Supplementary material 1

Details of study design I and II with preliminary (prevalidation) results

In initial phase two models were developed to estimate Rheumatoid Arthritis via two different inducing agents; Complete Freund's adjuvant (each ml contains 1 mg heat killed and dried *Mycobacterium tuberculosis* in 0.85 mL paraffin oil and 0.15 mL mannide monooleate, purchased from Sigma-Aldrich, Bangalore, India) and Bovine collagen type II (Chondrex Inc Redmond, WA, USA, supplied by Krishgen Biosystem Mumbai, India). Both the models were selected due to different mechanisms of developing RA and were compared to check the severity between the models. Here CFA was utilized as a single inducing dose of 0.1 mL/ animal by sub planter route on day 0 for induction of RA and disease progression was observed for 28 days. On the other hand, bovine collagen type II was also used in separate group of animals in 0.1ml dose (after forming emulsion of CFA and collagen in 1:1 ratio)(Miyoshi and Liu, 2024). Animals were sensitized with this emulsion by sub planter route for induction of RA once using 0.1mL dose/ animal on day 0 and disease progression was observed with and without LPS sensitization for 42 days.

Study protocol for CFA induced RA

After suitable acclimatization, animals were divided into five groups, where group I was considered as Normal control group and animals of this group were not sensitized with any agent. CFA induced RA study was designed for 28 days and group IIa was considered as a CFA model which received CFA 0.1 mL by sub planter route on left hind paw on day 0 for induction of RA. Groups IIIa, IVa and Va also received CFA 0.1 mL on left hind paw by sub planter route on day 0 of study. On day 14 to 28 animals of these groups (IIIa, IVa, and Va) were further sensitized with LPS in different doses by s.c. route. Group IIIa received CFA 0.1 mL + LPS 0.1 μg/mL. Group IVa received CFA 0.1 mL + LPS 0.5 μg/mL and Group Va received CFA 0.1 mL + LPS 10 μg/mL. All the evaluation parameters were performed for assessment of progression of RA at different time points. Paw volume was measured on days 1, 3, 5, 7, 9, 11, 14, 21, 28 by digital plathysmometer. Photography was done on alternate days and Arthritic index was calculated on the basis of paw volume and Arthritic score taken on day 5 and 21; ESR was

measured on days 1, 7, 14, 21 and 28. C-RP was performed on days1, 7, 14, 21 and 28. IL-6 and TNF-α were estimated on day 28. CBC, Homocysteine and Anti-CCP were performed on days 12 and 28 and at the end of the study X-Ray and histopathology were done for evaluating the development of model.

Study protocol for collagen induced RA

After suitable acclimatization, animals were divided into five groups where Group I was considered as Normal control group used here also for comparison.

CIA-induced RA protocol was designed for 42 days and groups IIb, IIIb, IVb and Vb were sensitized with collagen 0.1 mL (CFA1: CIA1). Bovine collagen type II was premixed with CFA before injecting in animals in 1:1 ratio to get a stable emulsion (tested by uniform droplet formation in cold water) and 0.1 mL of this prepared emulsion was injected by sub planter route on day 0 for induction of RA (Pietrosimone et al. 2015). Group IIb received CIA 0.1ml only on day 0. Group IIIb, IVb and Vb received CIA 0.1ml on day 0 and these groups were further sensitized with LPS from day 14 to day 42 with 0.1, 0.5 and 10 μg/mL of LPS doses respectively by s.c. route. Different parameters; biological ESR, CRP were preformed on days 7, 14, 21, 28, 35 and 42.

CBC, Anti-CCP and Homocysteine were performed on days 14 and 35. Paw volume of left hind paw was measured on days 1, 3, 5, 7, 11, 14, 17, 21, 28, 35, 42 and the arthritic score was assessed on days 5, 21 and 35. On the basis of paw volume, secondary lesions and severity Arthritic index was calculated. Photographic assessment was done on alternative days; radiographic (X-ray) were performed on days 14, 28 and 42, and at the end of the study, histopathological assessment of a required organ was done.

On the basis of observations in CFA sensitized groups; group Va (CFA 0.1 mL + LPS 10 μg/mL) represented higher severity among all the groups and produced statistically significant results, which were further used for comparison with CIA groups to get the best model. On the other hand, in CIA sensitized groups, group Vb (CIA 0.1 mL + LPS 10 μg/mL) represent the highest severity index among all other groups.

Model Development studies for Rheumatoid Arthritis (study-II)

Protocol for development of CVD in RA model

The final selected groups from study I – group Va (CFA + LPS 10 μg/mL) and group Vb (CIA 0.1 mL + LPS 10 μg/mL) – were used or further assessment here.

All the animals used for this protocol were fed with modified diet (HFD) throughout the study period to generate metabolic dysbiosis. This 30 % lard diet consists of normal pellet diet purchased from VRK Enterprises, Vadodara, Gujarat, India. Casein, corn starch and sucrose obtained from Spectrochem Pvt. Ltd, Mumbai, India. Along with vitamin mix purchased from Neelam enterprises for veterinary supplies, Vadodara, Gujarat, India, CFA and CIA were used here also for primary induction of RA, and for secondary sensitization LPS (0.1 mL s.c. dose for each animal for 14 days) was provided in different doses along with HFD. Two groups mentioned above were compared with the following group of animals: group VI (CFA 0.1 mL + HFD) which received CFA 0.1 mL on day 0 along with HFD for 28 days. Group VII (CFA 0.1 mL + HFD+ LPS 10 μg/mL) was sensitized with CFA 0.1 mL on day 0 and further sensitized with LPS 10 μg/mL; HFD was given to the animals for 28 days. Group VIII (CIA 0.1 mL + HFD) received CIA 0.1 mL on day 0 with HFD for 42 days, and group IX (CIA 0.1 mL + HFD + LPS 10 μg/mL) received CIA 0.1 mL on day 0 and further sensitized with LPS 10 μg/mL from day 14 to day 42; animals were fed with HFD throughout the study period (42 days). All the parameters for assessment of RA were performed at different time points for 28 days in CFA groups and 42 days in CIA groups, respectively. These groups were further analyzed for the progression of Atherosclerosis at different time points using Biological parameters (Triglyceride levels, Cholesterol, HDL, LDL levels, atherogenic index and TLR-4 activation) (Vargas-Caraveo et al. 2020), Perceptive indicators (Fibre length of Vastus Medialis and Biceps Femoris muscle) (Gizard, Fernandez, & De Vadder, 2020) were performed to check the development of Atherosclerosis. At the end of the study X-Ray and histopathology (H. Gerhard Vogel, 1997) of paw, aorta and Vastus Medialis and Biceps Femoris muscle were done for confirmation.

Kits for estimation of C – reactive protein (C-RP) obtained from ADI enterprises, Vadodara, Gujarat, India. ELISA kits for estimation of TNF-α, Interleukin-6, TLR-4 and homocysteine were purchased from Krishgen biosystems Mumbai, India. Kits for estimation of total cholesterol, triglycerides, HDL-C were purchased from Span Diagnostics Pvt. Ltd., Surat, Gujarat, India.

Clinical investigations of total WBC count and Anti-CCP were estimated by Dr. Kaushik A. Patel MD path& bact. Divine lab, Mangalkirti apartments, Fatehgunj, Vadodara, Gujarat, India. X-rays of rats were done at Dr. Angela Lobo's Veterinary Clinic, Kirti Mandir Compound, Tilak Road, opp. SSG Hospital Vadodara, Gujarat India. Receptor studies for TLR-4 and NLRP-3 were estimated by Deshpande Laboratories (DL) Pvt. Ltd. D, 25 Anushka Estate, Kalkheda-Neelbud, Bhopal, Madhya Pradesh, India.

Note: For better understanding, in succeeding sections the final compared groups will be denoted as

Va (CFA 0.1 mL + LPS 10 µg/mL) as Model I

Vb (CIA 0.1 mL + LPS 10 µg/mL) as Model II

VII (CFA 0.1 mL + HFD + LPS 10 µg/mL) as Model III

IX (CIA0.1 mL + HFD + LPS 10 µg/mL) as Model IV

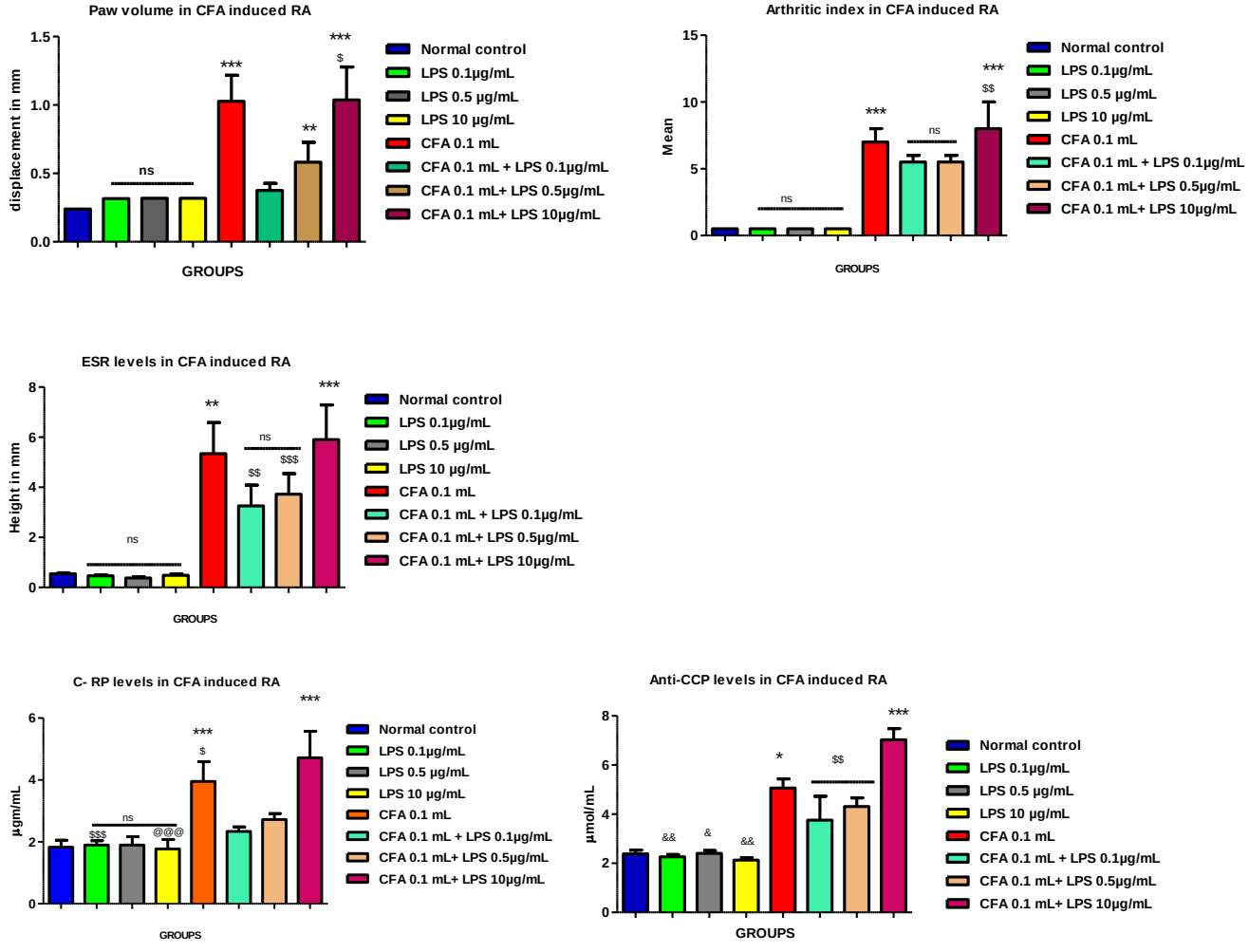
The estimated parameters were analyzed on the basis of observational outcomes (on the basis of physical estimations and the statistical data), pilot studies (for estimation of dose response and variability of different inducing agents), exploratory experiments (treatment responses, hypothesis correction via *p* value correction using post hoc tests in ANOVA), and confirmatory studies using the estimation parameters. Models with both the situations (RA and cardiovascular complications in RA) were statistically analyzed applying one way ANOVA and Repeated measure ANOVA using GraphPad Prism software, where values are expressed as Mean ± SEM. Boneferroni's, Dunnett and Tukey's Post hoc tests were used for comparison between each group. Significant values were compared at *$p<0.05$, **$p<0.01$, *** $p<0.001$.

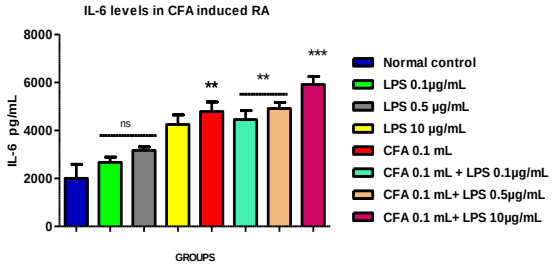
Results

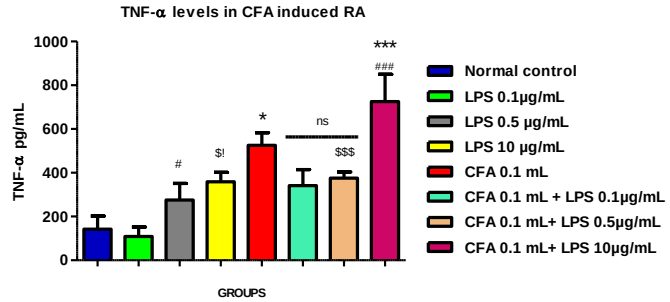
Results of designing and establishing translational competence between Preclinical and Clinical studies of Rheumatoid Arthritis model

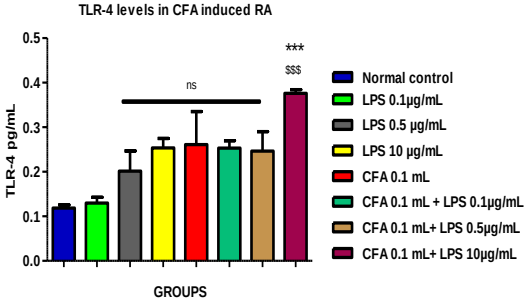
Groups representing CFA induced RA; group I (normal control), group IIa (CFA 0.1 mL), group IIIa (CFA 0.1 mL + LPS 0.1 µg/mL), group IVa (CFA 0.1 mL + LPS 0.5 µg/mL) and group Va (CFA 0.1 mL + LPS 10 µg/mL) were compared with groups developed for Collagen-induced RA (CIA); group IIb (CIA 0.1 mL), group IIIb (CIA 0.1 mL + LPS 0.1 µg/mL), group IVb (CIA 0.1 mL + LPS 0.5 µg/mL) and group Vb (CIA 0.1 mL + LPS 10 µg/mL). After comparing CFA sensitized groups with CIA sensitized groups, group Vb (CIA 0.1 mL+ LPS 10 µg/mL) showed the highest severity index among all other groups.

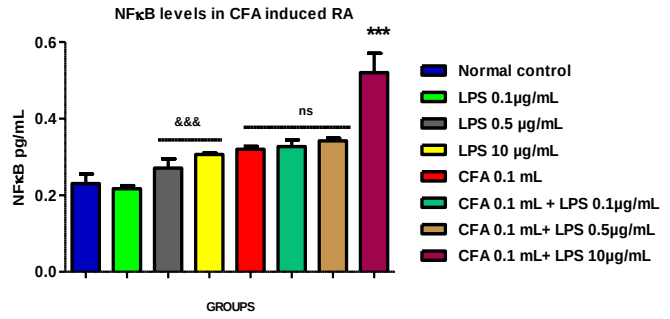
A

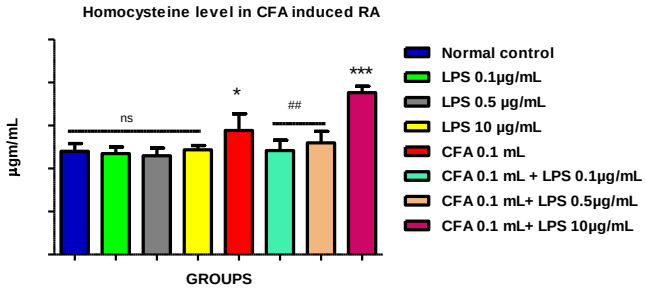



IL-6 levels in CFA induced RA
IL-6 pg/mL
8000
6000
4000
2000
0
ns
**
**
***
GROUPS
Normal control
LPS 0.1µg/mL
LPS 0.5 µg/mL
LPS 10 µg/mL
CFA 0.1 mL
CFA 0.1 mL + LPS 0.1µg/mL
CFA 0.1 mL+ LPS 0.5µg/mL
CFA 0.1 mL+ LPS 10µg/mL

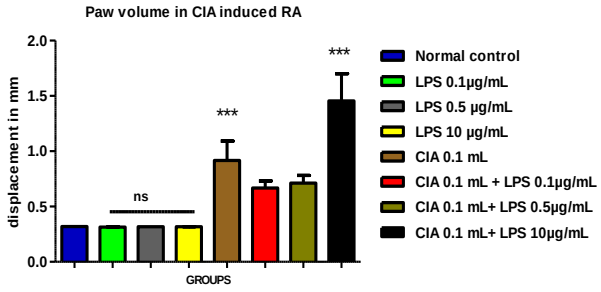

TNF-α levels in CFA induced RA
TNF-α pg/mL
1000
800
600
400
200
0
#
$!
*
ns
$$$
***
###
GROUPS
Normal control
LPS 0.1µg/mL
LPS 0.5 µg/mL
LPS 10 µg/mL
CFA 0.1 mL
CFA 0.1 mL + LPS 0.1µg/mL
CFA 0.1 mL+ LPS 0.5µg/mL
CFA 0.1 mL+ LPS 10µg/mL

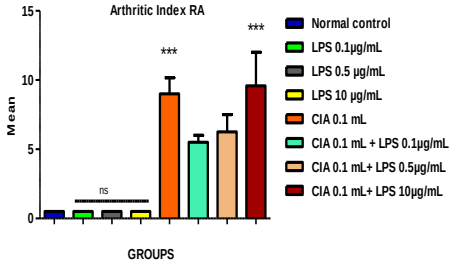

TLR-4 levels in CFA induced RA
TLR-4 pg/mL
0.5
0.4
0.3
0.2
0.1
0.0
ns
***
$$$
GROUPS
Normal control
LPS 0.1µg/mL
LPS 0.5 µg/mL
LPS 10 µg/mL
CFA 0.1 mL
CFA 0.1 mL + LPS 0.1µg/mL
CFA 0.1 mL+ LPS 0.5µg/mL
CFA 0.1 mL+ LPS 10µg/mL


NFκB levels in CFA induced RA
NFκB pg/mL
0.6
0.4
0.2
0.0
&&&
ns
***
GROUPS
Normal control
LPS 0.1µg/mL
LPS 0.5 µg/mL
LPS 10 µg/mL
CFA 0.1 mL
CFA 0.1 mL + LPS 0.1µg/mL
CFA 0.1 mL+ LPS 0.5µg/mL
CFA 0.1 mL+ LPS 10µg/mL


Homocysteine level in CFA induced RA
µgm/mL
ns
*
##
***
GROUPS
Normal control
LPS 0.1µg/mL
LPS 0.5 µg/mL
LPS 10 µg/mL
CFA 0.1 mL
CFA 0.1 mL + LPS 0.1µg/mL
CFA 0.1 mL+ LPS 0.5µg/mL
CFA 0.1 mL+ LPS 10µg/mL

B


Paw volume in CIA induced RA
displacement in mm
2.0
1.5
1.0
0.5
0.0
ns
***
***
GROUPS
Normal control
LPS 0.1µg/mL
LPS 0.5 µg/mL
LPS 10 µg/mL
CIA 0.1 mL
CIA 0.1 mL + LPS 0.1µg/mL
CIA 0.1 mL+ LPS 0.5µg/mL
CIA 0.1 mL+ LPS 10µg/mL


Arthritic Index RA
Mean
15
10
5
0
ns
***
***
GROUPS
Normal control
LPS 0.1µg/mL
LPS 0.5 µg/mL
LPS 10 µg/mL
CIA 0.1 mL
CIA 0.1 mL + LPS 0.1µg/mL
CIA 0.1 mL+ LPS 0.5µg/mL
CIA 0.1 mL+ LPS 10µg/mL

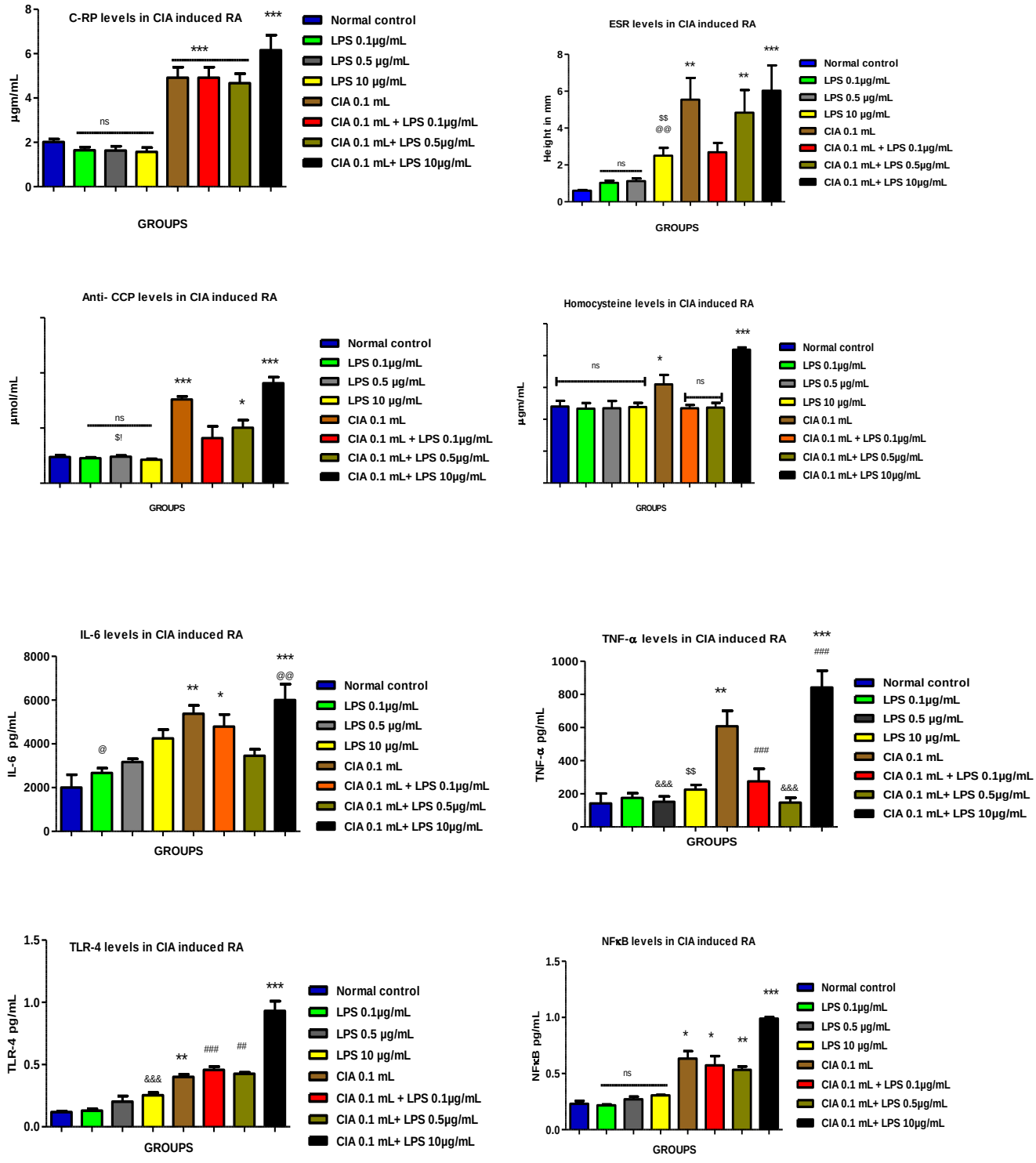


Figure 1. (A) Graphical representation of statistical evaluation for induction of RA using CFA

(B) Graphical representation of statistical evaluation for induction of RA using CIA

Note: Values are expressed as Mean ± SEM. Values are statistically evaluated using repeated measure ANOVA (Paw volume) and one way ANOVA analysis followed by Boneferroni's Post hoc test for other tests the comparison between groups were done by Bonferroni's, Dunnett's and Tukey's post hoc test. Significant values were compared with (*P<0.05, **p<0.01, *** P<0.001).

*, **, *** are used for comparison between normal and all groups

&, &&, &&& are used for comparison in between the CFA models.

$, $$, $$$ are used for comparison between LPS induced models.

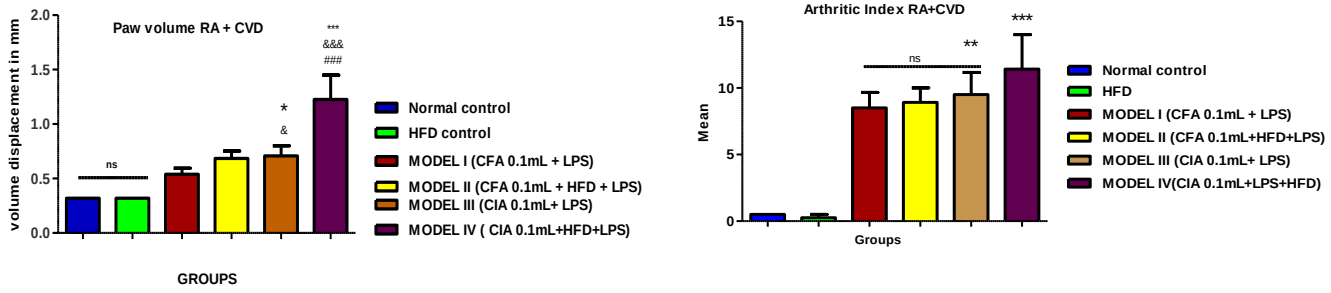
#, ##, ### are used for comparison between HFD and other groups.

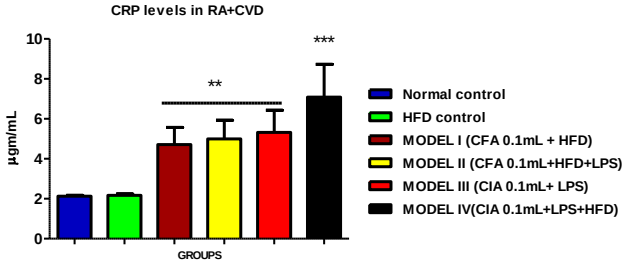
@, @@, @@@ are used for comparison of LPS and models in CIA groups.

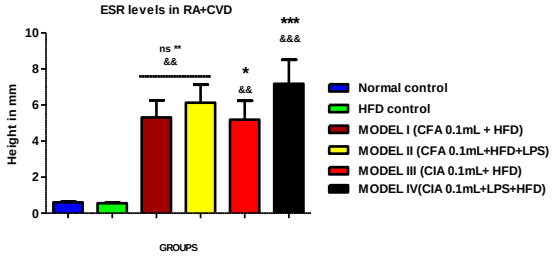
ns is non-significant differences between groups.

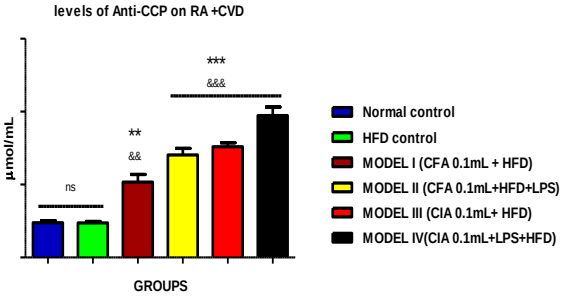
Results of designing and establishing translational competence between Preclinical and Clinical studies of Cardiovascular (CV) complications in RA model

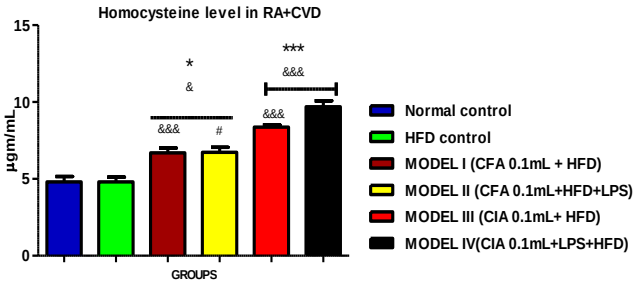
The results for this study were analyzed by evaluating the group VI (CFA 0.1 mL+ HFD), VII (CFA 0.1 mL + HFD+ LPS 10 µg/mL), Group VIII (CIA 0.1 mL + HFD) and group IX (CIA 0.1 mL + HFD+ LPS 10 µg/mL) which represents the final groups among both the situations. All the groups were compared statistically, with graphical representation of data depicted in Fig.3. Data for RF value were summarized in Table III and neutrophil count is given in Table IV. After comparing all the groups, model IV (CIA 0.1 mL + LPS 10 µg/m:L + HFD) showed the highest development of atherogenic conditions in rats as compared to other groups.

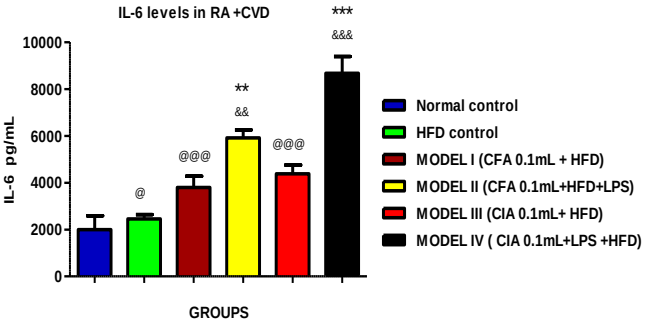


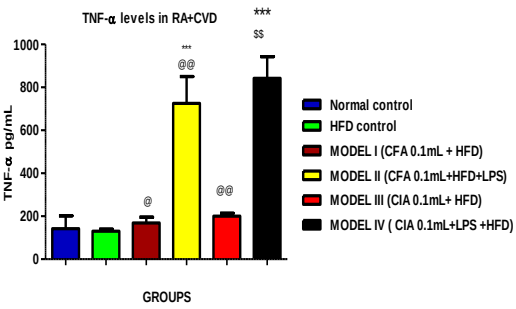

CRP levels in RA+CVD
µgm/mL
GROUPS
Normal control
HFD control
MODEL I (CFA 0.1mL + HFD)
MODEL II (CFA 0.1mL+HFD+LPS)
MODEL III (CIA 0.1mL+ LPS)
MODEL IV(CIA 0.1mL+LPS+HFD)

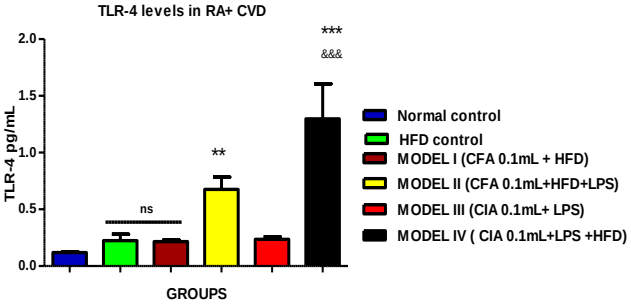

ESR levels in RA+CVD
Height in mm
GROUPS
Normal control
HFD control
MODEL I (CFA 0.1mL + HFD)
MODEL II (CFA 0.1mL+HFD+LPS)
MODEL III (CIA 0.1mL+ HFD)
MODEL IV(CIA 0.1mL+LPS+HFD)

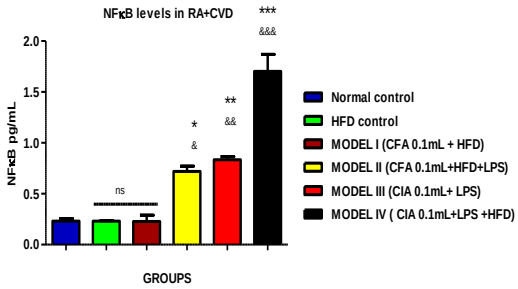

levels of Anti-CCP on RA +CVD
µmol/mL
GROUPS
Normal control
HFD control
MODEL I (CFA 0.1mL + HFD)
MODEL II (CFA 0.1mL+HFD+LPS)
MODEL III (CIA 0.1mL+ HFD)
MODEL IV(CIA 0.1mL+LPS+HFD)


Homocysteine level in RA+CVD
µgm/mL
GROUPS
Normal control
HFD control
MODEL I (CFA 0.1mL + HFD)
MODEL II (CFA 0.1mL+HFD+LPS)
MODEL III (CIA 0.1mL+ HFD)
MODEL IV(CIA 0.1mL+LPS+HFD)


IL-6 levels in RA +CVD
IL-6 pg/mL
GROUPS
Normal control
HFD control
MODEL I (CFA 0.1mL + HFD)
MODEL II (CFA 0.1mL+HFD+LPS)
MODEL III (CIA 0.1mL+ HFD)
MODEL IV (CIA 0.1mL+LPS +HFD)


TNF-α levels in RA+CVD
TNF-α pg/mL
GROUPS
Normal control
HFD control
MODEL I (CFA 0.1mL + HFD)
MODEL II (CFA 0.1mL+HFD+LPS)
MODEL III (CIA 0.1mL+ HFD)
MODEL IV (CIA 0.1mL+LPS +HFD)


TLR-4 levels in RA+ CVD
TLR-4 pg/mL
GROUPS
Normal control
HFD control
MODEL I (CFA 0.1mL + HFD)
MODEL II (CFA 0.1mL+HFD+LPS)
MODEL III (CIA 0.1mL+ LPS)
MODEL IV (CIA 0.1mL+LPS +HFD)


NFκB levels in RA+CVD
NFκB pg/mL
GROUPS
Normal control
HFD control
MODEL I (CFA 0.1mL + HFD)
MODEL II (CFA 0.1mL+HFD+LPS)
MODEL III (CIA 0.1mL+ LPS)
MODEL IV (CIA 0.1mL+LPS +HFD)

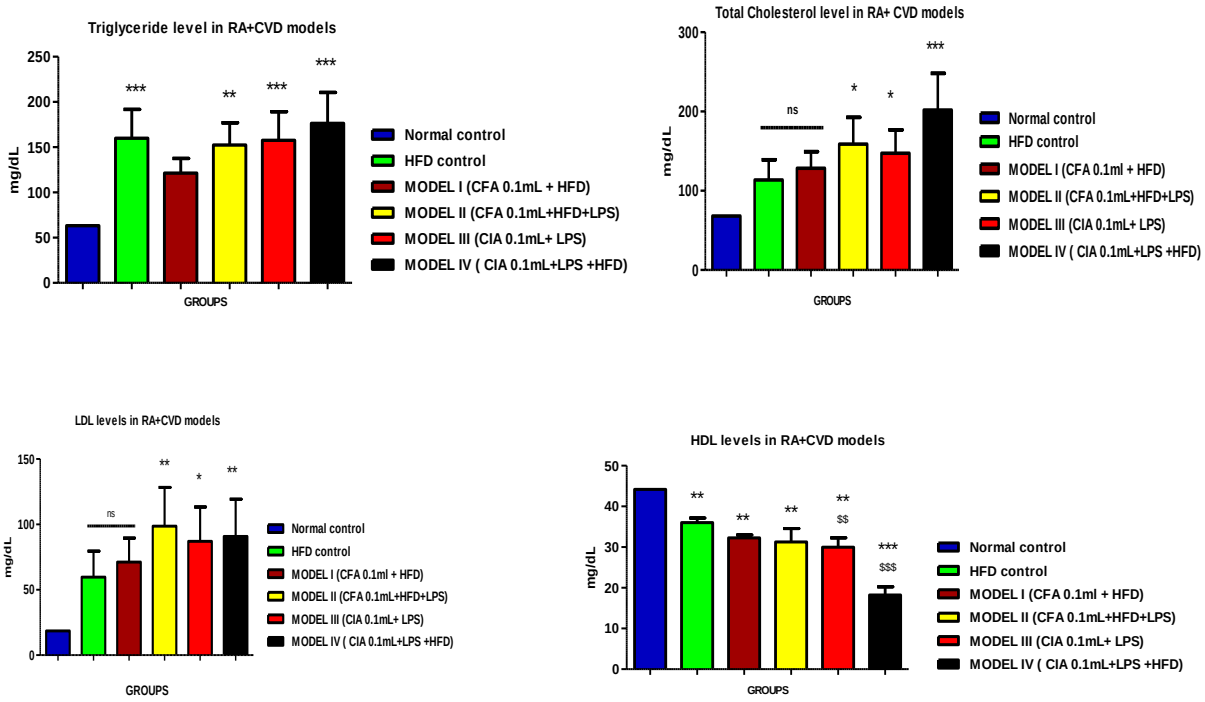


Figure 2. Graphical representation of statistical evaluation for induction of RA with CVD

Note: Values are expressed as Mean ± SEM. Values are statistically evaluated using repeated measure ANOVA (Paw volume) and one way ANOVA analysis followed by Bonferroni's, Dunnett's and Tukey's post hoc test. Significant values were compared with (*P<0.05, **p<0.01, *** P<0.001)

*,**, *** are used for comparison between normal and all groups

&, &&, &&& are used for comparison in between the models.

$, $$, $$$ are used for comparison between LPS induced models.

ns is non-significant differences between groups.

These are the summarized results for studies I and II

Sr. No.	**Parameter**	**Significance**	**Findings in our study**
1.	Paw Volume	To check the inflammation of RA induced Paw of rat	Paw volume was found to be increased in Model control (CIA 0.1 mL+ LPS 10 µg/mL) induced animals. There was a significant decrease in Paw volume in test group as compared to model control and standard control group.

2.	Arthritic Index	To check disease Progression through primary and secondary lesions	Arthritic Index in Model control group (CIA 0.1ml+ LPS 10µg/ml) was found to be increased as compare to normal control and treated groups. Standard control groups showed increased arthritic index as compare to normal and treatment control group.
3.	Arthritic Scoring	To check walking disability of animals	Scoring in model control group(CIA 0.1+ LPS 10µg/ml) and standard control group was found to be increased on day 5 and 21 as compare to normal control and test groups.
4.	CRP	To check inflammatory responses	There was a significant increase in CRP levels in model and Standard control groups as compare to normal and treatment groups from day 7 to day 21
5.	ESR	To check inflammatory Responses	ESR of model control groups was found to be Increased as compare to normal and treatment groups.
6.	Anti- CCP	To confirm RA in CFA induced arthritis	Model control and standard control groups shows increased levels of Anti-CCP which confirms that CFA induced model has developed Rheumatoid Arthritis in experimental animals.
7.	Homocysteine	To check the intraarticular manifestation	There was no significant increase was observed in any group which suggests no extra articular manifestation of disease.
8.	X-Ray	To confirm the evidences of RA	There was a significant change in edema and bone erosion was observed in X-Ray of model control animals as compare to normal control and treatment control groups
9.	Histopathology	For confirmation	Synovial erosion and structural deformity of tissues were noticed in model control and standard control groups which was absent in normal and treatment control groups.

Table 1 Values of statistics as Mean ± SEM for final model in comparison with standard drugs and test drugs

Parameter	Values					
	Normal control	**Model control**	**Std control**	**Test 1**	**Test2**	**Test 3**
Paw Volume	**0.32±0.002**	**0.89±0.03 *****	**0.59±0.034 **, ##**	**0.32±0.005 ###,$$**	**0.32±0.005 ###,$$**	**0.32±0.005 ###,$$**
Arthritic Index	**0**	**16.1±1.13 *****	**15.3±0.68 ***, #**	**6±0.91 ###,$$$**	**8.83±0.75 ###,$$$**	**8.83±0.34 ###,$$$**
Arthritic Scoring	**0**	**15.8±1.03 *****	**13±0.94 ***, ##**	**7.5±0.54 ###,$$$**	**7.5±0.54 ###,$$$**	**7.5±0.54 ###,$$$**
CRP	**2.2±0.34**	**6.6±0.17 *****	**6.3±0.23 *****	**2.4±0.21 ###,$$**	**2.2±0.33 ###,$$**	**2.7±0.23 ###,$$**
ESR	**0.6±0.22**	**10.4±0.61 *****	**5.8±0.34 ***, ##**	**0.66±0.21 ###**	**0.6±0.22 ###**	**0.67±0.23 ###**
Anti- CCP	**2.38±0.15**	**7.6±0.15 *****	**5.1±0.15 ***, ##**	**2.38±1.60 ###,$**	**2.38±1.74 ###,$**	**2.34±0.12 ###,$**
Homocysteine	**4.8±0.36**	**6.3±0.32 *****	**5.5±0.23 ***, ##**	**4.8±0.20 ###**	**4.8±0.50 ###**	**4.8±0.39 ###**

Results of study design 2

Sr. No.	Parameter	Significance	Findings in our study
1.	Paw Volume	To check the inflammation of RA induced Paw of rat	Paw volume was found to be increased progressively in Model control (CIA 0.1ml+HFD +LPS10µg/ml) induced animals from day 3 till day 28 as compared to normal control group. Standard control animals receiving MTX (0.6mg/kg) also showed increase in paw volume when compared with normal control and HFD fed rats but it was on the lower side when compared with model control. There was a significant decrease in all the three treatment groups.
2.	Arthritic Index	To check disease progression through primary and secondary lesions	Arthritic Index in Model control group was found to be increased when compared to normal control and HFD fed group. Standard control groups showed an increased arthritic index when compared to normal control and treatment groups but with a smaller increase then in the model control group.
3.	Arthritic Scoring	To check walking	Scoring in model control group and standard control group was

		disability of animals	found to be increased on days 5 and 21 when compared to normal control HFD and treatment groups. But standard control group did not show any secondary lesions. The groups treated with test drugs have significant results on secondary lesions, which shows their immunological intervention
4.	CRP	To check inflammatory responses	There was a significant increase in CRP levels in model and Standard control groups when compared to normal and HFD-fed groups from day 7 to day 21. The HFD-fed groups do not have any significant increase in CRP. All three test drugs have a significant decrease in CRP levels, which shows the anti inflammatory effects of the drugs.
5.	ESR	To check inflammatory responses	ESR of model control groups was found to be Increased when compared to normal control group. There was a slight elevation of ESR in standard control group. No significant change in HFD-fed groups was observed in ESR. The test drugs show the significant decrease in ESR levels when compared with model control group.
6.	Anti- CCP	To confirm RA in CFA induced arthritis	Model control and standard control groups show increased levels of Anti-CCP, which confirms that CFA-induced model has developed Rheumatoid Arthritis in experimental animals. But the ACCP levels were high in model control groups when compared to standard treated group. There was no significant change in ACCP in HFD-fed group and all the three drugs showed the significant decrease in ACCP levels when compared with Model and standard Control groups.
7.	Homocysteine	To check the extra articular manifestation	There was no significant increase in normal and HFD-fed groups in homocysteine but the significant elevation in model control suggests extra articular manifestation of disease, which is confirmed with other markers of atherosclerosis. There was no significant increase in treatment groups when compared to model control and standard control groups.
8.	Triglyceride (TG)	TG levels are primary indicator for Atherosclerosis	Groups having only HFD showed the increasing order of TG from day 7 till the bend of the study. The groups sensitized with CFA 0.1 mL+HFD + LPS 10 μg/mL (Model control) and standard control also showed the elevation of TG levels. The test groups showed the significant decrease in TG levels, which showed the hypolipidemic action of drugs but when compared with each other.
9	Low Density Lipoprotein (LDL)	LDL are primary raw material for plaque formation with help of TC deposition	Increase in LDL level shows the significant indication for the plaque bedding in arteries. Groups having only HFD showed the increasing order of LDL from day 7 till the end of the study. The groups sensitized with CFA 0.1 mL + HFD + LPS 10 μg/mL (Model control) and standard control also showed the elevation of LDL levels. The groups treated with test group showed the significant decrease in LDL levels when compared with HFD

			group
10.	Total cholesterol (TC)	Total cholesterol is responsible for plaque formation in arteries	Groups having only HFD showed the increasing order of TC from day 7 till the end of the study. The groups sensitized with CFA 0.1 mL+ HFD + LPS 10 μg/mL (Model control) and std control also showed the elevation of TC levels. The groups treated with test drugs showed the significant decrease in TC levels, which showed the hypolipidemic action of drugs against HFD-fed group
11.	High Density Lipoprotein (HDL)	Preventive marker for atherosclerosis	HDL was showed in increasing trends in test treated groups when compared with HFD group. The standard treatment MTX group and model control group showed the decreasing trends in HDL levels
12	Atherogenic score	Shows plaque formation in arteries	Atherogenic score was at lower side in treated groups when compared with model std and HFD-fed groups, which shows the preventive effects of test drugs against plaque formation
9.	X-Ray	To confirm the evidences of RA	There was a significant change in edema and bone erosion observed in X-Ray of model control animals when compared to normal control group. In standard control group, there was a low grade edema and inflammation when compared to model control group.
10.	Histopathology	Confirmatory test	The decreases fibre length of vistus medialis and biceps femoris muscle shows the atherogenesis in tissues. Histopathology of paws showed the generation of RA Aorta sections histopathology confirmed presence of atherosclerosis in model control group with RA

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