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Efficacy of Guaiacum Officinale on Rheumatoid Arthritis and its Possible Role on Inflammatory Pathway through an Experimental In-Vitro and Clinical Models

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***Abstract*: Rheumatoid arthritis (RA) is an autoimmune disorder characterized by symmetric, erosive synovitis along with extra articular involvement. Alternative medical intervention like Homoeopathy plays an essential role in the management of RA. The objective of the study highlights the anti-inflammatory and anti-oxidative potential of Homoeopathic mother tincture of Guaiacum Officinale (GUA)on raw cell line which have been extensively used to control the pain and inflammation of RA patients and also to evaluate the effect of the same in clinical setting. MTT assay was used to evaluate the cell viability of GUA. To establish a precise role in antioxidant activity, cellular reactive oxygen species (ROS) were quantified with DCFDA. The release of TNF-α, COX-2 was measured in the supernatants of PBMC cells treated with LPS in the absence or presence of GUA using ELISA kit. For clinical trial, 30 diagnosed cases of RA, attended on Out Patient Department of ANSS Homoeo Medical College Hospital and special medical camp were randomised to two Groups.(Group A: Homoeopathic Constitutional treatment; Group B:Homoeopathic Constitutional treatment along with Guaiacum tincture) In the present study, MTT assay of GUA at 12.5µg/mL concentration showed no cytotoxicity on hPBMCs cells with cell viability of 89%.We also confirmed the anti-inflammatory effect of GUA in LPS activation-mediated inflammatory response and the antioxidant potential of Guaiacum Officinale. From the study, Group A patients showed significant reduction in ESR among RA patients, but it is not effectively reducing RA factor and CRP. However, in case of Group B, there is significant reduction in ESR and RA factor. There was no considerable improvement in CRP values in both groups. And through in-vitro study it is evident that Guaiacum significantly acted as an anti-inflammatory and anti-oxidant agent that can be therapeutically have a role in treating RA.**

***Keywords*: Anti-inflammatory, Anti-oxidant, Guaiacum Officinale, Homoeopathy, Rheumatoid Arthritis.**

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disorder of unknown etiology characterized by symmetric, erosive synovitis and, in some cases, extra articular involvement. Most patients experience a chronic fluctuating course of disease that despite therapy, may result in progressive joint destruction, deformity, disability, and even premature death. Disability from RA causes major economic loss and can have a profound impact on families. RA affects 1% of the adult population. Current medical treatments do not consistently halt the long-term progression of these diseases, and surgery may still be needed to restore mechanical function in large joints. So, the patients with RA often take alternative treatments and Homeopathy is a popular alternative medical intervention for chronic conditions with patients reporting considerable satisfaction [1]. Homoeopaths treat disease using very low dose preparations administered according to the principle that "like should be cured with like." Practitioners would select a drug that if given to a healthy volunteer, produce the presenting symptoms similar to that of the patient. Prescribing strategies in Homoeopathy vary considerably.

Guaiacum, which is one of Hahnemann's antipsorics, is best known as a remedy in gout and rheumatism. Guaiacum extract is said to be useful for controlling pain and inflammation of Rheumatoid Arthritis. However significant gaps still exist in our knowledge, hence some recommendations are needed relating with the mechanism of action of Homeopathic Mother tincture Guaiacum in the management of acute exacerbation of RA, to prevent or control joint damage, prevent loss of function, and decrease pain. Homoeopathy has substantial scope in pre-clinical research where therapeutic and biological effects of homeopathic medicines with proper mechanism of action can be traced out with the use of modern molecular techniques, and *in -vitro experiments.*

The objective of this study is to make a systemic compilation of results of experimental pharmacological findings of homoeopathic mother tincture Guaiacum Officinale, in in-vitro, and to evaluate the effect of the same in clinical setting on Rheumatoid arthritis. Through this study I am trying to establish the mode of action of Homoeopathic mother tincture Guaiacum Officinale in the management of Rheumatoid Arthritis, and I hope, it will pave the way for the explanation of the mode of action of various Homoeopathic medicines in the

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management of similar autoimmune diseases.

2. Review of Literature

Rheumatoid Arthritis (RA) is a progressive, disabling, chronic multisystem disease which is characterized by pain, swelling and stiffness of the synovial joints, which is often worse in the morning and after periods of inactivity. Although there are varying degrees of systemic manifestations, the characteristic feature of RA is persistent inflammatory synovitis, usually involving peripheral joints in a symmetric distribution. In inflammatory reaction, increased cellularity of synovial tissue and joint damage are the pathological hallmarks of RA [2], [3].

Homoeopathy is a system of alternative treatment. It works on the principle of similarity, compounds which can produce symptoms (at high doses), can cure a disease with similar symptoms (when administered at low doses). Homoeopathic treatment aims to enhance and stimulate body's immune system and self-regulatory mechanisms. Homoeopathy not only focuses on the disease of the person but it also aims to improve person as a whole considering his mind, body and soul. As a Homoeopath, one has to have two tool kits: one for constitutional prescription and one for clinical prescription. Constitutional homoeopathy refers to treating a person as whole physically, mentally and emotionally including past and present symptoms. Constitutional treatment is quite often not successful where severe diseases are involved. In such cases, it is better, when looking for a homoeopathic remedy, to go by the pathology of the disease and its accompanying symptoms [4].

Guaiacum Officinale is a South American derivative. It was proved and introduced by Dr. Samuel Hahnemann in MM pura, Vol. 1 [5]. Its mother tincture extract is prepared from the resin obtained from the wood as given in HPI, Vol. 2, Page no. 72, Alcohol 93% v/v, Drug strength of 1/10 [6]. The resin is used in chronic gout and rheumatism. Its chief action on fibrous tissue, rheumatism and tonsillitis are clinically established. Dr. William Boericke, in his 'Homoeopathic Materia Medica' also states it is very valuable in acute rheumatism [7]. A previous study suggest that Homoeopathic preparations of G. Officinale possesses anti-rheumatic and anti-oxidant activity in experimental animal and these activities may be more significant in higher potencies [8]. My study here is an extended work of the above-mentioned study.

A systematic review of the clinical evidence for and against the effectiveness of Homoeopathic remedies in the treatment of patients with RA has been published. A pilot study conducted in private Homoeopathic practise with retrospective studies and case histories suggested that recovery or clinical improvement may be achieved with Homoeopathic treatment in RA [9]. Another study confirms, Homoeopathic treatment is effective in the control of patients with Rheumatoid arthritis, from the impression obtained from the double-blind clinical trials on 26 subjects with Rheumatoid arthritis [10]. In a 6-month, double-blind study of 112 people with Rheumatoid arthritis, treatment with individualized Homeopathic remedies have failed to prove more effective than a placebo. Due to a high rate of dropouts, however, these results carry little weight [11]. Another study published, reached a negative conclusion as to the effectiveness of Homoeopathy (individualised prescriptions) in Rheumatoid arthritis. It's authors concluded that, although the small number of randomised clinical trials conducted thus far tend to favour Homoeopathic treatment, they do not provide any conclusive evidence as to the effectiveness of Homoeopathic remedies in the treatment of RA [12]. The negative outcome of these trials suggest that the tested remedy cannot be effective if prescribed based only upon a diagnosis of disease. Another research review concludes that Homoeopathic remedies either individually prescribed or used in a Homoeopathic formula, can provide relief for people with Rheumatic disease [13], [14].

Though as a complementary medicine, Homoeopathy playing an increasingly prominent role in health care practices, there is a scarcity of controlled studies investigating their effectiveness. More and larger randomised controlled trials are thus needed to properly assess the role of Homoeopathy in managing immunological disorders and abnormal susceptibility to infections.

3. Objectives

1) To study anti-inflammatory and anti-oxidative effect of Guaiacum Officinale on raw cell line: In vitro.
2) To evaluate the efficacy of Guaiacum Officinale on RA patients in clinical setting.

4. Methodology

Experimental design for In-vitro model:

Materials and Methods:

Chemicals:

All the chemicals and bio chemical's used were high quality analytical grade reagents. Solvents were purchased from Merck, India. Lipopolysaccharide (LPS), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), RPMI and Fetal Bovine Serum (FBS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). ELISA kits were purchased from Cayman chemicals, USA.

Preparation of powdered form of Guaiacum Homoeopathic mother tinctures (GUA)

100 mL of mother tincture of GUA was taken in a conical flask. The solvent from the HMT of GUA was removed by using rotary vacuum evaporator. Finally, the residues were collected and refrigerated at 4 ºC until further analysis.

Isolation of human peripheral blood mononuclear cells (hPBMCs)

hPBMCs were isolated from heparinized venous blood via Ficoll density gradient centrifugation method. In brief, the blood sample was diluted with 2 x volume of 1x PBS (pH 7.4) and carefully layered over an equal volume of Ficoll-paque in a 15 mL-conical tube. The suspension was centrifuged (400 xg, 30 min, 20 °C) and the upper layer was aspirated, leaving the mononuclear cell layer (lymphocytes, monocytes, and thrombocytes) undisturbed at the interphase. The mononuclear cell layer was transferred to a new conical tube, and 1x PBS was added. Following centrifugation (300 xg, 10 min, 20 °C), the supernatant was carefully removed. The cell pellets were

resuspended with 1x PBS. Following centrifugation (200 xg, 10 min, 20 °C), the supernatant was carefully removed. The hPBMC pellets were obtained through centrifugation over Ficoll–Paque™ cushions of buffy-coat, and cell number was counted using a cell counter. The cell suspension at density of 5×10^6 cells/mL was prepared in RPMI 1640 medium supplemented with 10% fetal bovine serum (RPMI-FBS) and incubated at 37 °C for 3 h. About 100µl of the suspension were cultured in 96-well plates and incubated with different concentrations of GUA (6.25, 12.5, 25 and 50 µg/ml) for 24 h under identical environments.

Experimental design:

hPBMCs Cell lines were divided into different groups.

Group I was normal control

Group II was treated with 1µg/ml LPS

Group III was treated with 1µg/ml LPS + GUA (12.5 µg/ml)

And it maintained in culture for indicted time. The supernatant of cultures was collected for the determination of inflammatory markers like TNF-alpha, COX-2, Reactive oxygen species and morphological analysis.

Cytotoxicity assay:

Cytotoxicity was assayed by the modified tetrazolium salt 3-(4-5- dimethylthiozol-2-yl) 2-5- diphenyl-tetrazolium bromide (MTT) assay.

Reagents:

MTT solution

NADPH

Phosphate buffer saline (PBS)

Procedure:

PBMCs cells were plated with various concentrations of GUA extract in 96-well microtiter plates and then cultured for 24 h at 37C a 5% CO2 incubator. At culture termination, 10 µL of the MTT solution was added into each well, and then cultured for 4 h at 37^0C in a 5% CO2 incubator. Then 100 µL of solubilized solution was added into each well. The plate was allowed to stand overnight in the incubator after evaluation for complete solubilization of the purple formazan crystals and measurement of the optical density (OD) at 570 nm by a microplate reader.

Assay of total Cyclooxygenase activity (COX):

COX activity was assayed by the method of Shimizu et al[18] (Shimizu et al. 1981). Lysed monocytes isolated from control, standard and test groups were incubated with Tris-HCl buffer (pH 8), 5mM glutathione and 5mM haemoglobin for 1 min at 25ºC. The reaction was initiated by the addition of 200µM arachidonic acid and terminated after 20 min incubation at 37ºC by addition of 10% TCA in 1N HCl. Following centrifugal separation and addition of 1% thiobarbiturate, COX activity was determined by reading absorbance at 530 nm.

Morphological analysis:

The observation of morphological changes of monocytes cells was performed using a phase- contrast inverted microscope. Briefly, cells were incubated for 24 hours at 12.5 µg/ml concentration of GUA in a 60 mm diameter tissue culture dishes. The medium was discarded and cells were washed once with PBS. The changes in the morphology of cells were observed to determine the alterations in hPBMCs cells and the images of the cells were grabbed at 20x by using the phase-contrast inverted microscope (Labomed, USA).

Determination of intracellular ROS generation:

To study the total ROS content inside the cells, we use the H_2DCFDA probe. The attached monocytes cells in RPMI are treated with the GUA for 90s and the intracellular ROS content is measured on day 2, 4 and 8 with and without the presence of 1µM trolox (ROS scavenger), using 10mM of H_2DCFDA to each sample, and kept at 30 °C incubation for 1 h. Subsequently, the cells are washed twice with PBS and the intracellular ROS generation were determined by Phase contrast microscopy.

Enzyme linked Immunosorbent assay (ELISA):

The release of TNF-α, COX-2 was measured in the supernatants of PBMC cells treated with LPS in the absence or presence of GUA using ELISA kit (Cayman chemicals, USA). TNF-α, COX-2 concentration were expressed as pg/ml.

Procedure:

ELISA plate was divided into 6 different types of wells, i.e., blank, total activity (TA), non-specific binding (NSB), maximum binding (B0), standards (S) and sample wells. 100µl ELISA buffer was added to NSB wells, 50 µl ELISA buffer was added to B0 wells. 50 µl of standard was added to the standard wells from lowest concentration to highest. 50 µl samples were added per well. 50 µl AChE tracers were added to each well except TA and blank wells. 50 µl Monoclonal Antibody was added to all wells except TA, NSB and blank wells. The ELISA plate was covered with plastic film and kept for incubation for 18 hours at 4°C. After incubation the wells were rinsed five times with Wash buffer. 200 µl Ellman's Reagent was added to each well. 5 µl of tracer was added to TA wells. The plate was covered with the plastic film and kept for incubation in dark for 60-90 minutes. Then, the plate was read at a wavelength between 405-420nm.

Experiment model for clinical trial:

Population: Patients coming on OPD of ANSS Homoeo Medical College Hospital, Kottayam and special medical camp conducted by the Hospital

Sampling: In Phase I, 30 cases are selected according to inclusion and exclusion criteria and divided into two groups by randomization (simple Random Sampling) and gave treatment on each mode and reviewed on two-month interval.

Sample size: The sample size on each group was 15. For the study total sample size fixed as 30.

Study Design: Before and after Comparative trial.

Selection Criteria:

Inclusion criteria:

- Male and female subjects with age 18-60years.
- Diagnosed cases of RA based on ACR criteria.

Exclusion criteria:

- The subjects with more advanced pathology.
- Female of child bearing and lactating mother.
- Patients with uncontrolled diabetes and hypertension.

Mode of interventions:

1) Group A: Homoeopathic constitutional treatment as described.

Table 1
Group A: Constitutional Medicine Group

S.No.	Name	Age/Sex	Medicine	RA Factor Before	RA Factor After	ESR Before	ESR After	CRP Before	CRP After	ARA Score
1	Eliamma Vargheese	65/F	Thuja 200	8.4	5.6	14	13	0.97	1.4	4
2.	Sanitha	41/F	Calc Phos 200	9.6	8.9	17	20	1.4	0.9	4
3.	Radhika	52/F	Puls 200	118.9	76.2	27	14	0.82	0.92	5
4	Suchitra	30/F	Bry 1M	15.9	11.3	25	21	3.4	4.1	4
5	Asha Reji	46/F	Sep 200	17.7	13.1	17	10	1.04	1.3	4
6	Jaya Prabha	26/F	Ars 200	14.2	13.1	35	40	1.03	1.04	4
7	Jini	39/F	Puls 200	10.3	10.4	85	45	1.4	2.9	4
8	Thankamma	68/F	Calc 200	30.6	19.7	35	22	0.83	1.1	5
9	Mustafa	56/M	Sul 200	240	90	55	30	2.4	1.42	6
10	Paal Thangam	68/F	Med 200	16.7	14.3	51	53	1.12	1.3	5
11	Gouri	83/F	RT 200	14.9	12.2	48	27	0.97	0.82	4
12	Jancy	37/F	Puls 200	13.4	14.2	28	30	8.9	8.2	4
13	Saima Manu	34/F	Puls 200	230	198	48	41	0.4	0.4	4
14	Jaisamma	56/F	Puls 1M	5.2	5.8	15	22	0.37	0.24	4
15	Vijayamma	55/F	Actea 200	20	11.1	60	40	1.32	1.41	5

Table 2
Group B-Constitutional Medicine Combined with Guaiacum Tincture

S.No.	Name	Age/Sex	Medicine	RA Factor Before	RA Factor After	ESR Before	ESR After	CRP Before	CRP After	ARA Score
1	Valsamma	67/F	Puls 200+ Guiacum Tincture	8.6	8.6	14	16	0.87	0.78	4
2	Anandavalli	54/F	Calc Carb200+ Guiacum T	6.3	5.2	60	32	2.95	2.3	4
3	KG Cheriyan	65/M	Lyc 200+ GuiacumT	30.6	21.2	18	11	5.13	2.4	5
4	Mary Nesamani	63/F	Lach 200+ GuiacumT	14.9	11.2	65	38	27	14.1	5
5	Sali Mathew	59/F	Puls 200+ GuiacumT	7	5.6	24	13	0.9	0.01	4
6	Leela	72/F	Lyc200+ GuiacumT	17.7	14.6	48	48	2.19	2.12	5
7	Marykutty John	52/F	Phytolacca 200+ GuiacumT	34	6.3	22	6	3.4	0.87	5
8	Padma	56/F	Bry 1M+ GuiacumT	14	13.3	48	48	2.19	2.12	5
9	Rajamma	62/F	Lach200+ GuiacumT	11.7	10.7	62	36	2.09	2.5	4
10	Santha kumara	52/F	Rhus tox 1M+ GuiacumT	15.1	13.6	42	23	2.04	1.9	5
11	Monamma	48/F	Puls200+ GuiacumT	9.6	10.1	20	20	4.9	4.1	4
12	Valsamma	50/F	Rhus tox200+ GuiacumT	16.2	16.3	38	31	5.3	6.2	5
13	Kunjujamma	72/F	Sul 200+ GuiacumT	44.3	32.9	66	53	0.89	0.82	5
14	Elizebeth	58/F	Bry 200+ GuiacumT	12.7	7.6	32	24	4.51	4.47	4
15	Nirmala	55/F	Calc C Flour200+ GuiacumT	18	17.8	32	35	1.6	1.3	5

Constitutional medicines were selected according to the totality of symptoms. Frequency of administration and potency of constitutional medicines were decided according to the susceptibility of individual and severity of disease. The constitutional medicine was medicated and dispensed in lactose and was followed by placebo.

2) Group B: Homoeopathic constitutional treatment and Guaiacum tincture as add on treatment.

10 drops of Guaiacum tincture, two times per day was given to the second group along with constitutional medicine.

The different interventions were applied to the two groups and the entire study subject's treatments were last up to 2 months. In each subjects follow up was on two weeks interval.

Primary outcome measure:

Routine blood examinations were done on monthly basis. Improvement in blood parameter ESR, C reactive protein, RA factor and physical symptoms of each groups were noted for analysis.

The results were statistically processed by Paired t test to assess the significant difference between the before and after values of the above-mentioned parameters such as ESR, RA factor and CRP in Constitutional medicine group and Constitutional medicine with Guiacum Group.

5. Results

Results of in-vitro study are summarised below:

Evaluation of GUA Cell viability and cytotoxicity by MTT assay:

The effect of GUA on cell viability was confirmed by MTT (3-[4,5-dimethylthiazol-2-yl[-2,5 diphenyl tetrazolium bromide) assay. The non-cytotoxic character of GUA was evident when monocyte cells were incubated for 24 h with GUA at varying concentrations (Fig. 1). Since no short-term toxicity to GUA was observed from the trypan blue exclusion assay, LPS activated monocytes cells at concentrations below 50 µg/mL was selected for testing the in-vitro efficacy of GUA. More than 92% cells were viable when compared to the LPS treated group II. The effective dose was also determined by evaluating the inhibitory effect on total COX activity.

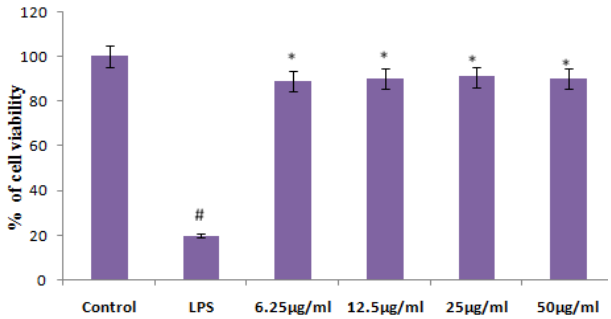

Fig. 1.

Cell viability assay done by MTT assay: hPBMCs cells were

incubated with different concentrations of GUA (6.25µg/ml, 12.5µg/ml, 25µg/ml, 50µg/ml) for 24 h. Compared with ox-LDL treated group, P ≤ 0.05. # denotes LPS treated group compared with control group, P ≤ 0.05. Values are expressed as mean ± SD (n=3).*

Effect of GUA on total COX activity:

At the end of the incubation period of 24hrs the hPBMCs cells were collected for the determination of total COX activity. The effective dose of GUA was also determined by evaluating the inhibitory effect on total COX activity. GUA at a concentration of 12.5µg/ml was found to be the minimum dose to exhibit maximum anti-inflammatory effect (89%). The results were shown in Fig. 2.

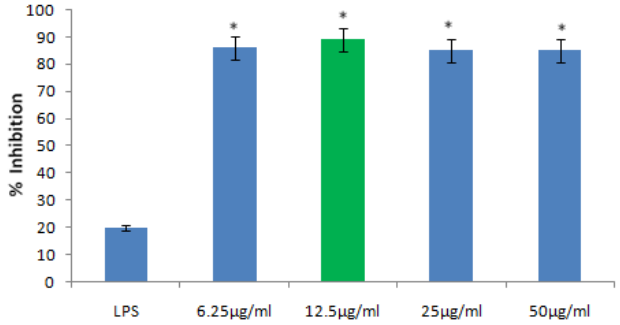

Fig. 2.

Effect of GUA on total COX inhibition on LPS activated hPBMCs cells: Comparison between groups, different symbols indicate significant difference at p ≤ 0.05. compared with LPS treated group, P ≤ 0.05. Values are expressed as mean ± SD (n=3).*

Effect of GUA on activity of COX-2 in hPBMCs cells:

At the end of the incubation period of 24hrs the *hPBMCs* cells were collected for the determination of COX-2 activity. LPS treated *hPBMCs* cells significantly (P≤0.05) increased the activity of COX-2 compared with the control group I. Supplementation with *GUA* significantly (P≤0.05) inhibited the LPS induced activity of COX-2 in *hPBMCs* cells in group III. The activity of COX-2 in *hPBMCs* cells are shown in Fig. 3.

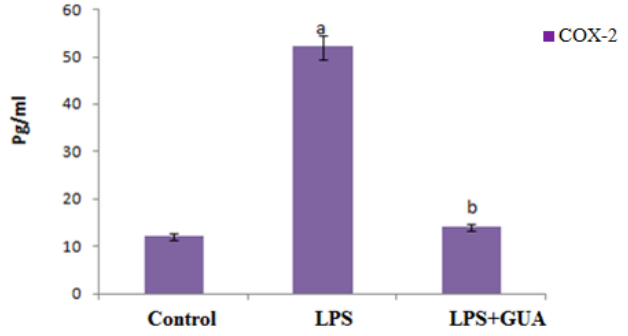

Fig. 3.

Effect of GUA on the COX-2 activity: Values expressed as average of 3 samples ± SD in each group. 'a' -Statistical difference with normal control group at P < 0.05. 'b' -Statistical difference with LPS treated group at P < 0.05.

Effect of GUA on the concentration of TNF-α:

LPS-induced hPBMCs were used to determine the inhibitory action of GUA on the production of pro-inflammatory cytokines like TNF-α. LPS significantly (P≤0.05) increased the concentration of TNF-α. Supplementation of GUA significantly (P≤0.05) inhibited the concentration of TNF-α in hPBMCs cells in a dose dependent manner. The results are shown in Fig. 4.

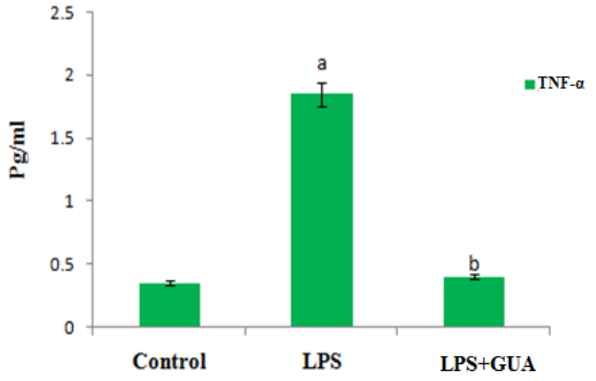

Fig. 4.

Effect of GUA on the TNF-α level: Values expressed as average of 3 samples ± SD in each group. 'a' -Statistical difference with control group at P ≤ 0.05. 'b' -Statistical difference with LPS at P ≤ 0.05.

Morphological analysis of GUA in hPBMC:

The cell morphology was changed in LPS treated hPBMCs cells as compared with control hPBMCs cells. The cell size was reduced and shrinkage occurs, necrosis and apoptosis are seen. Treatment with GUA revert normal cell morphology as compared with LPS treated group. There is no shrinkage, apoptosis, necrosis and the number of cells increased. The results were given in Fig. 5.

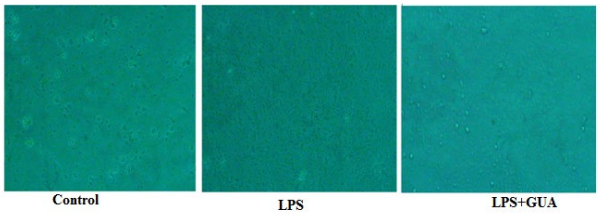

Fig. 5. Morphological analysis of GUA at 20x by using the phase- contrast inverted microscope

Determination of intracellular ROS generation by using green fluorescence assay:

To establish a precise role in antioxidant activity, cellular reactive oxygen species (ROS) were quantified with DCFDA. The results from the present study demonstrate that LPS treated monocyte cells generated a significant increase (P ≤0.05) in intracellular reactive oxygen species, as compared to the control. This oxidative stress-induced increase in ROS concentration decreased drastically (P≤ 0.05) on incubation with GUA at 12.5µg/ml concentration. The result was shown in Fig. 6.

Table 3
Results of clinical study

S.No.	Outcome Parameters	Calculated t value of Constitutional medicine with Guaiacum Group	Level of significance	Calculated t value of Constitutional medicine group	Level of significance
1	ESR	3.977	Significant at 0.05 level	2.575	Significant at 0.05 level
2	RA Factor	2.3	Significant at 0.05 level	1.8	Not Significant at 0.05 level
3	CRP	1.475	Not Significant at 0.05 level	0.516	Not Significant at 0.05 level

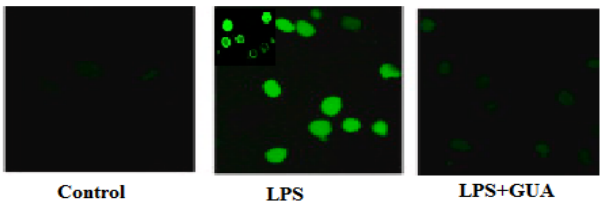

Fig. 6. Intracellular ROS generation by using green fluorescence assay

6. Discussion

The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. It depends both on the number of viable cells and on the mitochondrial activity of cells. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay is based on the assumption that dead cells or their products do not reduce tetrazolium. Tetrazolium salts are reduced only by metabolically active cells. Thus, MTT can be reduced to a blue coloured formazan by mitochondrial enzyme succinate dehydrogenase. The amount of formazan produced is directly proportional to the number of active cells. The principle involved is the cleavage of tetrazolium salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue colored product (formazan) by mitochondrial enzyme succinate dehydrogenase. In the present study, MTT assay of GUA at 12.5µg/mL concentration showed no cytotoxicity on hPBMCs cells with cell viability of 89%. The GUA did not show any cytotoxic effect. These results indicated that GUA is not toxic to mammalian cells and could be used to analyze other parameters of in vitro anti-inflammatory studies.

In this study, we confirmed the anti-inflammatory effect of GUA in LPS activation-mediated inflammatory response. LPS-activated human peripheral blood mononuclear cells, which represent appropriate model system to study anti-inflammatory effects of potential natural remedies, were utilized here to examine the role of inflammatory mediators as well as ROS generation in the observed anti-inflammatory effects of GUA supplementation. Lipopolysaccharide (LPS)-activated macrophages finds a widespread use in the evaluation of anti-inflammatory properties of potential anti-inflammatory herbal based extracts and compounds. The endotoxin LPS is a principle lipopolysaccharide of the outer membrane of Gram-negative bacteria that stimulates the production of pro-inflammatory cytokines such as IL-6, TNF-α, leukotrienes, and COX-2 (Sies, 1997)15. The anti-inflammatory effects of GUA was assessed here by measuring the cell cytotoxicity and cell viability, release of inflammatory markers like TNF- α levels and also the morphological and reactive oxygen species generation. Results obtained here show that GUA inhibit the LPS-induced inflammatory mediators production by decreasing the level of TNF- α. LPS treatment of PBMCs cells induced the secretion of a high level of TNF-α. The ability of GUA to modulate the inflammatory mediators in LPS-activated PBMCs represents an alternative treatment for inflammatory diseases.

Typically, inflamed tissues are associated with an elevated level of reactive species (reactive oxygen species (ROS)/reactive nitrogen species (RNS). These ROS/RNS are generated during the respiratory burst of immune cells and are important factors in defense against invading pathogens. It has been suggested that herbal-based antioxidants would be beneficial to human health and a lot of interest is focused on the determination of antioxidant capacity of natural products. Oxidative stress is defined as an increase in oxidation as a result of destruction of the balance between oxidation and antioxidant systems. SOD is a major factor in the defense against oxygen radicals. It prevents endothelial and mitochondrial dysfunction by inactivating nitric oxide and inhibiting peroxynitrite formation (Fukai et al, 2011)16. It also clears oxygen radicals produced in the respiratory chain. Dilated cardiomyopathy has been reported to be deficient or absent (Li et al, 1995)17, 18. The present study sheds light on this issue by showing that ROS generation, which constitutes the major defence system against oxygen radicals causing oxidative stress, was low in LPS treated hPBMC cells compared with that in the control group. LPS-activated inflammatory cells have also been shown to trigger the production of free oxygen radicals. With the production of these radicals, cell integrity deteriorates as various systems in the cell are damaged. By the treatment of GUA significantly decreased the levels of ROS generation and hereby diminished the oxidative stress condition as well as inflammation.

The results of the clinical study shows that Constitutional medicine alone is capable of reducing ESR in RA patients, but it is not effectively reduces RA factor and CRP in the first three months. In case of Guaiacum when it is given along with Constitutional medicine, can produce significant improvement in ESR and RA factor. There was no considerable improvement in CRP values in both groups. From this we can conclude that Guaiacum if used along with Homeopathically selected Constitutional medicine can be used effectively in RA patients especially in its acute stages with severe inflammatory signs. The observed findings in the present study should be regarded in the light of its limitations.

7. Conclusion

From the present study, we conclude that Guaiacum along with Homeopathically selected Constitutional medicine is found to be superior in the reduction of acute phase reactants of RA patients when compare with Constitutional medicine alone.

And through in-vitro experimental study it is evident that Guaiacum significantly acts as an anti-inflammatory and anti-oxidant agent that can be therapeutically have a role in treating RA.

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