Anti-inflammatory effect of lycopene rich paste extract in the presence of NSAID on carrageenan induced paw oedema

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ABSTRACT

Immense interest in nutraceuticals and their potential benefits has created the need to review the existing scientific information on their effect in preventing and managing inflammation that accompanies most chronic diseases. This study focuses on the basic mechanisms of inflammation and the potential of nutraceutical lycopene against the carrageenan induced oedema. The nutraceutical lycopene, when tested against carrageenan induced paw oedema showed significant anti-inflammatory activity in a dose dependent manner along with test drug. The nutraceutical at the dose of 5, 50 and 100 mg in combination with test drug at 5 mg/kg b. wt exhibited appreciable results and decreases the % swelling from 94.45% to 68%, 45% and 25% respectively. At the same dosage level, the MDA was decreased to 4.88, 4.07 and 3.77nmol/mg protein from Carrageenan induced rats (7.99nmol/mg protein). In addition, nutraceuticals along with the test drug increases the glutathione levels from 3.33 to 5.76, 6.01 and 7.98µg GSH/mg protein at the concentration of 5, 50 and 100mg/kg b wt.

Key words: Anti-Inflammatory; Lycopene; Carrageenan; antioxidant enzymes

INTRODUCTION

Free radical species derived from oxygen are the main chemical effectors in inflammatory response. Free radicals may exert direct or indirect damaging effects through their reaction with other chemical or structural components in target cells. The role of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in inflammation is well documented [1, 2]. ROS such as the superoxide anion (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radicals (OH) act as a physiological defense system against microbial infection and they are involved in maintaining normal cellular functions and intracellular signal transduction [3]. ROS and RNS further activate the cascade that triggers the production of pro-inflammatory cytokines and chemokines[4]. AsROS are capable of damaging lipids, proteins, DNA and the extracellular matrix, these reactions generate a complex network of responses culminating in outcome that may be detrimental or beneficial for the host. Studies indicate that the increased oxidative and/or defective antioxidant status has a big potential to contribute to the pathology of inflammation [5]. Inflammation leads to the perturbation in the oxidative modification of low density lipoprotein, the oxidative inactivation of α-1-protease inhibitor, and heat shock protein associated with the activation of neutrophil, nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) and endothelial cell xanthine dehydrogenase, which have been found to contribute significantly to the inflammatory processes. A vast array of therapies and drugs are employed for the alleviation of inflammation. Most of these drugs are targeted at non-specific suppression of the inflammation but these therapies are mostly loaded with severe side effects. Immense interest in nutraceuticals and their potential benefits has emerged with a need to review the existing scientific information on their effect in preventing and managing inflammation that accompanies most chronic diseases.

The present work is to assess the anti-inflammatory capability of lycopene rich tomato paste extract in combination with an established anti inflammatory drug Diclofenac sodium (Voveran).
Lycopene

Lycopene, the main carotenoid present in tomato and tomato products, has been reported to possess quenching ability of free radicals. Tomatoes are usually the major source of lycopene and provide the familiar red color to them. Low serum lycopene concentrations have been linked with increased risk of pancreatic and bladder cancers [6], whereas high dietary intakes and serum concentrations of lycopene appear to protect against cervical intraepithelial neoplasia [7]. Chronic ingestion of lycopene decreases spontaneous mammary tumor development in mice and enhances the immune response by increasing the number of helper T lymphocytes [8]. Evidence suggests that lycopene also functions as an anti-inflammatory agent [9, 10]. It has been demonstrated that lycopene can inhibit the expression of inflammatory cytokines and reverse the loss of antioxidant enzymes induced by inflammation [9,11]. Epidemiological studies describe an inverse relationship between a diet rich in tomatoes and tomato products and the incidence of Cardio-vascular disease. The cardio preventive effect of lycopene has been mainly associated with lipid peroxidation, including LDL oxidation [12,13]. The activity of Lycopene has been suggested to be considerably related to its free radical scavenging and antioxidant activity [14].

MATERIALS AND METHODS

Chemicals

The chemicals used in this study were of analytical grade and obtained from standard commercial sources in India. Bovine serum albumin, Hydrogen peroxide, tertiary butyl hydroperoxide (E. Merk), Thiobarbituric acid, Folinsciocalteus phenol reagent (CDH India) ethanol (Bengal chemical), Ferric nitrate, trichloroacetic acid, Sodium carbonate, Sodium Dihydrogen monophosphate, Sodium hydrogen Diphosphate, EDTA (Hi-Media), Sodium hydroxide, reduced glutathione,5,5-dithiobis-2-nitrobenxiox acid (DTNB), were obtained from Thomas Baker India. Diclofenac sodium from local chemist under the brand name voveran.

Preparation of diet extract

Tomato paste was purchased from the market and weighed nearly 50g. The tomato paste was weighed into 250ml flask and wrapped with aluminium foil to protect from the light. 100 ml of mixture of ethanol-acetone-hexane (1:1:1, v/v) was added to flask to dissolve carotenoids. The mixture was stirred with magnetic stirrer for 45 min, and then 20 ml of distilled water was added to the flask. The solution was left to separate into a distinct polar layer and a non-polar layer containing lycopene. The organic layer was decanted into a separating funnel. The organic solvent was removed under reduced pressure (8-10 mbar) at 40°C. The extract was kept at 4°C until use.

Animals

In present study pathogen free male Wistar rats weighing 180–200 g were used to induce inflammation using carrageenan which is injected in the rat hind paw. Animals were housed in an environmentally controlled room with 12hr light /12hr dark cycle throughout study and were acclimatized for a week before actual start of experiment.

Rats were divided into six groups, six rats in each group. The rats in group first served as a normal control, while as group second is carrageenan challenged group and the third group is drug treated group. Groups four, five and six are the test diet groups at various concentrations (5, 50 and 100 mg/kg bw) in combination with test drug at 5mg/kg bw.

The animals used in the present study were maintained in accordance with the guidelines prescribed by the National Institute of Nutrition, Indian Council of Medical Research, and the study was approved by the Ethical Committee of the University of Kashmir.

Anti-inflammatory activity

Anti-inflammatory activity of the diet and drug was determined by Carrageenan induced oedema described by [15]. A single injection of 0.1 ml of 1 % carrageenan solution produces acute inflammatory oedema leading to marked increase in the volume of limb. Paw volume was measured immediately after the injection of carrageenan (V_o or basal volume) and 6 hour later (V_6h) with the aid of plathysmometer.

Paw oedema was expressed as a percentage of increase in paw volume six hours after carrageenan injection relative to the basal values according to the equation:

\[
\text{Calculation of } \% \text{ swelling} = \frac{V_{6h} - V_o}{V_o} \times 100
\]

Where \(V_o\) is the volume of the hind paw immediately following carrageenan injection. \(V_{6h}\) is the volume of the paw after the 6hrs of carrageenan induced oedema at which time the swelling is maximum.
Preparation of tissue lysate for biochemical analysis

Animals were sacrificed by cervical dislocation under light ether anaesthesia in an ethically desired manner. The animals were immediately dissected and liver of each animal was removed and washed in ice cold saline (0.9 % NaCl). A portion of liver was cut homogenate was prepared in ice cold buffer (phosphate buffer) (0.1M, pH 7.4) using HeidolphDiax 900 homogenizer. The tissue lysate (10% homogenate) was subjected to centrifugation firstly at 800 x g for 10 minutes in a cooling centrifuge to remove nuclei and other cell debris. The aliquot so obtained was decanted in to a fresh centrifuge tube and subjected to one more step of centrifugation at 10,500 x g for 20 minutes to get postmitochondrial supernatant (PMS).

BIOCHEMICAL ANALYSIS

Superoxide dismutase (SOD)

Superoxide dismutase activity was determined according to the method of [16]. Enzyme activity was measured by mixing phosphate buffer, pH 7.8 containing 0.1 mM EDTA, L- methionine, Nitro blue tetrazolium and Triton X-100. Sample 100 µl was then added to the mixture, followed by addition of riboflavin (10 µl). The tubes were then illuminated for 7 min in a 100 W fluorescent lamp. The control tube, in which the sample was replaced by buffer, was also run and the absorbance measured at 560 nm.

Catalase

CAT activity was assayed by the method of [17]. The assay mixture consisted of 1.95 ml of phosphate buffer (0.05 M pH 7), 1.0 ml H₂O₂ (0.019 M), 0.05 ml of hepatic PMS in a final volume of 3 ml. Change in absorbance was recorded at 240 nm. CAT activity was calculated in terms of n moles of H₂O₂ consumed/min/mg of protein.

Glutathione peroxidase

The Glutathione produced as result of glutathione peroxidise activity is immediately reduced by glutathione reductase thereby, maintaining a constant levels of reduced glutathione in the reaction system, the assay takes advantage of concomitant oxidation of NADPH by Glutathione reductase ,which is measured at 340 nm. Specific activity was measured according to the procedure described by[18].The reaction mixture in a 3ml cuvette consisted of 1.53ml of phosphate buffer (0.05 M, pH 7.0) 0.1 ml of 1mM EDTA, 0.1 ml of 1mM NaN₃, 0.1ml of reduced glutathione, 0.1ml 0.2Mm NADPH, 0.01 ml 0.25mM H₂O₂ and 100µl PMS in a final volume of 2.0 ml. The activity was measured in terms of decrease in absorbance at 340nm suggestive of disappearance of NADPH at interval of 30 sec for 3min at room temperature. The enzyme activity was calculated as nmole NADPH oxidized/min/mg protein by using molar extinction coefficient of 6.22x 103M-1 cm-1.

Glutathione- S- transferase

GST activity was assayed using the method of [19]. The reaction mixture consisted of 1.67 ml sodium phosphate buffer (0.1 M pH 6.5), 0.2 ml of 1mM GSH, 0.025 ml of 1mM CDNB and 0.1 ml of PMS in a total volume of 2 ml. The change in absorbance was recorded at 340 nm and the enzyme activity was calculated as nmoles of CDNB conjugates formed/min/mg protein using € of 9.6 x 10⁻³ M⁻¹L⁻¹.

Lipid peroxidation

Lipid peroxidations in tissue fractions were estimated by the formation of thiobarbituric acid reactive substances (TBARS).The assay was done by the method of Nichans and Samuelson [20]. In brief 0.1ml of tissue homogenate (PMS; Tris- HCl buffer, pH 7.5) was treated with 2ml of (1:1:1 ratio) TBA-TCA-HCl reagent (0.37% thiobarbituric acid, 0.25 N HCl, and 15% TCA), placed in boiling water bath for 15 min, cooled and centrifuged at 2500 x g room at temperature for 10 min. The absorbance of the clear supernatant was measured against reagent blank at 535 nm. The results were expressed as a mole MDA (Malonaldehyde) formed/g of tissue using molar extinction coefficient of 1.56 x10⁵M⁻¹cm⁻¹

Reduced Glutathione

Reduced Glutathione in the liver was determined by the method of Jollow[21]. In this estimation the acid soluble sulphhydril group (of which more than 93% is reduced glutathione) forms a complex with DTNB. The complex, 5-thio-2-nitrobenzoate is yellow colored and can be detected at 412nm. Briefly, 1ml of tissue sample was precipitated with 1 ml of the tissue sample Sulphosalicylic acid (4 % w/v in distilled water).The samples were kept at 4°C for at least one hour and then subjected to centrifugation at 12000 g for 15min at 4°C. The assay mixture contained 0.1 ml of supernatant 2.7 ml of phosphate buffer (0.1 M pH 7.4) and 0.2 ml of freshly prepared 5,5-dithiobis-2-nitrobenzene (DTNB) (4mg/ml of 0.1M phosphate buffer of pH 7.4) in a total volume of 3.0 ml. The colour developed was read immediately at 412 nm in a spectrophotometer. The activity was calculated using GSH as standard and expressed as μ mole of GSH/ g tissue.
Nitric oxide
NO was determined by the method of Misko[22]. In this method nitrite was measured by using Greiss reaction. Briefly to 50µl of PMS sample diluted to 1 ml with distilled water and 1% sulphanilamide in 1N HCl, 0.02 NEDD (naphthalene ethylene diaminedihydrochloride) is added and immediately mixed and incubated at 37°C for 30 min. Reaction was read at 540 nm. The activity was calculated using sodium nitrate as standard and expressed as n mole of nitrate /ml of PMS.

STATISTICAL ANALYSIS
The values are expressed as mean ± standard deviation (SD). The results were evaluated by using the SPSS (version 12.0) and Origin 6 software and evaluated by one-way ANOVA followed by Bonferroni t-test. Statistical significance was considered when value of P was < 0.05

RESULTS AND DISCUSSION
Oxidative agents such as reactive oxygen species and reactive nitrogen species are produced in abundant quantities during every inflammatory response, independently of the causative agent, and in response to multiplicity of exogenous and endogenous stimuli [23]. The effect of test diet in the presence of test drug on paw edema induced by Carrageenan is shown in Figure 1. We observed 73.2% of swelling in the test drug at the concentration of 5mg/kg b wt, as compared with the Carrageenan induced group were 99.45% of swelling was observed. In the presence of test diet at the concentration of 5, 50 and 100mg/kg b wt, 68%, 40.5% and 24.5% of swelling were observed respectively.

BIOCHEMICAL ANALYSIS
Enzymatic Parameters
Inflammation induced by injection of carrageenan in rat paw has resulted in alteration in the number of biochemical factors. The perturbation in these parameters were evaluated in the presence and absence of the test drug and test diet extract under study. The carrageenan induced acute inflammation alters the level of all enzymatic as well as non-enzymatic antioxidant parameters. There is alteration in glutathione levels as well as in the activities of all antioxidant enzymes such as SOD, Catalase, Glutathione peroxidase and Glutathione-S-transferase[24]. Dose dependent effects of the test diet extract as observed indicate a role of free radical scavenging property to the test diet extracts.

Table 1: Shows the effect of Diclofenac sodium (Test Drug) and (LRTPE) Test Diet extract on the antioxidant enzymes following carrageenan induced edema

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (units/mg protein)</th>
<th>CAT (nmoles of H2O2 consumed/mg protein)</th>
<th>GPX (nmol NADPH oxidised/min/mg of protein)</th>
<th>GST (nmol of CDNB conjugated/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.92±0.24</td>
<td>10.78±0.99</td>
<td>6.93±0.66</td>
<td>122.20±7.40</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>1.27±0.11</td>
<td>2.10±0.24</td>
<td>1.99±0.145</td>
<td>48 ± 0.23</td>
</tr>
<tr>
<td>Test drug 5mg/kg b wt</td>
<td>2.55±0.33</td>
<td>4.99±0.38</td>
<td>3.10±0.145</td>
<td>65.12±1.99</td>
</tr>
<tr>
<td>Test drug+ LRTPE 5mg/kg b wt</td>
<td>2.59±0.22</td>
<td>4.01±0.33</td>
<td>3.5±0.87</td>
<td>70.01±1.88</td>
</tr>
<tr>
<td>Test drug+ LRTPE 50mg/kg b wt</td>
<td>2.65±0.27</td>
<td>4.57±0.3</td>
<td>3.77±0.89</td>
<td>95.01±2.33</td>
</tr>
<tr>
<td>Test drug+ LRTPE 100mg/kg b wt</td>
<td>2.87±0.31</td>
<td>6.35±0.39</td>
<td>4.77±0.86</td>
<td>100.26±2.88</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SD of 6 animals. $; p < 0.001, as compared with normal control group. $#; p < 0.001 as compared with Carrageenan, ns; non significant as compared with control, ns: non significant as compared with Carrageenan. The data were presented as means ± S.D of six parallel measures and evaluated by one way ANOVA followed by the Bonferroni t – test to detect inter group differences. Differences were considered to be statistically significant if p < 0.05

Table I represents the effect of different concentrations of test diet extract (LRTPE) and test drug (Diclofenac sodium) on various enzymatic parameters following carrageenan challenge. The test diet extract in a dose dependent manner was able to restore the SOD activity along with test drug. The test diet extract at the highest concentration 100mg/kg body weight in combination with the test drug 5mg /kg body weight was able to restore activity maximally from 1.27 ±0.11units/ mg protein to 2.87±0.31 units/ mg protein. The SOD activity in the presence of test drug alone was restored to 2.55±0.33 units/ mg protein. Superoxide dismutase the enzyme taking care of superoxide radicals that is one of the common radicals generated under both normal as well as pathological conditions plays a very important role in inflammation. Superoxide dismutase (SOD) enzymes may include Cu/Zn SOD in cytosol and on extracellular surfaces and Mn–containing SOD in mitochondria [25]. SOD is an important antioxidant enzyme having an antitoxic effect against superoxide anion. SOD is the first line of defense against ROS. It catalyzes the dismutation of the superoxide into hydrogen peroxide. In the inflammatory conditions, superoxide is produced at a
rate that overwhelms the capacity of endogenous SOD enzyme to remove it [26]. In combination with test drug our test diet exhibited good activity to restore SOD levels.

Figure 1: Shows the effect of Diclofenac sodium (Test drug) and LRTPE (Test diet extract) on Carrageenan induced paw oedema

The data were presented as means ± SD for six animals in each observation and evaluated by one-way ANOVA followed by Bonferroni t-test to detect inter group differences. Differences were considered to be statistically significant if p < 0.05

Figure 2: Shows the effect of Diclofenac sodium (Test Drug) and LRTPE (Test Diet extract) on Glutathione levels in carrageenan induced paw oedema

The data were presented as means ± SD for six animals in each observation and evaluated by one-way ANOVA followed by Bonferroni t-test to detect inter group differences. Differences were considered to be statistically significant if p < 0.05
Catalase and Glutathione peroxidase are the most important enzymes in the inflammation and serve as good indicators of compensatory metabolic response during carrageenan induced inflammation. Catalase, the major component of the anti-oxidant system catalyzing the decomposition of hydrogen peroxide plays a role in inflammation [27]. Although the tissue distribution of catalase is widespread, and the level of activity varies not only between the tissues but within the cell itself. The level of catalase as measured in acute model of inflammation, decreased when carrageenan was used. The decrease could be due to excessive production of H$_2$O$_2$ suggesting that catalase could provide an important line of defense against inflammation. Over expression of catalase in vitro and in vivo has been reported to directly affect the genes influencing inflammation [28]. Thus enhanced catalase activity might be compensatory response against inflammation, which acts by limiting the generation of ROS or by regulating the expression of genes involved in inflammation [29]. LRTPE improved the catalase activity suggesting that extracts might be inducing catalase to counteract the damaging effects of peroxides that are generated in abundant quantities during inflammation. The combination of diet in a dose dependent manner with drug yielded more effective results. An acute depression is observed in the catalase activity following carrageenan treatment. Test diet extract along with test drug was able to restore catalase activity from 2.10 ± 4.01 ± 0.33, 4.572 ± 0.3 and 6.35 ± 0.39 n moles of H$_2$O$_2$ consumed/mg protein) at the concentration of 5, 50 and 100mg/kg bw Table 1.

Glutathione peroxidase a selenium containing enzyme is another important protective enzyme involved in inflammation. GPx catalyses the reduction of a variety of hydroperoxides (ROOH and H$_2$O$_2$) using GSH, and has been shown to protect mammalian cells against oxidative damage [30]. The carrageenan induced inflammation resulted in the severe depression in GPx levels. LRTPE in combination with test drug restored the levels in a dose dependent manner.

Glutathione-S-transferase exerts a very critical role in protecting cellular structures from the oxidative stress. GST detoxifies a variety of compounds mainly oxidized lipids, oxidized DNA generated as a result of ROS-induced damage to the intra-cellular molecules [24]. Carageenan induced oedema decreased the levels of GST, however our test diet extract restored the decreased levels.

Following Carrageenan treatment Glutathione peroxidase activity was decreased to 1.99 ±0.145 nmoles NADPH oxidized/min/mg of protein. However in animals pre-treated with test drug and test diet extract showed
corresponding restoration of GPx activity. At the highest concentration of test diet extract (100mg/kg bw) in combination with test drug 5mg/kg bw the GPx activity was restored back to 4.77 ± 0.86. However in the presence of test drug only the GPx level was restored to 3.10 ± 0.145 nmol of NADPH oxidized/min/mg of protein only.

Glutathione–S-transferase also exhibited the similar restoration pattern in the presence of test drug and Test diet extract at various concentrations / Kg body weight. In the presence of test drug 5mg/kg body weight and test diet extract at 100mg/kg body weight the GST activity was restored to 100.26 ± 3.28 nmol of CDNB conjugated/mg protein from 48 ± 2.3 nmol of CDNB conjugated/mg protein in comparison to test drug alone where it is only 65.12 ± 1.99 nmol of CDNB conjugated/mg protein.

<table>
<thead>
<tr>
<th>Control</th>
<th>Carrageenan</th>
<th>Test Drug</th>
<th>Test Drug + Test Diet 5mg/kg BW</th>
<th>Test Drug + Test Diet 50mg/kg BW</th>
<th>Test Drug + Test Diet 100mg/kg BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO (nmol of nitrate/ml PMS)</td>
<td>250</td>
<td>500</td>
<td>350</td>
<td>300</td>
<td>250</td>
</tr>
</tbody>
</table>

Figure 4: Shows the effect of Diclofenac sodium (Test drug) and LRTPE Test Diet extract on Nitric oxides levels in carrageenan induced Oedema

The data were presented as means ± SD for six animals in each observation and evaluated by one-way ANOVA followed by Bonferroni t - test to detect inter group differences. Differences were considered to be statistically significant if p < 0.05.

NON ENZYMATIC PARAMETERS
Glutathione, Lipid peroxidation and NO.

The effect of test diet extract and test drug on the non-enzymatic parameters glutathione, Lipid peroxidation and Nitric oxide are depicted in fig 2, 3 and 4 respectively. As seen in Fig 2, GSH following carrageenan challenge was decreased to 3.33 ± 0.36 µg of GSH /g protein from 9.39±0.99 µg of GSH /g protein in control group. In the presence of test drug (Diclofenac sodium) GSH was restored back to 5.55/µg of GSH /µg protein. However when test drug was given in combination with test diet extract, GSH level increases in a dose dependent manner (5.75, 6.01 and 7.98µg of GSH/g protein) at the concentration of 5, 50 and 100 mg/kg bw. The Fig 3 shows the dose dependent decrease in lipid peroxidation with the increasing concentration of test diet extract I with a fixed dose of test drug 5mg/kg bw. The MDA levels were decreased to 3.77±0.35 nmol of MDA /mg protein from 7.99±0.77 (nmol of MDA /mg protein) when test drug was added up with 100mg/kg body weight of test diet extract I. In our studies the carrageenan induced inflammation increased the levels of NO from 298 nmol of nitrate/ml PMS in normal group to 484 nmol of nitrate/ml PMS as shown in Fig 4. The known anti-inflammatory drug, Diclofenac sodium decreased the NO levels to 357 nmol of nitrate/ml PMS. The test diet at the highest concentration 100mg/kg body weight and test drug at 5mg/kg body weight in combination were able to decrease the value to 338 nmol of nitrate/ml PMS. The level of hepatic glutathione has been reported to change under inflammatory conditions [30]. Glutathione (GSH), a ubiquitous tripeptidethiol, is a vital intra and extracellular protective antioxidant against oxidative stress. Reduced glutathione constitutes the major defense against radical damage that is of common occurrence during inflammatory conditions [30]. GSH serves a number of functions such as storing...
cysteine in a non toxic form and serving as a co-substrate for GSH peroxidase (GPx) and GSH acts as a hydrogen donor to reduce hydrogen peroxide and organic peroxides to water and alcohol. Besides it maintains the sulphhydryl residues of certain proteins and enzymes in reduced state, and forms conjugates with exogenous and endogenous toxic compounds in a reaction catalysed by glutathione-sulphur–transferase. The present study depicted a decrease in glutathione levels following carrageenan injection, however following the addition of test diet extract to the test drug there was perceptible improvement in glutathione levels. An increase in LPO following carrageenan treatment and hence, inflammation triggers some uncharacterized metabolic reactions in the liver, which increases the output of lipid peroxides. In studies with our test diet shows a good percentage of inhibition on LPO, suggesting that the anti-inflammatory effects could be also mediated through modulation of LPO. The lipid peroxidation in the membrane is known to occur in a number of pathological conditions. It generates a variety of hydroperoxides and aldehyde products that are highly reactive with the components of the cell and the extracellular matrix [31]. Our diet extract decreased the malondialdehyde levels in a dose dependent manner along with drug. An important mediator in inflammation is nitric oxide which is produced from activated cells and macrophages and its synthesis is basically controlled by inducible NOS and thus regulation of iNOS is an important feature of acute inflammation. iNOS has a most important role to play in the maintenance of acute inflammation and is supported by the observation that its expression is enhanced during the last stage of inflammation in paws and in dorsal horn neurons of the spinal cord after injection of carrageenan. NO is a potent vasodilator its involvement during an inflammatory response is related to its ability to increase vascular permeability and oedema through changes in local blood flow [32]. Furthermore, NO has been shown to increase the production of pro-inflammatory prostaglandins in vitro[33] ex vivo [34] and in vivo studies [35]. NO has been defined as a double edged sword that can actually mediates both induction as well as inhibition of inflammatory response. It has been reported to be a potent vasodilator, and its role in inflammatory response may be related to its ability to increase vascular permeability and oedema through changes in local blood flow. Additionally, NO has been shown to increase the production of prostaglandins in vitro and ex vivo [36]. Following carrageenan injection the NO levels increased dramatically. However in the presence of drug alone and in combination with test diet extract, the NO production is largely inhibited indicating the potential augmenting effect of test diet extract on the efficiency of test drug in decreasing the NO production and concomitant decrease in the inflammation.

CONCLUSION

Results from this study showed that nutraceutical lycopene against carrageenan induced oedema showed significant anti-inflammatory activity. Swelling percentage was decreased in a dose dependent manner. Levels of MDA and thus lipid peroxidation were significantly decreased in carrageenan treated rats. In addition nutraceuticals along with the test drug increases the glutathione levels and antioxidant enzymes at the concentration of 5, 50 and 100mg/kg bw. However, further chemical work and investigations at molecular level are required to establish the possible correlation among the mentioned activities of the nutraceuticals against the inflammation.

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