent. In combined extract, the powdered plant materials seeds and leaves at proportion (1:1) were subjected to soxhlet apparatus using ethanol. The ethanol was distilled at 40ºC temperature and concentrated on water bath to get the crude extract. The crude extract stored in desiccators for future use.

Animals

Healthy wistar albino rats of both sexes were used to evaluate the anti-arthritic activity. The animals were fed with standard pellet feed and water *ad libitum*. All the experimental animals and study was approved by the institutional animal ethical committee (IAEC) of CPCSEA (committee for the purpose of control and supervision of experiments on animals). Each type of experimental models divided into eight groups and individual group contained six rats.

Acute oral Toxicity

Oral toxicity study was performed according to the guideline of OECD 423. Ethanolic extract of all plants were administered orally to wistar albino rats dose of 2000 mg/kg body weight. The rats were observed of any sign of changes in behavior, acute and adverse effect such as salivation, convulsion, diarrhea and respiratory depression.[10]

Freund's Complete Adjuvant method

Each rat was injected with 0.1ml of Freund's complete adjuvant (FCA) into subplantar region of left hind paw on day 0 for induction of arthritis except group 1. All arthritis induced groups was started treatment daily orally from day 12 and continued till day 28. Anti-arthritic activity of different extracts was evaluated on following parameters: body weight, joint diameter, paw volume, pain withdrawal latency, fall off time and arthritic score on day 0, 4th, 7th, 10th, 12th, 14th, 17th, 19th, 21th and day 28th. On last day 28, blood was withdrawn by retro-orbital puncture for assessment of various hematological and biochemical parameters.[11, 12]The animals were divide into eight groups (n=6) each as follows:

Non-arthritic animals:

Group I: Vehicle normal animals, 1% aqueous solution of Tween 80, p.o. (non arthritic)

Arthritic animals:

Group II: Arthritic control, 1% aqueous solution of Tween 80, p.o.
Group III: Arthritic standard treated, 5mg/kg Dexame tha some, p.o.
Group IV: Arthritic animals treated with *Pongamia pinnta* Extract (low dose), p.o.
Group V: Arthritic animals treated with *Pongamia pinnta* Extract (high dose), p.o.
Group VI: Arthritic animals treated with *Bryophyllum pinnatum* Extract (low dose), p.o.
Group VII: Arthritic animals treated with *Bryophyllum pinnatum* Extract (high dose), p.o.
Group VIII: Arthritic animals treated with combination of *Pongamia* and *Bryophyllum pinnatum* Extract, (high dose) p.o.

Body weight

Body weight was measured of all groups from day 0 to till end 28 day and measured by single weighing balance.

Arthritic score

The morphological feature of the arthritis like redness, swelling and erythema was monitored by set visual criteria as follows: Normal paw = 0, Mild swelling and erythema of digits = 1, Swelling and erythema of the digits = 2, Severe swelling and erythema = 3, Gross deformity and inability to use the limb = 4. [13]

Paw volume

Paw volumes of all rats were measured on left hind on day 0 before FCA administration and different time intervals till day 28th using a Plethysmometer.[14]

Motor incoordination test (fall off time)

Rota rod apparatus was used for Motor incoordination test. The time taken for the falling of the rat from the roller, during the period of 1 min was recorded.[15]

Joint diameter

Joint diameter was measured by compressing the joint by rotating the screw of micrometer screw gauge till the pain elicited or leg withdrawal by animals. The distance moved by the screw gauge was recorded.[16]

Anti-nociceptive activity (Eddy's Hot Plate Method)

The apparatus consists of a hot plate on which the rats were placed for test. Pain threshold was determined by the latency for nociceptive response (withdrawal of any paw) with a maximum cut-off time 15 sec for all groups.[17]

Determination of haematological parameters

On the last day of experiment, the blood was withdrawn by retro-orbital puncture and collect into EDTA coated test tube for measurement of hematological and biochemical parameters. The hematological parameters analyzed were of follows: white blood cell (WBC) count, Red blood cell (RBC) count, Hemoglobin (Hb) concentration, Determination of erythrocyte sedimentation rate (ESR). Serum samples were collected after centrifugation of whole blood and analyzed biochemical serum parameters.[18]

Statistical analysis

Results which were presented as mean ± S.E.M. Statistical comparisons were made between treatment groups and FCA arthritic control group. The Statistical significance difference between two mean was determined by one-factor analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test by using Graph Pad Prism 7 software. The values of $P < 0.05$ were considered statistically significant.[19]

RESULTS

In this study the effect of different extracts and standard drug were observed on diseased rats and compared them with control group. Subject group included disease rats, which were categorized into 5 groups on the basis of different drugs administration.

Effect on body weight

All the groups administered with Freund's complete adjuvant (FCA) decrease in body weight. In this study, oral treatment with different extract of drug PBCE, PPEE and BPEE caused significant increased in body weight. Treatment with standard drug and PBCE (500 mg/ kg) were similar significant time dependent increase in the body weight from day 17 ($P< 0.01$) and highly significant increase in body weight from day 19 to 28 ($P< 0.001$) as compared to with FCA control group (Fig. 1).

Effect on arthritic score

FCA injection administered to all group of animals and showed sign of inflammation in one or more hind paw significantly from day 7 to 12. In On comparison from control group, the standard group and PBCE at 500 mg/kg showed significant ($P < 0.05$, $P < 0.01$ and $P < 0.001$) decrease in arthritic score from day 14 to till end of study 28 day. All the extracts showed moderately and highly significant decrease in arthritic score as compared to control group but on comparisons to PBCE and standard drug found to more than PPEE and BPEE (Fig. 2).

Effect on motor coordination

All FCA induced arthritis treated groups decrease in fall off time till day 12. All the extracts showed moderately and highly significant increase in fall off time as compared to control group. PBCE at 500mg/kg showed moderate to highly significant ($P < 0.05$, $P < 0.01$ and $P < 0.001$) increase in fall off time from 17 to 28 day and this significant increase in fall of time of rats was similar to that caused by 5mg/kg of standard drug as compared to control rats as shown in Fig. 3.

Effect on nociceptive activity

All the animals treated with FCA showed a consistent decrease in paw withdrawal from day 0 to 14. All the extracts showed moderately and highly significant increase in pain withdrawal time as compared to control group. On comparison from control group, PBCE at 500mg/kg dose showed significant increase in pain withdrawal latency on day 14 ($P < 0.05$), day 17 ($P < 0.0$) and 21 to 28 ($P < 0.001$) and this significant increase in pain withdrawal latency was similar to that caused by standard drug (Fig. 4).

Effect on paw volume

All FCA treated group produced an increase in rat paw volume compared to healthy control rats. Treatment with PPEE at dose 250mg/kg showed significant reduction from day 21 to 28, whereas PPEE at 500mg/kg showed significant reduction in paw volume from day 19 to 28. The rats treated with PBCE (500mg/kg) showed time dependent significant ($P < 0.05$, $P < 0.01$ and $P < 0.001$) decrease in paw volume from day 14 to 28 as compared to control group. Standard drug also showed similar significant reduction in paw volume as PBCE when on comparison to FCA control (Fig.5).

Effect on joint diameter

The joint diameter was increase in FCA control group to till the end of the study in 28 day. Treatment with standard drug showed significant ($P < 0.05$, $P < 0.01$ and $P < 0.001$) decrease in joint diameter from day 14 to 28 as compared to FCA control group. The reducing in joint diameter by the treatment of PBCE (500mg/kg) and standard drug showed the same efficacy when compared to control. Comparison all extracts showed as in Fig. 6.

Hematological Parameters

FCA induces rats showed decrease in RBCs along with Hb and increase in WBCs in control as compared to normal rats. Rats treated with PPEE and BPEE at 250mg/kg showed significant decrease in ESR value. Treatment with high dose of PPEE and BPEE showed significant increase in RBC and Hb values, while significantly decrease ESR and WBC in rat as compared to control rat. Treatment with Standard drug (5mg/kg) and PBCE (500mg/kg) both showed highly significant ($P < 0.01$) decrease in WBCs and ESR when compared to control rats whereas significantly ($P < 0.001$) increase in the RBCs and Hb level as compared to control rats. The hematological changes shown in Fig.7.

Biochemical parameters

In FCA induced arthritis rats, some biochemical blood parameters are such as ALP, AST, ALT and CRP increase significantly which are connected to the destruction of joint tissue. Treatment with low dose (250mg/kg) of PPEE and BPEE showed nonsignificant reduction as compared to control rat. The treatment with BPEE and PPEE at 500mg/kg caused significant decrease in the all serum parameters. Oral treatment with PBCE at 500mg/kg showed significant ($P < 0.001$) reduction in the all serum parameters and this significant ($P < 0.001$) reduction in ALP, CRP, AST and ALT similar that cause by standard drug (5mg/kg) as compared to control group.The changes in biochemical parameters shown in Fig. 8.

DISCUSSION

Rheumatoid arthritis is an inflammatory arthritis which is an auto immune disorder. Rheumatoid arthritis basically having three type of inflammatory process synovial proliferation, joint tissue destruction and complete dysfunction of joint. Recent study method used is Freund's complete adjuvant (FCA), FCA method is widely used for evaluation of various anti-arthritic drug effects for inflammatory condition because method is reliable on sent and early measurable.[20]In the present study, the results show that the *Pongamia pinnata* ethanolic extract (PPEE), *Bryophyllum Pinnatum* ethanolic extract (BPEE) and *Pongamia Bryophyllum* combined extract (PBCE) had anti-arthritic effect on behaviors, hematological and biochemical parameters. In study of behavior parameters, weight is an indirect index of development of arthritic syndrome and improvement health, during development of disease weight decreases and restoration of health weight increase.[18] The arthritic score is the index of inflammation in one or more hind paws. The arthritic score was significantly decreased with treatment by combined extract of *Pongamia Bryophyllum* in compare with control rat. Paw swelling is indicating to the increase in paw volume and it is sensitive parameter for assessment of therapeutic effect of various anti-arthritic drugs. The FCA induced arthritis showed increase in paw volume progressively throughout the experiment, whereas it was reduced after 14 days in dexamethasone and PBCE treated group. In the present study the observed PBCE showed significant improvement in nociceptive activity and highly increase pain threshold time compared to control rats. However a significant decrease in mechanical withdrawal threshold observed in all the animals treated with FCA. Treatment with PBCE and standard drug showed increase in fall off time compare to control.[19]

The hematological parameters like red blood cell count (RBC) and hemoglobin (Hb) level decrease in arthritic rats which indicate the anemia condition similar to that observed in arthritic control. Treatment with BPEE, PPEE, PBCE and standard drug exhibited a significant and potent effect in arthritis induced rats by increase the red blood cell count and hemoglobin level. White blood cell (WBC) count and erythrocyte sedimentation rate (ESR) value moderate increase in arthritis rats is mediated by IL-1β. The present study showed that the PBCE and standard drug to reduce the ESR and WBC value to near the normal level plant extracts have significant role in experimental arthritic conditions.[21]Biochemical parameters such as *alanine transaminase* (ALT), alkaline phosphatase (ALP) and aspartate transaminase (AST) significantly elevated in arthritic induced rats and these parameters indirectly connected to ankle joint destruction. C-reactive protein (CRP) level is valuable for measure the severity of arthritis in patient. CRP value higher in FCA administered treat arthritic rats. Treatment with PBCE and standard drug dexametahsone observed a similar effect on CRP and decrease CRP level was reported in arthritic rat. Treatment with PBCE reduces the all biochemical serum parameters similar as dexamethsone.[19]

CONCLUSION

In recent era, the synthetic drugs for the treatment of arthritis have several existing unavoidable side effects and limited arthritis drugs. Traditional herbal medicine has fewer side effects. The present study obtained on the basis of results and conclusion that the *Pongamia Bryophyllum* combined extract (PBCE) at specific dose 500mg/kg p.o. showed more potent anti-arthritic activity on comparison with standard, PPEE and BPEE. The current studies revealed that the presence of phytoconstituents such as antioxidant, flavonides, alkaloids and steroids of plants are may be responsible for anti-arthritic effect. Mechanism of action of plants was not be clear but have action on pro-inflammatory mediators and mediators will be carried out in future by this study.

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FIGURES

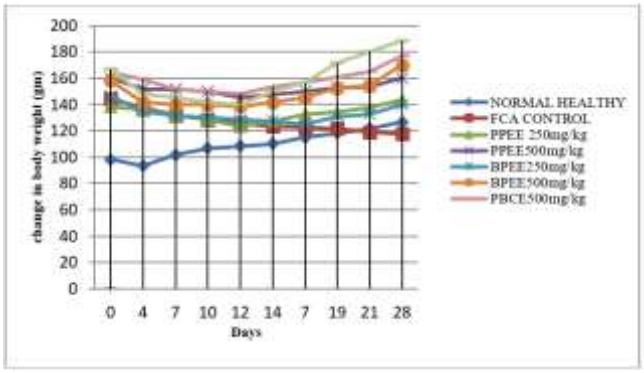

Fig. 1 Comparative effect on body weight by different ethanol extracts in FCA model. Values are plotted as the mean ± S.E.M, n=6 in each group. *P < 0.05, **P< 0.01 and ***P< 0.001 compared to FCA control.

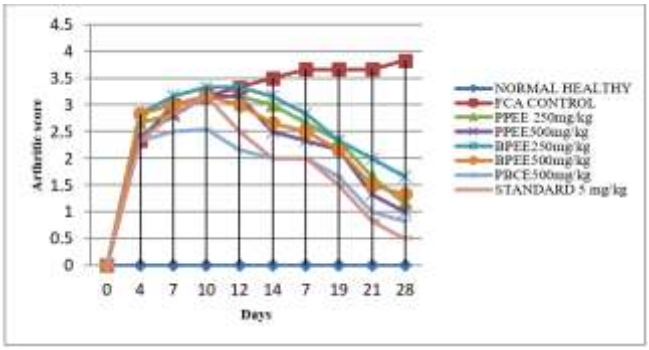

Fig. 2 Comparative effect on arthritic score by different ethanol extracts in FCA model. Values are plotted as the mean ± S.E.M, n=6 in each group. *P < 0.05, **P< 0.01 and ***P< 0.001 compared to FCA control.

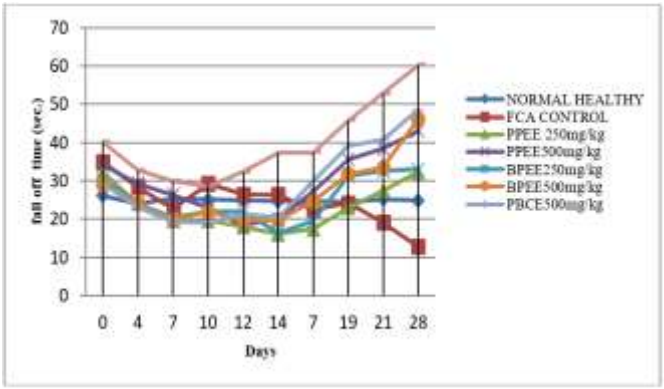

Fig. 3 Comparative effect on motor coordination by different ethanol extracts in FCA model. Values are plotted as the mean ± S.E.M, n=6 in each group. *P < 0.05, **P< 0.01 and ***P< 0.001 compared to FCA control.

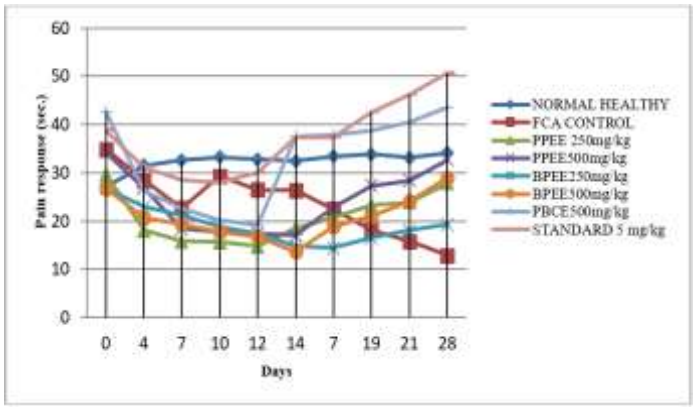

Fig. 4 Comparative effect on nociceptive activity by different ethanol extracts in FCA model. Values are plotted as the mean ± S.E.M, n=6 in each group. *P < 0.05, **P< 0.01 and ***P< 0.001 compared to FCA control.

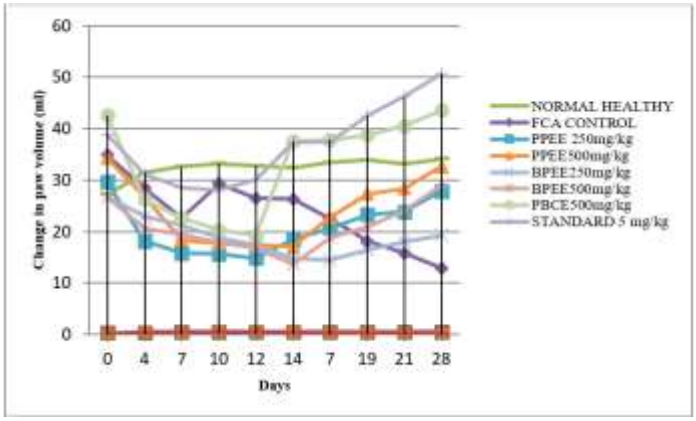

Fig. 5 Comparative effect on paw volume by different ethanol extracts in FCA model. Values are plotted as the mean ± S.E.M, n=6 in each group. *P < 0.05, **P< 0.01 and ***P< 0.001 compared to FCA control.

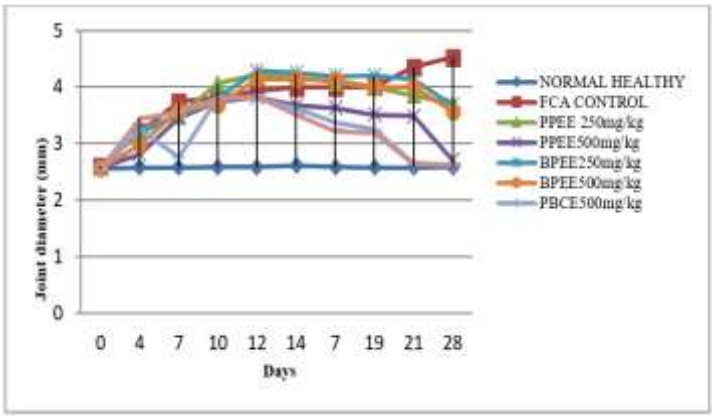

Fig. 6 Comparative effect on joint diameter by different ethanol extracts in FCA model. Values are plotted as the mean ± S.E.M, n=6 in each group. *P < 0.05, **P< 0.01 and ***P< 0.001 compared to FCA control.

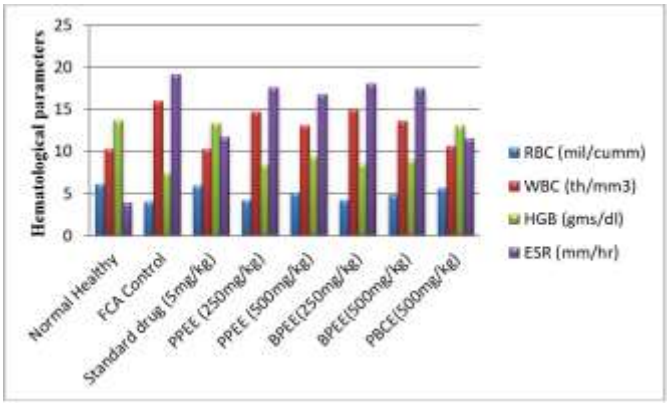

Fig. 7 Comparative effect on hematological Parameters by different ethanol extracts in FCA model. Values are plotted as the mean ± S.E.M, n=6 in each group. *P < 0.05, **P< 0.01 and ***P< 0.001 compared to FCA control.

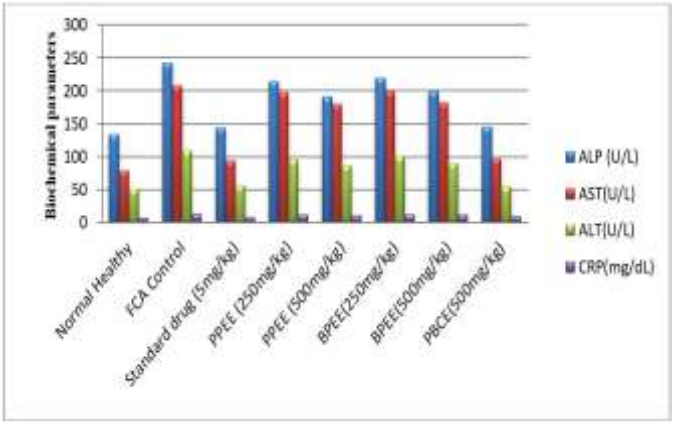

Fig. 8 Comparative effect on Biochemical parameters by different ethanol extracts in FCA model. Values are plotted as the mean ± S.E.M, n=6 in each group. *P < 0.05, **P< 0.01 and ***P< 0.001 compared to FCA control.