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ORIGINAL ARTICLE

Antioxidant and anti-inflammatory potential of some dietary cucurbits

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Lagenaria siceraria;
Luffa cylindrica

Abstract

Objective: The inflammatory problems and associated diseases in the gastrointestinal tract are known to exist in a sizable fraction of the population. It results due to complex interaction between food, enteric microbes and the host. Therefore, the cucurbits as an easily digestible diet were evaluated for their antioxidant and anti-inflammatory activities.

Methods: Antioxidant properties of cucurbits (*Lagenaria siceraria, Ls*; *Cucurbita pepo, Cp; Luffa cylindrical, Lc*) were evaluated *in vitro* following xylene orange or Amplex® Red dye method. Anti-inflammatory properties of cucurbits with respect to cyclooxygenase (COX)-1 and 2 were studied and interleukin (IL)-1β and tumor necrosis factor (TNF)-α in serum samples of lipopolysaccharide (LPS)-inflamed mice.

Results: The inhibition rate of superoxide radicals by *Ls*, *Cp* and *Lc* was 40, 36 and 31%, respectively, at a concentration of 0.08 mg/ml. The perhydroxyl radical scavenging activity was in a declining order and maximum effect was exhibited by Ls. With respect to COX-1 inhibition, IC_{50} for indomethacin, *Cp*, *Lc* and *Ls* were estimated to be 0.0154, 0.026, 0.0337 and 0.0335 mg/µl; and IC_{50} against COX-2 were 0.0427, 0.023, 0.0183 and 0.0150 mg/µl, respectively. *In vivo* study revealed that *Ls*, *Lc*, indomethacin and *Cp* correspondingly down-regulated the expression of IL-1β by 52, 68, 75 and 126 pg/100 µl, significantly less than control (260 pg/100 µl). TNF-α was also reduced significantly more by *Ls*, *Lc*, indomethacin and *Cp* by 12, 16, 20 and 26 pg/100 µl, respectively, than control (46 pg/100 µl).

Conclusion: The dietary cucurbits seem to have potential for developing a non-toxic therapeutic product.

INTRODUCTION

Reactive oxygen species (ROS) are regularly generated in the body during metabolic activities. Pollutants, radiations, pathogens and gut microbiome also generate free radicals in our bodies. The free radicals interact with biomolecules like DNA, proteins and membranes. Interaction of radicals with biological membranes generates various peroxides and malonaldehyde [1], and alters membrane permeability, signal transduction, and even leads to the cell rupture.

The free radicals activate various pro-inflammatory mediators and transcription factors like tumor necrüosis factor (TNF)-α, interleukin (IL)-1β, IL-6, nuclear factor (NF)-κB *etc*, which in turn activate various cytokines and subsequent inflammatory events at initial levels [2]. In the gastrointestinal (GI) tract, free radicals interact with gut lining and alter the intracellular redox status. Free radicals activate receptors and non-receptor kinase cascades like tyrosine kinase, protein kinase C and mitogen-activated protein kinase (MAPK), which in turn activates various inflammatory cytokines, tumor growth factors, lymphocytes, neutrophils and prostaglandins. These inflammatory molecules generate pain and fever [3]. During inflammation, membrane bound enzymes cyclooxygenase (COX)-1 and COX-2 are activated. COX-1 is a constitutive and cytoprotective enzyme [4], while COX-2 activates phospholipase A_2, releases arachidonic acid from cell membrane and biosynthesizes prostaglandins (PG) which activates inflammation.

There are in fact a large number of natural and synthetic compounds namely SAID (steroidal anti-inflammatory drugs) and NSAID (non-steroidal anti-inflammatory drugs) used for relieving inflammation. Unfortunately, both classes of these agents have adverse reactions which may sometimes prove even fatal. NSAID is indeed a weak inhibitor of COX-2 but strong inhibitor of COX-1 and therefore leads to GI toxicity. For controlling inflammation in the GI tract inhibition of COX-1 should be as low as possible in comparison to COX-2.

Some dietary cucurbits, *i.e. Lagenaria siceraria* (Molina) Standl, *Cucurbita pepo* Linn *and Luffa cylindrica* (Linn) M. Roem, were investigated for anti-inflammatory activities. Extracts of these cucurbits contain biomolecules like flavonoids, terpenoids, alkaloids *etc*, which have been well documented free radical scavenging properties. Such agents have been reported for enhancing the potential of endogenous antioxidants such as glutathione peroxidase (GPx),

superoxide-dismutase (SOD) and catalase (CAT) [5]. *Lagenaria siceraria* (*Ls*), has been reported for antioxidant and anti-inflammatory attributes [6, 7]. Similarly, *Cucurbita pepo* (*Cp*) has been shown to have antioxidant [8], anti-inflammatory [9] and anti-ulcer properties [10]. *Luffa cylindrica* (*Lc*) has been documented for antioxidant [11], anti-inflammatory [12], anti-bacterial and anti-fungal properties [13].

The present study aims to investigate the effect of some dietary cucurbits (fruit extracts) both *in vitro* and *in vivo* against lipopolysaccharide (LPS)-induced inflammation as a challenge in the GI tract.

MATERIAL AND METHODS

Reagents

Nutrient broth, eosin methylene blue, trypan blue, gallic acid, and xylene orange were procured from HiMedia (Mumbai, India); Tri-sodium citrate, citric acid, sodium chloride, disodium phosphate hydrogen phosphate, calcium chloride, magnesium chloride, sodium-potassium tartrate, dimethyl sulphoxide (DMSO), methanol and ethanol were procured from Merck (Mumbai); Sodium dihydrogen phosphate, aluminum chloride, dextrose, potassium chloride, potassium dihydrogen phosphate, hydrogen peroxide (H_2O_2), sodium carbonate and sodium bicarbonate were procured from Qualigens (Mumbai); WST-1 (2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) was procured from Interchim (Montlucon, France); Indomethacin was procured from MP Biomedicals (Strasbourg, France); RPMI-1640, Histopaque®-1077 and Histopaque®-1119, were procured from Sigma Aldrich (St. Louis, MO, USA); Quercetin was procured from Sisco Research Laboratories (Mumbai); Folin-Ciocalteu was procured from Loba Chemie (Mumbai); Amplex® Red assay kit was procured from Molecular Probes® (Germany); TMPD (N',N',N',N'-tetramethyl-*p*-phenylenediamine), COX inhibitor screening kit, TNF-α and IL-1β kits were procured from Cayman (Ann Arbor, MI, USA).

Bacterial culture

Escherichia coli procured from Jamia Milia Islamia University (New Delhi, India) were grown on selective growth media eosin methylene blue at 37ºC in a sterilized environment and were harvested after 24 h of inoculation. *E.coli* were identified by gram reaction, cell morphology and catalase reaction [14].

Preparation of cucurbits extracts

Fresh fruits of cucurbits *Ls* (bottle gourd), *Cp* (pumpkin) and *Lc* (sponge gourd) procured from local market were washed thoroughly with triple distilled water (TDW) several times and 1 kg of each plant material was crushed and spun in homogenizer. The homogenate of each plant material was kept separately in 100 ml solvent (TDW and absolute alcohol; 50:50, v/v for 24 h) at ambient temperature. Twenty four hours later the homogenate was centrifuged for 15-20 min at 5000 rpm and supernatant was collected and filtered using 0.22 µ membrane filters and further concentrated by using Rotavapor. Finally the concentrated extract of each cucurbit fruit was stored at 4ºC [15].

Isolation of human neutrophils

Three milliliters of Histopaque®-1119 was added to 15 ml capped centrifuge tubes. 3 ml of Histopaque®-1077 was poured gently on the Histopaque®-1119 and thereafter 6 ml of anticoagulated blood collected from healthy volunteers was added. The blood on the Histopaque® solutions was centrifuged at 700 rpm for 30 min at room temperature (18-26ºC). Neutrophils precipitated and formed a layer (2 ml from the bottom) in the centrifuge tube. These neutrophils were carefully separated using a micropipette and were washed with 10 ml of phosphate buffer saline. This mixture was further centrifuged for 10 min at 200 rpm and the concentrated cell mass was converted into pellet. The pellets were re-suspended in Hank's balanced salt solution (HBSS) and the viability of cells was determined by trypan blue [16].

LPS induced inflammation in mice

LPS from *E.coli* (1 mg/ml) was stored as stock solution at -20ºC. For experiments the solution was diluted (20 µg/100 µl saline) immediately before intraperitoneal (i.p.) injection. Control mice received 20 µg of LPS in 100 µl. In double control, 100 µl of saline was injected to mice. In experimental group 2 h after LPS injection, prebiotic was administered orally (100 µl) wherever needed. In double control, control and test samples, after 4 h of LPS injection, mice were anesthetized and blood samples were taken by cardiac puncture and kept overnight at 4ºC for clot formation [17]. Serum was collected after centrifugation (5000 rpm) for 5 min and stored at -20ºC until used for the measurement of IL-1β and TNF-α using ELISA.

Determination of phenols and flavonoids

The phenolic contents were determined as equivalent to gallic acid and expressed as mg/ml. Following the method as adopted by Hodzic *et al* [18], 1 ml aliquot of a cucurbit extract (*Ls*, *Lc* or *Cp*) taken in a test tube was made up to a volume of 3 ml by adding TDW. To this solution, Folin-Ciocalteau solution (0.5 ml of 1 part Folin-Ciocalteau and 1 part TDW) and 2 ml of 20% sodium carbonate solution in TDW were added in each test tube and was kept 40ºC for 1 min. Each test tube was cooled and absorbance was measured at 650 nm. Gallic acid was taken as a reference for the determination of total phenolic contents.

Flavonoid content was determined as equivalent to

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quercetin and expressed as mg/ml. Following the method as adopted by Khatiwora *et al* [19], 1 ml aliquot of each cucurbit extract (*Ls*, *Lc* and *Cp*) was taken in different test tubes and made up to the volume of 3 ml with methanol (analytical grade). 0.1 ml of 10% aqueous solution of aluminum chloride (10%) and 0.1 ml of sodium-potassium tartrate (1 M aqueous solution) and 2.8 ml TDW were added. Contents in each test tube were shaken and were incubated for 30 min at ambient temperature and absorbance was measured at 415 nm. Quercetin was taken as reference for determination of flavonoids.

Superoxide radical scavenging assay

The superoxide radicals in neutrophils were determined following the method adopted by Jalil *et al* [20]. The neutrophils were stressed by addition of the cells of *E.coli*, three times of the number of neutrophils. The stressed neutrophils were transferred to the microplate well. WST-1 dye was added at this stage and maintained at ambient temperature. Ten minutes later different concentration of a cucurbit extract (*Ls*, *Cp* or *Lc*) or indomethacin was transferred separately to a microplate well having activated neutrophils. The stressed neutrophils were incubated for 30 min at 37ºC and the concentration of decomposed superoxide radicals were measured at 450 nm using microplate reader.

Catalase estimation

Xylene orange method: The decomposition of perhydroxyl radicals by endogenous catalase present in neutrophils was evaluated by using xylene orange as an indicator following the method adopted by Zmijewski *et al* [21]. Neutrophils (620 cells/250 µl of HBSS) were stressed by addition of hydrogen peroxide (50 µl of 150 mM), *E.coli* (34×10^3/50 µl broth) or both the agents together. The effect of a cucurbit extract (*Ls*/*Cp*/*Lc*; 0.05 mg/ml) or NSAID (indomethacin) on scavenging the concentration of perhydroxyl radicals was investigated by adding these agents individually to neutrophils after 10 minutes of stress induction. This aliquot thereafter was incubated at 37ºC for 60 min and the perhydroxyl radicals were measured spectrophotometrically at 585 nm.

Amplex® Red method: The production of hydrogen peroxide in neutrophils (750 cells /250µl of Hank's solution) was induced by the stressors hydrogen peroxide (40 mM) or *E.coli* (11×10^3/50 µl in medium) individually or in combination in equal volumes. The decomposition of hydrogen peroxide by cucurbit extracts or indomethacin was determined by using Amplex® Red dye and horse radish peroxidase following the method adopted by Zhao *et al* [22]. Cucurbit extracts (*Ls*/*Cp*/*Lc*) or indomethacin was added in microplate well after 10 min of induction of stress in neutrophils. This was then incubated at 37ºC for 30 min and the decomposition of hydrogen peroxide was measured spectrophotometrically at 585 nm.

COX-1 and COX-2 inhibition assay

10 µl of COX-1 or COX-2 was transferred to each well of the microplate followed by addition of 10 µl of each agents (*Ls*, *Cp* , *Lc* or indomethacin) separately and was incubated for 5 min at 25ºC. Thereafter, 20 µl of TMPD dye was added and read at 585 nm using microplate reader as the method followed by Alberto *et al* [23]. The data was represented as percent inhibition of COX-1 or COX-2. The IC_{50} for COX-1and COX-2 as a result of the addition of various agents was calculated as follows:

IC_{50} of cucurbit / IC_{50} of indomethacin x 100

Measurement of IL-1β and TNF-α in LPS induced inflammation in mice serum *(in vivo*)

In 96 well plates, each well was pre-coated with monoclonal antibody specific for IL-1β. 50 µl of serum samples was added in a well followed by addition of 50 µl of IL-1β-acetylcholinesterase Fab' conjugate and incubated overnight at 4ºC. Non-adherent cells from well plates were rinsed using wash buffer and 100 µl of Ellman's reagent was added and incubated for 30 min in dark. Absorbance was measured at 415 nm.

For TNF-α measurement, a monoclonal antibody specific for TNF-α was pre-coated in 96 well plates. Fifty microliter of serum samples were added in different wells followed by addition of 50 µl of acetylcholinesterase Fab' conjugate and incubated overnight at 4ºC. Non adherent cells were removed by rinsing with wash buffer and 100 µl of Ellman's reagent was added and incubated for 30 min in dark and absorbance was measured at 415 nm as the method followed by Jaegle *et al* [17].

Statistical analysis

Each experiment was an average of three sets of experiments and the data has been calculated as the mean ± standard error of the mean (SEM). One way ANOVA was applied to test the significance which was defined as $P < 0.05$.

RESULTS

Total phenols and flavonoids

Spectrophotometric measurement revealed that *Lc* has maximum phenolic contents followed by *Ls* and *Cp* in a decreasing order (Table 1). On the other hand, *Ls* has maximum flavonoid concentration as compared to *Lc* and *Cp*.

Superoxide radical scavenging activity

In the unstressed and stressed neutrophils the values for superoxide radicals were 0.123 ± 0.009 and 1.623 ± 0.1, respectively, and the data obtained for

stressed neutrophils was used as the 'control' for this study. With the increasing concentration of the cucurbits extracts (0.01, 0.02, 0.06 and 0.08 mg/ml) added to a well containing 250 µl of the neutrophils in HBSS, the SOD activity was enhanced and the superoxide radicals were appreciably scavenged and reduced. Addition of 0.08 mg/ml of each of the cucurbit extracts reduced the superoxide radicals as follows: *Ls* 40%, *Cp* 36% and *Lc* 31% (Fig.1). However, an increase in the concentration of cucurbit extract to the extent of 0.12 mg/ml did not increase the SOD further and rather decreased to some extent. The superoxide radicals therefore remained in more abundance at this concentration of cucurbit.

Administration of indomethacin also followed the same pattern. An increase in the concentration of indomethacin from 0.01 to 0.08 mg/ml rendered progressive scavenging of superoxide radicals up to 44%. However, the presence of 0.12 mg/ml of indomethacin decreased SOD to some extent and therefore the decrease in scavenging of superoxide radicals was observed as 40% only.

Catalase activity (in presence of xylene orange)

The absorbance of neutrophils without stress measured as 0.738 ± 0.009 was taken as control. The stressed neutrophils exhibited the absorbances 0.662 ± 0.01 for H_2O_2, 0.578 ± 0.04 for *E.coli* and in case of H_2O_2 and *E.coli* given together the value was 0.63 ± 0.003 (Fig.2).

Presence of *Ls* or *Cp* had scavenged the perhydroxyl radical formed in stressed neutrophils within 30 min and almost completely neutralized within 60 min (*Ls* 0.736 ± 0.01; *Cp* 0.735 ± 0.007). Stressed neutrophils were partially neutralized by *Lc* (0.7 ± 0.009) or indomethacin (0.649 ± 0.01) in 60 min (Fig.2). Statistical analysis revealed that presence of *Ls*, *Cp* and *Lc* in stressed neutrophils enhanced the CAT activity significantly compared to control ($P < 0.05$).

Catalase activity (in presence of Amplex® Red)

The excess of H_2O_2 formed in unstressed neutrophils was measured as absorbance and found to be 0.108 ± 0.005 which acted as its control. However, interaction with stressors like *E.coli*, H_2O_2 or both together, the presence of H_2O_2 in neutrophils increased to 0.166 ± 0.01, 0.161 ± 0.02 and 0.15 ± 0.01, respectively. On addition of cucurbit extract or indomethacin the presence of excess of H_2O_2 was found to decrease; maximum effect was observed by addition of *Ls* (0.124 ± 0.008) followed by *Cp* (0.134 ± 0.01), *Lc* (0.136 ± 0.005) and indomethacin (0.146 ± 0.003) as depicted in Fig.3. The statistical analysis by ANOVA revealed that the presence of *Ls* *Lc* and *Cp* in stressed neutrophils enhanced the CAT activity significantly compared to control ($P < 0.05$).

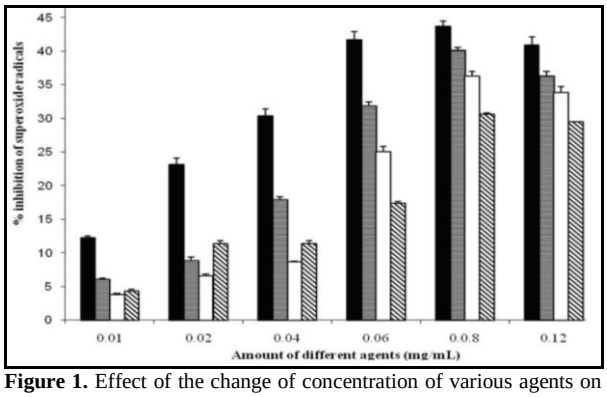

Figure 1. Effect of the change of concentration of various agents on inhibition of superoxide radicals generated in stressed neutrophils The data were averaged for three independent experiments. The *Ls* and *Cp* were highly significant in scavenging superoxide radicals than control ($P < 0.05$). [The agents in columns are listed in following order; indomethacin, *Ls*, *Cp*, *Lc*.]

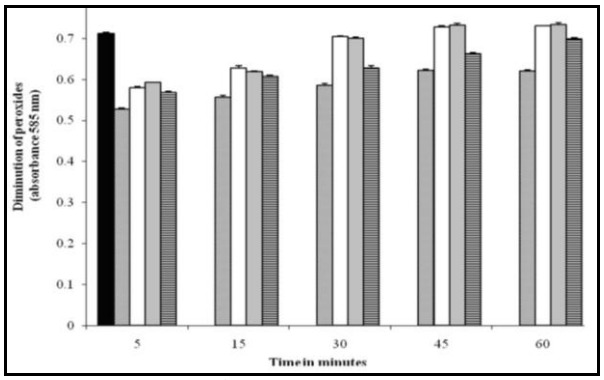

Figure 2. Diminution of peroxide radicals by CAT (using xylene orange method) generated in neutrophils stressed by addition of a combination of H_2O_2 or *E.coli* or a combination of the two in equal quantities of the culture medium in equal volume. The modulatory effect of various cucurbit extracts and indomethacin was observed at different post-treatment time. Data from three independent experiments were averaged. The *Ls*, *Cp* and *Lc* were significant in scavenging peroxide radicals compared to the control ($P < 0.05$). [The black column represents the control group; for the other columns, the order is the same as in Fig.1.]

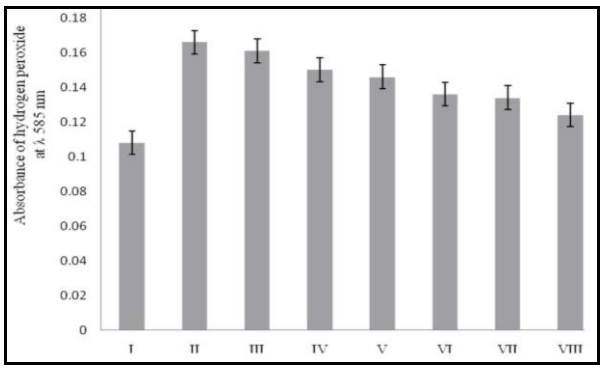

Figure 3. Estimation of CAT activity generated in neutrophils due to the addition of stressors H_2O_2 or *E.coli* individually or their combination in equal quantity. Data from three independent experiments were averaged. The *Ls*, *Cp* and *Lc* were significantly effective in scavenging peroxide radicals than control ($P < 0.05$). [I, double control (un-stressed neutrophils); II, *E.coli*; III, H_2O_2; IV, H2 H_2O_2 + *E.coli* (control); V: H_2O_2 + *E.coli* + indomethacin; VI, H_2O_2 + *E.coli* + *Cp*; VII, H_2O_2 + E.*coli* + *Lc*; and VIII, H_2O_2 + *E.coli* + *Ls*]

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COX-1 and COX-2 inhibition

Fig.4 depicts the IC_{50} values for various agents studied here. Indomethacin rendered IC_{50} for COX-1 as 0.0154 mg/µl, followed by *Cp* (0.026 mg/µl), *Lc* (0.0337 mg/µl) and *Ls* (0.0335 mg/µl). Similarly, as to see in Fig.5, indomethacin revealed an IC_{50} for COX-2 as 0.0427 mg/µl, followed by *Cp* (0.023 mg/µl), *Lc* (0.0183 mg/µl) and *Ls* (0.015 mg/µl).

Statistical analysis revealed that the IC_{50} of COX-1 by *Ls*, *Lc* and *Cp* were 2.17, 2.18 and 1.68 times less effective than indomethacin. However the IC_{50} of COX-2 by *Ls*, *Lc* and *Cp* were 0.35, 0.428 and 0.53 times more effective than indomethacin ($P < 0.05$).

***In vivo* studies in LPS induced mice serum**

The specific cytokines involved in inflammation were studied in response to the administration of cucurbit extracts orally. Various assays were done to evaluate the concentration of IL-1β and TNF-α in LPS-inflamed mice serum.

IL-1β: the amount of IL-1β in control (mice serum) was 260 pg. However, IL-1β measured in serum of mice which were subjected to inflammation by LPS administration (*i.p.*) revealed that treatment with *Cp*, *Lc*, *Ls* or indomethacin individually was 126, 68, 52 or 75 pg, respectively, as displayed in Fig.6. Each experiment was performed three times independently and statistical analysis revealed that *Ls* was most effective in reducing the expression of IL-1β followed by *Lc*, indomethacin and *Cp* than control ($P < 0.05$).

TNF-α: the amount of TNF-α quantified in control (mice serum) was 46 pg. However, the mice which were administered LPS, revealed that treatment with *Cp*, *Lc*, *Ls* or indomethacin rendered 26, 16, 12 or 20 pg of TNF-α, respectively, as displayed in Fig.7. Each experiment was performed three times independently and statistical analysis revealed that *Ls* was most effective in reducing the expression of TNF-α followed by *Lc*, indomethacin and *Cp* than control ($P < 0.05$).

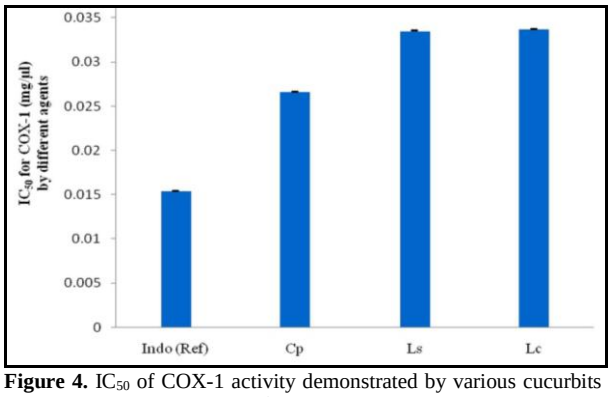

Figure 4. IC_{50} of COX-1 activity demonstrated by various cucurbits or indomethacin. The data of three independent experiments were averaged. The indomethacin was highly significant ($P < 0.05$) than *Lc* and *Ls* for inhibiting COX-1.

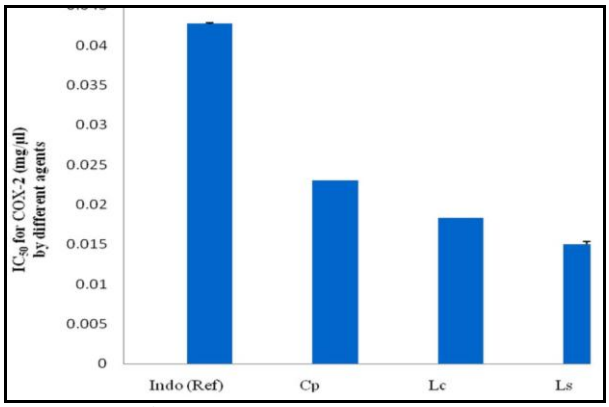

Figure 5. IC_{50} of COX-2 activity demonstrated by various cucurbits or indomethacin. The data of three independent experiments were averaged. The *Ls*, *Lc* and *Cp* were highly significant ($P < 0.05$) than indomethacin for inhibiting COX-2.

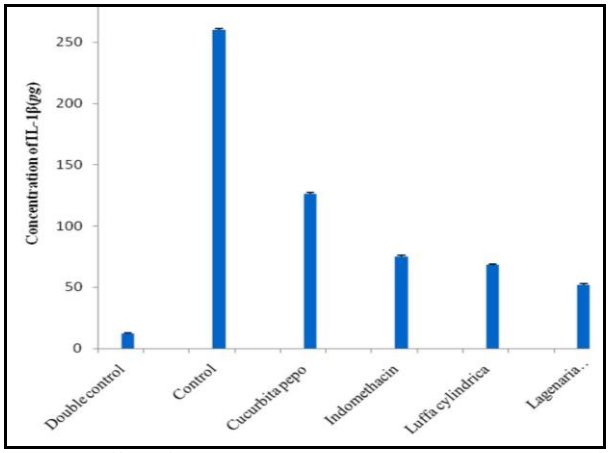

Figure 6. Effect of cucurbits and indomethacin on the expression of IL-1β. These agents were administered to LPS-inflamed serum of the data represented to average of three independent experiments. *Ls* was highly significant in reducing the expression of IL-1β followed by *Lc*, indomethacin and *Cp* than control ($P < 0.05$).

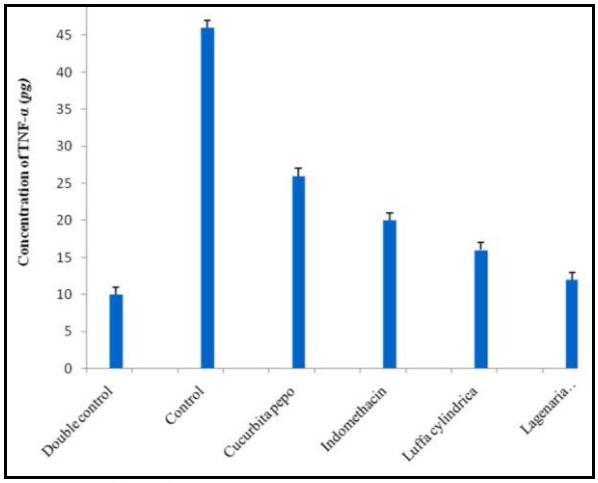

Figure 7. Effect of cucurbits and indomethacin on the expression of TNF-α. These agents were administered to LPS-inflamed serum of the data represented to average of three independent experiments. *Ls* was highly significant in reducing the expression of TNF-α followed by *Lc*, indomethacin and *Cp* than control ($P < 0.05$).

Table 1. Total Phenols and flavonoids of various cucurbit extracts

Cucurbit species	Total phenols (mg/ml)	Total flavonoids (mg/ml)	Flavonoids (% of total phenols)
Lagenaria siceraria	0.256 ± 0.005	0.052 ± 0.003	20%
Luffa cylindrica	0.295 ± 0.007	0.042 ± 0.001	14%
Cucurbita pepo	0.234 ± 0.003	0.018 ± 0.001	7%

DISCUSSION

The oxidative stress to an organism is responded by scavenging or neutralization of oxidative radicals with the help of endogenous antioxidants such as SOD, GPx and CAT [24]. In the gut the prebiotic microbiota remains in a dynamic state and keeps generating oxidative species. The prebiotics interact with the oxidative and antioxidant molecules in the metabolome and in the body. The cucurbit extracts (prebiotics) display high antioxidant activities through variations in hydroxylic groups and spatial arrangement of phenolic compounds could be looked in for explaining variations in the inhibition potential of oxidative radicals (Table 1). The total phenolic contents of *Lc* were higher than that of *Ls*. However in *Ls* the higher antioxidant activity may be attributed to the increased proportion of flavonoids as compared to *Lc* and *Cp* (Table 1). The free radical scavenging efficacy of various cucurbits as seen above may be attributed to the differences in the genetic composition also.

The addition of cucurbits extract to the stressed neutrophils led to enhancement of free radical scavenging activity (Fig.1). This activity of cucurbits may be attributed to the phenolic compounds like flavonoids [25-28] which are known to act as antioxidants because of the donation of an electron or hydrogen atom [29]. The increase in concentrations of different cucurbit extracts (from 0.01 to 0.08 mg/ml) quenched the superoxide radicals in stressed neutrophils in a proportionate manner. Further increase in the concentration of cucurbit extracts (0.12 mg/ml) however did not inhibit the superoxide radicals in an increasing order. It appears that active biomolecules up to in 0.08 mg/ml of the cucurbit extracts scavenged superoxide radicals in stressed neutrophils almost completely (Fig.1). At 0.12 mg/ml the presence of some other molecules in the whole extract did not enhance the effect and rather decreased in this context bringing down the SOD activity in comparison to the 0.08 mg/ml concentration.

The CAT activity in neutrophils induced by *E.coli* was more pronounced in terms of peroxides in comparison to the one induced by hydrogen peroxide or *E.coli* alone or in combination (Fig.2). In fact, hydrogen peroxide is a known per-oxidizing agent [30]; yet, in the present study it evoked much less effect than was done by *E.coli*. The stress generated by the presence of *E.coli* (LPS) augmented the production of endogenous CAT (Fig.2) for neutralizing the perhydroxyl radicals. In stressed neutrophils, neutralization of perhydroxyl radicals by endogenous catalase could not be achieved completely within 15 or 30 min (post-treatment). The addition of cucurbits to neutrophils at this concentration of hydrogen peroxide increased the CAT activity for scavenging of perhydroxyl radicals. This study also indicates that some biomolecules present in cucurbit extracts (*Ls, Cp, Lc*) could have enhanced the scavenging activity of perhydroxyl radicals independently or in synergy with catalases [31]. Our results indicate that for production of endogenous CAT there has to be some time delay; therefore, up to 30 min the scavenging of perhydroxyl radicals displayed an increasing trend. However this activity becomes stable at 45 min and beyond. Bioactive molecules may require some time to activate a phenomenon and this time dependence varies from product to product. Many bioactive molecules have been shown to act in the time dependent manner [32].

The Amplex® Red assay also corroborates the results of CAT activity as shown above. The activity of CAT was exhausted in the process of perhydroxyl radicals scavenging. However, addition of cucurbits (*Ls*, *Cp* and *Lc*) enhanced the peroxide scavenging activity further (Fig.3) in a more effective manner than indomethacin and thus decreased the peroxide stress. The study implies that *Ls* has maximum flavonoid contents (Table 1) out of the three cucurbits studied here. Therefore *Ls* had shown maximum anti-peroxide activity. *Cp* though had less flavonoid contents, yet enhanced the catalase activity comparable to *Ls* and it implies that molecules other than flavonoids present in *Cp* could have also enhanced the CAT activity.

The cyclooxygenases form another set of important bioactive molecules: COX-1, a constitutive and cyto-protective enzyme; and COX-2, an inducible enzyme regulated by various cytokines are two important cyclooxygenases. COX-2 leads to the formation of prostaglandins which further induce the inflammatory response [33]. The results revealed that indomethacin inhibits COX-1 [34] substantially more than the one manifested by administration of *Ls*, *Lc* and *Cp*. The cucurbits selectively inhibited COX-2 activity more efficiently than indomethacin. Therefore, cucurbits can provide very useful clues towards the production of anti-inflammatory agents for the cure of chronic inflammatory disorders.

LPS induces free radical generation which activates the cellular kinase cascades [3]. Activated kinases

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phosphorylate IκB, a nuclear factor (NF)- κB inhibitor, and detach IκB from the IκB/NF-κB complex. The released NF-kB acts as a transcription factor and induces the expression of many cytokines such as IL-1β and TNF-α, and prostaglandins [35]. For the present investigations, gut was used as a model system for inflammation because we intended to study the anti-inflammatory effects of some dietary cucurbits in the GI tract.

Administration of LPS (20 µg/100 µl) to mice (Figs.6&7) induced the production of IL-1β (260 pg) and TNF-α (46 pg). These indicated that immuno-competent cells (macrophages, lymphocytes, *etc*) followed the caspase-1 pathway which induced the production of IL-1β. The secretion of IL-1β and TNF-α behave independently and their regulatory control with respect to each other could not be evidently observed. Post-treatment with *Ls* significantly reduced the expression of both IL-1β (52 pg) and TNF-α (12 pg). This effect may be attributed to the presence of various phenolic compounds like flavonoids, triterpenoids (cucurbitacin), *etc*. The *Ls* acts as a primary free radical scavenger and thus inhibits various inflammatory molecules like cytokines (IL-1β, IL-6, TNF-α) and prostaglandins [6, 36, 37]. Therefore, the cucurbits need to be looked for reduction of gut inflammation and related disorders.

The above study concluded that commonly consumed dietary cucurbits (fruits) are rich in various pharmacologically active molecules like phenols, flavonoids, terpenes and saponins having a wide spectrum of biological functions and may be exploited for developing non-toxic anti-inflammatory and antioxidant products.

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