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11 Acknowledgment

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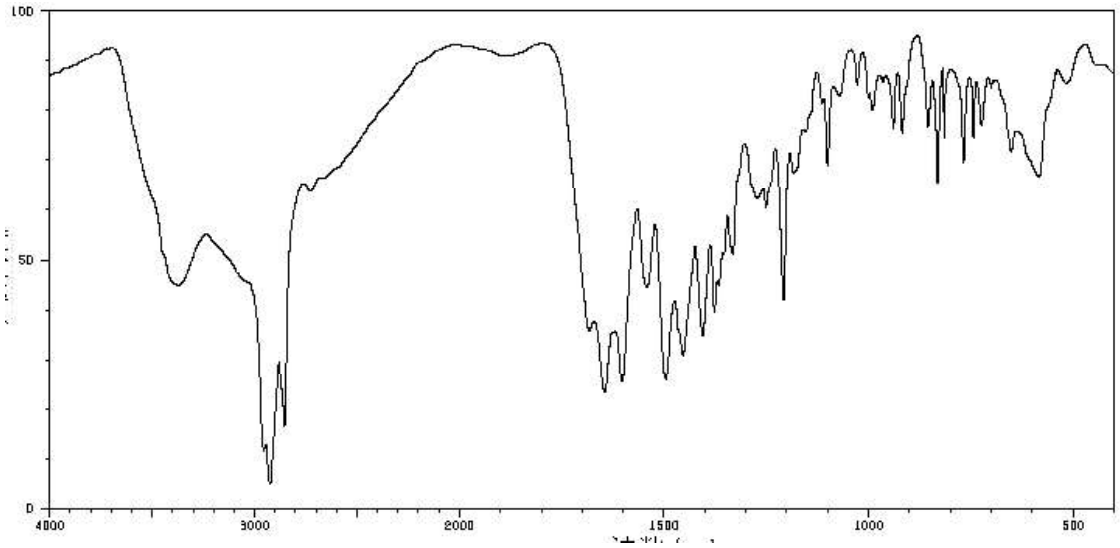
My sincere gratitude to **Dr. Vishal Patel** and **Dr. Hardik Patel** from **Vasu healthcare Pvt. Ltd.** for their kind support and guidance for formulation development and standardization of formulation.

12 Annexure

Test IR spectra of Methotrexate



Standard IR spectra of Methotrexate

Annexure

Institutional Animal Ethics Committee
Pharmacy Department
Old: 404/01/a/CPCSEA (25th April, 2001)
New: 404/PO/Re/S/01/CPCSEA (28th October,2015)
The Maharaja Sayajirao University of Baroda
Vadodara

सत्यं शिवं सुन्दरम्

CERTIFICATE

This is to certify that the following project has been approved by the Institutional Animal Ethics Committee (IAEC), Pharmacy Department.

Protocol No.	:	MSU/IAEC/2016-17/1661
Project title	:	Pharmacological evaluation of some herbs in experimentally induced rheumatoid arthritis along with cardiovascular complications
Chief Investigator	:	Trupti Dubey
Research Guide	:	Dr. Kirti V. Patel

PHARMACY DEPARTMENT M.S IAEC

Dr. Bharat Gajjar
(CPCSEA Main Nominee)

Shri S.P. Rathod
(Chairman, IAEC)

Date of Approval:

Institutional Animal Ethics Committee
Pharmacy Department
Old: 404/01/a/CPCSEA (25th April, 2001)
New: 404/PO/Re/S/01/CPCSEA (28th October,2015)
The Maharaja Sayajirao University of Baroda
Vadodara

CERTIFICATE

This is to certify that the following project has been approved by the Institutional Animal Ethics Committee (IAEC), Pharmacy Department

Protocol No.	:	MSU/IAEC/2018-19/1802
Project title	:	Pharmacological evaluation of some herbs in Rheumatoid Arthritis along with cardiovascular complications
Chief Investigator	:	Trupti Dubey
Research Guide	:	Dr. Kirti V. Patel

PHARMACY DEPARTMENT M.S.U. IAEC

02/08/18

Dr. Bharat Gajjar
(CPCSEA Main Nominee)

(Chairman, IAEC)

Date of Approval: 02/08/18

Institutional Animal Ethics Committee
Pharmacy Department
Old: 404/01/a/CPCSEA (25th April, 2001)
New: 404/PO/Re/S/01/CPCSEA (28th October,2015)
The Maharaja Sayajirao University of Baroda
Vadodara

सत्यं शिवं सुन्दरम्

CERTIFICATE

This is to certify that the following project has been approved by the Institutional Animal Ethics Committee (IAEC), Pharmacy Department

Protocol No.	:	MSU/IAEC/2019-20/1904
Project title	:	Pharmacological evaluation of some herbs in Rheumatoid Arthritis along with cardiovascular complications
Chief Investigator	:	Trupti Dubey
Research Guide	:	Dr. Kirti V. Patel

Dr. Bharat Gajjar
(CPCSEA Main Nominee)

(Chairman, IAEC)

Date of Approval: 29/Aug/2019

PHARMACY DEPARTMENT M.S.U.

SIGMA-ALDRICH

3050 Spruce Street, Saint Louis, MO 63103, USA
Website: www.sigmaaldrich.com
Email USA: techserv@sial.com
Outside USA: eurtechserv@sial.com

Product Specification

Product Name:
Freund's Adjuvant, Complete – cell suspension

Product Number: **F5881**

MDL: MFCD00131105

Storage Temperature: 2 - 8 °C

TEST	Specification
Appearance (Color)	Light Yellow to Yellow
Appearance (Form)	Liquid
with particulates	
Appearance (Turbidity)	Clear to Slightly Hazy
Emulsification	Pass
Forms emulsion with 0.85% NaCl	
Note	

Each mL contains 1 mg Mycobacterium tuberculosis(H 37RA, ATCC 25177), heat killed and dried, 0.85 mL paraffin oil and 0.15 mL mannide monooleate.

Specification: PRD.1.ZQ5.10000016267

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1 of 1

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Introduction:

The GENLISA™ ELISA kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma and cell culture supernatant as validated with the kit. The kit employs a sandwich ELISA

THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA
FACULTY OF SCIENCE
DEPARTMENT OF BOTANY
VADODARA
e-mail: dmnagar@gmail.com

Dr. P. S. Nagar
Asst. Prof.

Ref: Bot/38317/aut/1 Date: 30/03/2017

CERTIFICATE OF AUTHENTICATION

This is to certify and authenticate that the herbarium specimens provided by **Trupti Dubey**, Faculty of Pharmacy, The Maharaja Sayajirao University of Baroda are of *Cichorium intybus* L. (root), *Carica papaya* L. (seed) and *Nigella sativa* L. (seeds).

(P.S. Nagar)

Dr. Padamnabh S. Nagar
Assistant Professor
Department of Botany
The M. S. University of Baroda
Vadodara - 390002

DEPARTMENT OF BOTANY
FACULTY OF SCIENCE
THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA
VADODARA- 390 002, Gujarat, (INDIA)
Telephone: (0265) 2791891
E- mail : drnagar@gmail.com

Dr. P. S. Nagar (M.Sc. Ph.D)
Associate Professor and Garden Superintendent

Date: 22/06/2022

CERTIFICATE OF PLANT AUTHENTIFICATION

This is to certify that the plant material (seeds) TD04 provided by Miss Trupti Dubey of Faculty of Pharmacy of The Maharaja Sayajirao University of Baroda, Vadodara is that of

1. *Momordica charantia* L. (Cucurbitaceae)

Verified by

Padamnabhi S. Nagar

DR. PADAMNABHI S. NAGAR
ASSOCIATE PROFESSOR
DEPARTMENT OF BOTANY, FACULTY OF SCIENCE,
THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA,
VADODARA - 390 002

AMSAR PRIVATE LIMITED

47, Laxmibai Nagar, Industrial Estate, Fort, INDORE - 452006

Certificate of Analysis

1. Sample : **NIGELLA SATIVA STANDARDIZED DRY EXTRACT**
2. Batch No. : 3606 3. No.F/D : 542
4. Supplier : AMSAR 5. Category : INTERMEDIATE
6. Date of Receipt in Lab : 03/08/2022
7. Date when test completed : 08/08/2022

8.	PROTOCOLS OF TEST APPLIED	STANDARD TO WHICH COMPARED	ACTUAL RESULT
1	DESCRIPTION	BROWN POWDER WITH CHARACTERISTIC ODOUR	COMPLIES
2	ORGANOLEPTIC TEST	BITTER TASTE	COMPLIES
3	WATER SOLUBLE EXTRACTIVES BY API METHOD TAKING 1 GM SAMPLE	NOT LESS THAN 70%	84.34%
4	BITTER	NOT LESS THAN 1.5%	1.86%
5	HEAVY METALS		
	a) As	NOT MORE THAN 03PPM	COMPLIES
	b) Pb	NOT MORE THAN 10 PPM	COMPLIES
	c) Cd	NOT MORE THAN 0.3 PPM	COMPLIES
	d) Hg	NOT MORE THAN 1 PPM	COMPLIES
6	MICROBIAL PROFILE		
	a) TOTAL PLATE COUNT	NOT MORE THAN 1000 CFU/G	370CFU/G
	b) YEAST & MOULDS	NOT MORE THAN 100 CFU/G	ABSENT
	c) E.COLI	ABSENT	ABSENT
	d) SALMONELLA	ABSENT	ABSENT

Opinion of Analyst: The sample Conforms to the standard compared.

Statement: MANUFACTURING DATE : AUGUST - 2022
EXPIRY DATE : 3 YEARS FROM THE DATE OF MANUFACTURE

Sample Tested by:
Report Authorised by:

Manisha
Testing Chemist

FOR AMSAR PRIVATE LIMITED

Dr. (Mrs.) C. P. Bhatt
Q.C Manager

AMSAR PRIVATE LIMITED

47, Laxmibai Nagar, Industrial Estate, Fort, INDORE - 452006

Certificate of Analysis

1. Sample : **CARICA PAPAYA (PAPAYA) SEED STANDARDIZED DRY EXTRACT**
2. Batch No. : 3777.
3. No F/D : 714
4. Supplier : AMSAR
5. Category : INTERMEDIATE
6. Date of Receipt in Lab : 15/09/2022
7. Date when test completed : 20/09/2022

8. PROTOCOLS OF TEST APPLIED	STANDARD TO WHICH COMPARED	ACTUAL RESULT
1 DESCRIPTION	BROWN POWDER WITH CHARACTERISTIC ODOUR	COMPLIES
2 ORGANOLEPTIC TEST	SLIGHT BITTER TASTE	COMPLIES
3 ALCOHOL SOLUBLE EXTRACTIVES BY API METHOD TAKING 1 GM SAMPLE IN 50% ALCOHOL	NOT LESS THAN 30%	30.62%
4 LOSS ON DRYING	NOT MORE THAN 6%	4.94%
5 ASH	NOT MORE THAN 20%	17.56%
6 SIEVE TEST POWDER PASSES TROUGH 40 MESH	NOT LESS THAN 90%	COMPLIES
7 HEAVY METALS		
a) As	NOT MORE THAN 03PPM	COMPLIES
b) Pb	NOT MORE THAN 10PPM	COMPLIES
c) Cd	NOT MORE THAN 0.30PPM	COMPLIES
d) Hg	NOT MORE THAN 1PPM	COMPLIES
8 MICROBIAL PROFILE		
a) TOTAL PLATE COUNT	NOT MORE THAN 1000 CFU/G	390CFU/G
b) YEAST & MOULDS	NOT MORE THAN 100 CFU/G	ABSENT
c) E.COLI	ABSENT	ABSENT
d) SALMONELLA	ABSENT	ABSENT

Opinion of Analyst: The sample Conforms to the standard compared

Statement: MANUFACTURING DATE : SEPTEMBER - 2022
EXPIRY DATE : 3 YEARS FROM THE DATE OF MANUFACTURE

Sample Tested by:

Report Authorised by:

FOR AMSAR PRIVATE LIMITED

AMSAR PRIVATE LIMITED

47, Laxmibai Nagar, Industrial Estate, Fort, INDORE - 452006

Certificate of Analysis

1. Sample : **MOMORDICA CHARANTIA SEED STANDARDIZED DRY EXTRACT**
2. Batch No. : 3871 3. No.F/D : 813
4. Supplier : AMSAR 5. Category : INTERMEDIATE
6. Date of Receipt in Lab : 10/10/2022
7. Date when test completed : 15/10/2022

8.	PROTOCOLS OF TEST APPLIED	STANDARD TO WHICH COMPARED	ACTUAL RESULT
1	DESCRIPTION	BROWN POWDER WITH CHARACTERISTIC ODOUR	COMPLIES
2	ORGANOLEPTIC TEST	BITTER TASTE	COMPLIES
3	ALCOHOL SOLUBLE EXTRACTIVES IN 90% ALCOHOL BY API METHOD TAKING 1 GM SAMPLE	NOT LESS THAN 40%	46.34%
4	BITTER	NOT LESS THAN 3.5%	3.56%
5	LOSS ON DRYING	NOT MORE THAN 8%	4.46%
6	HEAVY METALS		
	a) As	NOT MORE THAN 03PPM	COMPLIES
	b) Pb	NOT MORE THAN 10PPM	COMPLIES
	c) Cd	NOT MORE THAN 0.30PPM	COMPLIES
	d) Hg	NOT MORE THAN 1PPM	COMPLIES
7	MICROBIAL PROFILE		
	a) TOTAL PLATE COUNT	NOT MORE THAN 1000 CFU/G	380 CFU/G
	b) YEAST & MOULDS	NOT MORE THAN 100 CFU/G	ABSENT
	c) E.COLI	ABSENT	ABSENT
	d) SALMONELLA	ABSENT	ABSENT

Opinion of Analyst: The sample Conforms to the standard compared.

Statement: MANUFACTURING DATE : OCTOBER - 2022
EXPIRY DATE : 3 YEARS FROM THE DATE OF MANUFACTURE

Sample Tested by:

Report Authorised by:

Testing Chemist

FOR AMSAR PRIVATE LIMITED

Dr. (Mrs.) C. N. Dhatt
Q. C. MANAGER
Q C Manager

13 Supplementary file

FMID based model validation approaches for Rheumatoid Arthritis and associated cardiovascular complications: An attempt towards translational competence

The proposed study was hypothesized to develop an animal model which can give a better insight of Rheumatoid Arthritis with pathophysiological interventions similar to human etiology of the disease. The design of study was progressed with the complexity of the disease similar to the human disease events. Here, different inducing agents were selected for the induction of Rheumatoid Arthritis and the extra organ manifestations similar to human conditions.

CFA (Complete Freund's Adjuvant) was taken as primary inducing agent as this is the most frequently used agent for development of RA in rats. To see the advancement in disease stages CIA (Collagen type II) was opted as secondary inducing agent to cross link the inflammatory pathway with the immunological manifestations. LPS (Lipopolysachcharide) and HFD (High Fat Diet) was incorporated to mimic the cardiovascular complications generally occurring in RA. Clinically, in patients taking NSAIDs for long term or the patients having stable RA progressed CVD complications with alteration of autoimmune and inflammatory responses.

The study was carried out using Wistar male rats. Different groups were evaluated for selected inducing agents alone and with combination. All the groups were primarily evaluated using ANOVA as statistical tool for two situations 1) Rheumatoid Arthritis induction and 2) Cardiovascular complication associated with Rheumatoid Arthritis. After getting the suitable groups these optimized groups were carried forward for the validation of models to check the human resemblance of the disease generated in rats for above mentioned two conditions.

Validation

Decision regarding selection of an optimum/appropriate dose of LPS (among three concentrations 0.1, 0.5 and 10µg/ml) was made by processing the obtained data for significance using ANOVA as primary evaluation tool for pharmacological comparison. The selected models were carried forward for validation based on the Framework to Identify Models of Disease (FIMD) given by Guilherme S. Ferreira et.al. This is a questionnaire based validation system where were models were further compared and evaluated for eight domains; Epidemiology, Symptomatology and Natural History–SNH, Biochemical validation, Aetiological validation, Pharmacological validation, Histological validation, Endpoint validation and Genetic validation.

The framework was adopted and the questions were framed using reference of the framework but some extra points were added according to the need of the study. The questions were more focused on the Rheumatoid Arthritis and Cardiovascular complications in the selected models with the questions of clinical relevance.

Instructions given for scoring and calculations for the final radar plot reading were followed to fit the validation criteria in the proposed models of RA and RA along with CVD using following steps-

The questions were framed according to disease severity and progression using the mentioned domains.

Each domain has given the same weighing (score) which was calculated using 100 as total score and equally divided 100 by 8 domains which comes 12.5 for each domain.

Now all the domains may be having sub sections so the same weighing for each domain (12.5) was

equally divided to get the score for each sub section.

Suppose question no. 1 has two subsections so the score will be
Weighing score 12.5
score 6.25
score 6.25
The subsections may also have other sub subsections like question 1.1 has total score 6.25 but it has 2 more sub subsections so the score will be calculated as-
score 6.25
score 3.125
score 3.125
All the section, sub section and sub subsection total should be 100
Now how to give answer of the questions mentioned in each domain and sections-
There are total five options to answer each question with their weighing score in percentage-
Yes which has 100% score
If your Answer for question 1.1.1 is yes you have to take percentage of the given score (3.125) with 100
Yes completely 100% score but it has a grading system where we have to give a grade to the question on the basis of its severity or the weight. Here in this study we have taken three grades; A (70%), A^{+} (80%) and A^{++} (90%)
If your answer is yes completely with 70% you have to calculate the 70 % of obtained score and if it is A^{+} or A^{++} with 80 or 90 % weight you have to calculate the percentage accordingly.
Yes Partially has 50% weight you have to calculate 50% of the obtained score
No has 10% weight
Unclear 0%
After answering the questions with suitable grade all the factors were added to get the final score and the final score was subtracted with the actual domain score to get ratio for plotting radar chart.
Que. 1 Weighing score for domain- 12.5
score 6.25
score 3.125 Answer is yes partially so the factor will be 50% and the final score will be 3.125*50/100= 1.56
score 3.125 answer is A then the factor will be 70% and the final score will be
3.125*70/100= 2.18

8. Now to get the Radar value (Ratio), all final scores obtained after taking percentage were added and subtracted with the score of domain i.e. 12.5
   So the Radar value for the Que. 1 will be- 1.56+2.18+6.25/12.5= 0.7

How to prepare a Radar Plot

Radar chart or web chart is one of the comparative tools for analyzing multivariate data. Here the radar chart give each domain an axis and we can compare the models by putting the ratio obtained after giving suitable score to each question and calculated through the steps mentioned above.

How to interpret a Radar plot

Here, Microsoft excel was used to generate a Radar plot and values (calculated ratio) for each domain were analyzed.
While we put the ratio in radar plot using excel data sheet we get the graph.
In this graph we can see the domains on different axis as this graph is 2 dimensional representations.
On the basis of the ratio value which move towards the axis and the dispersion from the axis we can get results in the form of similarity factor and uncertainty factor.

The values moves towards the axis and having the similar intersecting points with other domains values have the interconnectivity between them
The values which are not intersecting each other and having higher dispersion from the axis towards the edges of graph are having higher ratio and they are having higher dissimilar or uncertainty with compared groups.

Design of questionnaire
Questionnaire were made for two situation as per the need of study-
Rheumatoid Arthritis model
Questionnaire 1 (Model I RA induction with CFA 0.1ml+ LPS 10µg/ml)
Questionnaire 2 (Model II RA induction with CIA 0.1ml+ LPS 10µg/ml)
Rheumatoid Arthritis with co morbid conditions
Questionnaire 3 (Model III RA and CVD induction with CFA 0.1ml+ LPS 10µg/ml+ HFD)
Questionnaire 4(Model IV RA and CVD induction with CIA 0.1ml+ LPS 10µg/ml + HFD)

The FIMD questionnaire was prepared and each section and sub section was answered using the following instructions to get final radar score.

How to answer the questions
Here in this study the questions were answered by using the five criteria **Yes, Yes completely, Yes partially, No and unclear**. In this section each domain wise answer and their justification for suitable answer is given with details.

1. Epidemiological Validation
This section is similar for both the models RA as well as RA and associated complications

13.1 Nature of population (Inbred/Out bred)

Here the question is answered by ***yes partially*.**
Justification
The animals were obtained from the different research organizations who are registered breeders and these animals were inbreeded for experimental purpose only. The place where the experiment was conducted is different from their breeding place bit for this laboratory the animals were out bred, so the answer is ***yes partially.***

13.2 Is the model able to simulate the disease in relevant age groups (e.g. juvenile, adult or ageing)

Answer for the question is ***Unclear***
Justification
In humans for clinical presentation of symptoms age is one of the criteria to see the prevalence of disease in particular age (e.g. juvenile, adult and elderly). Some diseases have onset at particular stage of life in clinical situations. It is important to incorporate such criteria in preclinical situation but it will not be able to give similar results as the life span of rat and human is different but we can compare that whether the disease develops totally, partially or not at all in the same developmental phases. In the present study RA was developed in the rats which were selected on

the basis of their maturity but age was not the major criteria as compared to the human rheumatoid conditions where elder people and adults are more prone towards the disease.

Here wistar male rats were taken for the studies were approximately of 6-8 wk old. But the specific age was not adopted for the model development and comparison between the models, so the *in-vivo* data is not sufficient to answer this question; hence the answer is ***Unclear***

13.3 Is the model able to simulate the disease in the relevant genders?

Here the question was answered differently for different questionnaires

In RA only using CFA and LPS as inducing agent this question was answered as *yes partially*

Justification

This particular study was performed on wistar male rats but the data for the RA development were present in both the genders using CFA and CIA. Here the model which was induced RA with CFA 0.1ml sub planter and CIA 0.1ml sub planter were answered *yes partially* because the literature search suggests that female rats are equally prone towards the disease induction using the same inducing agent. LPS was not having any accounts with both of these inducing agents.

Clinically also firstly RA was considered as the disease of female and elder people but as the clinical manifestations were occurred equally in the adult males also and now, RA is no longer considered gender or age specific disease.

In other models of RA along with cardiovascular complications (questionnaire 3, 4) this que was answered as ***Unclear.***

Justification

All the models have incorporation of LPS with CFA or CIA with or without HFD which is uniqueness of this model and no such models have been come to our knowledge for inducing these situations in wistar male rats. Moreover here study was performed on male rats only and data of this particular combination is not available for female rats so these questions were answered as ***Unclear*** in questionnaire 3 and 4 in RA along with CVD models.

2. Symptomatology and natural history

This section has different sub sections and sub subsections on the basis of models and questionnaire prepared for that particular model.

Is model is able to Mimic human disease symptoms, if so which one

13.4 In this section the major symptoms according to the progression of disease in model developed for Rheumatoid Arthritis and questions were framed accordingly.

13.5 Inflammation is one of the major markers in RA and immunological intervention is connecting link between RA and extra organ manifestations.

Inflammatory markers

Inflammation is the primary indication of RA as the definitions says RA is a chronic inflammatory autoimmune disorder. Here in this part the core symptoms generated in clinical situation are tries to compare. Physical inflammatory markers like paw volume and biochemical markers like ESR and CRP were measures for assessment of inflammatory responses.

In questionnaire 1 (RA only with CFA and LPS) was answered as ***yes partially*** because the inflammation was not constant throughout the study period and when compared with other groups it was lesser then the CIA induced groups.

In **questionnaire 2 and 3** in which collagen was used as inducing agent were answered as ***yes***

Due to high grad persistent inflammation in these two groups

In **questionnaire 4** the question was answered as ***yes completely*** as the inflammation was very high and it was similar with clinical situations which represents walking

Immunological markers

Immunological markers such as TNF-α, IL-6 and ACCP were measured for assessment. Where-

In questionnaire 1 (Model I) was answered as ***yes partially*** because the immune markers were expressed but not significantly different with normal control and test groups.

In **questionnaire 2 and 3** in which collagen was used as inducing agent were answered as ***yes*** with A^+ (70 %) grade as the expression of immune markers in these two groups was high as compared to other groups.

In **questionnaire 4** the question was answered as ***yes completely*** (100%) as the immune responses were very high and it was similar with clinical situations.

Crosslinking markers for cardiovascular complications

Cross linking markers such as homocysteine and TLR-4 were estimated in both the situations which are the indication of initiation of extra organ manifestations in stable RA.

In Model I and II there was no significant estimation was observed so the answer was given as ***unclear*** in these groups

In model III the values of these markers were expressed at some extant so this model was answered as ***yes partially***

In model IV the expression of these markers were significantly high so this question was answered in this group as ***yes*** with grade A

3. Natural History criteria matching with human disease onset

As the patient history cannot be taken in the animal models due to species specific criteria this question was assessed and answered on the observational measures only. In this section the questions were kept similar with clinical conditions and on the basis of observation done by researcher they were answered for all four models.

Time of onset

The time of onset of disease in CFA induced models was answered as ***yes partially*** as this model was of 28 days and the disease specific markers were elevated significantly after day 14, so the model was practically induced in later phase of the study and they were not stable throughout the study period.

In model III as the onset of disease was early as compared to group I but it was not matched completely with human conditions it was scored with grade A (70%) and answered as ***yes***

In model IV the disease induction was fast and persistent throughout the study and the progression of disease just like human condition; primary and secondary lesions were noticed prominently this group was scored with 100% grade with ***yes completely***

Disease Progression

Disease progression in model I was matched partially with human conditions so answered as ***yes partially***

In model II the progression was high as compared to group I so it was answered as ***yes*** but with grade A (70%) as the progression was fluctuating.
In model III again the progression was partial (on /off) effects in symptoms, so the answered as ***yes partially***. Rat in CFA model do not show immunological intervention as it was seen in CIA model so the Ans for specific group will be yes partially. As the species is different this Ans will not be count as bias.
In group IV this question was answered with **yes completely** as the progression was symmetrical, persistent and constant which mimics the clinical situations fully. But the biasness will be there as human and rat belongs to different species and exact physiological changes are not possible

Duration of Symptoms
Disease symptoms in model I was matched partially with human conditions so answered as ***yes partially***
In model II the progression was high as compared to group I so it was answered as ***yes*** but with grade A^+ (80%) as they are not constant throughout study. Model III A (70%) and IV also showed the higher duration of symptoms with grade A^+ (80%)

Severity
Describe whether the severity of the symptoms manifested in the model is similar to the ones manifested in humans. This shall be done comparatively, with a brief description of both the human and animal situation.
This question was answered as ***yes partially*** in model I ***yes*** with 70% in model II, in model III ***yes partially*** 50% and in model IV ***yes*** with 80%

Metabolic dysbiosis (Obesity, TG, TC, LDL, HDL and fat accumulation in stool)
This question was answered ***unclear*** in model I and II where diet modification was not done and these models were developed only for RA.
In model III this question was answered with ***yes partially*** as the changes were 50% similar with human conditions and in model IV the question was answered with ***yes*** with grade A.

Co-Morbid Conditions replicated in model similar to human conditions? If yes which one
In this section models were compared with clinical situations in terms of severity, extra organ manifestation and constant pathological changes.

Secondary lesions
Secondary lesions were observed on day 21 in CFA induced groups and on day 21 and 35 in CIA induced groups. In this section the model I was not resemble the secondary lesions only primary lesions were seen in these models so the answer is ***unclear***.
In model II and III this changes were observed with grade A^+ (80%) so answered as ***yes***

4. Biochemical Validations
Pharmacodynamic biomarkers mimic the pathophysiology of the human disease
Biochemical validation was done for different biomarker elevation with the physical changes occurred in the animals with the progression of disease. In these sections questions were framed for inflammatory Markers (CRP, ESR, and Arthritic Index), immunological markers (TNF-α, IL-6, ACCP, and Hyc), atherogenic markers (TG, TC, Cholesterol, Atherosclerotic index).
How to score

In this section the models were scored on the basis of answers given yes I, II III and yes completely in model IV on the basis of elevation of these biochemical.

Do these PD markers behave similarly to human?

The comparison of human condition with 100% symptomatology is not possible but comparison in between the models and the resemblance with the clinical situation given in next section were compared and assessed in this section. And answered as ***yes partially*** and ***yes*** in respective groups

Known prognostic markers related to pathophysiology of the disease

This section was to assess the physical, biochemical and perceptive changes in the models in context to RA as well as RA with CVD in form of body weight, paw volume, biochemical elevation with inflammation nodule formation symmetric progression of disease, increase in secretions and walking disability in animals of different groups

5. Aetiological Validation

Aetiological validation was done for both the two situations; RA and RA with CVD for different situations in the form of different physical, perceptive changes and biochemical changes.

In this section the questions were designed for Cytokine Activation (TNF-α, IL-6) Cell infiltration (ACCP generation) and Radiographic changes seen in the models and they were answered according to the results for different models of RA.

Disability, Receptor Activation (TLR-4), Extra organ manifestation, Fibre length of Vistus medialis, Fibre length of Biceps Femoris were framed for RA with CVD.

The Aetiology of the disease in the model is similar to humans regarding both genetic and environmental (including lifestyle) factors. The hypothesized biomarker activation is also included in this section for comparison of progression of disease via different sensitizing agents. A brief review of what is known of the aetiology of the disease shall be included together with a comparative discussion on the animal model's disease aetiology. Genetic factors shall be cited in reference to the Genetic Validation domain, and environmental factors shall also be described.

If there are known Pharmacodynamic (PD) biomarkers related to the pathophysiology of the disease, are they also present in the model?

The known Pharmacodynamic markers for RA were taken from literature survey and they were analyzed in the developed model to see the mimicking in model with resemblance with human condition. The most relevant markers in pathophysiology of the disease in humans were selected for the comparison (ACCP, CRP, Hcy) because these are the molecules which can describe the current state of a disease. These markers can also be prognostic biomarkers which can give a better insight of disease and we can also define the severity of disease by evaluating them on different time points.

How to score?

If more than one biomarker is identified, a subsection shall be created for each relevant biomarker and individually analyzed. The total score for this question shall be calculated by the sum of each subsection (which can be answered according to the pre-specified answers).

Examples

Yes.

Here we can take C-RP as one of the markers if it is high as similar to human Ans will be yes

No.

If the C-RP levels were not increased the Ans will be No

Do these PD biomarkers behave similarly to humans'?

Describe whether the behavior of such biomarkers (higher or lower levels) is similar to the one seen in humans. If relevant, temporal (i.e. relevant only during a specific stage of development of disease phase) and spatial (i.e. present only in specific tissues) aspects of expression must also be considered.

How to score?

If more than one biomarker is identified, a subsection shall be created for each relevant biomarker and individually analyzed. The total score for this question shall be calculated by the sum of each subsection (which can be answered according to the pre-specified answers).

Examples

Yes.

Like humans, rats have increased levels ACCP

No.

Unlike humans, rats have normal levels of ACCP

If there are known prognostic biomarkers related to the pathophysiology of the disease, are they also present in the model?

Prognostic markers similar to human condition of RA and RA along with CVD were identified with different quantitative and qualitative methods which were analyzed during model development and they are kept here as sub sections and sub sub sections in questionnaire

How to score?

If more than one biomarker is identified, a subsection shall be created for each relevant biomarker and individually analyzed. The total score for this question shall be calculated by the sum of each subsection (which can be answered according to the pre-specified answers).

Do these prognostic biomarkers behave similarly to humans'?

Describe whether the behavior of such biomarkers (higher or lower levels) is similar to the one seen in humans. If relevant, temporal (i.e. relevant only during a specific stage of development of disease phase) and spatial (i.e. present only in specific tissues) aspects of expression must also be considered.

How to score?

If more than one biomarker is identified, a subsection shall be created for each relevant biomarker and individually analyzed. The total score for this question shall be calculated by the sum of each subsection (which can be answered according to the pre-specified answers).

How to score?

In this section the hypothesized biomarker activation; TLR 4 or NLRP activation were answered as **yes partially**

And Known biomarkers like ACCP and Metabolic dysbiosis, walking difficulties, Arthritic Index, symmetric progression of disease were answered as **yes completely** according to suitable grading.

6. Pharmacological Validation

Pharmacological validation was done on the basis of evaluation of test drugs in different groups

In this study three herbal drugs were evaluated against the models given in this study. And all the treatment groups were also evaluated on the parameters mentioned in different sections in the questionnaire.

As the treatment protocol was not part of this study, and the study was more focused for model comparison the results for treatment groups were not presented in this study. And code for the test drugs was given as Test drug 1 (MPTN02), Test drug 2 (MPTN01) and Test drug 3 (MPTN04). These test compounds were evaluated individually as well as in the combination in finalized models to evaluate their ameliorative effects on RA and RA with CVD.

Here MTX was used against the model group for RA and Atorvastatin and Telmisartan were used in RA with CVD groups to see the effects on severity of disease and the drug was proved to reduce the disease severity in treated models, so the answer of this que is given as- ***Yes and Yes Partially*** in suitable models.

Justification

Here in the Rheumatoid Arthritis model Methotrexate was taken as standard drug for comparison which is most frequently used DMARDs in treatment of RA in clinical situations. The pathophysiological events are quite similar as per the result assessment in human and rat RA development the effective drug MTX in human condition here also proved to reduce the severity in standard group as compared to model control animals.

All the criteria mentioned in the source article were followed while giving answer of this Que.

Are ineffective drugs in humans also ineffective in this model?

As per the source literature the ineffective drug should be listed in the drugs withdrawn from the market after suitable clinical trials.

No such drug was evaluated here in this study so the answer is No and unclear for the relevant groups

Have the drugs with different mechanism of action and acting on different pathway been tasted? If yes which one?

Herbal drugs were evaluated in this study and they were having different mode of action as compared to existing therapies for the disease.

6. Histological Validation

The major Histopathological changes generated in human RA conditions and cardiovascular complications progressed in RA patients were taken as a reference via review literature and the affected organs like bone, heart and targeted muscles (Vistus medialis and biceps Femoris) were studied to see the changes as per the disease progression and were compared with normal and treatment groups along with standard human treatment drugs to see the changes and recoveries.

7. Endpoint Validation

Some of the methods were similar in estimation of the endpoints like estimation of biochemical markers which are comparable with clinical methods and some of the methods were different like paw edema measurement but the purpose of estimation was similar (i.e. for estimation of inflammation) In this section the endpoints were validated differently for both the conditions

The endpoints in context of similarity of developed model are compared with the clinical symptoms generate in selected disease in humans to see that whether the disease is translated or not. Here as per the reference at least one study included in the pharmacological validation are the same or can be translated to the clinical endpoints commonly used in clinical trials, indicating their feasibility in the model.

In the Rheumatoid Arthritis these Translatable endpoints were, symmetric progression of disease, walking disability, nodule formation, change in X-Ray and Histopathological changes. And For cardiovascular complications associated with RA the endpoints were Gut infiltration, TLR and NLRP activation, fibre length of Vistus medialis and biceps Femoris muscle and histopathology of heart

How to score?

The evaluation parameters which were hypothetized were answered as ***Yes Partially*** to avoid the biasness due to species difference

And the biomarkers which are confirmed in human and seen in the models also were answered as Yes complitely with the grading according to severity in different models sensitized with different inducing agents.

8. Genetic Validation

Genetic validation is one of the methods which can confirm the disease, its complications and the root cause of that particular pathophysiological event in the form of genes involved in activation of disease. Due to limitations in facility, funds or ethical consideration the genetic validation is not possible in each type of research. Here in this study also the genetic aspects of Rheumatoid Arthritis as well as RA with its complications were not studied on genetic validation measures hence each and every model is answered accordingly.

Does this species also have orthologous genes and/or proteins involved in the human disease?

In this section all the models were answered as ***yes partially***

Justification

In this particular disease wistar rats were used as models and the literature search suggests that the proteins and the genes are similar in the pathophysiological condition of RA and RA along with associated complications in both the species (rat as well as human).

In this study the TLR-4 Protein was hypothesized to get activated in the representative models of RA along with co-morbid conditions and the expression was checked by the ELISA methods. The data of ELISA methods were not sufficient to give the gene activation accounts and there is a scope of proper detection and identification of genes involved for both the conditions in this study with advanced techniques. However the literature search revealed the mechanisms of primary inducing agents and role of some similar genes and heat shock proteins incorporated in both the species for the selected condition. On this account this section was answered as ***yes partially*** in all the developed models

If so, are the relevant genetic mutations or alterations also present in the orthologous genes/proteins?

This question was answered as ***unclear*** in all the model groups

Justification

The supporting studies which can represent the mutation or alterations in the relevant proteins or gene were not performed here and so the question was answered as ***unclear***

If so, is the expression of such orthologous genes and/or proteins similar to the human condition?

This question was answered as ***unclear***

Despite the involvement of similar proteins and genes the similarity was not accounted fully similar and this will be counted as partial as there is a species variation is present in rat and human. Moreover genetic alteration was not evaluated according *to in-vitro* and *in-vivo* studies suggested by the experts in this area of research so the question was answered as ***unclear***