Curative and protective properties of crude gel of *Aloe vera* from sub-Himalayan West Bengal in chronic and acute inflammatory rat models

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*Aloe vera* (L.) Burm. f. (Xanthorrhoeaceae) has been used traditionally as a wound healer and an anti-inflammatory medication. The *Aloe* gel has natural combination of different bio-active compounds and is commonly consumed or applied in crude condition. We have used unprocessed *Aloe* gel homogenate in acute and chronic inflammatory Wistar albino male rat models to investigate its properties in a systematic way. Chronic inflammatory arthritic model was established by injecting 0.1 ml Freund’s Complete Adjuvant (FCA) in the hind paw of rat. Radiograph, histology, hematological and biochemical properties of the serum were used to determine the ameliorative role of crude *Aloe* gel homogenate in the rat experimental groups in vivo. Acute inflammatory rat model was established using sub-cutaneous cotton pellet induction. The weight of dried cotton pellets were determined to estimate the extent of granuloma formation. *A. vera* crude gel inhibited paw swelling up to 65.59 % in experimental groups of arthritic rats and decreased the granuloma formation up to 25 % in cotton pellet induced inflammatory rats. It is well evidenced from the experiments that unprocessed *A. vera* crude gel possesses a good anti-inflammatory property for both chronic and acute types when fed at a dose of 0.40 gm/kg body weight.

**Keywords:** Paw edema, Rheumatoid arthritis, Freund’s complete adjuvant, Cotton pellet

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Inflammation is the immediate manifestation of the triggering of immune system against any infiltrating pathogen in the host body, expressed by means of redness, swelling, pain and heat generation. The etiology of inflammation and inflammatory diseases such as rheumatoid arthritis (RA) is not well understood till date. The clinical symptoms of the inflammatory RA are inflammation and swelling of the joints. This leads to the damage of bone and cartilage and as a result finally disrupts the joint structure and function. Recent treatment strategies like cytokine therapy (anti-TNFα therapy) or disease modifying anti-rheumatic drugs (DMARDs) regime can only suppress the disease. The disease flares up whenever the treatment is stopped. In past few decades researchers have shown interest in the herbal products which are reported to ameliorate disease in ancient Ayurvedic system. The intention of the present work was to reveal the synergistic effect of crude *Aloe* gel on structural, biochemical and hematological parameters in RA. *Aloe vera* (L.) Burm. f. (Xanthorrhoeaceae) is a stem-less or short-stemmed succulent plant growing up to 60–100 cm (24–39 in) tall, spreading by offsets. The leaves are thick and fleshy, green to grey-green, with some varieties showing white flecks on their upper and lower stem surfaces. The margin of the leaf is serrated and has small white teeth. The flowers are produced in summer on a spike up. It is found all over the North-Eastern India and sub-Himalayan region and is being traditionally used in various skin ailments. The gel is consumed or applied topically to reduce joints pain. Phytochemical screening of *A. vera* has confirmed the presence of flavonoids, alkaloids, resins, tannins, steroids and other chemical substances. Anti-inflammatory properties of the crude *A. vera* gel in wound healing, carrageenan induced paw oedemal model of rats have also been documented. Experiment-based chronic and acute anti-inflammatory properties of this agent are not well...
documented though it is used traditionally as anti-inflammatory agent in Ayurveda. Therefore, we have tried to investigate the role of wild A. vera raw gel in both chronic and acute inflammation by studying appropriate parameters in appropriate rat model, which are established standard models for research in experimental inflammation. The gel was used in crude form as we hypothesize that the bioactive compounds present in A. vera in different proportions may act synergistically for better result. In other words, isolated bioactive chemical(s) may not act optimally. Moreover, ethnic people consume it in crude condition as medicine and therefore it is important to know the effect of this gel in a crude condition.

Therefore, the present study was designed to evaluate the anti-inflammatory activities of raw or unprocessed Aloe gel and its effects in FCA-induced arthritic rats and cotton pellet induced inflammatory rats through radiology, hematology, biochemical and biometric parameters.

**Methodology**

**Collection of plant specimens**

Naturalized Aloe vera (L.) Burm. f. (Family: Xanthorrhoeaceae) plants were collected from the sub-Himalayan Terai areas of northern West Bengal, India and were identified in the Department of Botany, University of North Bengal. The voucher specimen was deposited in the NBU herbarium (Accession No. NBU09884).

**Preparation of extract**

The leaf gel was collected by peeling out the outer cuticle and cutting out the gel aseptically into small pieces. The gel was mixed with distilled water (1:5 w/v) and then homogenized to create a crude homogenate. The sample was freshly prepared every time before use. The experimental animal groups were fed with 125 µl and 250 µl of the homogenates.

To determine the dry weight, pieces of A. vera gel was weighed and then dried separately in an air oven at 37 ºC for 48 hrs.

**Experimental animals**

Wistar albino male rats (80-100 gm) used for all the experiments were procured from an authorized animal dealer (Ghosh Enterprise, Kolkata, India). Animals were kept in polypropylene cages (max. 4 rats per cage) and maintained at a room temperature of 25 ± 3ºC. Rats were maintained in the laboratory with standard feed and water ad libitum. 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.

**Induction of chronic and acute inflammation**

Adjuvant induced Arthritis (AIA) model was produced as chronic inflammatory model as per the method described by Bendele et al. (1999)\(^8\). All the animals were administered a dose of 0.1ml of FCA in the left hind paw interplanetary except the positive control group. The animals were administered a dose of 0.1ml FCA again on the same region to boost up the immune response on the 14\(^{th}\) day. The paw diameter was measured with the help of a vernier caliper and recorded at regular intervals of 2 or 3 days. Acute inflammation was induced by cotton pellet granuloma model. This method was adopted from Penn (1963)\(^9\) with slight modifications, which was carried out by using sterilized cotton pellet implantation method in rats\(^10\). Under light ether anesthesia subcutaneous tunnel was made by using blunted forceps and the sterilized cotton pellets (25 ± 1 mg) were implanted in one side of the vertebra of each rat.

**Experimental setup**

Wister male rats were considered as the experimental animals in both the anti-arthritic and anti-inflammatory experiments. For chronic inflammatory test, in anti-arthritic rat models, animals were divided into 5 groups, each containing 10 rats. The first two groups were considered as the Control groups. Among them one group was considered as non-treated or positive control (PC) having neither FCA nor A. vera and the other group was considered as negative control (NC) in which only FCA injection was given but no treatment was administered. The third and the fourth groups were considered as experimental groups (EGs). These groups were treated with A. vera doses of 125µl (125D) and 250 µl (250D) respectively corresponding to 25 gm wet gel/60 kg body weight (20 mg dry weight/kg body weight) and 50 gm wet gel/60 kg body weight (40 mg dry weight/kg body weight). The doses were empirically calculated on the basis of daily consumption of 50 gm of A. vera in a man weighing 60 kg, although the absorption rates may differ considerably between rat and human systems. The fifth group was the Protective group (PG) in which
125 µl of A. vera (25gm wet gel /60 kg body weight or 20 mg dry weight/kg body weight) was given for 7 days prior to FCA injection. Five rats from all the groups were sacrificed on the 21st day of experiment, while the rest were sacrificed on the 28th day as per our experimental design to measure the different parameters in different times. The left hind paws of all the animals of 5 groups were separated out from the body after sacrifice and were subjected to radiological and histological analyses. Blood samples were collected using insulin syringes from the hearts and were subjected to different hematological and biochemical tests.

Acute inflammation model was established by cotton pellet induced granuloma formation in rat. Four groups of animals, including a control and a standard group, were selected for the experiments, each group comprising of 4 rats. The control group (Control) was left without any treatment. The standard group (Standard) animals were fed with indomethacin, as a control drug, at a concentration of 4 mg/kg body weight. Third and fourth groups were experimental groups namely AV1 and AV2, treated with 125 µl and 250 µl A. vera crude gel homogenates respectively as in anti-arthritic experiments. The experimental animals were sacrificed on the 8th day and the dry weight of each granuloma was measured.11

Parameters for anti-arthritic tests

Biometric studies

Body weight was recorded at regular intervals. Rats from all the groups were subjected to radiographic study. After dissection, the joints were taken immediately for radiography. Measurement of paw circumference was done with the help of a vernier caliper and circumference was measured using the formula: \(2\pi \left[\sqrt{A^2+B^2}\right]/2\), where A and B are measures of diameter at two different planes of paw taken with the help of Vernier Caliper)12,13 (Fig. 1).

Histological studies

Samples were preserved in 10% formaldehyde after radiographic analysis. Decalcification of the histological samples was done in 3% HCl for 4 days followed by paraffin block preparation. Histological sections were made along the longitudinal plane of joint axis and were stained in hematoxylin-eosin stain following standard protocols.

Hematological studies

Hematological parameters included RBC total count, WBC total count using hemocytometer and hemoglobin estimation by using Sahli’s hemoglobinometer.

Biochemical studies

Total blood glucose estimation was done by ortho-toluidine method14 to estimate the effects of Aloe gel on blood glucose level of rats. Total protein was estimated by Coral Total Protein Kit (Coral Clinical Systems, Goa, India) (Biuret method) according to the manufacturer’s protocol. Total albumin estimation was done using the Coral Total Albumin Kit (Coral Clinical Systems, Goa, India) (BCG method). Serum ceruloplasmin estimation was done using p-phenylenediamine oxidase activity spectro-photometrically15. The levels of serum creatinine were measured spectrophotometrically by studying reactions between creatinine and alkaline picrate.8

Parameters for anti-inflammatory tests

Animals of all groups were sacrificed on the 8th day by cervical dislocation and the pellets were removed, freed from extraneous tissue and dried at 60 °C for 24 hrs. The percentage inhibitions of the dry weight of the granuloma were calculated and compared11.

Statistical analysis

All statistical analyses were done using the MS-Excel 2007 and Kyplot ver 2.0 Beta. In Kyplot analysis, the data represented mean ± SD which was analyzed by one way ANOVA. The results were considered significant when p≤0.05.

Results

There was significant increase in paw circumference in every arthritic group except the non-arthritic positive control group (Fig. 1). But paw circumference in the experimental groups showed significant reduction after treatment with A. vera crude gel homogenate. 125 µl doses showed the maximum reduction rates, whereas 250 µl dose elicited greater protection rates after the booster dose of FCA. 125D, 250D and PG groups showed 23.30%, 65.59% and 83.79% less swelling after the 28th day of experiment commencement. In the PG, 125 µl doses showed very less paw swelling after FCA injection in both initial and booster dose injections signifying the protective property of the plant against arthritis. Radiographs further confirmed the role of A. vera gel in arthritis (Fig. 2). Histological observations showed less affected cartilage and joints in all experimental groups when compared to negative control. Cellular
infiltration and cartilage damage was less in all the experimental groups (Fig. 3). The hematological parameters showed decrement in RBC and hemoglobin counts in arthritic condition, whereas WBC count increased significantly. The treatment with *A. vera* in experimental groups brought back the parameters toward their normal levels. However, protective group (PG) showed more or less normal levels of all the parameters in both experimental time points (day 21 and 28) (Table 1).

There was no significant change in the blood sugar level in both arthritic and non-arthritic rats (CG, EG and PGs) (data not shown). In all the groups, the blood glucose level remained <100 mg/dl. Arthritic rats showed a significant decrease in the total protein level that was restored within their normal ranges after *Aloe* gel treatment. In both 21st and 28th day of experiment, the protective group showed increased total protein compared to other EGs (Fig. 4 & Table 2). Confirming the role of *Aloe* gel treatment regime on serum protein level, the experimental groups showed elevated levels of serum albumin on 21st day than the negative control group. The protective group animals showed greater level of albumin that approached towards the normal value showing the protective property of the plant (Table 2). The experimental groups restored the elevated levels of serum ceruloplasmin after treating with *A. vera* gel. In all the cases the protective group showed normal ceruloplasmin level (Fig. 4). Arthritic rats showed an increase in serum creatinine level on the 21st day. However, the value of creatinine decreased significantly towards the normal value in experimental groups. Protective group also showed decreased level of serum creatinine compared to the negative controls (Table 2). Standard group had a mean weight of dry cotton pellet 0.051±0.006 gm. AV1 and AV2 measured for 0.053±0.007 and 0.057±0.005 gm. Control group had the mean weight of 0.062±0.007 gm (Fig. 5).

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![Fig. 1](image1.png)

**Fig. 1**—Comparison of rat paw edema expressed in circumference (mm) in different experimental groups. PG shows maximum protection against the paw swelling than that of the NC.

![Fig. 2](image2.png)

**Fig. 2**—Radiographs of control and experimental rats on the 21st (upper panel) and the 28th (lower panel) days of FCA injection comparing effects of *Aloe vera* treatment.

![Fig. 3](image3.png)

**Fig. 3**—Longitudinal sections of rat hind paw joints stained in hematoxylin-eosin on the 21st day of treatment in different groups of rats. Black arrows (↑) indicate cartilage breakdown and hollow arrows (↓) indicate immune cell infiltration in the joint. Inset indicates 40X magnification of a region within the 10X region.
Table 1—Hematological parameters of different experimental rat groups expressed as mean value of the group. Data represented in the form of mean ± standard deviation.

<table>
<thead>
<tr>
<th>Days</th>
<th>PC</th>
<th>NC</th>
<th>125D</th>
<th>250D</th>
<th>PG</th>
</tr>
</thead>
<tbody>
<tr>
<td>21st Day</td>
<td>9270000±490408</td>
<td>5430000±485283</td>
<td>8440000±674907</td>
<td>7920000±442436</td>
<td>8760000±571182</td>
</tr>
<tr>
<td>28th Day</td>
<td>9270000±732802</td>
<td>6240000±693181</td>
<td>8880000±737054</td>
<td>8840000±763544</td>
<td>8450000±325960</td>
</tr>
<tr>
<td>21st Day</td>
<td>5100±503</td>
<td>7200±489</td>
<td>5700±382</td>
<td>6200±516</td>
<td>5300±382</td>
</tr>
<tr>
<td>28th Day</td>
<td>5500±683</td>
<td>6900±600</td>
<td>5900±600</td>
<td>6600±765</td>
<td>5400±516</td>
</tr>
</tbody>
</table>

RBC (7-10x10^6/mm^3) | WBC (6-17x10^4/mm^3) | Hemoglobin (11-18 gm/dl)

PC = comparison between NT (+ control) and other groups, NC = comparison between NC (- control) and other groups, *** = significant at P ≤ 0.001; ** = significant at P ≤ 0.01; * = significant at P ≤ 0.05; ( ) = normal ranges in rats according to CPCSEA, MoEF, India

Table 2—Measurement of serum total protein, albumin and creatinine in different experimental rat groups (mean ± S.D.)

<table>
<thead>
<tr>
<th></th>
<th>Total protein (gm/dl)</th>
<th>Total protein (gm/dl)</th>
<th>Albumin (gm/dl)</th>
<th>Creatinine (mg/dl)</th>
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</thead>
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<tr>
<td>21st Day</td>
<td>9.12±0.50</td>
<td>4.43±0.48</td>
<td>3.4</td>
<td>2.92±0.22</td>
</tr>
<tr>
<td>28th Day</td>
<td>9.87±0.52</td>
<td>5.82±0.16</td>
<td>3.73±0.12</td>
<td>3.75±0.03</td>
</tr>
<tr>
<td>PC</td>
<td>6.95±0.02</td>
<td>3.95±0.05</td>
<td>3.82±0.07</td>
<td>3.90±0.12</td>
</tr>
<tr>
<td>250D</td>
<td>2.92±0.02</td>
<td>2.97±0.06</td>
<td>3.825±0.07</td>
<td>3.90±0.12</td>
</tr>
</tbody>
</table>

Fig. 4—Comparison of total protein on the 21st day (a) and the 28th day (b) in different groups or rats. Comparison of ceruloplasmin in different groups of rats is shown on 21st day (c) and 28th day (d). NS: Non significant (*** = significant at P ≤ 0.001; ** = significant at P ≤ 0.01; * = significant at P ≤ 0.05).

Discussion

The measurements of rat paw circumference clearly indicated that the swelling was regressed rapidly in the experimental groups (Fig. 1). The difference in the mean paw circumference between the experimental and the negative control groups started to diverge from the 3rd day and swelling approached almost normal state within 10th day in all the experimental groups, while negative control rats retained the swellings. The same feature was also observed after the booster dose of FCA. At the end of the 28th day treatment schedule, all the experimental groups showed significant less swelling, respectively when compared with the NC group, which strongly supports the ameliorative property of the crude extract. Moreover, the paw swelling in the protective group (PG) was negligible compared to the negative control (NC) group. In the radiography data, the intact structures of the joint appeared loosened and the increment of space in between the joints (Fig. 2) confirmed the diseased state in the arthritic rats. The treated groups showed a tendency to restore the joint structure towards normalcy (Fig. 2). Histological observations clearly showed that the treated groups had less cellular infiltration in the cartilage compared to the negative controls (Fig. 3). The structure of the cartilage layer showed more breaks and uneven lining in the negative control group rat joints. These observations confirmed that the cartilage layer is protected when treated with A. vera. Interestingly, PG showed a very well formed joints structure with very little cellular infiltration with even and clear cartilage lining (Fig. 3). RBC count usually decreases in arthritic conditions. This is because the bone marrow loses its normal functioning and fails to respond to anemic condition. The RBC count increased significantly in experimental groups after 21st and 28th days (Table 1). There was no significant difference between the experimental groups and the positive control group (PC). The results indicated that the RBC count reached almost the normal level when
treated with A. vera. In contrast, WBC count increased in arthritic condition. This is because of the boosting of the immune system with antigens\textsuperscript{18} (here FCA). The levels of WBC in experimental groups reached the normal values and showed no significant difference with the normal rats (Table 1). The hemoglobin count also showed similarity with the value of RBC, as low RBC count results in low hemoglobin levels. The experimental groups showed significant increase in hemoglobin when compared with the negative control group (Table 1). Blood glucose level >100 mg/dl is known to be normal in rats. The blood glucose level (data not shown) remained normal in every group of rats confirming that the physiology of glucose metabolism in not affected by the development of RA. No significant change within the groups is seen, as also reported by other researchers\textsuperscript{3}. The level of total protein usually decreases during arthritis\textsuperscript{18}. Rats of the negative control group showed a significant decrease in the total protein level (P≤ 0.05) in comparison to the positive control rats in both the experimental time points (21\textsuperscript{st} and 28\textsuperscript{th} days). All the experimental groups showed the tendency to increase the protein level with statistical significance. The levels of total protein between positive control and the protective group reflected no (or very less on 28\textsuperscript{th} day) significance. It reflects the role of A. vera gel in maintaining total protein level of serum during RA (Fig. 4 & Table 2). However, there was an increase in the amount of total protein recorded between 21\textsuperscript{st} to 28\textsuperscript{th} days of treatment. When compared to the increment of the body weight in these treatment groups, the role of protein synthesis in gaining the body weight is apparent. We have observed that the total albumin of all the rat groups in 21\textsuperscript{st} day correlated with the total protein values. The total serum albumin level is reported to decrease in arthritic condition\textsuperscript{17}. It can be postulated that the increased permeability of vascular cells during the arthritic inflammation may increase the diffusion of albumin into vascular tissue and thereby may decrease the level of serum albumin. The albumin level increased 1.15 folds in the protective group when compared to the negative controls, suggesting a role of Aloe crude gel homogenate in the restoration of serum albumin level (p>0.05) (Table 2). The experimental group rats also showed an increased albumin concentration when compared to the negative control group rats. However, the experimental results did not show much significant alteration in the albumin levels of arthritic group. This may be due to the small interval between the booster dose (day 14) and the day of the experiment (day 21), which was probably insufficient to restore the normal albumin levels in rats (Table 2). Longer study duration may establish a clearer picture. Ceruloplasmin is a major superoxide scavenger and increases during the rheumatoid arthritis condition\textsuperscript{18}. The experimental rat groups showed a restoration of elevated serum ceruloplasmin levels after the treatment with A. vera gel (Fig. 4). In all the cases, the protective group (PG) showed normal ceruloplasmin levels (Fig. 4). In both the 21\textsuperscript{st} and 28\textsuperscript{th} days, there was significant increase of ceruloplasmin levels in negative control group (Fig. 4). For example, negative control showed 1.5 fold increase in ceruloplasmin level on 21\textsuperscript{st} day whereas 125D, 250D and PG showed 1.1 fold, 1.2 fold and 1.1 fold increase, respectively, confirming the efficacious role of A. vera gel in the decrement of ceruloplasmin level (P≤0.05). Interestingly, both on 21\textsuperscript{st} and 28\textsuperscript{th} day, the protective group rats showed no significant difference in the ceruloplasmin levels when compared with that of the normal (PC) group rats confirming the significant protective role of A. vera in the amelioration of RA. Arthritic rats showed an increase in the serum creatinine level on 21\textsuperscript{st} day (Table 2). However, serum creatinine concentration increased in the negative control rats when compared to the normal rats (P<0.05) but all the other rats of the experimental group showed no significant change when compared to the positive control rats which indicated that the level of creatinine is normal (P>0.05). Protective group rats also showed normal creatinine level when compared to the positive control (P>0.05) (Table 2).

From the results, it is clearly seen that oral administration of A. vera crude leaf gel can reduce
granuloma formation (Fig. 5). It was found that the extract elicited significant anti-inflammatory activities in AV1 and AV2 experimental group rats by reducing the dry weight of granuloma and inhibiting the migration of WBC. AV1 showed a better result with 25% reduction in granuloma than the control followed by 15% reduction in AV2 (Fig. 5). Hematological and serum biochemical parameters determine the protective role of the plant in the animal body. However, restoration of ceruloplasmin and creatinine levels indicates the restoration of non-toxic environment of the body. In our earlier work, toxicity of A. vera unprocessed gel on rat spleenocytes and macrophages were shown to be absent, rather the gel showed protective properties on the cells in 4 hrs cell culture system.

It is well evidenced from the experiments that unprocessed A. vera crude gel possessed a good anti-inflammatory property for both chronic and acute types. Keeping in mind that all the physiological parameters need different time to restore, most of the critical parameters studied in the arthritic rats have shown significant changes during disease state and that were brought back to normal or near normal states during Aloe gel feeding. These biochemical experiments were strongly supported by the biometric, radiographic, hematological and histological parameters. Our results thus confirm that consumption of crude Aloe gel not only ameliorates RA conditions in the bone joints but also shows no notable side effects. The protective value of the gel was also demonstrated in animal model against subsequent development of RA. Changes in the immunological and cell biological parameters will be done in future to confirm the role of the plant in rheumatoid arthritis as well as inflammation.

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