Analyses of Human and Rat Clinical Parameters in Rheumatoid Arthritis Raise the Possibility of Use of Crude *Aloe vera* Gel in Disease Amelioration

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Abstract

Rheumatoid Arthritis (RA) is a chronic inflammatory condition affecting the joints causing swelling, stiffness and pain which finally leads to substantial loss of functioning and mobility in its advanced stages. In the present study we have monitored important serological parameters of fifty RA patients and also have discussed the justification of using rat as a model for human RA researches by comparing their respective serological parameters. We have also evaluated the anti-arthritic roles of raw *Aloe vera* gel and its effects in rat model where arthritis was induced by using Freund's Complete Adjuvant (FCA). Three essential conclusive statements were derived from the study. Firstly, the six clinical parameters that we have selected for the study namely, RA factor, CRP, ASO, ESR, ceruloplasmin and serum creatinine were all essential for the differential diagnosis of Rheumatoid Arthritis during its early and later stages, RA factor being the most sensitive of all parameters (92% sensitivity). Secondly, this study has supported the use of the rat as a model for designing therapeutic strategies against RA. Lastly, as evident from our study, *Aloe vera* extracts can be beneficial for the reduction of inflammatory edema and also for the reduction of ceruloplasmin in RA condition in rat model. However, further investigations are necessary for more refined therapeutic usage of *Aloe vera* for the treatment of RA in human.

Keywords: Rheumatoid arthritis; RA factor; North Bengal; *Aloe vera*

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory condition affecting the bone joints causing swelling, stiffness and pain that gradually leads to the substantial loss of functioning and mobility in the advanced stages [1]. Effects of RA are not only limited to extreme physical distress but also cause mental distress. As the etiology behind RA prognosis is not well understood confirmed curative measures are not discovered till date. All treatment regimes and therapies presently applied, such as cytokine therapy (anti-TNFα therapy) or Disease Modifying Anti-Rheumatic Drugs (DMARDs) are only limited to the reduction of symptoms of the disease and delay of pathogenesis [2]. Thus RA has become an undeniable threat to human life and therefore preventive measures must be developed to cure this disorder.

In the recent times, a large body of research has been directed towards finding herbal solutions to the treatment of the diseases. In Indian Ayurveda, one such promising herbal candidate having anti-arthritic effect is *Aloe vera* (Family Xanthorrhoeaceae), a perennial succulent xerophytic plant. In this plant water is held in the form of viscous mucilage within the thin walled parenchymatous cells in the innermost part of the leaves. *Aloe vera* has been used for many centuries for its curative and therapeutic properties [3]. It has been traditionally used in various skin ailments [4] and has well known wound healing and anti-inflammatory activities [5,6]. *Aloe vera* gel has been traditionally consumed or applied dermally to reduce joint pains. Phytochemical screening of *Aloe vera* confirms the presence of flavonoids, alkaloids, resins, tannins, steroids and other chemical substances [7]. The anti-inflammatory activity of the crude unprocessed gel has also been documented [8]. It has been documented that *Aloe vera* contains anthroquinone that may play a key role in anti-arthritic activity [9]. Many of the medicinal effects of Aloe leaf extracts have been attributed to the polysaccharides found in the inner leaf parenchymatous tissue [5,6], but it is believed that these biological activities should be assigned to a synergistic action of the compounds contained therein rather than a single chemical substance [7].

In the present study we have estimated the levels of certain clinical parameters in Rheumatoid arthritic patients of northern West North Bengal, India compared to the control subjects and also have discussed the possibility of using *Aloe vera* crude gel in the treatment of RA by evaluating the effects of crude leaf gel in experimental arthritic conditions in rat groups induced by Freund's complete adjuvant.

Materials and Methods

This section has been compartmentalized into separate categories for better understanding as follows:

Human based studies

Sample collection: Blood samples of 50 RA patients and 50 controls subjects were collected from an authorized diagnostic laboratory of Siliguri and also from North Bengal Medical College and Hospital (NBMCH, Sushrutnagar, West Bengal, India) under the guidance of a medical practitioner. Both patients and control subjects have provided their written consent after knowing the purpose of the study. The patients were diagnosed on the basis of physical examinations, clinical symptoms,, disease progression studies and were confirmed of having RA based on the reports of Anti-CCP assays.

Estimation of RA clinical parameters: Each blood sample was

divided into two parts, the anti-coagulated (EDTA added) part for ESR estimation and the clotted part for serum extraction for the estimation of RA factor, CRP assay, ASO titre estimation, Ceruloplasmin and creatinine assays.

Erythrocyte Sedimentation Rate (ESR) of each blood sample was immediately estimated by Westergren method by measuring the rate of gravitational settling of anti-coagulated erythrocytes in 1 hour from a fixed point in an upright calibrated tube of predefined dimensions [10]. Its normal upper limit for males is 15 mm/hr, and for females is 20 mm/hr [11]. ESR is an indirect measure of the acute phase reaction, it being a simple and inexpensive laboratory test for assessing inflammation.

Rheumatoid (RA) factor, C-Reactive Protein (CRP) and Anti-streptolysin O (ASO) were estimated by quantitative turbidometric assay and their normal range reference values in the serum were considered to be up to 20 IU/ml, 6 mg/dl, and 200 IU/ml respectively. RA factor is a very potent marker of RA as majority of the patients have this abnormal antibody in their serum at a range higher than normal.

Furthermore, we analyzed ceruloplasmin and creatinine concentration in the serum samples of the RA patients and the control subjects. Ceruloplasmin estimation was done spectrophotometrically by using p-phenylenediamine oxidase activity [12]. The levels of serum creatinine were measured spectrophotometrically by studying reactions between creatinine and alkaline picrate [13].

Rat model based study

Experimental setup: Swiss Albino male rats weighing 60 ± 10 gm each were used for all the experiments under this section and were procured from an authorized animal dealer (Ghosh Enterprise, Kolkata, India). Animals were maintained under standard laboratory conditions. The study was approved by the Institutional Animal Ethical Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) of the University of North Bengal, West Bengal, India.

The animals were divided into 6 groups of 4 male rats in each. The first group was considered as Non-treated or control group (NT) as arthritis was not induced in the rats of this group. The second group was considered as Arthritic group or Negative Control (AG) as arthritis was induced in the members of this group as per the method proposed by Bendele et al. [14]. All the animals of AG were administered a dose of 0.1 ml of Freund's Complete Adjuvant (FCA) in the left hind paw and a

booster dose of 0.1 ml was given on the 15th day of the experiments. The animals of three other groups were also induced with FCA following the methods as mentioned above. These three groups were designated as the Experimental Groups (EGs) and were fed raw *Aloe vera* gel as mentioned below.

Wild *Aloe vera* plants collected from the sub-Himalayan Terai regions of Northern West Bengal were used for the experiments. The plants were identified by the Department of Botany, University of North Bengal [accession no. 09884 (NBU)]. They belong to class Magnoliopsida under order Asparagales and family Xanthorrhoeaceae. The crude gel was obtained by peeling out the outer cuticle layer and cutting the gel aseptically into small pieces. The sample homogenate was freshly prepared with distilled water (1:5 w/v) every time before use. Each piece of gel was weighed and then dried separately in an air oven at 37°C for 48 hours to know the dry weight of the gel doses.

Three experimental groups (EGs) were treated with different doses of *Aloe vera* doses viz., 125 μl (EG-125), 250 μl (EG-250) and 500 μl (EG-500) respectively corresponding to 25 gm wet gel/kg body weight (20 mg dry weight/kg body weight), 50 gm wet gel/kg body weight (40 mg dry weight/kg body weight), and 100 gm wet gel/kg body weight (80 mg dry weight/kg body weight). The above mentioned doses were selected on the basis of the quantity of *Aloe vera* gel that should be taken by a person per day for therapeutic use. However, the absorption rate may be different in case of rat and human systems.

The sixth group was considered as the Protective group (PG) where animals were fed with 250 μl *Aloe vera* (50 gm wet gel/kg body weight or 40 mg dry weight/kg body weight) 7 days prior to the FCA injection (arthritis induction).

Parametric studies: Two rats from each of the six groups were sacrificed on 21st day of the experiment and the rest were sacrificed on the 28th day to know the levels of modulatory activities of crude *Aloe vera* after the booster doses of FCA. The left hind paws of all the six rat groups were amputated from the body after their sacrifice and were subjected to radiological analysis. The measurements of paw circumference was done at regular intervals of 2 or 3 days with the help of a vernier caliper following methods of Paquet et al. and Rathore et al. [15,16]. Body weight was also recorded at regular intervals. Blood samples were collected using insulin syringes from the tail vein for serum isolation for biochemical tests. Serum ceruloplasmin and creatinine estimation were done by applying the procedures followed
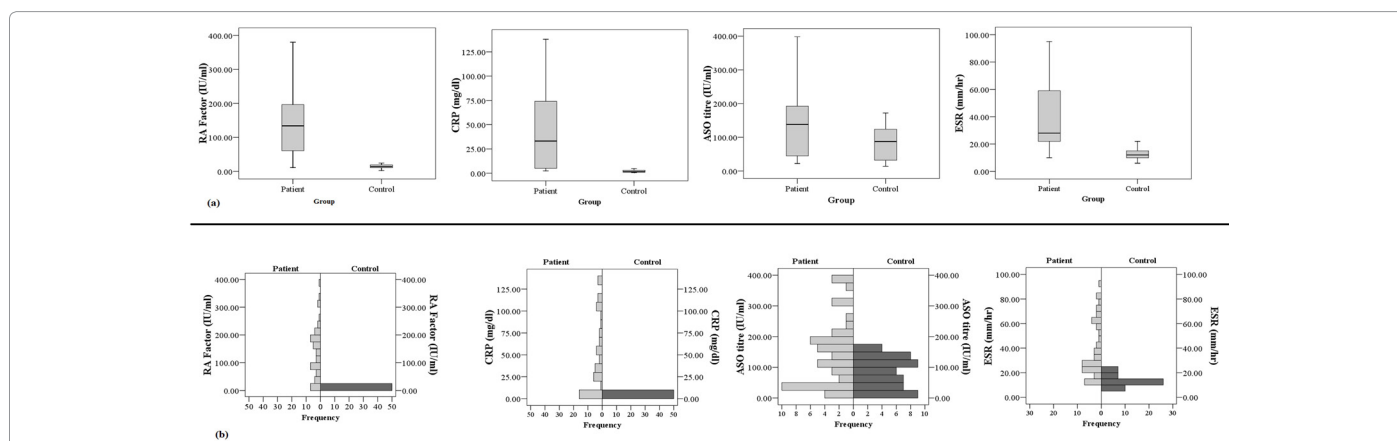
Figure 1: (a) Box plot analyses show the differences in the distribution of the different clinical parameters in the Rheumatoid Arthritic patients and the control samples. **(b)** Pyramid charts compared the distribution of the levels of different clinical parameters in the RA patients and the control samples.

in case of the human samples for reducing complications during further analyses.

Statistical analysis

All the statistical calculations were performed using the softwares MS-Excel and Kyplot ver. 2.0 β and SPSS ver. 16.0. In the Kyplot analysis the data represented mean ± SD which was analyzed by T-test for finding the significance. The results were considered significant when $p > 0.05$. Box plots and the frequency plots were constructed using SPSS ver. 16.0.

Results

Out of 50 selected anti-CCP positive RA patients 92 % were found to be positive for the Rheumatoid (RA) factor test having RA factor values above the normal range of 0-20 IU/ml. The RA factor values fluctuated within a very wide range of 11-380 IU/ml having mean ± SD value of 135.9 ± 91.4 IU/ml (Table 1). In contrast, 88% of the control patients (anti-CCP negative) were found to be RA factor negative with a mean value of 14.0 ± 5.5 IU/ml. The calculated t value of patients versus control for the RA factor was found to be 9.45 ($p \leq 0.001$) indicating significant differences in the distribution of the RA factors in the RA patients and control samples. The sensitivity and specificity of the RA factor estimation were found to be 92% and 88% respectively. The mean CRP value in the RA patients was found to be 46.4 ± 42.9 mg/dl whereas in the control samples it was found to be 2.2 ± 1.6 mg/dl which is well within the normal clinical range (Table 1). Thus considerable differences also exist in case of CRP levels among the patients and the control samples, which is supported by the t-test. The estimated ASO titre also showed considerable differences among the RA patients and the controls with mean ± SD values of 147.5± 107.8 IU/ml and 83.1 ± 49.7 IU/ml respectively (Table 1). The estimated ESR values in the RA and the control subjects were found to be 37.6 ± 23.0 and 12.7 ± 4.1 mm/hr respectively.

The box plot analyses were also performed based on the quantitative estimation of the four essential clinical parameters of Rheumatoid Arthritis namely RA factor, CRP, ASO titre and ESR (Figure 1a). The box plots revealed that there exist considerable differences in the distribution of each of the four clinical parameters among the patients and the control samples. It can also be observed that each of the four clinical parameters have shown considerable fluctuations in 50% of the RA patients, whereas such wide range distribution could not be observed for the control samples except for the moderate fluctuations in the ASO titre. From Figure 1b it can be seen that the frequencies of the distribution of the above mentioned four parameters varied over a very wide range of values compared to that in the control samples. However, as evident from the Figure 1b, minor variations in the frequency distribution of two parameters namely ASO titre and ESR were also observed in case of the controls.

The two other parameters i.e. plasma ceruloplasmin and serum creatinine, which are generally not considered as conventional parameters for RA diagnosis, were then compared with RA factor level in the RA patients and the control samples with the help of scatterplot (Figure 2). Surprisingly in both the cases the scatterplots showed positive correlation with an upwardly directed linear trendline.

Correlation analyses were also performed among the different parameters for the RA patients (Table 2). From the correlation table it was evident that the correlation coefficients between RA factor and other five parameters were all greater than 0.5 with RA versus CRP value

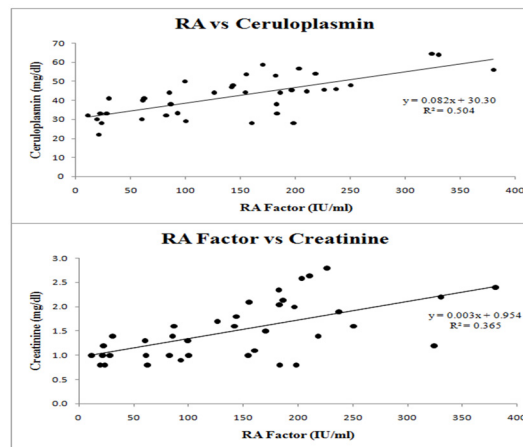**Figure 2**: Scatterplot analyses to show RA factor vs Ceruloplasmin and RA factor vs. creatinine levels respectively in RA patients.
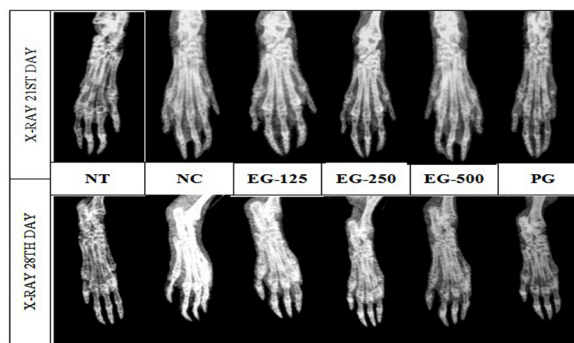

Figure 3: X ray photographs of the rat paw on the 21st and the 28th days of the experiment in different rat groups.
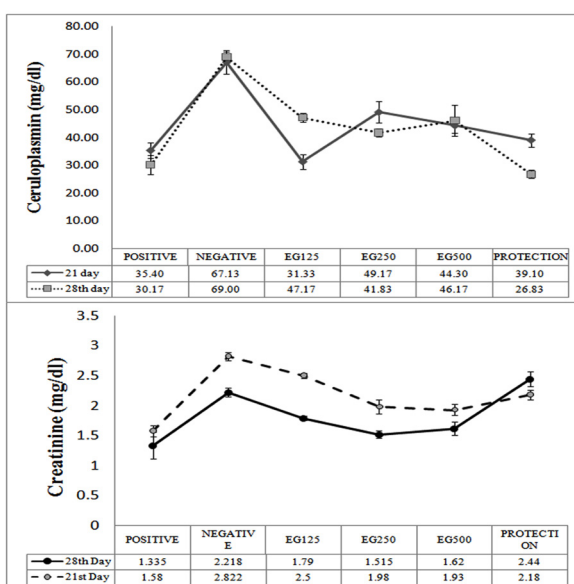

Figure 4: Line diagram shows (a) the ceruloplasmin level on the 21st and 28th day respectively in the different rat groups and also (b) creatinine level on the 28th day.

leading the chart. One interesting observation was that no negative correlation was evident among the six clinical parameters. However, it was also found that among the RA patients, the lowest correlation coefficient was evident between ASO titre and creatinine level.

In rat model based analyses, it was observed that with the exception of non-arthritic positive control (NT) group, there was significant increase in paw circumferences in the FCA-induced arthritic group. The paw circumference in all the groups was presented in Table 3. Interestingly the paw circumference in treatment groups showed significant reduction after treatment with *Aloe vera* crude gel homogenate. Among the three experimental groups, 125 µl dose, corresponding to 20 mg dry gel/kg body weight, showed the maximum reduction rate of paw edema. Whereas 250 µl and 500 µl doses, corresponding to 50 gm and 100 gm wet weight/kg body weights respectively, showed greater protection rates after the booster dose of FCA. However in the protective group (PG), 250 µl doses showed very minor paw swelling even after FCA injection in both the initial and booster doses. Similar results were also evident from the x-ray photographs where it was observed that in the protection group, the paw swelling was significantly lower compared to the other experimental groups (Figure 3).

The levels of ceruloplasmin and serum creatinine in the control and the experimental rat groups have also been presented in Figure 4. It was clearly observed that the ceruloplasmin level was much more elevated in the arthritic group in comparison to the positive control group. However the administrations of *Aloe vera* plant extract in different doses have shown considerable reduction in the ceruloplasmin levels in varying degrees. In the EG125 group, it was found that the ceruloplasmin level was almost reduced to normal level when measured on the 21st day whereas the ceruloplasmin level went high on the 28th day. In case of EG250 and EG500 groups, we found that the ceruloplasmin level was not only reduced from that of the AG but was also maintained at a steady state both on the 21st and 28th day. Interestingly, it was found that the Protection Group (PG) did not show any elevations in their plasma ceruloplasmin during both the 21st and 28th day of the experiments. In serum creatinine assay, it was found that all the experimental groups showed lowered levels compared to the Arthritic Group (AG). However, unlike the ceruloplasmin results, we found no significant reduction in creatinine level in the PG group compared to the negative control group.

Discussion

A good number of observations were derived from the results of the estimation of the different clinical parameters that we have conducted on the blood samples of 50 RA patients and 50 control subjects. Interestingly it was observed that none of the four clinical parameters were 100% positive (above the threshold value) in all the affected individuals as were also evident from their sensitivity and specificity measures. These discrepancies have been explained below. In case of RA factor, it was found that 92% of the patients had values above the threshold level while 8% had lower values. When compared to data of other populations, it was found that in case of the Korean population, 80.56% of the RA patients were found to be RF positive while 19.44 were RF negative [17]. In a similar study conducted on the Iranian population, it was found that 66.5% of the RA patients were RF positive while 33.5 were negative [18]. In another study based on Turkish patients, it was found that 53.335 were RF positive while 46.67 were RF negative [19]. These variations may be the outcome of some peculiar fundamental facts. RA factor is the antibody against the FC portion of the IgG and it may belong to different isotypes e.g. IgM, IgE, IgG, IgA and IgD and thus their detection may vary based on the proportion of the different isotypes present in the serum. Moreover, diagnosis of the RA factors in the patients suffering for not more than 6 months may result as seronegative, who may become positive during the progression of the disease. In fact three patients in our study population, who were diagnosed as seronegative, had a history of only 4 month suffering. However the sensitivity and specificity of the RA factor test as calculated in our study are 92% and 88% respectively. Therefore it could be said that the test of RA factor may prove beneficial and is an essential clinical parameter for evaluating the disease prognosis provided it is accompanied by other parametric evaluations for confirmation of the results. Moreover from Table 1 it is observed that the odds of exposure to RA factor were greater by 84.33% among the RA patients compared to the control. The CRP and ESR are the two primary parameters which are frequently used for the clinical detection of acute phase reactions like inflammations. The concentration of these two parameters become relatively high during inflammation compared to normal level. Moreover they have a relatively short time lag from the moment of stimulus, and are cost-effective. CRP is a very sensitive parameter in detection of inflammation and therefore was included in our study. In a study conducted in a Pakistani population it was found that the CRP values ranged between 11.2 to 108 mg/dl with a mean value of 39.1mg/dl in severe RA condition, while the ESR values ranged between 12 to 146 mm/hr with a mean value of 62.5 mm/hr [20]. In another study conducted in Chandigarh, the mean CRP and ESR values were found to be 22.8 mg/dl and 51.3 mm/hr respectively [21]. Thus these already published results are very much comparable to our reports. CRP is produced in the liver induced by monocytes and macrophages derived pro-inflammatory responses. These proinflammatory responses triggers increased secretion of interleukin –1β (IL-1β) and tumor necrosis factor – α(TNF-α), which via the release of interleukin 6 (IL-6) stimulate the liver to secrete CRP. Recent studies have suggested the direct contribution of the CRP in the inflammatory activities, where it stimulates secretion of inflammatory cytokines such as IL-1β, IL-6 and TNF-α from the monocytes and also directly provides pro-inflammatory stimulus to phagocytic cells [22,23]. CRP is also the only independent determinant of microvascular endothelial dysfunction in patients with RA [24]. On the other hand, the basic principle underlying the estimation of ESR is that the erythrocytes normally repel each other due to the net negative charges. However, at the time of acute phase reactions, positively charged high molecular weight proteins such as fibrinogens, present in the blood, increase in amount and promote rouleaux formation which further increases the ESR. Inflammatory processes play a pivotal role in the pathogenesis of RA and therefore these two parameters are also considered important measures of RA prognosis. However, sometimes severe inflammation does not corroborate the prognosis of RA and clinicians have to depend on other diagnostic features for determining RA. As evident from our results, both CRP and ESR are not always present above the threshold value in case of the RA patients though they are positive for 74% and 78% respectively. This may happen due to the lack of prominent acute phase reactions in some of the patients. This may also occur because of the tendency of these parameters to return to lower values if measured during early phases of the disease. However the sensitivities and specificities of both these tests were quite high thereby assuring us to keep our faith on these tests in RA diagnostic purposes. The ASO titre is another essential parameter for the diagnosis of RA. This test was employed in our experiments to detect the rheumatic fever caused by environmental triggers like streptococci infection. It measures the plasma levels of anti-streptolysin O antibodies produced against

Parameters	Normal Range	RA patients (n=50)	Control Subjects (n=50)	T-value	Odds Ratio	Relative Risk	Sensitivity (%)	Specificity (%)
Age		47.2 ± 14.0	35.1 ± 11.4					
RA factor (IU/ml)	0-20.0 IU/ml	135.9 ± 91.4	14.0 ± 5.5	9.45***	84.33	7.67	92	88
CRP (mg/dl)	0-6 mg/dl	46.4 ± 42.9	2.2 ± 1.6	7.32***	44.59	12.33	74	94
ASO (IU/ml)	0-200 IU/ml	147.5 ± 107.8	83.1 ± 49.7	3.66***	29.41	23.00	22	100
ESR (mm/hour)	0-20 mm/hour	37.6 ± 23.0	12.7 ± 4.5	8.01***	31.91	7.80	78	90
Ceruloplasmin (mg/dl)	20-35 mg/dl	42.1 ± 11	23.8 ± 6.1	8.96***	34.62	12.50	65.79	94.74
Creatinine (mg/dl)	0.6-1.2 mg/dl	1.5 ± 0.6	0.9 ± 0.2	5.79***	11.69	5.50	57.89	89.47

*p<0.05, **p<0.01, ***p<0.001.

Table 1: Statistical analyses of the different clinical parameters of the Rheumatoid Arthritic patients and control subjects.

	RA	CRP	ASO	ESR	Cerulo plasmin	Creatinine
RA	1.00					
CRP	0.73	1.00				
ASO	0.65	0.48	1.00			
ESR	0.71	0.72	0.63	1.00		
Ceruloplasmin	0.57	0.52	0.38	0.40	1.00	
Creatinine	0.60	0.65	0.22	0.48	0.62	1.00

Table 2: Correlation coefficient of the different clinical parameters in the Rheumatoid Arthritis patients.

	Days								
Groups	**1**	**4**	**7**	**10**	**13**	**16**	**19**	**23**	**27**
+ Control(NT)	22.57	23.76	22.46	23.24	22.84	22.56	22.29	22.14	21.99
- Control(AG)	22.38	32.00	28.28	26.84	26.02	25.14	32.85	30.80	26.98
EG-125	21.63	27.17	25.54	23.63	23.21	23.27	29.21	27.55	25.04
EG-250	22.75	27.67	25.84	24.75	24.00	23.44	26.53	26.42	24.36
EG-500	22.86	26.00	25.47	25.17	23.94	22.92	26.43	26.25	24.69
Protective (PG)	22.21	22.39	22.14	22.24	21.98	21.78	24.18	22.92	22.95

Table 3: Measurement of paw circumference (mm) of different rat groups in different days.

streptolysin of different streptococcal strains. It has been observed that out of 50 samples only 11 samples had higher than 200 IU/ml ASO titre values. However it is clearly evident that the mean ASO titre value was higher in case of the RA patients compared to the control samples (Table 1) which may have resulted due to the presence of ASO positive patients having ASO titre above threshold value (200 IU/ml). Francois (1965) [25] has shown significantly higher prevalence of 3-haemolytic streptococci of Groups A, C, and G at any point of the study duration in the RA patients compared to control samples which he assumed to be due in part to a slightly higher acquisition rate, but mainly to a reduced elimination rate. Thus due to lower elimination rate, prolonged infection may lead to higher antibody response and this statement may justify the facts that although raised anti-streptolysin-O titres (>150 IU/ml) were found in both the groups, their numbers were higher in RA patients compared to the controls and also more higher titres (>300 U) were significantly found in the RA patients but such high values of ASO titre were not observed in the control samples (Figure 1b). One interesting observation was that the sensitivity of the test was as low as 22% which may result due to the presence of only 11 patients having positive titre result i.e. ASO>200 IU/ml, but on the contrary, the specificity was 100% indicating the negative outcome of the test result in absence of acute phase reactions which is an well-established pathophysiological condition in case of rheumatoid arthritis. Thus ASO titre may also prove useful in the diagnosis of RA.

It is interesting to note that the plasma ceruloplasmin and creatinine showed significant differences in their levels in the RA patient and the control blood samples though they are not generally considered as essential diagnostic tool for RA diagnosis. Serum ceruloplasmin was selected for the study because of its high correlation with serum antioxidant property which have already been reported elsewhere [26] and may have considerable protective role [27,28] especially in presence of tissue damage or destruction. In a study conducted in Poland, mean ceruloplasmin level was found to be 0.3 g/L which is very much comparable to our data [29]. Serum creatinine estimation has been carried out in this study as it is an important metabolic by-product of creatine which in turn is a very crucial amino acid for building and repairing of muscular tissues.

Increased level of plasma ceruloplasmin level during inflammation may occur due to an increased production of interleukins such as IL1 and IL6 in the RA patients which stimulate the hepatocytes to release and increase the amount of ceruloplasmin into the blood. As evident from our study, such rise of ceruloplasmin level in case of RA patients was also evident from other previously published reports [26]. Previous reports have also shown highly significant correlation between the serum ceruloplasmin and serum antioxidant activity [26] and thereby supporting the concept that ceruloplasmin may be responsible for actively governing the serum antioxidant activity [30] which when increased may be an important component of the systemic inflammatory response. On the other hand the creatinine level showed moderate differences in between the RA and the control blood samples. Blood creatinine generally shows a rise only after marked damage of nephrons which is observed in patients having long term RA and inflammatory responses. Thus it may not serve as a very potent tool for early diagnosis of RA but can be useful for detection and treatment of nephropathy during late Rheumatoid Arthritis. Moreover these two tests have considerable sensitivity and specificity indicating that their results may prove affirmative with that of the other above mentioned tests for RA diagnosis.

In case of the rat model it was found that considerable foot swelling

and increased paw circumference induced by FCA injection in the Arthritic rat group were also accompanied by significant increase in serum ceruloplasmin and creatinine levels in this group compared to that of the non-treated or the positive control groups. In both the 21st and 28th days, there was significant increase of ceruloplasmin levels in arthritic group. Thus the above mentioned similarity between human and the rat models in the distribution of ceruloplasmin and creatinine in the arthritic and the normal population strongly suggest the efficacy of the rat model for various pharmaceutical and clinical experiments targeted towards the Rheumatoid Arthritis therapy in the humans.

It was found that the paw circumference was decreased towards the normal state on applying *Aloe vera* gel in the experimental rat groups. It clearly indicated the rapid regression of the foot swelling in the experimental groups compared to the negative control or the arthritic groups (Figure 3 and Table 3). The differences in the mean paw circumference between the members of the experimental and the negative control (AG) groups started to increase from the 3rd day. Paw swelling reverted back almost to the normal condition within 10th day in all the experimental groups while the swelling was retained in the negative control (Arthritic group) rats throughout the experiment. The same feature was also observed after the booster injection of FCA. Moreover, X-ray photographs (Figure 3) have clearly indicated that in case of the arthritis induced rats, the hind paw joints appeared loosened and the spaces increased in between the joints, confirming the diseased state in the arthritic rats [31]. Thus, it can be clearly said that these two parameters can be used as effective visual tool for understanding the disease severity. The treatment groups showed a tendency to restore the joint structure towards the normal state (Figure 3). Moreover, the decreased levels of ceruloplasmin in the experimental groups were evident compared to the negative controls which confirm the efficacious role of *Aloe vera* gel in lowering the ceruloplasmin level. Interestingly, the protective group showed no significant difference in the ceruloplasmin levels when compared with the Non-Treated (NT) group rats, both on 21st and 28th day, indicating the strong protective role of *Aloe vera* in the amelioration of rheumatoid arthritis.

Serum creatinine concentration increased in the negative control arthritic group rats when compared to the normal rats ($p > 0.05$) but rats of all the other experimental groups showed no significant change when compared to the positive control rats. This observation indicated that the level of creatinine is normal ($p > 0.05$). However the protective group did not show any effect on the creatinine level probably because this factor increases due to increasing nephropathy in case of long term RA. In correlation with other biochemical parameters examined in this part of the study, it can be said that *Aloe vera* plays a significant role in amelioration of rheumatoid arthritis induced paw edema.

In conclusion, the work can be summarized with three principal sentences. Firstly, six clinical parameters that we have selected for the study like, RA factor, CRP, ASO, ESR, ceruloplasmin and serum creatinine, are all essential for the differential diagnosis of RA during its early and later stages. Secondly, this study has supported the justification of the experimentation on rat model for designing the therapeutic strategies against Rheumatoid Arthritis in humans. Lastly, as evident from our study, *Aloe vera* extracts can be beneficial for the reduction of inflammatory edema and also for the reduction of ceruloplasmin in RA condition in rat model. However, further investigations are necessary for more refined therapeutic usage of *Aloe vera* for the treatment of RA in humans.

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