Anti-arthritic activity of *Glycyrrhiza glabra*, *Boswellia serrata* and their synergistic activity in combined formulation studied in freund’s adjuvant induced arthritic rats

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ABSTRACT

The present investigation was undertaken to assess the anti-arthritic activity of *Glycyrrhiza glabra and Boswellia serrata*. Then *Glycyrrhiza glabra* and *Boswellia serrata* extracts were combined to study the synergistic property for potent anti-arthritic activity. The methanolic extract of *Glycyrrhiza glabra* was administered orally at a dose of 150 mg/kg and n-hexane extract of *Boswellia serrata* was administered 50mg/kg body weight for 21 days to the experimental animals after the induction of adjuvant arthritis. Further the combined formulation containing both *Glycyrrhiza glabra* and *Boswellia serrata* 100mg/kg was administered in separate group. Different biochemical estimations were carried out like paw edema volume, lysosomal enzyme activity such as ACP, membrane bound enzymes like SGPT, SGOT and ALP, Total WBC count, different in vitro parameters like Proteinase inhibitory action and inhibition of Protein denaturation for the evaluation of anti-arthritic property. The anti-arthritic activity of *Glycyrrhiza glabra and Boswellia serrata* were assessed by significant reduction of paw edema volume and it’s capacity to stabilize lysosomal enzyme activity such as ACP significantly (P < 0.01). Furthermore the combined formulation containing both *Glycyrrhiza glabra and Boswellia serrata* at proportion (1:1) showed significant synergistic action. As a better synergistic activity was observed in combined formulation it may be tried for therapeutic use clinically.

Key words: Lysosomal enzyme, ACP, ALP, WBC count, Freund’s complete adjuvant.

INTRODUCTION

Typically arthritis is a common inflammatory joint disease characterized by inflammation of the synovial membrane, pain and restricted joint movement.1 Rheumatoid arthritis (RA), one of the commonest autoimmune diseases, is a chronic, progressive, systemic inflammatory disorder affecting the synovial joints and typically producing symmetrical arthritis that leads to joint destruction, which is responsible for the deformity and disability. The consequent morbidity and mortality has a substantial socio-economic impact.2 The prevalence of arthritis is approximately in the West.3 The prevalence of RA in India subcontinent is 1.5-2 percent of population. The epidemiological ratio of arthritis in female: male is 3:1 and the prevalence is 1% of the world population. Adjuvant induced arthritis (AIA) in rats, a chronic inflammatory disease characterized by infiltration of synovial membrane in association with destruction of joints resembles RA in humans.4

Anderson (1976) reported that the increase in edema of hind paw after adjuvant infection in rat is paralleled by increased extra cellular activities of lysosomal enzymes. These enzymes are involved in the degradation of structural macromolecules in connective tissue and cartilage proteoglycans. They are also capable of destroying extra cellular activities by increased extra cellular activities of lysosomal enzymes. They are also capable of destroying extra cellular structures and may participate in mediating tissue injury in rheumatic diseases.5 Freund’s complete adjuvant (FCA) containing 1.0 mg dry heat-killed *Mycobacterium tuberculosis* per milliliter sterile paraffin oil. As *Mycobacterium tuberculosis* in chronic infectious condition produces arthritis in human hence the model is suitable for the evaluation of arthritic activity. Acute inflammatory response induced by FCA is associated with leukocyte...
infiltration, mast cell activation and release of cytokines and free radicals. [6] This process gets aggravated with macrophage activation and secretion of bioactive product that play an important role in tissue destruction, vascular and fibrosis over a period of time. [7] Presently many non steroidal, steroidal and immunosuppressive drugs are used to control inflammatory symptoms and pain, they are associated with certain undesirable side effects.[8] With these difficulties, the field of arthritis research has progressed exponentially towards herbal therapies that have been considered safe and effective in all elevating chronic pain associated with arthritis. [9] Glycyrrhiza glabra (Liquorice) has been used in medicine for more than 4000 years. The plant is distributed in the subtropical and warm temperature region of the world. In India it is reported to be cultivated in Jammu and Kashmir. The root and rhizome of the plant has been used as anti-inflammatory, anti-oxidant, anti-spasmodic, expectorant. Similarly, Boswellia serrata (Burseraceae) consists of the oleo-gum-resin, has been used as immunomodulatory, anti-inflammatory and anti-tumor activity.[10] The significant of selection of Glycyrrhiza glabra and Boswellia serrata is that, arthritis is an autoimmune and inflammatory disorders which may be controlled by both the drugs in combination. No work has been still done about the anti-arthritis activity. Hence the present study has been carried out to evaluate the anti-arthritis activity of methanolic extract of Glycyrrhiza glabra and n-hexan extract of Boswellia serrata and determination of synergestic activity in combination containing both Glycyrrhiza glabra and Boswellia serrata at equal proportion.

MATERIALS AND METHODS

Collection of plant materials:

Rhizomes of Glycyrrhiza glabra and gum resin of Boswellia serrata were collected from Yucca enterprises, Mumbai, India and the plant product was identified by Prof. M. Mishra, Dept of Botany, Berhampur University, Specimen no.542 for further studies. After identification of rhizomes and gum resin were subjected to drying in normal environmental condition under shade.

Preparation of extract:

The dried rhizomes and gum resin were powdered by pulverization and were stored in air tight container. The powdered material was passed through sieve of 16 to obtain uniform particle size for extraction. Glycyrrhiza glabra was extracted by Soxhalation Process by taking 550 g powder with 2000 ml methanol of analytical grade. Distillation was carried out to recover maximum solvent. The yield of Glycyrrhiza glabra was found to be 16.07%. The extraction of Boswellia serrata was carried on by Cold Maceration Process. 500 g Boswellia serrata was taken in a stainless steel beaker, treated with 500 ml of n-hexane, stirred to form a homogenous mixture, and allowed to stand at room temperature for 24 hr. The supernatant liquid was filtered by decantation through a fritted, medium porosity funnel under vacuum. The highly resinous marc was washed by being suspended in remaining amount of n- hexane for 1 hr and filtered as above. The solvent from the combined filtrate and washing was stripped off under reduced pressure and left under vacuum overnight to get the n-hexane extract. The marc that remained after extraction with hexane was left in the hood overnight for drying. The dried marc was triturated with methanol 500 ml until it was dispersed completely to form a homogenous mixture. The mixture after being stirred mechanically at room temperature for 24 hr was left to stand at room temperature to allow the residue to settle down and was then filtered by decantation under vacuum as described above. The marc was washed with 500 ml of methanol. Both final fractions were evaporated under elevated temperature and under reduced pressure until a constant weight were obtained to ensure complete removal of the extraction solvent.[11] The yield of Boswellia serrata was found to be 6%. Both the extract was administered orally as suspension by triturating with 1% Carboxy Methyl Cellulose.

Animals:

Male albino rats of Wistar strain weighing 160-180 g were taken from Ghosh Enterprises, Calcutta. The male rats were selected which helps an economic pharmacokinetic process of the test drug through out the experimental periods. The animals were housed in solid-bottomed polypropylene cages and acclimatized to animal house conditions. The rats were fed with commercial pellets and water ad libitum. The standard pellet diet was supplied by Rayan’s biotechnologies Pvt. Ltd, Hyderabrd, (A.P.). The experiments were designed and conducted in accordance with the ethical norms. The
study protocol was submitted before Institutional Animal Ethics Committee (IAEC) (Regd. No.926/ab/06/CPCSEA dated: 22-02-2006) of Roland Institute of Pharmaceutical Sciences. The IAEC has approved this protocol (Approval No. 27 dated on 06-03-2010).

**Acute oral toxicity study:**

Acute toxicity test were performed on rat of either sex weighing 160-180 g body weight. As per the OECD guidelines, the individual and combined herbal extracts at different doses up to 2000 mg/kg were administered and the animals were observed for behavioral changes, toxicity and motility up to 48 h. In case of *Glycyrrhiza glabra* and *Boswellia serrata* the dose was adjusted 150 mg/kg and 50 mg/kg respectively depending upon the animal behavioral and toxicity study. Where as in case of combined extract, at ratio (1:1) the dose was adjusted to 100 mg/kg for the evaluation of synergistic activity.

**Complete Freund’s adjuvant induced Arthritis:**

Arthritis was induced by a single intra-dermal injection (0.1 ml) of Complete Freund’s adjuvant (CFA) containing 1.0 mg dry heat-killed *Mycobacterium tuberculosis* per milliliter sterile paraffin oil into a foot pad of the left hind paw of male rats.[12] A glass syringe (1 ml) with the locking hubs and a 26G needle was used for injection. The rats were anesthetized with ether inhalation prior to and during adjuvant Injection, as the very viscous nature of the adjuvant exerts difficulty while injecting. The swelling paws were periodically examined (up to 21 days) in each paw from the ankle using Digital Plethysmometer.[13] The rats were divided in to five groups of six each. The first group served as normal control and received 1% CMC solution (10ml/kg). The second group was taken as arthritic control group. The third group was administered prednisolone (10mg/kg p.o) as the standard drug. The fourth group was administered extract of *G. glabra* (150 mg/kg body weight/rat/day for 21 days). The fifth group was administered with extract of *B. serrata* (50 mg/kg body weight/rat/day for 21 days). The sixth group was administered combine formulation containing equal proportion (1:1) of *G. glabra* and *B. serrata* (100 mg/kg body weight/rat/day for 21 days). Results were expressed as change in paw volume (in ml) by using following formula

\[
\text{Increased Volume of edema} = \text{Final Paw Volume} - \text{Initial Paw Volume}
\]

**Biochemical Analysis:**[14]

Different biochemical parameters like Alkaline phosphatase(ALP) marker for bone destruction, Acid Phosphatase (ACP) the lysosomal enzyme activity, Serum glutamate oxalo acetate transaminase (SGOT) and Serum glutamate pyruvate transaminase (SGPT) were estimated by using ALP, ACP, SGOT and SGPT kit in Autoanalyser. Bood samples were collected by sublingual rout, centrifused and supernatant serum was collected. Different enzyme reagents were added to the serum and estimated in an auto analyser. For the estimation of Total WBC count blood samples were added with WBC diluting fluid and by the help of Neubauer’s chamber total numbers of WBC was calculated by using formula, Total WBC count = Total no. of cells X Volume correction factor X 20.

**In- vitro test Analysis:**

**Inhibition of protein denaturation**

The reaction mixtures (0.5 ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of *G. glabra*, *B. serrata* and combine extract (500ìg/ml of final volume) and standard prednisolan (100 ìg/ml of final volume). pH was adjusted at 6.3 using a small amount of 1 N HCl. The samples were incubated at 37°C for 20 min and then heated at 57°C for 3 min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660 nm. For control tests 0.05 ml distilled water was used instead of extracts while product control tests lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows:

\[
\text{Percent inhibition} = \left[90 - (\text{O.D. of test} - \text{O.D. of product control}) / \text{O.D. of control}\right] \times 100
\]

The control represents 100% protein denaturation. The result was compared with prednisolone treated samples.[15]

**Proteinase Inhibitory Action**

The reaction mixtures (2.0 ml) contained 0.06 mg trypsin, 1.0 ml. 25 mM tris-HCl buffer (pH 7.4) and 1.0 ml aqueous solution of *G. glabra*, *B. serrata* and combine extract (500ìg/ml of final volume) and standard prednisolan (100 ìg/ml of final volume). The mixtures were incubated at 37°C for 5 minutes. Then 1.0 ml of 0.8% (w/v) casein
was added. The mixtures were incubated for an additional 20 minutes. 2.0 ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged. Absorbance of the supernatant was read at 280 nm against buffer as blank. 

The percentage inhibition is calculated as follows: Percent inhibition = \[100 - \frac{\text{O.D. of test-O.D. of product control}}{\text{O.D. of control}}\] x 100

Statistical analysis:

All the grouped data were statistically evaluated with PRISM/Version-4, April 3, 2003 software and Microsoft excel. Hypothesis testing methods include one way analysis of variance (ANOVA) along with post-hoc test. P d°0.05 and 0.01 were considered to indicate statistical significance. All the results were expressed as Mean ± SEM for six animals in each group.

RESULTS

Preliminary photochemical screening showed the presence of steroidal saponins, flavonoids, carbohydrates and alkaloids in the methanolic extract of *Glycyrrhiza glabra*. Similarly Cold Macerated Extraction by n-hexane of *Boswellia serrata* showed the presence of Carbohydrates, terpinoids, Gums and Mucilages. After calculation of Maximum Tolerated dose 150mg/kg (*Glycyrrhiza glabra*) and 50mg/kg (*Boswellia serrata*) was found to be effective dose.

Table-1 shows change in paw volume measurement after the administration of *G. glabra*, *B. serrata* and combine extract treated rats. For more than 20 days gradually the adjuvant injected paw became swollen. The change in paw volume could be divided in to two phases. In the first phase, edema rate of the injected foot pad increase and reached a peak during first 5-10 days. There after, swelling slowly subsided until 15th day when the paw began to swell again and picked in the 3rd week (second phase). Administration of Prednisolon, *G. glabra*, *B. serrata* and combination (*G. glabra* and *B. serrata*) was started after the adjuvant injection, significantly (p d° 0.01) suppress the secondary increasing in swelling of the injected foot in all groups as compare to arthritic control (Group II). The Group VI (combine extract) produced a better suppression of paw volume as compare to Group IV and Group V.

<table>
<thead>
<tr>
<th>DAYS</th>
<th>Normal Control</th>
<th>Arthritic Control</th>
<th>Prednisolone (10mg/kg)</th>
<th>Glycyrrhiza (150 mg/kg)</th>
<th>Boswellia (50 mg/kg)</th>
<th>Combination (100 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.53±0.05</td>
<td>0.60±0.02</td>
<td>0.60±0.06</td>
<td>0.56±0.01</td>
<td>0.60±0.02</td>
<td>0.52±0.01</td>
</tr>
<tr>
<td>5</td>
<td>0.52±0.04</td>
<td>0.78±0.01</td>
<td>0.68±0.01</td>
<td>0.69±0.01</td>
<td>0.71±0.02</td>
<td>0.61±0.01**</td>
</tr>
<tr>
<td>7</td>
<td>0.54±0.02</td>
<td>0.88±0.02</td>
<td>0.60±0.01**</td>
<td>0.64±0.02**</td>
<td>0.63±0.02**</td>
<td>0.65±0.01</td>
</tr>
<tr>
<td>10</td>
<td>0.56±0.05</td>
<td>0.95±0.01</td>
<td>0.53±0.01**</td>
<td>0.57±0.02**</td>
<td>0.59±0.03**</td>
<td>0.52±0.01**</td>
</tr>
<tr>
<td>13</td>
<td>0.47±0.03</td>
<td>0.96±0.3</td>
<td>0.44±0.01**</td>
<td>0.52±0.02**</td>
<td>0.52±0.03**</td>
<td>0.58±0.01**</td>
</tr>
<tr>
<td>15</td>
<td>0.48±0.02</td>
<td>0.99±0.02</td>
<td>0.36±0.01**</td>
<td>0.45±0.01**</td>
<td>0.46±0.03**</td>
<td>0.43±0.01**</td>
</tr>
<tr>
<td>18</td>
<td>0.44±0.01</td>
<td>1.03±0.02</td>
<td>0.24±0.02**</td>
<td>0.38±0.02**</td>
<td>0.38±0.03**</td>
<td>0.23±0.03**</td>
</tr>
<tr>
<td>21</td>
<td>0.44±0.01</td>
<td>1.05±0.02</td>
<td>0.17±0.02**</td>
<td>0.30±0.02**</td>
<td>0.30±0.04**</td>
<td>0.16±0.03**</td>
</tr>
</tbody>
</table>

Values are expressed as mean± SEM (standard error of the mean) of 6 determinants. **P<0.01, compared to Arthritic control.

Table 2: Effect of Glycyrrhiza glabra, Boswellia serrata and combined formulation on change in membrane marker enzymes of control and treated rats.

<table>
<thead>
<tr>
<th>DAYS</th>
<th>Normal Control</th>
<th>Arthritic Control</th>
<th>Prednisolone (10mg/kg)</th>
<th>Glycyrrhiza (150 mg/kg)</th>
<th>Boswellia (50 mg/kg)</th>
<th>Combination (100 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP</td>
<td>73.5±2.44</td>
<td>442±14.92*</td>
<td>139.3±12.59**</td>
<td>222.8±7.67**</td>
<td>234.6±6.02**</td>
<td>210.6±16**</td>
</tr>
<tr>
<td>SGOT</td>
<td>36.5±0.921</td>
<td>110.6±4.08*</td>
<td>49 ± 2.64**b</td>
<td>64.16 ± 1.55**</td>
<td>65.0±1.29**</td>
<td>50.16±2.63**</td>
</tr>
<tr>
<td>SGPT</td>
<td>37.83±0.401</td>
<td>169±3.396*</td>
<td>42.5 ± 2.077***</td>
<td>66.66 ± 2.74**</td>
<td>68.66±1.89**</td>
<td>0.65±0.01**</td>
</tr>
</tbody>
</table>

Values are expressed as mean± SEM (standard error of the mean) of 6 determinants. *P<0.05, compared to normal and **P<0.01, compared to Arthritic control.
A marked increase in the activity of membrane marker enzymes (ALP, SGOT and SGPT) were observed in the serum of arthritic rats (Group II). Hepatic cells were also affected in arthritic rat. Arthritic rats treated with G. glabra, B. serrata and combination of G. glabra and B. serrata showed a significant (P < 0.01) decrease in the activity of membrane marker enzyme (Table 2). Acid phosphatase is one of the lysosomal enzyme responsible for cartilage destruction in arthritic condition. It’s concentration was significantly reduced by treated groups as compare to arthritis control (Table 3). Migration of leucocytes into the inflamed area of arthritic rats were significantly (p <0.01) prevented with the treatment of G. glabra, B. serrata and combination of G. glabra and B. serrata (Table 4).

Table 3: Effects of Glycyrrhiza glabra, Boswellia serrata and combined formulation on change in acid phosphatase level of control and treated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ACP(U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Normal control</td>
<td>4.6 ± 0.073</td>
</tr>
<tr>
<td>II Arthritis Control</td>
<td>25.68 ± 2.02*</td>
</tr>
<tr>
<td>III Prednisolone</td>
<td>6.495 ± 0.62**</td>
</tr>
<tr>
<td>IV Glycyrrhiza 150mg/kg</td>
<td>7.25 ± 0.46**</td>
</tr>
<tr>
<td>V Boswellia 50mg/kg</td>
<td>8.22 ± 0.22**</td>
</tr>
<tr>
<td>VI Combination 100mg/kg</td>
<td>7.166 ± 0.464**</td>
</tr>
</tbody>
</table>

Values are expressed as mean± SEM (standard error of the mean) of 6 determinants. *P<0.05, compared to normal and **P<0.01, compared to Arthritic control.

Table 4: Total leucocyte count of different groups on 21st day.

<table>
<thead>
<tr>
<th>Groups</th>
<th>WBC Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10498.33 ± 101.502</td>
</tr>
<tr>
<td>CFA</td>
<td>29441.67 ± 101.502*</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>13540 ± 101.502**</td>
</tr>
<tr>
<td>Glycyrrhiza 150mg/kg</td>
<td>21291.67 ± 387.499**</td>
</tr>
<tr>
<td>B. serrata 50mg/kg</td>
<td>2110.45 ± 224.30**</td>
</tr>
<tr>
<td>Combination 100mg/kg</td>
<td>18473.33 ± 39.1081**</td>
</tr>
</tbody>
</table>

* P d<0.05 as compared to Normal and ** P d<0.01 as compared with Complete Freund’s adjuvant (CFA).

From the in-vitro study the result indicating a significant inhibition of protein denaturation and control of proteinase activity. The standard prednisolan concentration at 100 µg/ml showed 80.65% and 75.8%, Glycyrrhiza at 500 µg/ml showed 61.29% and 65.8%, Boswellia at 500 µg/ml showed 58.22% and 60.20% and Combined Formulation at 500µg/ml showed 70.97% and 68.43% inhibition of protein denaturation and control of proteinase activity respectively (Table 5).

Table 5: In-vitro test for inhibition of protein denaturation and proteinase inhibition by different treated groups in percentage.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Inhibition of Protein denaturation (%)</th>
<th>Proteinase inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycyrrhiza 500 µg/ml</td>
<td>61.29± 3.44</td>
<td>65.8± 2.88</td>
</tr>
<tr>
<td>Boswellia 500 µg/ml</td>
<td>58.22± 3.84</td>
<td>60.20±1.56</td>
</tr>
<tr>
<td>Prednisolone 100 µg/ml</td>
<td>80.65±2.58</td>
<td>75.8±3.34</td>
</tr>
<tr>
<td>Combined Formulation 500µg/ml</td>
<td>70.97±3.31</td>
<td>68.43±2.32</td>
</tr>
</tbody>
</table>

Values (Mean± SEM) are presented in comparison to control tests.

DISCUSSION

Most of the investigators have reported that inhibition of adjuvant-induced arthritis in rats is one of the most suitable test procedures to screen anti-arthritic agents since it closely resembles human arthritis. Freund’s adjuvant induced arthritis is thought to occur through cell-mediated autoimmunity structural mimicry between mycobacteria and cartilage proteoglycan in rats. It activates macrophages and lymphocytes by adjuvant inoculation or their products like monokines, cytokines, and chemokines may be involved in abnormal lipid and protein metabolism. The CFA administered rats showed soft tissue swelling around the ankle joints during the development of arthritis which was considered as edema of the particular tissues. As the disease progressed, a more diffused demineralization developed in the extremities. [16]

Reduction of paw swelling in the Glycyrrhiza glabra 150 mg/kg, Boswellia serrata 50 mg/kg and combined formulation contain both Glycyrrhiza glabra and Boswellia serrata 100 mg/kg treated rats from the second week onwards may be due to immunological protection rendered by the plant extract.

Lysosomes are membrane enclosed cytoplasmic organelles, which possess an acidic interior that contain many hydrolytic enzymes. Lysosomal enzymes are widely distributed in tissue and circulating blood cells and are responsible for intracellular breakdown of complex macromolecules. They also degrade endothelial membrane...
glycol-conjugates. The altered enzyme activities in arthritis can be regarded as an index of lysosomal enzyme activation occurring in response to metabolic need of degrading various constituents of cells such as mucopolysaccharides and glycoproteins accumulated in tissue due to arthritis associated with vasculopathies.\[^{17}\]

Acid phosphatase seem to be an important index for the examination of the integrity of the lysosomal membrane and are responsible for the tissue damage and necrosis of hepatic tissue. Cytoplasmic cellular enzymes, such as alkaline phosphatase (ALP) membrane bound indicator of type II cell secretary activity or the lysosomal enzyme \(\beta\)-glucuronidase, an indicator of phagocytic activity, can also be used as sensitive markers of cellular integrity and cellular toxicity induced by pathological conditions. Increased activities of plasma acid phosphates were observed in arthritic rats. This may be attributed towards persistent inflammation. These changes are due to decreased lysosomal stability in adjuvant induced arthritis.\[^{18}\] The cytokines IL-1, IL-6, and TNF are the most important mediators of the acute phase reactions. These cytokines are produced by leucocytes and other cell types in response to infection, or to immune and toxic injury, and are released systemically. TNF can induce the production of IL-1, which in turn induces the production of IL-6. IL-6 stimulates the increased activity of liver. So enzymes like SGPT, SGOT, which are abnormality markers, are synthesized in the body.\[^{19}\]

In the present study, the activity of lysosomal enzymes, liver enzymes in plasma was markedly increased in the adjuvant induced arthritic rats and significantly reduced after treatment with \textit{Glycyrrhiza glabra} 150 mg/kg, \textit{Boswellia serrata} 50 mg/kg and combined formulation contain both \textit{Glycyrrhiza glabra} and \textit{Boswellia serrata} 100 mg/kg.

An important mechanism of anti-arthritic activity is the membrane stability modulating effect.\[^{20}\] The administration of \textit{Glycyrrhiza glabra} 150 mg/kg, \textit{Boswellia serrata} 50 mg/kg and combined formulation contain both \textit{Glycyrrhiza glabra} and \textit{Boswellia serrata} 100 mg/kg may exert its effects by modifying the lysosomal membrane in such a way that it is capable of fusing with the plasma membrane and there by preventing the discharge of acid hydrolase or by inhibiting the release of lysosomal enzymes.\[^{21}\] The activity of \textit{Glycyrrhiza glabra} may be due to presence of flavonoids i.e. liquiritin and isoliquiritin.

Increased white blood cell count is a common feature of inflammatory reactions, especially those induced by microbial infection. The leukocyte count usually increases to 15,000 or 20,000 cells/\(\mu\)l (normal = 4000 to 10,000 cells/\(\mu\)l) but exceptionally may climb as high as 40,000 to 1000,000 cells/\(\mu\)l, a so called leukemoid reaction. Leukocytosis initially results from the release of cells from the bone marrow (caused by IL-1 and TNF) and is associated with an increased number of relatively immature neutrophils in the blood.\[^{22}\] So in arthritic group an increase in total leukocyte number was found. A significant reduction (\(p < 0.01\)) in total leukocyte number was found in case of treated groups. In our study it was found that the administration of \textit{Glycyrrhiza glabra}, \textit{Boswellia serrata} and combined formulation lead to inhibition leukocyte migration which may have beneficial effect for joint preservation. The activity may be due to presence of boswellic acid in \textit{Boswellia serrata} and steroidal glycoside i.e. glycyrrhetinic acid in \textit{Glycyrrhiza glabra}.

Denaturation of proteins as one of the causes of rheumatoid arthritis is well documented. Production of auto antigens in certain rheumatic diseases may be due to in vivo denaturation of proteins. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding.\[^{22}\] From the results of the present study it can be stated that \textit{Glycyrrhiza glabra} 150 mg/kg, \textit{Boswellia serrata} 50 mg/kg and combined formulation contain both \textit{Glycyrrhiza glabra} and \textit{Boswellia serrata} 100 mg/kg is capable of controlling the production of auto antigens due to in vivo denaturation of proteins in rheumatic diseases.

Proteinases have been implicated in arthritic reactions. Neutrophils are known to be a rich source of proteinases which carry in their lysosomal granules many neutral serine proteinases. Leukocyte proteinases play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors.\[^{23}\] \textit{Glycyrrhiza glabra} 150 mg/kg, \textit{Boswellia serrata} 50 mg/kg and combined formulation contain both \textit{Glycyrrhiza glabra} and \textit{Boswellia serrata} 100 mg/kg exhibited significant anti-proteinase activity. After investigation of all the parameters a better synergistic activity has been
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