### MATERIALS

Chemicals used for the present studies were obtained from the following sources

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agarose</td>
<td>Koch Light Laboratories</td>
</tr>
<tr>
<td>Bathocuproine disulphonate</td>
<td>Aldrich Chemical Co., USA</td>
</tr>
<tr>
<td>Bovine serum albumin</td>
<td>Sigma Chemical Co., USA</td>
</tr>
<tr>
<td>Catalase</td>
<td>Sigma Chemical Co., USA</td>
</tr>
<tr>
<td>Chrysin</td>
<td>Sigma Chemical Co., USA</td>
</tr>
<tr>
<td>Deoxyribonucleic acid (Calf thymus type I)</td>
<td>Sigma Chemical Co., USA</td>
</tr>
<tr>
<td>5-5-Dithiobis-2-nitrobenzoic acid (DTNB)</td>
<td>Prepared in this laboratory according to the method of Baker</td>
</tr>
<tr>
<td>Diphenylamine</td>
<td>B.D.H., India</td>
</tr>
<tr>
<td>Ethylene diaminetetra-acetic acid</td>
<td>B.D.H., India</td>
</tr>
<tr>
<td>Genistein</td>
<td>Aldrich Sigma Chemical Co., USA</td>
</tr>
<tr>
<td>Glutathione</td>
<td>Sigma Chemical Co., USA</td>
</tr>
<tr>
<td>7-hydroxyflavone</td>
<td>Aldrich Sigma Chemical Co.,USA</td>
</tr>
<tr>
<td>6-hydroxyflavone</td>
<td>Aldrich Sigma Chemical Co.,USA</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>Sigma Chemical Co., USA</td>
</tr>
<tr>
<td>Luteolin</td>
<td>Carl Roth, GmbH, Karlsruhe, Germany</td>
</tr>
<tr>
<td>Nitroblue tetrazolium (NBT)</td>
<td>Sisco Research Labs., Bombay</td>
</tr>
<tr>
<td>Quercetin</td>
<td>Sigma Chemical Co., USA</td>
</tr>
</tbody>
</table>
Sodium azide  
Sodium benzoate  
S$_1$-nuclease  
Supercoiled plasmid pBR322  
Merck, Germany  
Merck, Germany  
Sigma Chemical Co., USA  
Prepared in this laboratory according to the procedure of Maniatis et al. (1982)  
Sigma Chemical Co., USA  
Sigma Chemical Co., USA  
Fluka, Switzerland  
B.D.H., India  
All other chemicals were commercial products of reagent grade.
METHODS

Stoichiometric Titration of Cu(I) Production

The concentration of Cu(I) produced in the flavonoid-Cu(II) reaction mixture was determined by titrating with bathocuproine as follows: flavonoid (10 or 20 μM) in 10 mM Tris HCl, pH 7.4 was mixed with varying concentration of Cu(II) and 120 μl of 10 mM stock bathocuproine aqueous solution was added to a final bathocuproine concentration of 400 μM, in a total volume of 3.0 ml. Absorbance at 480 nm was recorded.

Reactions of Flavonoids and Cu(II) with pBR322 DNA

The reaction mixture (20 μl) contained 10 mM Tris HCl pH 7.5, 0.5 μg pBR322 DNA and 0.1 mM flavonoids in presence of 0.1 mM Cu(II). The mixtures were incubated at 37°C for time periods indicated in the legends after which 8-10 μl of the solution containing 50% glycerol (W/V), 40 μM EDTA and 0.05 % bromophenol blue (W/V) were added. The samples were electrophoresed on 1.4% agarose gels, which contained 0.5 μg/ml ethidium bromide.

S1 Nuclease Hydrolysis

The enzyme assay was done by estimating the acid soluble nucleotides released from DNA as a result of enzymatic digestion. The reaction mixture (0.5 ml) contained 10mM Tris HCl, pH 7.4 and 500 μg
calf thymus DNA (native, denatured or flavonoid treated DNA). The reactions were started by the addition of Cu(II) (0.1 mM). S1 nuclease digestion of the mixture was carried out in a total volume of 1.0 ml by addition of acetate buffer 0.1 M, pH 4.5, 1 mM ZnSO4 and 20-30 units of S1 nuclease. The reaction mixtures were incubated for 2 hours at 48°C and the reactions stopped by additions of 0.2 ml of 10 mg/ml bovine serum albumin and 1.0 ml of 14% perchloric acid. The acid soluble material was determined by the diphenylamine method (Schneider, 1957).

Detection of Superoxide Anion (O2−)

Superoxide anion was detected by the reduction of nitroblue tetrazolium (NBT) essentially as described by Nakayama et al (1983). A typical assay contained in a total volume of 3.0 ml, 50 mM potassium phosphate buffer, pH 7.8, 33 μM NBT, 0.1 mM EDTA and 0.06% triton X-100. The reaction was started by the addition of 40 μM flavonoids (quercetin; kaempferol; luteolin, chrysin, 7,8 dihydroxyflavone, 7 and 6 hydroxyflavone and an isoflavonoid, genistein). Immediately after mixing the absorbance at 560 nm was measured under various experimental conditions against a blank which did not contain flavonoids. To confirm the formation of O2−, superoxide dismutase (SOD) was introduced into the reaction mixture before adding flavonoids.

Estimation of Hydroxyl Radical (OH·)

The assay is based on the ability of OH· radical to hydroxylate (attack) aromatic rings and the measurement of hydroxylated products by
simple colorimetric method using salicylate (α hydroxybenzoate) as a
detector molecule (Richmond et al., 1981). The reaction mixture (2.0
ml) contained the following reagents at indicated concentrations: 2.0
mM salicylate, 0.1 mM EDTA, 0.1 mM Cu(II) and 150 mM KH₂PO₄.KOH
buffer pH 8.0. The reactions were initiated by adding 60 μM flavonoids
(quercetin, kaempferol, luteolin, chrysin, 7,8 dihydroxyflavone, 7
hydroxyflavone, 6 hydroxyflavone and genistein) and tubes were
incubated at room temperature for 2 hours. Reactions were stopped by
adding 80 μl of 11.6 N HCl and 0.5 gms NaCl, followed by 4.0 ml of
chilled diethylether. The contents were mixed by vortexing for 1
minute. Next, 3.0 ml of upper ether layer was pipetted out and evaporated
to dryness in a boiling tube at 40°C. The tubes were cooled and the
residue dissolved in 0.25 ml of cold distilled water to which the
following reagents were added in order stated: (a) 0.125 ml of 10% W/V
TCA dissolved in 0.5 N HCl. (b) 0.25 ml of 10% W/V sodium
tungstate in water. (c) 0.25 ml of 10% W/V sodium nitrite (freshly
prepared). After standing for 5 minutes, 0.5 ml of 0.5 M KOH was
added and absorbance at 510 nm was read exactly after 1 minute.

Estimation of H₂O₂

H₂O₂ was determined quantitatively according to the method of Hozumi
(1969) with modifications. A sample containing approximately 0.2
μmol of flavonoid in 0.5 ml dimethyl sulfoxide, 1.5 ml distilled water
and 2 ml of 50 mM sodium phosphate buffer, pH 7.2 were mixed
vigorously for 1 minute at room temperature. The reaction mixture was
centrifuged at 9000 rpm for 10 minutes. One half milliliter of the
solution was added rapidly either to 2 ml of 1% titanium sulphate
solution in 2.5 N H$_2$SO$_4$ (as a sample) or to 2 ml of 2.5 N H$_2$SO$_4$ (as a blank) and the absorbance was determined at 410 nm. The amount of H$_2$O$_2$ was calculated from a calibration curve of H$_2$O$_2$.

**Spectroscopy:**

The absorption spectra were obtained by using Beckman DU-40 spectrophotometer. All spectrophotometric experiments were done at pH 7.4 and ambient temperature.

**Determination of GSH:**

Glutathione was determined by reaction with 5-5' dithiobis-2-nitrobenzoic acid (DTNB) and reading the absorbance at 412 nm as described by Sedlak and Lindsay.

**Synthesis of 7-8 Dihydroxyflavone**

7,8-Dihydroxyflavone was synthesised by the method of Baker. In outline, gallacetophenone (2,3,4-trihydroxyacetophenone) and excess benzoyl choride (24 h, room temperature in dry pyridine) yielded gallacetophenone tribenzoate, melting point (m.p) 118-119°C, which was stirred and recovered with dry K$_2$CO$_3$ in toluene (100°C for 6 h). The product was treated with boiling acetic acid/ethanol to yield 0,3,4-tribenzoyl gallacetophenone (m.p. 193-194). This is the "Baker rearrangement" in which a benzoyl group migrates from the 2-O to the less hindered acetophenone C. Ring closure was achieved by boiling with aqueous sodium acetate/acetic acid for 6 h; the intermediate (benzoyl
protected) product was not recovered and the solution was diluted with water and heated with methanolic KOH. The product, 7,8-dihydroxyflavone, was recrystallised from ethanol, m.p. 245°C (literature value 243°C). The structure was confirmed by 'H NMR (300 MHz) in DMSO-d$_6$: the spectrum was assigned as follows, δ6.87 (1H, s, H3), δ6.93 and δ7.43 (1H, d, J = 9Hz, H5 and H6), δ7.53 (3H, m, H3', 4', 5'), δ8.13 (2H, dd, J$_1$ = 9Hz, J$_2$ = 2, H2', H6'). δ9.5 (1H, s, OH7), δ10.4 (1H, s, OH8).