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# Original Article

# Anti-inflammatory activity of Dimethyl cardamonin on formalin induced paw edema in albino rats

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ABSTRACT

Inflammation is a reaction of a living vascularised tissue to an injury. Conventional or synthetic drugs used in the treatment of inflammatory diseases are inadequate, it sometimes have serious side effects. **Aim:** The present study is aimed to evaluate the anti inflammatory activity of DMC on formalin induced paw edema in rats as for controlling inflammatory disorders. **Methods:** For anti inflammatory activity, albino rats were used and divided into 5 groups and treated accordingly: Normal control, Formalin induced group, Formalin+ DMC (100mg/kg bw), Normal+ DMC (100mg/kg bw) and Formalin+ Indomethacin (10mg/kg bw).After the experimental period of 7 days, the blood and tissue samples were collected and biochemical parameter and hematological studies were carried out. **Result:** Oral administration of formalin to the experimental animals produced reduction in the levels of SOD,GSH, GPX ,GR,GST,CAT, serum protein, total RBC and Hb. The animals treated with DMC extract at dose levels of 100mg /kg bw were significantly increased the levels of the above parameters. A significant increase in the length of the paw thickness, in the level of serum enzymes (SGOT, SGPT, ALP, CK) glucose and Lipid peroxide (LPO) was noted in the rats induced with formalin, while these levels were normalized by treatment with DMC **conclusion:** From the present observation, it is evidenced that Dimethyl cardamonin would be an effective drug for the treatment of inflammatory reactions.



## Introduction

Plants, which have one or more of its parts having substances that can be used for treatment of diseases, are called medicinal plants [1]. Medicines derived from plants are widely famous due to their safety, easy availability and low cost [2]. Herbal medicines may include whole parts of plant or mostly prepared from leaves, roots, bark, seed and flowers of plants. They are administered orally, inhaled or directly applied in the skin [3]. Medicinal herbs are more significant to the health of individual and community. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body [4]. Some of the most important bioactive phytochemical constituents are alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds and many more [5]. These natural compounds formed the foundations of modern prescription drugs as we know today [6]. The knowledge of medicinal plants was gradually developed and passed on from one individual to other, which foundation for traditional medicine throughout the world. India is one of the Nations with the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants [7].

**Dimethyl cardamonin (2,4 Dihydroxy 6 Methoxy 3,5 Dimethyl Chalcone)**

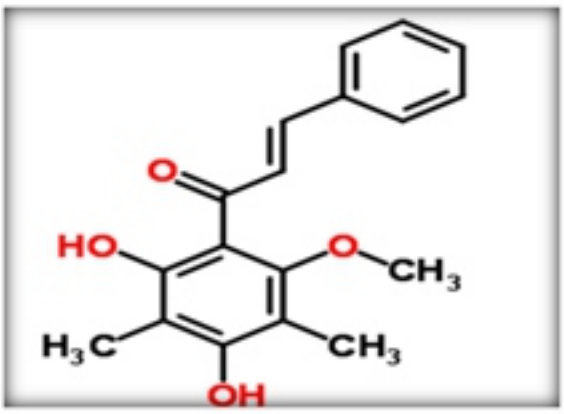


**Fig 1: Dimethyl Chalcone**

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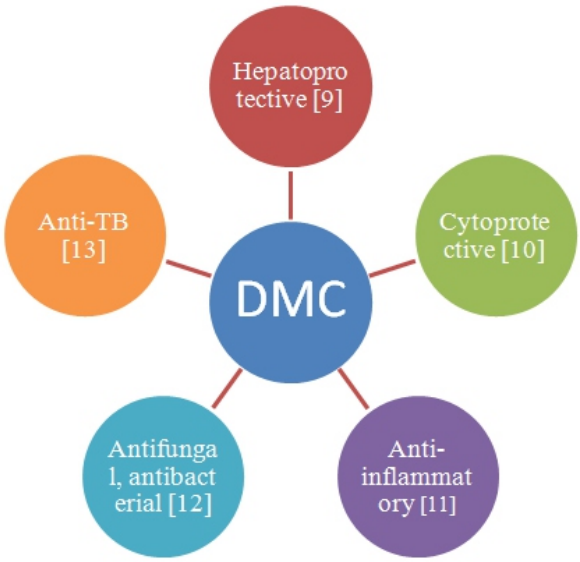
| | |
|---|---|
| Molecular formula | : $C_{18}H_{18}O_4$ |
| Average Mass | : 298.3310 |
| Monoisotopic Mass | : 298.121 |

A member of the class of chalcones that is *trans*-chalcone substituted by hydroxy groups at positions 2' and 4', a methoxy group at position 6' and methyl groups at positions 3' and 5'. Chalcones are precursor compounds for flavonoids biosynthesis in plants, and they can also be synthesized in laboratory. Changes in their structure have offered a high degree of diversity that has proven useful for the development of new medicinal agents having improved potency and lesser toxicity and good pharmacological actions. Chalcones became an object of continued interest in both academia and industry. Nowadays, several chalcones are used for treatment of viral disorders, cardiovascular diseases, parasitic infections, pain, gastritis and stomach cancer, as well as like food additives and cosmetic formulation ingredient [8].

**Biological activity of DMC**




**Inflammation**

Inflammatory diseases remain one of the world's major health problems [14]. More recently, inflammation was described as "the succession of changes which occurs in a living tissue when it is injured provided that the injury is not of such a degree as to at once destroy its structure and vitality" or "the reaction to injury of the living microcirculation and related tissues" [15].Inflammation is an important physiological reaction which occurs in response to a wide variety of injurious agents (e.g., bacterial infection, physical trauma, chemicals or any other phenomenon) ultimately aiming to perform the dual function of limiting damage and promoting tissue repair [16]. Inflammatory processes are required for immune surveillance, optimal repair and regeneration after injury [17]. Inflammation is a host defence mechanism of the body and it's an essential immune response that enables the body to survival during infection or injury and maintains tissue homeostasis in noxious conditions [18]. In other words "Inflammation is the major and complex reaction of the body against infection upon tissue injury." The role of inflammation as a healing, restorative process, as well as its aggressive role, is also more widely recognized today. But in some conditions appears to be no resolution and a chronic state of inflammation develops that may last the life of the individual. Such conditions include the inflammatory disorders rheumatoid arthritis, osteoarthritis, inflammatory bowel diseases, retinitis, multiple sclerosis, psoriasis and atherosclerosis [19].

**MATERIALS AND METHODS**

DMC was purchased from Loba Chemie, Mumbai, India. All the other chemicals used were of analytical grade and purchased from commercial sources.

**Experimental Animals**

Healthy adult wistar strain of albino rats of both sexes, two to three months old and weighing 150g-200g were obtained from Sivasubramanian Nadar College, kelambakkam, Chennai. The animals were allowed to acclimatize under laboratory conditions for a period of 5 days prior to the experiment. Animals were housed in standard polypropylene cages. Six animals were housed per cage, so as to provide them with sufficient space and to avoid unnecessary morbidity and mortality. Animals were maintained under standard condition of 12: 12- hour's light/ dark cycle and at an ambient temperature at 23 ± 2°C, with 65 ± 5 % humidity. Animals were fed with standard rat chow pellet obtained from Sai Durga Foods and Feeds, Bangalore, India and water *ad libitum*. All the studies were conducted according to the ethical guidelines of IAEC after obtaining necessary clearance from the committee (Approval No: 06/013/2013/ IAEC).

**Experimental Design**

The rats were divided into five groups containing six rats in each group. In both the control and test groups, chronic inflammation was produced by injecting 0.1 ml of 2 % formalin in to the left hind paw of rat[20].The DMC was administrated to the rats 1hr before formalin injection. Different groups were treated as follows:

Group I : Normal control rats received saline (0.5ml/kg)

Group II : Disease control rats

Group III : Formalin + DMC (100mg/kg b.wt)

Group III : DMC only (100mg/kg b.wt)

Group IV : Formalin + Indomethacin (10mg/kg b.wt)

The paw thickness was measured using vernier calipers before and 7 days after formalin injection. Increase in paw thickness was calculated using the formula Pt – Po, where Pt is the thickness of paw at time 't' (i.e. 7 days after formalin injection) and Po is the paw thickness at '0' time. Percentage edema was calculated using the formula [(Pt–Po) / Pt] x 100

**Collection of blood and separation of serum and liver tissue**

Blood and tissue samples were collected and serum was separated by centrifuging at 3000 rpm for 10 minutes and subjected for the determination of Serum enzymes like Alanine transaminase , Alkaline phosphatase , Aspartate transaminase [21], Creatine kinase [22], Enzymatic and non enzymatic antioxidants like superoxide dismutase [23], Glutathione reductase [24], Glutathione peroxidase [25], Reduced glutathione [26], lipid peroxide [27],

hematological parameters like total leucocytes count, Total Red Blood Corpuscles,Total White Blood Corpuscles, Haemoglobin [28], biochemical parameters like Blood glucose [29], Serum protein [30]. After the experimental period (7 days), animals were sacrificed by cervical dislocation under mild anesthesia. Blood was collected in plain and heparinized tubes immediately and centrifuged for 10min at 2500 rpm and the serum was separated and used for the analysis of various biochemical parameters.

### Statistical analysis

The data are expressed as mean ± SEM. Results were analyzed using one – way ANOVA followed by Dunnet's *t*- test. Differences were considered as statistically significant at p < 0.05, when compared with control

## RESULTS

Administration of formalin in rats resulted in a significant increase in the levels of LPO, total WBC, serum enzymes, blood glucose and also in the length of the paw thickness while, these increase in the levels of above parameters were inhibited by the treatment of DMC. Formalin to the experimental animals produced reduction in the levels of SOD, GSH, GPX, GR, GST, CAT serum protein and total RBC and Hb. The animals treated with DMC at dose levels of 100mg/kg bw were significantly increased the levels of SOD, GSH, GPX, GST, CAT and GR (Table 1-5).

**Table 1: Effect of DMC on Paw edema induced by formalin in rats**

| | | | |
|---|---|---|---|
| Normal control | 1.38±0.62 | 2.69±0.21 | 3.80±0.24 |
| Disease control (Formalin induced) | 1.25±0.71* | 2.01±0.90* | 2.26±0.20* |
| Formalin + DMC (100mg/kg) treated | 1.27±0.57 [a] | 1.82±0.38 [a] | 1.28±0.15 [a] |
| Normal + DMC (100mg/kg) | 1.18±0.89 | 1.69±0.21 | 1.10±0.20 |
| Indomethacin 10(mg/kg) | 1.20±0.74 [b] | 1.71±0.34 [b] | 1.11±0.16 [b] |

*Denotes significant difference in comparing with normal control at P<0.001.[a,b]Denotes significant difference in comparing with diseases group at P<0.05.

| Groups | Methods of treatment | RBC(×10⁶ /mm³) | WBC(×10³ /mm³) | Hb (g/dl) | Glucose(mg/dl) | Protein(g/dl) |
|---|---|---|---|---|---|---|
| I | Normal control | 4.21±0.21 | 6.56±0.45 | 17.5±1.2 | 87.9±2.5 | 6.91±0.5 |
| II | Disease control (Formalin induced) | 2.56±0.12* | 14.7±1.2* | 11.6±0.9* | 198.4±11.4* | 4.35±0.21* |
| III | Formalin + DMC (100mg/kg) treated | 3.12±0.14 [a] | 6.67±0.58 [a] | 14.8±1.3 [a] | 117.5±4.8 [a] | 6.73±0.51 [a] |
| IV | Normal + DMC (100mg/kg) | 4.16±0.23 | 6.78±0.61 | 16.5±1.5 | 91.4±1.6 | 7.01±0.65 |
| V | Formalin+ Indomethacin treated | 4.56±0.31 [b] | 7.13±0.59 [b] | 15.9±2.1 [b] | 143.6±9.4 [b] | 6.76±0.51 [b] |

*Denotes significant difference in comparing with normal control at P<0.001.[a,b]Denotes significant difference in comparing with diseases group at P<0.05.

**Table 3: Effect of DMC on Liver Enzymes in control and experimental animals**

| Groups | Methods of treatment | AST(IU/L) | ALT(IU/L) | ALP(IU/L) | CK(IU/L) |
|---|---|---|---|---|---|
| I | Normal control | 34.12±1.8 | 37.6±1.4 | 273.4±19.6 | 97.22±5.3 |
| II | Disease control (Formalin induced) | 76.13±5.4* | 82.9±6.6* | 386.45±21.9* | 217.6±18.4* |
| III | Formalin + DMC (100mg/kg) treated | 48.77±3.4 [a] | 57.8±4.3 [a] | 296.58±19.5 [a] | 178.5±12.8 [a] |
| IV | Normal + DMC (100mg/kg) | 33.9±2.1 | 38.4±2.5 | 270.6±16.4 | 112.9±10.3 |
| V | Formalin+ Indomethacin treated | 47.6±3.6 [b] | 56.3±4.8 [b] | 283.14±19.3 [b] | 164.3±15.8 [b] |

*Denotes significant difference in comparing with normal control at P<0.001.[a,b]Denotes significant difference in comparing with diseases group at P<0.05.

**Table 4 : Effect of DMC on Anti oxidant enzymes in control and experimental animals**

| Groups | Methods of treatment | LPO(n of MDA/g tissue) | SOD(mμ epinephrine oxidized/min/mg protein) | GSH(mg/g tissue) | GPX (mg of GSH reduced / g tissue) | GR (mg of GSH oxidized / g tissue) | GST(μmoles of CDNB conjugated /min/mg protein) | CAT(nmoles of H2o2 decomposed/min/mg protein) |
|---|---|---|---|---|---|---|---|---|
| I | Normal control | 3064±119.6 | 10.08±0.97 | 2.23±0.16 | 48.9±1.9 | 6.45±0.56 | 5.02±0.43 | 7.42±0.64 |
| II | Disease control (Formalin induced) | 7289.4±203.5* | 4.18±0.25* | 1.1±0.09* | 12.18±0.98* | 2.17±0.19* | 2.87±0.17* | 3.92±0.24* |
| III | Formalin + DMC (100mg/kg) treated | 5138.4±116 [a] | 8.54±0.73 [a] | 1.87±0.15 [a] | 37.4±2.1 [a] | 4.76±0.35 [a] | 4.65±0.38 [a] | 5.98±0.32 [a] |
| IV | Normal + DMC (100mg/kg) | 3073±204.6 | 10.09±0.51 | 2.1±0.18 | 47.99±2.5 | 6.24±0.46 | 4.99±0.23 | 7.33±0.54 |
| V | Formalin+ Indomethacin treated | 5872.5±118.9 [b] | 8.67±0.64 [b] | 1.96±0.14 [b] | 38.1±1.9 [b] | 4.89±0.22 [b] | 4.57±0.28 [b] | 6.04±0.59 [b] |

*Denotes significant difference in comparing with normal control at P<0.001.[a,b]Denotes significant difference in comparing with diseases group at P<0.05.

**Fig 2: Effect of DMC on Hematological and biological values in control and experimental animals**

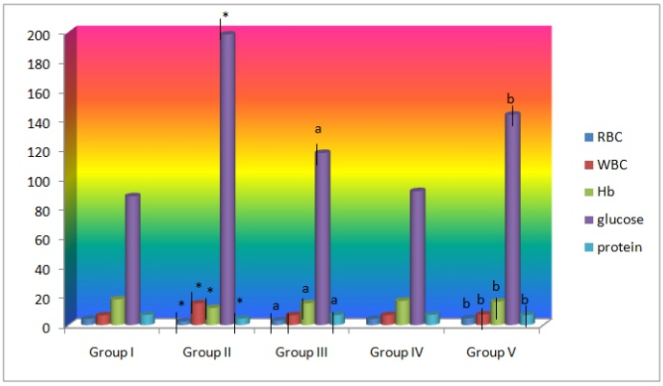


**Fig 3 : Effect of DMC on Liver Enzymes in control and experimental animals**

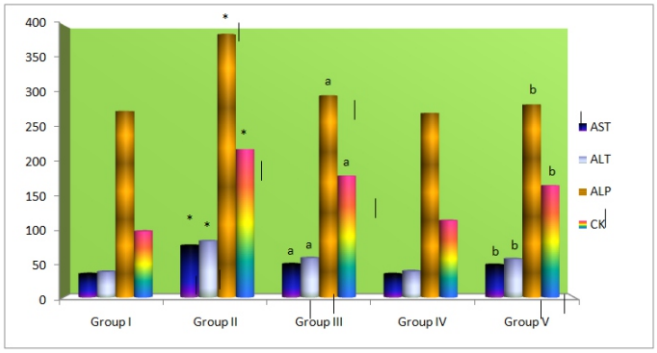
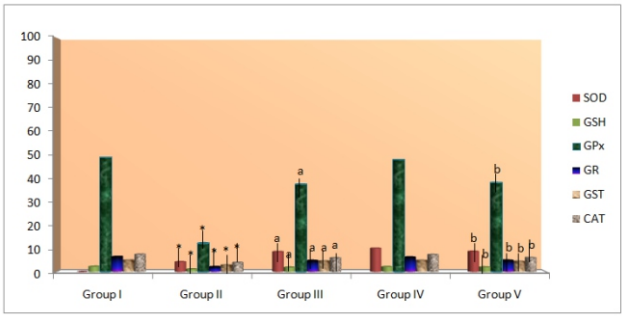



**Fig 4: Effect of DMC on Anti oxidant enzymes in control and experimental animals**




## DISCUSSION

### Effect of DMC on Hematological and Biological values

The hematopoietic system is one of the most sensitive parameters to assess the effect of drugs in animals and human. In the present study hematological and biochemical analysis of formalin induced paw edema of control and diseases treated rats showed significant difference and is in the normal range. Literature has shown that oral ingestion of medicinal compounds or drugs can alter the normal range of haematological parameters [31, 32]. These alterations can either be positive or negative.

Lymphocytes are the predominant cell in chronic inflammation. It can cause permanent distortion of the tissue, interfying its function.Total WBC 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 was also reported by Eric and Lawrence [33]. Hence in the present study the level of WBC was found to be higher in formalin induced inflammation. Oral treatment with the DMC at the dose levels 0f 100mg/kg bw significantly decrease the WBC count that indicate the significant recovery from the inflammatory process.

Goel *et al.*, [34] found that the 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, IL-5) and granulocyte macrophage stimulating factor that contribute pro inflammatory functions.

Hemoglobin and RBC play a major role in the oxygen transport. Formalin induction causes the significant decrease in the RBC and Hb which leads to anemia. However, treatment with the DMC at dose levels of 100mg.kg brought back these levels to near normalcy which was on par with the standard drug treated groups.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 Swingle and Shideman [35].

Glucose is a major source of energy for most cells of the body. Insulin facilitates glucose entry into the cells. Glucose is also a key molecule in carbohydrate metabolism. It is formed as a result of the digestion of complex carbohydrates or as a result of its synthesis in the body (gluconeogenesis) [36]. The present data showed significant increase in the serum glucose of formalin treated rats (Table 2).

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 [37]. In addition Weissman [38] reported that the level of serum protein content is lowered in rheumatoid arthritis. The proteins were clearly changed the perception of the pathogenesis of inflammation.Chronic inflammation is known to stimulate protein metabolism in animals [39]. Hence in the present study, DMC treated rats showed significant improved in the protein level in formalin induced rats.

### Effect of DMC on Liver Enzymes

Serum Aspartate transaminase (AST), Alanine transaminase (ALT) and alkaline phosphatase (ALP) activities are reliable indicators for assessing liver cellular lesions because they are excessively released into the bloodstream after disruption of hepatocellular membrane [40]. The aminotransferases liver enzymes (ALT and AST) are commonly used as diagnostic markers for damage and necrosis of liver cells. However, ALT is more specific for liver injury than AST which is present in a diversity of different tissues [41]. Furthermore, ALT is present only in the cytosol while AST present in both the mitochondria and cytosol of hepatocytes [42]. In the present study, the elevation of ALT, AST and ALP levels was observed in the formalin induced rats and indicates the hepatic damage.

In liver injury, the transport function of the hepatocytes is disturbed, resulting in the leakage of plasma membrane [43], thereby causing an increased enzyme level in serum. The elevated activities of SGOT and SGPT in serum are indicative of cellular leakage and loss of the functional integrity of cell membranes in liver .Although serum enzymes levels are not a direct measure of hepatic injury they show the status of liver [44].

Apple *et al.*, [45] showed that the biochemical enzymes such as Creatine kinase are well established biomarkers that are often released upon cellular degeneration such as necrosis and inflammation .Increase in Creatine kinase concentrations are related to the irreversible injury which was also well observed in the present investigation in experimental animals after formalin induction which on oral treatment with DMC showed reversion to normalcy.

**Effect of DMC on Anti oxidant enzymes**

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 [46] 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 chemostatic 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 [47]. The present study also demonstrated a cumulative decrease in the levels of anti oxidant enzymes and considerable increase in the levels of lipid peroxide which is well indicative of tissue damage on induction of formalin in experimental animals. However treatment with DMC resumed the levels of antioxidants to normalcy accompanied with a notable decrease in the lipid peroxide levels. The results showed a significant increase in the enzymatic antioxidant values at a dose of 100 mg/kg bw DMC.

Glutathione and glutathione-related enzymes play a key role in protecting the cells against the effects of reactive oxygen species. Reduced glutathione can act as a reductant, reducing hydrogen peroxide and lipid hydroperoxides directly to $H_2O$, a reaction catalyzed by GSH-Px. Depletion of intracellular GSH, under conditions of continuous intracellular oxidative stress, leads to oxidation and damage of lipids, proteins and DNA by the reactive oxygen species [48,49].

Babior *et al*., [50] explained that the toxicity produced by free radicals, bone cells maintain a well-coordinated free radical scavenging system formed by antioxidants like superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase. Superoxide dismutase and catalase are the first and second line defense antioxidant system, which interacts with superoxide toxicity and forms hydrogen peroxide. Catalase reacts with hydrogen peroxide to liberate water and oxygen with no free radical formation.

Glutathione peroxidase scavenges lipid peroxides in the cell membrane and act as a primary defense in mitochondria. The decreased glutathione peroxidase activity observed in arthritic rats might be due to the accumulation of hydrogen peroxide and deficiency of selenium. The glutathione redox cycle is an essential part of the antioxidant system which comprises enzymatic and non-enzymatic glutathione. Glutathione acts as a substrate for glutathione peroxidase and glutathione-S-transferase during the removal of $H_2O_2$ and lipid peroxides. Thus, a decrease in the level of glutathione will lead to the reduction in glutathione peroxidase and glutathione-S-transferase activity[51].Glutathione-S-transferase is involved in the detoxification of xenobiotics. This enzyme plays a role in catalyzing the glutathione to electrophilic substrates [52].

**SUMMARY AND CONCLUSION**

The present study proves that DMC has a protective role against formalin induced alteration on all hematological and biochemical parameters of blood in adult rats. The increased activities of ALT, AST, ALP and Creatine kinase the formalin group of rats may be primarily due to the leakage of these enzymes from liver cytosol into blood stream as a consequence of the hepatotoxic effect of formalin. However, formalin group of rats treated with DMC for 7 days

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, IL-5) and granulocyte macrophage stimulating factor that contribute pro inflammatory functions.

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**Effect of DMC on Liver Enzymes**

diminished the activity of these enzymes to their basal levels, suggesting the tissue protective nature of DMC. Administration of formalin in rats resulted in a significant increase in the levels of LPO, and decreased in the levels of SOD, GSH, GPX, GST, CAT and GR. The animals treated with DMC, were significantly increased the levels of above mentioned parameters. In consideration with the results, it was clearly indicated that the isolated compound has the tendency to prevent the liver tissue from the damage caused by the oxidative stress during inflammation in formalin induced rats. These studies have shown that the DMC contain some active ingredients with the potential of being good anti-inflammatory agent. NSAIDs like indomethacin, used as standard drug in anti inflammatory study, is having good anti inflammatory, but is also having side effects on gastrointestinal complications. Chalcones act as an excellent and universally accepted pharmacophore for development and design of novel medicine for treatment of pathological conditions

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