3.1. INTRODUCTION

3.1.1. Inflammation

Inflammation is defined as the reaction of vascularised living tissue to injury (Cooke T. 1903). The inflammatory response is closely intertwined with the process of repair. Inflammation serves to destroy, dilute or wall off the injurious agent, but in turn it sets into motion a series of events that as far as possible heal and reconstitute the damaged tissue. Repair begins during the early phases of inflammation but reaches completion usually after the injurious influence has been neutralized. Inflammation, however, if runs unchecked, leads to onset of vasomotor rhinorrhoea, rheumatoid arthritis, hypersensitivity reactions, fetal renal disease and atherosclerosis (Shah GL. 1978). Inflammation may be acute or chronic depending on the disease course. Acute inflammation is characterized by heat, erythema, pain, swelling and loss of function. Pain is a common and distressing feature of many diseases and analgesics relieve pain by acting in the central nervous system or on peripheral pain mechanisms, without significantly altering consciousness. Chronic inflammation on the other hand results in a progressive shift in inflammatory cells characterized by simultaneous destruction and healing of the injured tissue.

The acute inflammatory response to a physical or chemical stress can last up to three days, with the whole repair process taking up to six weeks. If inflammation from a given stress lasts beyond this timeframe, it may have a negative impact on our health, and is typically indicative of some imbalance – one that can be corrected through lifestyle. For some people, the presence of inflammation is obvious. But most people with inflammation are not aware of it – the signs and symptoms may not be apparent.

The best test for inflammation is called C-reactive protein (CRP), which is measured from the blood. None of the other tests may be as accurate as the CRP, which can detect very low levels of inflammation. This test can also predict future risk of coronary heart disease and stroke even in healthy individuals. The erythrocyte sedimentation rate (ESR) is a common blood test for inflammation. It can be
performed when blood is taken for other tests, or with a finger prick. The white blood cells may indicate inflammation. This test is part of the Complete Blood Count (CBC) commonly performed.

Body temperature is a general indicator of inflammation. However, it is not the best test as only more severe inflammation will elevate temperature. The best suggestion is to have a CRP performed yearly. If it is not normal, a re-test every three to six months (once you start taking the appropriate supplements, making dietary changes, etc.) until it is normal is a good way to monitor the effectiveness of your program.

Different types of trauma or microtrauma, infections, and toxins can produce inflammation. Even excess body fat can produce inflammation. It results from the production of large quantities of proinflammatory chemicals such as cytokines and eicosanoids. Trauma, such as a fall, is a common cause of significant inflammation. Microtrauma, which includes such subtle actions as walking, typing and any repetitive motion, normally produces inflammation. Intense physical activity, such as anaerobic exercise – high intensity training, weight lifting and competition – also causes significant inflammation.

Chemicals – food allergy, hay fever, handling or breathing harsh chemicals (cleaners, gasoline, etc.), air pollution and others can cause inflammation. Even cosmetics and toiletries. Infections also cause inflammation – from bacteria, virus, fungus, or yeast. Increased body fat can cause inflammation because fat cells produce inflammatory chemicals. This is especially serious in those who are obese. As discussed below, nutritional imbalances can cause chronic inflammation. This is especially true with dietary fats – herein lies the key to controlling inflammation from a dietary standpoint.

There is a full spectrum of problems associated with chronic inflammation. These include functional problems such as fatigue, hormone imbalance and reduced immunity, and may be precursors to more severe conditions that lead to disease in many individuals. Fatigue may be among the more common results of chronic inflammation.
Other problems may include the following

**Lowered immunity**

This can result in frequent infections, including colds and flu, yeast and fungal infections such as Candida. Asthma, allergies and other problems may also be due to low immunity. These conditions further aggravate or cause more inflammation, maintaining a vicious cycle.

**Hormonal imbalance**

This can include many aspects of the hormone system, especially the adrenal stress hormones, reducing one's ability to cope with stress. Sex hormones can also be adversely affected – estrogen, progesterone and testosterone balance – resulting in diminished sex drive and reproductive function. Reduced thyroid function can also result due to inhibition of thyroid-stimulating hormone.

**Nervous system imbalance**

This includes increased activity of the sympathetic nervous system, potentially leading to increase tension, rising blood pressure, disturbed blood sugar, anxiety or depression, or other neurological problems.

**Digestive distress**

Among the problems that result include poor digestion, gas formation, heartburn, and various inflammatory conditions. Poor absorption of nutrients can create a whole series of potential problems throughout the body.

**Chronic pain**

Inflammation produces pain-stimulating chemicals throughout the body. Individuals with more body fat produce more inflammation and have a reduced pain threshold.

Many drugs including steroidal and non-steroidal anti-inflammatory drugs are employed to control the inflammatory reaction and pain associated with it. Out of the anti-inflammatory drugs, the nonsteroidal anti-inflammatory drugs (NSAIDs) are preferred and commonly used in clinical practice. These drugs have wide range of chemical nature and share, the common mechanism of action, pharmacological actions and adverse effect profiles. Although these drugs are effective in controlling
signs of inflammation, numbers of adverse effects encountered are the biggest limitations to their use. Because of the side effect profile of NSAIDs, patients are inclined to choose the alternative system of treatment. Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly employed drugs for the treatment of inflammatory conditions but the adverse effect profile is the limitations in medicinal plants.

3.1.2. Clinical Correlations

3.1.2.1. Serum (Lysosomal) Enzymes level

SGOT, SGPT, ALP are the lysosomal enzymes. There is increasing evidence that lysosomal enzymes play an important role in the development of acute and chronic inflammation (Anderson et al., 1971). Most of the anti-inflammatory drugs exert their beneficial effect by inhibiting either release of lysosomal enzymes or by stabilizing lysosomal membrane which is one of the major events responsible for the inflammatory process (Nair et al., 1998).

3.1.2.2. Lipid peroxidation

Lipid peroxidation has been implicated in the pathogenesis of various diseases including arthritis. It is well established that bioenzymes are very much susceptible to LPO, which is considered to be the starting point of many toxic as well as degenerative processes. LPO level was increased during inflammation (Bonata et al., 1980). Administration of formalin produced an elevated level of LPO, which may due to the free radicals and is responsible for damaging cell membranes there by further intensifying inflammatory damage (Telang et al., 1990). The inflammatory tissue damages could be due to the liberation of reactive oxygen species from phagocytes invading the inflammation sites (Conner and Grisham, 1996). SOD is the most important mitochondrial antioxidant enzymes and it provides defense against super oxide anions. In inflammatory condition, there is excess activation of phagocytes and production of super oxide radical (Gillham et al., 1997) which can harm surrounding tissue either by a powerful direct oxidizing action or indirectly as with hydrogen peroxide and hydroxy radicals formed from ROS, which initiate LPO resulting in membrane destruction. The membrane destruction then provokes inflammatory response by the production of mediators and chemotactic factors. Glutathione is an important endogeneous antioxidant, which plays an important role
in protecting cells against oxidative stress via glutathione redox system. Tissue glutathione depletion seems to be responsible for the induction of LPO (Lewis. 1989).

3.1.2.3. Levels of Leucocytes

Leucocytes play a major role in the development and propagation of inflammation. Neutrophils play a crucial role in the development and manifestation of inflammation and they are the major source of free radicals at the site of inflammation. Neutrophil derived free radical is known to because of inflammation and cytokines produced by neutrophils are also responsible for inflammation. Eosinophils are granule containing leucocytes that differentiate from stem cell precursors. It synthesizes and release lipid derived mediators which stimulate responses in tissues. In addition, it produces cytokines such as interleukins (IL-3 and IL-5) and granulocyte macrophage stimulating factor that contribute pro inflammatory functions. Lymphocytes are the predominant cell in chronic inflammation. It can cause permanent distortion of the tissue, interfering its function. Total WBCs which plays a major role in body defense mechanism. The increase in WBC count during inflammation may be due to the release of interleukins, responsible for the production of both granulocytes and macrophage colony stimulating factor (Eric and Lawrence, 1996).

3.1.2.4. Levels of Hemoglobin and Erythrocytes

Hemoglobin and RBC play a major role in the oxygen transport. The low concentration of Hb is noted in chronic inflammatory disease such as rheumatoid arthritis which is usually associated with the anorexia and weight loss. Such a decline in Hb level has been reported earlier (Swingle and Shideman, 1972).

3.1.2.5. Levels of Connective tissue components

Collagen induction causes the changes in connective tissue metabolism, is one of the major biochemical events during the process of inflammation. These changes are effected in the alteration of relative composition of various constituents of connective tissue such as mucopolysaccharides, glyco protein, hexosamine and hydroxy proline, sialic acid (Houck and Jacob. 1969). Hence the levels of hexosamine and hydroxyproline were found to be higher in inflammation induced mice.
3.1.2.6. Levels of Total Proteins

Proteins are the building block of amino acids. The propagation of free radical can bring many adverse reactions leading to extensive tissue damage. Lipids, proteins, DNA are very susceptible to attack by free radicals (Yu et al., 1992). The level of serum protein content is lowered during inflammation. The proteins were clearly changed the perception of the pathogenesis of inflammation which has been reported earlier (Weissman, 1967). Chronic inflammation is known to stimulate protein metabolism in animals (Mercier et al., 1921).

3.1.3. Astaxanthin

Astaxanthin is a red-pigment carotenoid occurring naturally in a wide variety of living organisms and classified as a xanthophyll. It has a chemical structure similar to that of the familiar carotenoid \( \beta \)-carotene. It is commonly found in crustaceans (e.g. shrimps, crawfish, crabs and lobster) and produced by microalgae. The pink flesh of a healthy wild salmon is due to the presence of astaxanthin. It has been suggested that astaxanthin protects muscle cells from damaging effects of active oxygen produced upon swimming upstream. Meanwhile, astaxanthin contained in salmon roe is considered to protect the roe from reactive oxygen species generated by UV rays. The antioxidant of astaxanthin is stronger than \( \beta \)-carotene and vitamin E by 40x and 1,000x respectively. Besides, astaxanthin differs from other antioxidants in its ability to penetrate the blood brain and retina barriers. Therefore, it is believed to protect the brain and nervous system from neurodegenerative diseases (e.g. cerebral thrombosis and stroke) and aging. Meanwhile, astaxanthin has been documented to prevent age-related macular degeneration (AMD) and enhance immune functions. Furthermore, recent studies revealed the wrinkling and moisturizing effect of astaxanthin which suggest its potential cosmeceutical applications in protection against skin aging.

![Structure of Astaxanthin](image)

**Figure 9 : Structure of Astaxanthin**
3.1.3.1. Antioxidant effect of Astaxanthin

Astaxanthin has unique chemical properties based on its molecular structure (Goto, S. et al., 2001) and W. Miki, 1991. The presence of hydroxyl (OH) and ketone (C=O) moieties on each ionone ring along with an extension of conjugated double bond system explained the potency of astaxanthin with higher antioxidant activity compared to β-carotene and vitamin E. The 2 most prominent antioxidant activities of astaxanthin are quenching of singlet oxygen and inhibition of lipid peroxidation.

![Figure 10: Astaxanthin & its antioxidant chemical site](image-url)

3.1.3.2. The Effect of Astaxanthin on Cell Membrane

Lipid peroxidation occurs when free radicals such as reactive oxygen species oxidizes cell membrane which leads to cell damage. There are 3 main protective antioxidants in the human body, namely, vitamin E, vitamin C & beta-carotene. These antioxidants supplement each other in protecting the cell membrane from multiple oxidative chained reactions (Yoshikawa S, 1996). Vitamin E is present in the hydrophobic region of the cell membrane. Upon lipid peroxidation by free radicals, Vitamin E will donate its free electrons to neutralize free radicals. Oxidized Vitamin E will be recycled by Vitamin C which is located in the hydrophilic region of the cell membrane. Hence, Vitamin E continues its antioxidant activity while oxidized Vitamin C is metabolism and excreted from the body. Beta-carotene as a lipophilic compound, is present in the hydrophobic region of cell membrane and trap free radicals generated within the cell membrane.
Astaxanthin has been reported to span the cell membrane bilayer (fat /water) because of its unique structure with polar terminal rings (H.Y. Yamamoto et al., 1978). The polar OH-group in two terminal rings of astaxanthin is likely to be oriented at/near the membrane surface while the polyene chain in the interior of the membrane (Goto, S., Kogure, K., Abe, K., et al., 2001 and W. Miki, 1991). Accordingly, astaxanthin could be effective in scavenging reactive oxygen species at membrane surface while its polyene chain inhibit oxidative chain reaction in the membrane (W. Miki, 1991). Astaxanthin is an excellent antioxidant that protects the entire cellular components and cells from free radicals damage and degradation.

3.1.3.3. Anti-inflammatory Effect

The anti-inflammatory effect of astaxanthin has been documented (Ohgami, K., Shiratori, K., et al., and Lee, S.J., Bai, S.K., et al., 2003). Inflammation is the very initial response of the immune system to infection or irritation. It is characterized by redness, heat, swelling, pain and dysfunction of organs involved. Phagocytosis occurs upon invasion of pathogens where pathogens are ingested by macrophages followed by the release of cytokines IL-1 and TNF-α at inflammation site. Meanwhile, enzyme iNOS is stimulated to produce NO (carbon monoxide). Illustration on cascade of inflammation is shown in Fig. IL-1 and TNF-α further activate NF-κB to increase the production of IL-1. Increased IL-1 in turn activated COX-2 and produces PGE2
(prostaglandin E2). NO, TNF-α and PGE2 are inflammatory factors that mediate the immune system. However, elevated blood NO level may result in tissue disorders such as cancer and aging. Meanwhile, excessive production of PGE2 can result in feverish symptoms and pain such as rheumatoid arthritis (RA).

Study found that astaxanthin prevent the anti-inflammatory response of macrophages. In a mice experimental model, the effect of astaxanthin on inflammation induced by lipopolysaccharides (LPS) was evaluated and compared with prednisolone, a steroidal anti-inflammatory drug. Findings indicated that astaxanthin regulated the production of NO, TNF-α and PGE2 while its activity was 1/10 of prednisolone. In vitro, astaxanthin demonstrated inhibitory effect against NF-kB and IL-1 in mice’s RAW cells which are similar to macrophages.

![Image of mechanism of macrophages during inflammation and the effect of Astaxanthin](image)

**Figure 12 :** The mechanism of macrophages during inflammation and the effect of Astaxanthin

### 3.2. MATERIALS AND METHODS

#### 3.2.1. Plant materials

The leaves of *Delonix elata* war collected from collected from Kolli hills, Namakkal District.
3.2.2. Preparation of crude extract

The plant material(s) was allowed to shadow dry and afterwards pulverized by using mortar and pestle. 10 grams pulverized material were dissolved in 100 ml of solvent (Methanol) and kept in a shaker for overnight. The obtained extracts were filtered with Whatmann No.4 filter paper and the filtrate was collected and used for acute study (Kokate, C.K. 1994).

3.2.3. Toxicity level of Crude extract of the plant and Astaxanthin

In an attempt to arrive at suitable doses for pharmacological studies, a pilot study was conducted by the method of Miller and Tainter. The LD50 value of the methanolic extract of Delonix elata was found to be 1.995 g/kg using probit analysis (Manimekalai K, et al., 2011). LD50 of astaxanthin is deduced to be >2000mg/kg in mice.

3.2.4. Animals and arthritis induction

Eight to ten weeks old male Swiss albino mice weighing 25 - 30g bred in the EASMA BIOTECHNOLOGY were used in this study. The animals were fed on the standard pellet diet (Agro Corporation Private Limited, Bangalore, India). Water was given ad libitum. The animals were housed in plastic cages under controlled condition of 14 h light / 10 h dark cycles, 50% humidity and at 23 ± 2°C. The animals used in the present study were maintained in accordance with the guidelines of institutional animal ethics committee (DIC (2008/33/014/00049/BEYA) TN/1695 (BSS)/2013).

Chick type II collagen was purchased from Sigma. In acute study (7 days), mice were injected at the base of the tail with 50 µg of collagen in a 100 µl emulsion of Complete Freunds Adjuvant (Sigma Chem. Co., St. Louis, USA) as described previously (Anderson et al., 2009).

3.2.5. Experimental design for dose fixation

Animals were randomized and divided into eleven experimental groups (n=6) as follows;

- Group I : Normal mice with i.v. injection of saline served as control.
- Group II : These mice received tail i.v injection of Chicken Type II Collagen of 50µg.
Group III: These mice were treated with 20mg/kg body weight of crude methanolic leaf extract of *Delonix elata* orally and i.v injection of Chicken Type II Collagen of 50µg.

Group VI: These mice were treated with 40mg/kg body weight of crude methanolic leaf extract of *Delonix elata* orally and i.v injection of Chicken Type II Collagen of 50µg.

Group V: These mice were treated with 60mg/kg body weight of crude methanolic leaf extract of *Delonix elata* orally and i.v injection of Chicken Type II Collagen of 50µg.

Group VI: These mice were treated with 80mg/kg body weight of crude methanolic leaf extract of *Delonix elata* orally and i.v injection of Chicken Type II Collagen of 50µg.

Group VII: These mice were treated with 2mg/kg body weight of astaxanthin orally and i.v injection of Chicken Type II Collagen of 50µg.

Group VIII: These mice were treated with 4mg/kg body weight of astaxanthin orally and i.v injection of Chicken Type II Collagen of 50µg.

Group IX: These mice were treated with 6mg/kg body weight of astaxanthin orally and i.v injection of Chicken Type II Collagen of 50µg.

Group X: These mice were treated with 8mg/kg body weight of astaxanthin orally and i.v injection of Chicken Type II Collagen of 50µg.

Group XI: These mice were treated with 15mg/kg body weight of Dichlofenac (Standard drug) orally and i.v. injection of Type II Collagen of 50µg.

### 3.2.6. Experimental design for acute study

In dose dependent study four different doses of crude methanolic leaf extract of *Delonix elata* (20, 40, 60 and 80 mg/kgbw). It was observed that after the experimental period of 7 days, methanolic crude extract of *Delonix elata* pretreatment at the doses of 20, 40, 60 and 80 mg/kgbw appreciable decrease in the hind paw oedema and near normal biochemical parameters. From the results it was observed
that 60 and 80 mg/kgbw of crude methanolic extract of Delonix elata administration showed similar results but 20 and 40 mg/kg are very less significant. As consequence, we have chosen the optimum dose 60 mg/kg for our acute and chronic study. In case of astaxanthin (Pure) 6 mg/kg concentration was chosen.

Animals were divided into five experimental groups (n=6) as follows;

<table>
<thead>
<tr>
<th>Group I</th>
<th>Normal mice with i.v. injection of saline served as control.</th>
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</thead>
<tbody>
<tr>
<td>Group II</td>
<td>These mice received tail i.v injection of Chicken Type II Collagen of 50µg on first day of 7 days treatment.</td>
</tr>
<tr>
<td>Group III</td>
<td>These mice were treated with 60mg/kg body weight of crude methanolic extract of Delonix elata orally and i.v injection of Chicken Type II Collagen of 50µg.</td>
</tr>
<tr>
<td>Group IV</td>
<td>These mice were treated with 6mg/kg body weight of Astaxanthin orally and i.v injection of Chicken Type II Collagen of 50µg for seven days.</td>
</tr>
<tr>
<td>Group V</td>
<td>These mice were treated with 15mg/kg body weight of Dichlofenac (Standard drug) orally and i.v injection of Chicken Type II Collagen of 50µg for seven days.</td>
</tr>
</tbody>
</table>

3.2.7. Mean Paw Volume

The collagen induced oedema and increase in paw thickness will be measured by Vernier caliper method (Brownlee, 1950).

3.2.8. Blood sampling and Hematological parameters

Blood was collected in heparinised tubes and centrifuged at 2000 × g for 10 min and hematological parameters using serum were analyzed on the same day.

3.2.9. Biochemical Assay

3.2.9.1. Estimation of Serum SGOT (IFCC, 1986)

SGOT catalyses the transfer of amino group from L-Aspartate to 2-Oxoglutarate with the formation of oxaloacetate and L-glutamate. The rate of this
reaction is monitored by an indicator reaction coupled with Malate dehydrogenase (MDH) in which the oxaloacetate formed is converted to malate ion in the presence of NADH (Nicotinamide Adenine Dinucleotide). The oxidation of NADH in this reaction is measured as a decrease in the absorbance of NADH at 340 nm, which is proportional to SGOT activity.

\[
\text{L- Aspartate + 2- Oxoglutarate} \xrightarrow{\text{AST}} \text{Oxaloacetate + L- glutamate} \\
\text{Oxaloacetate + NADH} \xrightarrow{\text{MDH}} \text{L- Malate + NAD} \\
\text{Sample + NADH} \xrightarrow{\text{LDH}} \text{L- Lactate + NAD}
\]

**Solutions**

<table>
<thead>
<tr>
<th>Volume (µl)</th>
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<tbody>
<tr>
<td>Working reagent</td>
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<tr>
<td>Sample</td>
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### 3.2.9.2. Estimation of Serum SGPT (Brodie, 1968)

SGPT catalyses the transfer of amino group from L-Alanine to 2-Oxoglutarate with the formation of pyruvate and L-glutamate. The pyruvate so formed is allowed to react with NADH to produce L-lactate. The rate of this reaction is monitored by an indicator reaction coupled with LDH in the presence of NADH (Nicotinamide Adenine Dinucleotide). The oxidation of NADH in this reaction is measured as a decrease in the absorbance of NADH at 340 nm, which is proportional to SGPT activity.

\[
\text{L- Alanine + 2- Oxoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate + L –Glutamate} \\
\text{Pyruvate + NADH} \xrightarrow{\text{LDH}} \text{L- Lactate + NAD}
\]

**Solutions**

<table>
<thead>
<tr>
<th>Volume (µl)</th>
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<tbody>
<tr>
<td>Working reagent</td>
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<tr>
<td>Sample</td>
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</table>

As mentioned above blank, standard and sample was prepared by considering 500 µl of working reagent and 50 µl each of distilled water, standard and sample respectively. Then all were incubated at 37°C and absorbance was noted at 340 nm.
3.2.9.3. Estimation of serum alkaline phosphatase (IFCC, 1986)

Serum alkaline phosphatase hydrolyses p-Nitrophenyl phosphate in the presence of oxidizing agent Mg\(^{2+}\). This reaction is measured as absorbance is proportional to the ALP activity.

\[
P\text{-Nitrophenyl phosphatase} + \text{H}_2\text{O} \xrightarrow{\text{ALP}, \text{Mg}^{2+}} \text{p-nitrophenol}
\]

<table>
<thead>
<tr>
<th>Solutions</th>
<th>Volume (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working reagent</td>
<td>500</td>
</tr>
<tr>
<td>Sample</td>
<td>10</td>
</tr>
</tbody>
</table>

As mentioned above table blank, standard and sample was prepared by considering 500 µl of working reagent and 10 µl each of distilled water, standard, sample respectively, later all the samples were incubated at 37°C and absorbance was recorded at 405 nm.

3.2.9.4. Evaluation of lipid peroxidation

The extent of lipid peroxidation in the samples was estimated by thiobarbituric acid reactive substances.

3.2.9.4.1. Thiobarbituric acid reactive substances (TBARS)

TBARS were estimated by the method of Niehaus and Samuelsson (Niehaus and Samuelsson, 1968).

Principle

In this method, malondialdehyde was measured by their reaction with thiobarbituric acid in an acidic condition to generate a pink colored chromophore which was read at 535 nm.

Reagents

1. Tris-HCl buffer [pH 7.5; 0.025]
2. TBA-TCA-HCl reagent (1:1:1 V/V)
3. Trichloroacetic acid (TCA) 15% W/V
4. 0.375% Thiobarbituric acid in 0.25 N HCl (W/V)
5. Standard (1, 1’, 3, 3’-tetramethoxy propane)

0.16 mL of 3 M standard tetramethoxy propane solution was made upto 100 mL with double distilled water. 1.0 mL of this was taken and made upto 100 mL with double distilled water, which served as working standard.

**Procedure**

To 0.5 mL of the supernatant/ tissue homogenate, 0.5 mL of double distilled water was added and then 2.0 mL of TBA-TCA-HCl reagent was added and mixed well. The mixture was kept in a boiling water bath for 15 min. After cooling, the tubes were centrifuged at 1000x g for 10 min and the supernatant was estimated. A series of standard solutions in the concentration of 2-10 nmol were treated in a similar manner. The absorbance of the chromophore was read at 535 nm against reagent blank. The values were expressed as mg/ dL.

**3.2.9.5. Estimation of Total Proteins**

Protein in the enzyme extract was determined after trichloroacetic acid precipitation by the method of Lowry *et al.*, (1951).

**Principle**

Protein reacts with the folin phenol reagent to give a color complex. The color so formed is due to the reaction of the alkaline copper with the protein and the reduction of phosphomolybdate by tyrosine and tryptophan present in the protein. The intensity of the color developed is proportional to the concentration of protein in the sample.

**Reagents**

1. Alkaline copper reagent:
   - Reagent A: 2% sodium carbonate in 0.1 N NaOH
   - Reagent B: 0.5 % copper sulphate in 1% sodium potassium tartarate.
   - Reagent C: 50 mL of Reagent A was mixed with 0.5 mL of Reagent B just before use.
2. Folin’s phenol reagent - Dilute 1:2 with distilled water.
3. Stock standard -100 mg of bovine serum albumin/ 100 mL of water.
4. Working standard -10 mL of the stock standard was diluted to 100 mL to get a working standard containing 0.1 mg/ 100 mL.
Procedure

0.5 mL of tissue homogenate/ supernatant was mixed with 0.5 mL of 10% TCA and centrifuged for 10 min. The precipitate was dissolved in 1.0 mL of 0.1 N NaOH. A aliquot was taken, and 4.5 mL of alkaline copper reagent added and allowed to stand at room temperature for 10 min. 0.5 mL of Folin’s phenol reagent was added and the blue color developed was read after 20 min at 640 nm. A standard curve was obtained with standard bovine albumin.

3.2.9.6. Estimation of hexosamine

To 1 ml of tissue homogenate, 9ml of 90% alcohol was added. The mixture was kept for 10 minutes before it was centrifuged. The supernatant was discarded. The precipitate was dissolved in 0.5ml of 0.1N NaOH. To this 1.8ml of 6N HCl was added. The mixture was hydrolyzed in water bath at 100°C for 2 hours. The hydrolysate was neutralized by 5N NaOH using phenolphthalein as indicator and the volume was made upto 4.5ml with distilled water and using for the estimation of hexosamine as described below. 0.5ml of the hydrolysate fraction was taken. To this 0.5ml of acetylaceton reagent was added. The mixture was heated in boiling water bath at 60°C for 20 minutes, and then cooled under running tap water. 1.5ml of 90% alcohol was added and allowed for 30 minutes. The colour intensity was measured in Systronics UV-VIS spectrophotometer- 180 at 540nm against blank prepared by using distilled water instead of hydrolysate. Hexosamine content was determined from the standard curve prepared by using D(+)-glucosamine hydrochloride and concentration has been expressed in ìg/ml of protein.

3.2.9.7. Estimation of hydroxyproline

The paw oedema tissue were homogenized in saline and hydrolyzed with 2N NaOH for 30 minutes at 120°C and then we determined hydroxyproline content with Chloramine T and Ehrlich's reagent with a hydroxyproline standard curve measuring at 550 nm. Values were expressed as micrograms of hydroxyproline per milligrams of protein (Reddy and Enwemeka, 1996).

3.2.10. Hematological assay

3.2.10.1. Enumeration of Leucocytes (WBC COUNT)

Leucocytes were enumerated according to the procedures of Lehmann (1998)
Principle

Glacial acetic acid in the WBC diluting fluid lyses the RBC cells in the blood while the gentian violet slightly stains the nuclei of the leucocytes. The blood specimen is diluted 1:200 in a WBC pipette with the diluting fluid and the cells are counted under in a microscope by using neubauer chamber. The number of cells in undiluted blood is reported per cubic mm of whole blood.

Reagents

1. WBC diluting fluid
   Glacial acetic acid : 1.5mL
   1% Gentian violet : 1.0mL
   Distilled water : 98 mL

Procedure

The anticoagulated blood was taken into WBC pipette carefully without any air bubble. Immediately the WBC diluting fluid, was drawn. Mix both the blood and diluting fluid in the pipette carefully and after five minutes the diluted blood was carefully layered on the edge of Neubeur chamber. The cells were allowed to settle for 2-3min and counted.

Calculation

The area of each large square = 1 sq. mm
The volume of square is = 0.1 mm$^3$
Volume of four corner squares = 0.1 x 4= 0.4
Number of cells in four corner squares = N
0.4 mm$^3$ contain = N cells
1 mm$^3$ contains = N x 20/0.4 (dilution factor)
= N x 50
Normal count = 5000 to 10,000 cells/ mm$^3$ of the blood

3.2.10.2. Differential count of WBC

To determine the relative proportion of WBC
Reagents

1. Sterilized needle
2. Clean microscope slide
3. Leish man’s stain
4. Distilled water.

Procedure

The finger was pricked with sterile needle; the first drop was wiped off. The second drop was taken at the end of the thin slide. The slide was placed on a smooth surface and it was held steadily in the left hand. The second slide was placed at 45° in front of the blood drop this causes the blood to run away along the spaces. The second slide was pushed slightly along the first slide without exerting any pressure. A thin film of blood was made on the first slide and it was dried in air. Few drops of Leishman’s stain was added to the above blood film and kept for few minutes, cover with petridish to prevent drying. Few drops of distilled water were added and the slide was rocked for few minutes with the stain do not run the stain or water over the edges. Don’t block the slide it was left for 10 minutes and the stain was drained off with water. The slide was examined under high power microscope. In the longitudinal strip of the film various kinds of WBC was counted from top to the bottom of the slide. 100 to 200 WBC were counted and each type WBC was noted on the paper and their percentage was calculated.

The percentage of the WBC present in the given blood smear contains

1. Basophils-----
2. Neutrophils----
3. Eosinophils-------
4. Lymphocytes-----
5. Monocytes ---------

3.2.10.3. Determination of ESR

To determine the erythrocytes sedimentation rate of the given blood sample using westergen method.
Principle

When the anticoagulated blood is allowed to stand undisturbed, the RBC gradually settles down at the bottom of the tube containing a clear layer of the plasma. Red cells settle down, because of their higher specific gravity. The rate at which red cells settle down is known as ESR. It is expressed in millimeter at the end of one hour.

Apparatus

1. Westergren tube

It is a glass tube of uniform bore (2mm) open at both the ends. Its length is 300mm. From its lower end it is graduated in millimeters from 0 to 200.

2. Westergen stand or rack

This accommodates six westergren tubes. At the base there is a rubber cushion on which the lower end of the westergren’s tube rests. At the upper end there is a screw cap, which fits upon the upper end of westergren tube. It is screwed down to exert sufficient amount of pressure on the tube so as to prevent leakage of blood. When fitted in the stand, westergren tube remains in vertical position.

3. Anticoagulant & blood

0.4 ml of 3.8% sodium citrate is added to 1.6 ml of venous blood & mixed well.

Reagents

Sodium citrate 3.8%. This is used as an anticoagulant.

Procedure

1. With usual method of venepuncture, venous blood is collected. It is placed in a tube containing 3.8% sodium citrate. It is mixed properly.
2. Blood is sucked slowly in westergren’s tube up to 0 mark. Outer surface of the tip is wiped off.
3. Keeping the finger on the upper end, lower end of the tube is pressed on the rubber cushion of the stand, taking care that there is no leakage of blood from the lower end.
4. Finger from the upper end of the tube is released and tube is fixed with screw cap. Tube is fitted exactly vertical.
5. Vertically fitted tube in the rack is kept undisturbed for one hour.
6. At the end of one hour, height of the clear plasma (in mm) is read. This indicates ESR.

3.2.10.4. Enumeration of Erythrocytes (RBC COUNT)

Erythrocytes were enumerated according to the procedures of Lehmann (1998)

**Principle**

The number of RBC in a known volume of diluted blood is counted via hemocytometer and the number of cells in one cubic mm of undiluted blood is calculated. Sodium chloride and sodium sulphate together keeps the isotonicity of fluid. Sodium sulphate also prevents clumping of red cells. Mercuric perchloride fixes the cells and acts as a preservative.

**Reagents**

1. RBC diluting fluid (Hayem’s fluid)

   Sodium chloride 0.5 g, Sodium sulphate 2.5 g, and Mercuric perchloride 0.25 g were weighed and dissolved in 100mL of distilled water.

**Procedure**

The heparinised blood was carefully pipetted into RBC pipette till 0.5 mark without air bubble and immediately RBC diluting fluid was taken. The blood and diluting fluid was mixed thoroughly by gently rolling the pipette horizontally. The diluted blood was carefully layered on a Neubauer chamber and the diluted blood was spread over the chamber by placing a cover slip. The cells were allowed to settle for 2-3 minutes and counted using light microscope.

**Calculation**

\[
\text{Total area of the whole large central square} = 1 \text{ sq. mm} \\
\text{The smallest square length} = 1/20 \text{ mm} \\
\text{Width of the smallest square} = 1/20 \text{mm} \\
\text{Therefore the volume of the small square is} = 1/400 \times 1/10 = 1/4000 \text{mm}^3 \\
\text{The dilution of the blood is} = 1/200
\]
Total RBCs  = \frac{Number \ of \ cells \ counted \times \ dilution \ factor \times \ Depth \ factor}{Area \ counted}

Number of cells counted = N

Dilution Factor = 1/200

Depth factor = 1/10

\[
\text{RBC/cubic mm} = \frac{N \times 80/400 \times 200 \times 10}{1/5}
\]

\[
= \frac{N \times 2000 \times 5}{1} = 10000 \times N
\]

3.2.10.5. Estimation of haemoglobin

Principle

When the blood is mixed with the drabkin’s reagent containing potassium cyanide and potassium ferricyanide hb reacts with the ferricyanide to form methHb which is converted into stable cyanomethHb(HicN)by the cyanide. the intensity of the colour is proportional to Hb concentration and is compared with the a known cyanomethHb standard at 540nm (reen filter)

Requirements

1. Drabkin’s reagent

   **It contains:**

   1. Distilled water:1000ml
   2. Potassium ferricyanide:400mg
   3. Potassium dihydrogen phosphate:280mg
   4. Potassium cyanide:100mg
   5. Nonidet:1ml

2. Cyanomethemoglobin (HicN) standard .its OD is measured at 540nm. The reading is obtained corresponds to 15g/dl,Hb.

3. Hb pipette (20 ml calibrated)

4. Test tubes.

5. Photophometer or spectrophometer.
Procedure

Mix the contents in the tube labelled as ‘Test’ thoroughly and wait for 5 minutes.

Read the absorbance of test by setting blank to 100% T at 540nm.

Read the absorbance of standard by pipetting it directly in a cuvette.

\[
\text{Calculation Hb (g/L) } = \frac{\text{OD of Test} \times 15}{\text{OD of Std.}}
\]

Statistical Analysis

All the results were expressed as mean ± S.E. The data were statistically analyzed by one-way analysis of variance (ANOVA) and P values <0.05 were considered as significant.

3.3. RESULTS AND DISCUSSION

3.3.1. Dose Fixation

3.3.1.1. Mean paw volume

Table 12: Mean paw volume

<table>
<thead>
<tr>
<th>S.No</th>
<th>Groups</th>
<th>Paw Circumference in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GROUP I – Control</td>
<td>39.61±3.02</td>
</tr>
<tr>
<td>2</td>
<td>GROUP II – Collagen (50µg)</td>
<td>59.22±4.51</td>
</tr>
<tr>
<td>3</td>
<td>GROUP III – Collagen (50µg) + Crude D.elata (20mg)</td>
<td>52.08±3.97</td>
</tr>
<tr>
<td>4</td>
<td>GROUP IV – Collagen (50µg) + Crude D.elata (40mg)</td>
<td>47.22±3.60</td>
</tr>
<tr>
<td>5</td>
<td>GROUP V – Collagen (50µg) + Crude D.elata (60mg)</td>
<td>43.03±3.28</td>
</tr>
<tr>
<td>6</td>
<td>GROUP VI – Collagen (50µg) + Crude D.elata (80mg)</td>
<td>42.91±3.27</td>
</tr>
<tr>
<td>7</td>
<td>GROUP VII – Collagen (50µg) + Astaxanthin (2mg)</td>
<td>51.08±3.89</td>
</tr>
<tr>
<td>8</td>
<td>GROUP VIII – Collagen (50µg) + Astaxanthin (4mg)</td>
<td>48.22±3.67</td>
</tr>
<tr>
<td>9</td>
<td>GROUP IX – Collagen (50µg) + Astaxanthin (6mg)</td>
<td>44.03±3.35</td>
</tr>
<tr>
<td>10</td>
<td>GROUP X – Collagen (50µg) + Astaxanthin (8mg)</td>
<td>43.81±3.15</td>
</tr>
<tr>
<td>11</td>
<td>GROUP X1 – Collagen (50µg) + Diclofenac (15mg)</td>
<td>38.89±2.96</td>
</tr>
</tbody>
</table>
3.3.1.2. Assay of Serum (Lysosomal) Enzymes in Experimental Animals

Table 13: Assay of Serum (Lysosomal) Enzymes in Experimental Animals

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Groups</th>
<th>SGOT U/ml</th>
<th>SGPT U/ml</th>
<th>ALP U/ml</th>
<th>Lipid peroxidation Moles/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GROUP I – Control</td>
<td>54.24±4.13</td>
<td>20.11±1.53</td>
<td>64.61±4.92</td>
<td>59.61±4.54</td>
</tr>
<tr>
<td>2</td>
<td>GROUP II – Collagen (50µg)</td>
<td>99.06±7.54</td>
<td>62.05±4.72</td>
<td>109.13±8.31</td>
<td>121.23±9.23</td>
</tr>
<tr>
<td>3</td>
<td>GROUP III – Collagen (50µg) + Crude <em>D.elata</em> (20mg)</td>
<td>94.14±7.17</td>
<td>55.08±4.19</td>
<td>94.09±7.16</td>
<td>92.09±7.01</td>
</tr>
<tr>
<td>4</td>
<td>GROUP IV – Collagen (50µg) + Crude <em>D.elata</em> (40mg)</td>
<td>90.13±6.86</td>
<td>47.02±3.58</td>
<td>88.08±6.71</td>
<td>87.02±6.63</td>
</tr>
<tr>
<td>5</td>
<td>GROUP V – Collagen (50µg) + Crude <em>D.elata</em> (60mg)</td>
<td>85.13±6.48</td>
<td>34.03±2.59</td>
<td>75.03±5.71</td>
<td>72.03±5.48</td>
</tr>
<tr>
<td>6</td>
<td>GROUP VI – Collagen (50µg) + Crude <em>D.elata</em> (80mg)</td>
<td>84.60±6.44</td>
<td>33.90±2.50</td>
<td>74.72±5.54</td>
<td>72.94±5.33</td>
</tr>
<tr>
<td>7</td>
<td>GROUP VII – Collagen (50µg) + Astaxanthin (2 mg)</td>
<td>81.13±6.18</td>
<td>48.08±3.66</td>
<td>88.08±6.71</td>
<td>87.08±6.63</td>
</tr>
<tr>
<td>8</td>
<td>GROUP VIII – Collagen (50µg) + Astaxanthin (4 mg)</td>
<td>78.12±5.95</td>
<td>33.02±2.51</td>
<td>71.22±5.42</td>
<td>70.02±5.33</td>
</tr>
<tr>
<td>9</td>
<td>GROUP IX – Collagen (50µg) + Astaxanthin (6 mg)</td>
<td>58.13±4.43</td>
<td>25.02±1.91</td>
<td>69.03±5.26</td>
<td>64.03±4.88</td>
</tr>
<tr>
<td>10</td>
<td>GROUP X – Collagen (50µg) + Astaxanthin (8 mg)</td>
<td>57.60±4.39</td>
<td>25.70±1.88</td>
<td>68.72±5.23</td>
<td>63.41±4.83</td>
</tr>
<tr>
<td>11</td>
<td>GROUP XI – Collagen (50µg) + Diclofenac (15mg)</td>
<td>56.08±4.27</td>
<td>23.88±1.82</td>
<td>65.06±4.95</td>
<td>61.19±4.66</td>
</tr>
</tbody>
</table>

In dose dependent study four different doses of crude leaf extract of _Delonix elata_ (20, 40, 60 and 80 mg/kgbw) were given. It was observed that after the experimental period of 7 days, Crude extract pretreatment at the doses of 20, 40, 60 and 80 mg/kgbw appreciable decrease in the hind paw oedema and near normal biochemical parameters.

From the results it was observed that 60 and 80 mg/kgbw of crude extract of _Delonix elata_ administration showed similar results but 20 and 40 mg/kg are very less significant. As consequence, we have chosen the optimum dose 60 mg/kg for our acute study. In case of astaxanthin (Pure) 6 mg/kg concentration was chosen.
3.3.2. Acute Study

3.3.2.1. Mean paw volume

Table 14: Mean paw volume

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Groups</th>
<th>Paw Circumference in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GROUP I – Control</td>
<td>39.61±3.02</td>
</tr>
<tr>
<td>2</td>
<td>GROUP II – Collagen (50µg)</td>
<td>59.22±4.51</td>
</tr>
<tr>
<td>3</td>
<td>GROUP III – Collagen (50µg) + Crude <em>D.elata</em> (60mg)</td>
<td>51.03±4.01</td>
</tr>
<tr>
<td>4</td>
<td>GROUP IV – Collagen (50µg) + Astaxanthin (6mg)</td>
<td>44.03±3.35</td>
</tr>
<tr>
<td>5</td>
<td>GROUP V – Collagen (50µg) + Diclofenac (15mg)</td>
<td>38.89±2.96</td>
</tr>
</tbody>
</table>

Figure 13: Mean paw volume

3.3.2.2. Biochemical parameters

Assay of Serum (Lysosomal) Enzymes in Experimental Animals

Increase in the levels of lysosomal enzymes is a notable change in acute and chronic inflammation. There are several evidences are there implicating that the levels of SGOT, SGPT and ALP increased in acute and chronic inflammation. In general anti inflammatory drugs acts by either stabilizing the lysosomal membrane or by inhibiting the release of lysosomal enzyme. In the experimental groups collagen
treated mice have shown increased levels of lysosomal enzyme (SGOT, SGPT and ALP) in serum, compared to the normal group. Crude methanolic extract of Delonix elata treated mice (60 mg/bw) exhibits reduced levels in lysosomal enzymes compared to collagen treated mice group which clearly elucidates the anti-inflammatory effect of Delonix Elata. The restoration levels of SGPT and ALP are better than SGOT.

**Table 15 : Assay of Serum (Lysosomal) Enzymes in Experimental Animals**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Groups</th>
<th>SGOT U/ml</th>
<th>SGPT U/ml</th>
<th>ALP U/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GROUP I – Control</td>
<td>54.24±4.13</td>
<td>20.11±1.53</td>
<td>64.61±4.92</td>
</tr>
<tr>
<td>2</td>
<td>GROUP II – Collagen (50µg)</td>
<td>99.06±7.54</td>
<td>62.05±4.72</td>
<td>109.13±8.31</td>
</tr>
<tr>
<td>3</td>
<td>GROUP III – Collagen (50µg) + Crude D.elata (60mg)</td>
<td>85.13±6.48</td>
<td>34.03±2.59</td>
<td>75.03±5.71</td>
</tr>
<tr>
<td>4</td>
<td>GROUP IV – Collagen (50µg) + Astaxanthin (6mg)</td>
<td>58.13±4.43</td>
<td>25.02±1.91</td>
<td>69.03±5.26</td>
</tr>
<tr>
<td>5</td>
<td>GROUP V – Collagen (50µg) + Diclofenac (15mg)</td>
<td>56.08±4.27</td>
<td>23.88±1.82</td>
<td>65.06±4.95</td>
</tr>
</tbody>
</table>

**Figure 14 : Assay of Serum (Lysosomal) Enzymes in Experimental Animals**
3.3.2.3. Evaluation of Lipid Peroxidation in Experimental animals

Lipid peroxidation is believed to contribute to the development of diseases such as inflammation, atherosclerosis, aging and cancers. Peroxidation of unsaturated fatty acids destroy structure of cell membrane leading to functional disorders of receptors and proteins within cell membrane. Lipid Peroxidation is a free radical mediated process and acts as a potential marker of susceptibility of tissue damage. Due to oxidative stress during inflammation in rheumatoid arthritis, collagen induced mice group shown a notable increase in TBARS compared to control and pretreated (*D.elata* treated) mice. It clearly shows that crude methanolic extract of *Delonix elata* (60mg/bw) inhibits the lipid peroxidation triggers as a result of oxidative stress in inflammation.

Table 16 : Evaluation of Lipid Peroxidation in Experimental animals

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Groups</th>
<th>Lipid peroxidation moles/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GROUP I – Control</td>
<td>59.61±4.54</td>
</tr>
<tr>
<td>2</td>
<td>GROUP II – Collagen (50µg)</td>
<td>121.23±9.23</td>
</tr>
<tr>
<td>3</td>
<td>GROUP III – Collagen (50µg) + <em>D.elata</em> Crude (60mg)</td>
<td>72.03±5.48</td>
</tr>
<tr>
<td>4</td>
<td>GROUP IV – Collagen (50µg) + Astaxanthin (6mg)</td>
<td>64.03±4.88</td>
</tr>
<tr>
<td>5</td>
<td>GROUP V – Collagen (50µg) + Diclofenac (15mg)</td>
<td>61.19±4.66</td>
</tr>
</tbody>
</table>

Figure 15 : Evaluation of Lipid Peroxidation in Experimental animals
3.3.2.4. Estimation of Proteins

Table 17: Levels of Total protein in experimental animals

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Groups</th>
<th>Total Protein gm%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GROUP I – Control</td>
<td>8.60±0.65</td>
</tr>
<tr>
<td>2</td>
<td>GROUP II – Collagen (50µg)</td>
<td>4.50±0.34</td>
</tr>
<tr>
<td>3</td>
<td>GROUP III – Collagen (50µg) + Crude <em>D.elata</em>(60mg)</td>
<td>5.0±0.38</td>
</tr>
<tr>
<td>4</td>
<td>GROUP IV – Collagen (50µg) + Astaxanthin (6mg)</td>
<td>5.21±0.40</td>
</tr>
<tr>
<td>5</td>
<td>GROUP V – Collagen (50µg) + Diclofenac (15mg)</td>
<td>5.83±0.44</td>
</tr>
</tbody>
</table>

Collagen induction causes the significant decrease in the proteins in arthritic animals. Pretreatment with the methanolic crude extract of *Delonix elata* (60 mg/bw) increase the levels of protein to normal in pretreated animals.

3.3.2.5. Estimation of Hydroxy proline and Hexosamine

Alterations in connective tissue metabolism is one the important process in inflammation, this lead to bring out changes in the relative composition of various constituents of connective tissues like mucopolysaccharides, hexosamine,
glycoprotein and sialic acid. Collagen induced oedematous tissue homogenates showed increased levels of hexosamine and hydroxyproline compared to normal and treated groups, which indicates the protective effect of methanolic crude extract from *Delonix elata* (60 mg/bw) against inflammation.

**Table 18 : Levels of Hydroxy proline and Hexosamine in experimental animals**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Groups</th>
<th>Hydroxy proline µg/gm</th>
<th>Hexosamine µg/gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GROUP I – Control</td>
<td>185.03±14.09</td>
<td>680.34±51.81</td>
</tr>
<tr>
<td>2</td>
<td>GROUP II – Collagen (50µg)</td>
<td>370.06±28.18</td>
<td>1280.55±97.51</td>
</tr>
<tr>
<td>3</td>
<td>GROUP III – Collagen (50µg) + Crude <em>D.elata</em> (60mg)</td>
<td>210.04±15.99</td>
<td>728.12±55.44</td>
</tr>
<tr>
<td>4</td>
<td>GROUP IV – Collagen (50µg) + Astaxanthin (6mg)</td>
<td>212.04±16.15</td>
<td>715.12±54.45</td>
</tr>
<tr>
<td>5</td>
<td>GROUP V – Collagen (50µg) + Diclofenac (15mg)</td>
<td>203.16±15.47</td>
<td>730.66±55.64</td>
</tr>
</tbody>
</table>

**Figure 17 : Levels of Hydroxy proline and Hexosamine**
3.3.2.6. Hematological Parameters

3.3.2.6.1. Enumeration of Leucocytes

Table 19: Enumeration of Leucocytes

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Groups</th>
<th>Lymp %</th>
<th>Neut%</th>
<th>ESR Mm after 1hr</th>
<th>T.WBC Cu.mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GROUP I – Control</td>
<td>38.90±2.96</td>
<td>56.11±4.27</td>
<td>1.89±0.14</td>
<td>7102.07±540.80</td>
</tr>
<tr>
<td>2</td>
<td>GROUP II – Collagen (50µg)</td>
<td>61.13±4.65</td>
<td>65.20±4.96</td>
<td>7.12±0.54</td>
<td>10008.74±762.13</td>
</tr>
<tr>
<td>3</td>
<td>GROUP III – Collagen (50µg) + <em>D.elata</em> Crude (60mg)</td>
<td>44.60±3.40</td>
<td>64.56±4.80</td>
<td>2.91±0.22</td>
<td>8198.96±624.32</td>
</tr>
<tr>
<td>4</td>
<td>GROUP IV – Collagen (50µg) + Astaxanthin (6mg)</td>
<td>41.60±3.17</td>
<td>61.60±4.69</td>
<td>2.59±0.20</td>
<td>8101.94±616.93</td>
</tr>
<tr>
<td>5</td>
<td>GROUP V – Collagen (50µg) + Diclofenac (15mg)</td>
<td>42.08±3.20</td>
<td>61.08±4.65</td>
<td>2.07±0.16</td>
<td>8301.45±632.12</td>
</tr>
</tbody>
</table>

Figure 18: Enumeration of Lymphocytes and Neutrophils

![Figure 18](image-url)
The increase in WBC count during inflammation may be due to the release of interleukins, responsible for the production of both granulocytes and macrophage colony stimulating factor (Eric and Lawrence, 1996). Hence in the present study the level of WBC was found to be higher in collagen induced inflammation. Pre treatment with the methanolic crude extract of *Delonix elata* (60 mg/bw) significantly decrease the WBC count that indicate the significant recovery from the inflammatory process.
Especially the restoration of lymphocytes level (which play significant role in inflammation) in the pretreated groups is well comparable with standard treated (Diclofenac 15 mg/bw) group.

3.3.2.6.2. Estimation of Hemoglobin and enumeration of Total RBCs in Experimental Animals

Table 20: Levels of Hemoglobin and Total RBCs in experimental animals

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Groups</th>
<th>Hb%</th>
<th>RBC Million/cu.cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GROUP I – Control</td>
<td>88.90±6.77</td>
<td>5.0±0.38</td>
</tr>
<tr>
<td>2</td>
<td>GROUP II – Collagen (50µg)</td>
<td>71.13±5.42</td>
<td>3.00±0.23</td>
</tr>
<tr>
<td>3</td>
<td>GROUP III – Collagen (50µg) + D.elata Crude (60mg)</td>
<td>79.60±6.06</td>
<td>4.30±0.33</td>
</tr>
<tr>
<td>4</td>
<td>GROUP IV – Collagen (50µg) + Astaxanthin (6mg)</td>
<td>81.60±6.21</td>
<td>4.70±0.36</td>
</tr>
<tr>
<td>5</td>
<td>GROUP V – Collagen (50µg) + Diclofenac (15mg)</td>
<td>82.08±6.25</td>
<td>4.20±0.32</td>
</tr>
</tbody>
</table>

Figure 19: Levels of Hemoglobin and Total RBCs in experimental animals

Collagen induction causes the significant decrease in the RBC and Hb which leads to anaemia. Pretreatment with the methanolic crude extract of Delonix elata (60 mg/bw) altered these levels to normal.
3.4. CONCLUSION

Administration of collagen in mice resulted in a significant increase in the levels of serum enzymes, LPO, total leucocytes, hydroxy proline, hexosamine and also in the length of the paw thickness while, the increase in the levels of above parameters were inhibited by the pretreatment of crude methanolic leaf extract of *Delonix elata* (60 mg/bw). Collagen induced experimental animals showed reduction in the levels of total protein, RBC and Hb, whereas the animals pretreated with *Delonix elata* leaf extract at dose level of 60 mg/kgbw showed enhanced levels of total protein RBC and Hb.

In conclusion, the present experimental findings of hematological and biochemical parameters suggest that crude methanolic leaf extract of *Delonix elata* possessing a promising anti-inflammatory agent in the treatment of inflammation.