Protective effect of standardized extract of *Glycine max* seeds against experimentally induced gastroesophageal reflux disease in rats

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Gastroesophageal reflux disease (GERD) is a condition in which the stomach contents flow upward into the oesophagus. The molecules having flavonoid like structure reported to have radical scavenging activity and the antioxidant activity are useful in the treatment of GERD. Kaempferol is one such flavonoid found in plants including soybean. In the current study, we investigated the effect of standardized extract of *Glycine max* (soybean) seeds on gastroesophageal reflux disease in rats. The standardized extract (50-200 mg/kg) was administered orally twice daily for 5 days and omeprazole 1 h prior to the induction of gastroesophageal reflux disease. The levels of gastric wall mucus increased and of plasma histamine and H⁺-K⁺-ATPase significantly decreased in groups treated by standardized extract. The standardized extract reduced lipid peroxidation and superoxide dismutase and increased the levels of catalase and reduced glutathione. The results suggest that the *Glycine max* seeds are effective against gastroesophageal reflux disease and it protects esophageal in rats.

**Keywords:** Antisecretory, GERD, Kaempferol, Soybean

Gastroesophageal reflux disease (GERD) is caused by the ascent of gastric or gastroduodenal content above the gastroesophageal junction, which causes esophageal lesions that affect the health and quality of life of individuals¹,². On the other hand, flavonoids, a class of secondary phenolic compounds found scattered in nature and concentrated mainly in fruits, vegetables, wines, teas and cocoa, have been reported for radical scavenging activity; antioxidant activity, such as glutathione-peroxidase, glutathione reductase, superoxide dismutase and catalase; and prevention of lipid peroxidation and subsequently preservation of membrane integrity³,⁵.

Until now, the structure-activity relationship of flavonoid against GERD was not elucidated. Here, we tried to achieve a structure-antioxidant activity relationship of flavonoids. Literature reveals the correlation between flavonoids structure and their radical scavenging activity. It seems to depend robustly on the molecular structure, the number and the substitution pattern of hydroxyl groups on the benzene ring (Fig. 1)⁴,⁵. The occurrence of hydroxyl groups in ring A and C are more concerned in the process of a radical-capturing than freshly thought⁶.

The C2-C3 double bond conjugated with a 4-keto group in ring C, which is responsible for electron delocalization from the ring B, increases the radical scavenging activity⁷. The occurrence 5-OH group in ring A also enhances the radical scavenging activity of flavonoids⁸. Earlier, cell-free studies have demonstrated the capacity of flavonoids to trap free radicals⁹. In addition, flavonoids prevent lipid peroxidation level in egg-laying chickens⁹.

*Glycine max* L. Merrill (Soybean) is an imperative legume, has high protein content with nutritionally balanced amino acid profile and considered a chief part of diet worldwide. Its consumption lessens the risk of cancer by modulating the detoxifying and antioxidative enzymes¹⁰. It has biologically active phytoconstituents such as vitamin E, coumestrol, lecithin, phytosterols, phytate, saponins and isoflavones that are beneficial for health and

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Fig. 1—Chemical Structure of kaempferol
protective against oxidative stress\textsuperscript{11}. Its phytoestrogens are known to have estrogenic, anticarcinogenic, antiviral, antifungal, antisteoporotic and antioxidant activity. Phytate and saponins have been examined to show antioxidant and anticarcinogenic properties\textsuperscript{12}.

\textit{G. max} seeds also have been investigated for free radical scavenging and antioxidant activity, such as total superoxide dismutase, catalase, and glutathione peroxidise\textsuperscript{13}. However, there are no reports on the role played by \textit{G. max} seeds on GERD. \textit{G. max} seeds are known to contain the flavonoid kaempferol. In the current study, we explored the effect of kaempferol standardized extract of \textit{Glycine max} seeds on gastroesophageal reflux disease in rats.

### Materials and Methods

#### Plant material and extraction

Seeds of \textit{Glycine max}, cultivated under uniform cultural conditions at the National Research Centre for Soybean, Khandwa Road, Indore, Madhya Pradesh (India), were procured, dried, powdered (40-mesh) and stored in polythene bags. Powdered samples (200 g) were extracted thrice with 65\% methanol (HPLC grade) containing 2 g/L TBHQ at 70°C on a waterbath using soxhlet extractor for 3 h and filtered, concentrated on rotavapour (Buchi, USA) to get an aqueous extract containing flavonoids.

#### Phytochemical screening and HPTLC analysis

Methanolic extract of \textit{G. max} seeds was screening done for presence of phenolic compounds, tannin, saponins and flavonoids. HPTLC analysis was processed on pre-activated (100°C) Aluchroep silica gel 60F254 HPTLC plates (S.D. Fine-Chem Ltd, Mumbai, India) along with kaempferol. Plates were eluted in solvent system toluene:ethyl acetate:methanol:formic acid (6:4:0.5:1) for flavonoids. The isolated flavonoid was characterized on the basis of phytochemical analysis (Shinoda test, zinc hydrochloride reduction test) and spectroscopic studies. (UV, IR, Mass and \textsuperscript{1}HNMR).

#### Isolation and characterization of flavonoids

The resulted aqueous extract was subsequently extracted thrice with petroleum ether, diethyl ether and ethyl acetate in a separating funnel. Petroleum ether fraction (Fr-I) was discarded (due to presence of fatty substances, diethyl ether fraction (Fr-II) was used for analysis of free flavonoids and ethyl acetate fraction (Fr-III) was hydrolyzed (acid hydrolysis) to cleave glycosides by refluxing with 7\% H\textsubscript{2}SO\textsubscript{4} (10 ml/g plant material) for 2 h at 85°C for analysis of bound flavonoids. Resultant mixture was filtered and re-extracted thrice with ethyl acetate. All ethyl acetate layers were pooled together and neutralized by adding 5\% NaOH. Then diethyl ether fraction and ethyl acetate fraction were evaporated in rotavapour to 1/10 of the initial volume, dried in lyophilizer, weighed and stored at −19°C until it was used. Completion of acid hydrolysis of ethyl acetate fraction was confirmed by spraying agent (i.e. 5\% Fehling solution and 1\% AlCl\textsubscript{3} solution) during TLC. Flavonoids, free from sugar fraction reacted with the spraying agent and gave colour reactions during TLC analysis while flavonoids with sugar part didn’t react with the spraying agent and didn’t give any colour reaction during TLC analysis. Diethyl ether fraction gave colour reaction with spraying agent and it didn’t need acid hydrolysis.

The ethyl acetate fraction of \textit{G. max} seeds (GMEF) had the highest amount of flavonoids. Hence, 15 g of this fraction was chromatographed over silica gel column to obtain purified fractions using various mobile phases in increasing polarity. Flow of mobile phase was maintained at 6 drops/min. TLC analysis of column chromatography (CC) fractions were carried out on silica gel plates using EtOAC-MeOH-H\textsubscript{2}O (65-10-15) as a mobile phase. Flavonoid spots were visualized under UV lamp and also by staining with iodine vapour. Chromatographically identical fractions were combined and concentrated. Main flavonoids of each fraction group was further purified by preparative TLC on silica gel using toluene:ethyl acetate:formic acid:methanol (6:4:1:0.5) which resulted in isolation of flavonoids. The isolated flavonoid was characterized on the basis of phytochemical analysis (Shinoda test, Zinc hydrochloride reduction test) and spectroscopic studies. (UV, IR, Mass and \textsuperscript{1}HNMR).

#### Animals

Wistar rats (100-150 g) of either sex were purchased from the animal house of the National Laboratory Animal Centre, Lucknow, India. They were put under controlled conditions of temperature 24±5°C and relative humidity 40-46\%, light/dark cycles of 12 h, respectively for one week before and during the experimental study. They were given standard rodent pellet diet (Amrut, India) and the food was withdrawn 18-24 h before the experiment though water was allowed \textit{ad libitum}. All experimental works were performed in accordance with the guide for the care and use of laboratory animals, as approved and promoted by the Institutional Animal Care Committee, CPCSEA, India (Reg. No. 1732/GO/Re/S/13/CPCSEA).
Induction of GERD and treatment
GERD model was induced in Wistar rats according to methods described by Rao & Vijayakumar. According to this method, rats were fasted for 24 h under pentobarbitone sodium anesthesia (50 mg/kg, i.p.), the abdomen of the animal was opened by a median incision of about 2 cm; then the transitional region between the fore stomach and corpus was ligated very carefully with a 2-0 silk thread, and continuously the pyloric portion was ligated. A longitudinal cardiomiotomy (1 cm length) across the cardiac sphincter was performed to enhance reflux from the stomach into the oesophagus (Fig. 2 A and B). Immediately, the incised regions were sutured and the animals were kept in recover chamber (Medi HEAT, UK) and returned to their home cages. After 6 h, the animals were sacrificed by cervical decapitation and the chest was opened with a median incision and the tissue oesophagus and stomach were removed. The tissue organs were opened along the greater curvature of the stomach, and the oesophagus was dissected out by extending the dissection line along the major axis. The tissues were washed with physiological saline and were examined for GERD. GMEF in doses of 50, 100 and 200 mg/kg were administered orally twice daily at 10:00 and 16:00 h, respectively, for 5 days and kaempferol (100 mg/kg) or OMZ in the dose of 30 mg/kg one hour prior to the induction of GERD. Control groups received suspension of 1% carboxymethyl cellulose (CMC) in distilled water (10 mL/kg).

Estimation of histamine
The animals were sacrificed by cervical dislocation, their abdomen opened with a median incision and the blood was collected from the supraorbital plexus using microcapillary technique and plasma was separated. The separated plasma was treated with 0.2 M perchloric acid and centrifuged at 10000×g for ½ h at 4°C. Then, the clear supernatant was used for determination of histamine content by HPLC and expressed as IU/mg protein.

Assay of H+-K+-ATPase
The H+-K+-ATPase activity was assayed in medium consisting of 70 mM Tris-HCl buffer, pH 6.8, 5 mM MgCl₂ and enzyme solution in the presence of 10 mM KCl in a total volume of 1 mL, and incubated for 1 h. The reaction was initiated by adding 2 mM ATP Tris salt. The reaction was terminated by adding 10% trichloroacetic acid after incubation for 20 min at 37°C. After centrifugation, 2.5 mL ammonium molybdate and 0.5 mL 1-amino-2-naphthal-4-sulfonic acid were added to the supernatant and the absorbance was read at 620 nm. Results were expressed as mmol of Pi liberated/min/mg protein.

Estimation of gastric wall mucus
Gastric wall mucus was measured by the method of Hung et al. After removal of glandular segments from stomach, it was weighed and incubated in tubes containing 0.1% Alcian blue solution (a solution of 0.16 M sucrose and 0.05 M sodium acetate with pH adjusted to 5.8 with hydrochloric acid) for 2 h. The Alcian blue binding extract was centrifuged and the absorbance of supernatant was measured at 498 nm. The quantity of Alcian blue extracted (g/g of glandular tissue) was then calculated.

Antioxidant assay
Thiobarbituric acid reactive substances (TBARS), a measure of lipid peroxidation (LPO), was estimated by method and expressed as nmol MDA eq/g protein. Superoxide dismutase (SOD) activity was estimated by the inhibition of nicotinamide adenine dinucleotide (reduced)-phenazine methosulfate-nitrobluetetrazolium reaction system as adapted by Kakkar et al. and the results were expressed as units (U) of SOD activity/mg protein. The decomposition of H₂O₂ in the presence of catalase (CAT) was followed at 240 nm and one unit of catalase was defined as the amount of enzyme required to decompose 1µmol of H₂O₂ min⁻¹, at 25°C and pH 7.0 and results were expressed units (U) of catalase activity per mg protein. Reduced glutathione (GSH) was determined according to the method of Ellman et al. and expressed as nmol/g protein.

Statistical analysis
All the data were presented as mean ± SEM for six rats and were analyzed by one-way analysis of
variance (ANOVA) followed by Newmann Keuls test. \( P \) values <0.05 were considered as significant.

**Results**

**Phytochemical screening and HPTLC analysis**

Phytochemical results found the presence of flavonoids, tannins and phenolic compounds. HPTLC analysis showed the presence of kaempferol in ethyl acetate fraction of *Glycine max* seeds (GMEF) (Fig. 3).

**Isolation and characterization of flavonoids**

Elution of column with EtOAc:MeOH 9.9:0.1 (Fr. 17-19); EtOAc:MeOH 9.8:0.2 (Fr. 20-22); afforded yellowish mixture. Fr. 19-21 on preparative TLC on silica gel using toluene: ethyl acetate: formic acid: methanol (6:4:1:0.5) yielded two compounds, out of which one is identified as kaempferol (yield 35.02 µg/g) on the basis of spectroscopy (Fig. 4 A-D).

**Induction of GERD and treatment**

Gastroesophageal reflux disease (GERD) developed 6 h after the surgery in 100% of the animals. Administration of GMEF, significantly reduced oesophageal index from 1.95±0.09 to 1.17±0.11 and kaempferol and OMZ inhibited the oesophageal index to 1.12±0.50 and 0.95±0.17, respectively as compared to the control group (0.92±0.36). Effects of GMEF at dose of 50-200 mg/kg, twice a day for 5 days prevented GERD in a dose related manner. The GERD group resulted in the...
decrement in gastric wall mucus level (139.8±14.6) and increment in levels of plasma histamine (286.3±17.72) and H+-K+-ATPase (1.51±0.05). The gastric wall mucus level was increased (from 176.2±17.2 to 257.2±10.9 g/g wet glandular tissue) and level of plasma histamine (from 257.6±10.10 to 187.2±13.25 IU/mg protein) and H+-K+-ATPase were significantly decreased (from 1.13±0.04 to 0.49±0.08 mmol of Pi liberated/min/mg protein) in extract treated group. OMZ showed significantly enhancement in gastric wall mucus level (269.3±16.8 g/g wet glandular tissue) and decrement in levels of plasma histamine (191.5±16.56 IU/mg protein) and H+-K+-ATPase (0.46±0.07 mmol of Pi liberated/min/mg protein) (Table 1).

The lipid peroxidation is an indicator for the generation of reactive oxygen species (ROS) in the oesophageal tissue in rats. GERD induced animals showed elevation in lipid peroxidation (0.51±0.01 nmol MDA eq/g protein) and SOD (199.5±12.5 units of SOD activity/mg protein) and reduction in catalase (20.1±1.5 units of catalase activity per mg protein) and and GSH (42.9±2.1 nmol/g protein). GMEF @ 50-200 mg/kg significantly reduced the lipid peroxidation (from 0.47±0.03 to 0.41±0.07 nmol MDA eq/g protein) and SOD (from 168.8±18.5 to 95.7±14.8 units of SOD activity/mg protein) and increased in levels of catalase (from 23.6±1.8 to 31.5±1.7 units of catalase activity per mg protein) and GSH (from 45.1±3.6 to 57.6±4.3 nmol/g protein) (Table 2).

The UV spectrum of GMEF exhibited two major absorption bands at 365 and 264 nm, which confirmed the flavonol structure (Fig. 4A). The above mentioned spectral data were in close agreement with literature value of kaempferol. The melting point, phytochemical test and spectral data of IR, NMR, and Mass suggested that the isolated compound was flavonoid, kaempferol (Fig. 4 B-D).

**Discussion**

In the animal models of esophagitis as well as those on human esophageal tissue, ROS that are generated in the process of reflux esophagitis are responsible for esophageal tissue damage. These findings are further supported by studies which further interpret that such tissue damages could be prohibited with the use of antioxidants22. Free oxygen radicals in general and superoxide radical (O−2) in particular were revealed to raise in animals with esophagitis and it was claimed that free radical scavengers like superoxide dismutase (SOD) could stop the tissue damage23. Studies performed in adults with reflux esophagitis are in support of the experimental esophagitis models showing that free oxygen radicals do take part in the pathogenesis of reflux esophagitis24.

The investigative study on the structure-activity relationship (SAR) of the inhibition of lipid peroxidation by flavonols was started by characterizing the influence of substituents on the

<table>
<thead>
<tr>
<th>Groups/treatment</th>
<th>Oesophageal Index</th>
<th>Histamine (IU/milligram protein)</th>
<th>H+-K+-ATPase (mmol of Pi liberated/min/mg protein)</th>
<th>Gastric wall mucus (g/g wet glandular tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.92±0.36</td>
<td>185.7±11.51</td>
<td>0.44±0.03</td>
<td>262.9±12.2</td>
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<td>GERD</td>
<td>2.10±0.51</td>
<td>286.3±17.72</td>
<td>1.51±0.05</td>
<td>139.8±14.6</td>
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<tr>
<td>GMEF (50 mg/kg)</td>
<td>1.95±0.09</td>
<td>257.6±10.10</td>
<td>1.13±0.04</td>
<td>176.2±17.2</td>
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<tr>
<td>GMEF (100 mg/kg)</td>
<td>1.72±0.67</td>
<td>198.9±12.10</td>
<td>0.84±0.02</td>
<td>217.0±13.2</td>
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<tr>
<td>GMEF (200 mg/kg)</td>
<td>1.17±0.11</td>
<td>187.2±13.25</td>
<td>0.49±0.08</td>
<td>257.2±10.9</td>
</tr>
<tr>
<td>Kaempferol (100 mg/kg)</td>
<td>1.12±0.50</td>
<td>192.6±10.27</td>
<td>0.51±0.08</td>
<td>250.3±15.7</td>
</tr>
<tr>
<td>OMZ (30 mg/kg)</td>
<td>0.95±0.17</td>
<td>191.5±16.56</td>
<td>0.46±0.07</td>
<td>269.3±16.8</td>
</tr>
</tbody>
</table>

*Mean ± SEM; n=6; *P<0.001 vs. Control group; *P<0.01 and *P<0.001 vs. GERD group*

<table>
<thead>
<tr>
<th>Groups/treatment</th>
<th>LPO (nmol MDA eq/g protein)</th>
<th>CAT (units/mg protein)</th>
<th>SOD (units/mg protein)</th>
<th>GSH (nmol/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.40±0.08</td>
<td>32.6±1.6</td>
<td>96.2±8.3</td>
<td>62.7±3.5</td>
</tr>
<tr>
<td>GERD</td>
<td>0.51±0.01</td>
<td>20.1±1.5</td>
<td>199.5±12.5</td>
<td>42.9±2.1</td>
</tr>
<tr>
<td>GMEF (50 mg/kg)</td>
<td>0.47±0.03</td>
<td>23.6±1.8</td>
<td>168.8±18.5</td>
<td>45.1±3.6</td>
</tr>
<tr>
<td>GMEF (100 mg/kg)</td>
<td>0.44±0.02</td>
<td>26.5±1.3</td>
<td>127.2±20.5</td>
<td>51.3±2.7</td>
</tr>
<tr>
<td>GMEF (200 mg/kg)</td>
<td>0.41±0.07</td>
<td>31.5±1.7</td>
<td>95.7±14.8</td>
<td>57.6±4.3</td>
</tr>
<tr>
<td>Kaempferol (100 mg/kg)</td>
<td>0.38±0.03</td>
<td>32.6±1.2</td>
<td>99.5±13.7</td>
<td>50.3±2.2</td>
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<tr>
<td>OMZ 30mg/kg</td>
<td>0.42±0.05</td>
<td>30.6±1.1</td>
<td>103.5±8.6</td>
<td>57.1±3.1</td>
</tr>
</tbody>
</table>

*Mean ± SEM; n=6; *P<0.05 and *P<0.001 vs. Control group, *P<0.01 and *P<0.001 vs. GERD group*
activity of phenol. It was revealed that the nature of the substituents as well as its position determine the activity of flavonols. These findings can be explained by the different electron-donating effect of the various substituents at different positions.

Structure-activity analysis of flavonol molecule suggests that the polyhydroxylated substitutions on rings A and B and C2-C3-double bond conjugated with a 4-keto group in ring C would confer its antiperoxidative properties. The scavenging activity increases with the number of hydroxyl groups substituted in the ring B. It is suggested that the overall antioxidant activity of flavonoids on lipid peroxidation may be due to their hydroxyl radical (OH) and superoxide radical (O^{-2}) scavenging properties and the reaction with peroxy radicals (RO_{2}·)27. The antioxidant potential of the kaempferol on lipid peroxidation due to the metal chelation, proton radical and hydroxyl radical scavenging has been demonstrated by Singh et al.28.

In general, the balance of aggressive and defensive factors plays a pivotal role in integrity of gastrointestinal wall29. The aggressive factors encompass the rise in acid output and subsequent lipid peroxidation, which is due to the reaction between oxy-radicals and the polyunsaturated fatty acids. The defensive factors are gastroprotective in nature and involve the antioxidative enzymes; superoxide dismutase (SOD, superoxide-scavenging enzyme) which catalyses the dismutation of superoxide radical (O^{-}) into less noxious hydrogen peroxide (H_{2}O_{2}), and catalase (CAT) or glutathione peroxidase (GSH) that inactivate hydrogen peroxide (H_{2}O_{2}) to water (H_{2}O) and oxygen (O_{2})30.

It has been found that oxygen-derived free radicals are drawn in the mechanism of acute and chronic ulceration in the gastric mucosa and scavenging free radicals can play an appreciable role in healing ulcers. Histamine is widely distributed in the gastrointestinal tract in different cells and involves in the pathogenesis of gastroduodenal ulceration, gastric inflammation and gastric acid secretion whereas, a significant increase in plasma histamine concentration was observed after development of GERD. Earlier studies have revealed that flavonoids could stop the secretion of histamine. Antigen binding to the mast cell-attached immunoglobulin E (IgE) then triggers the mast cell to take action and this response results in histamine secretion. The flavonols significantly inhibited IgE, able to mediate histamine release in RBL-2H3 cells. Flavonoids are known to hinder the enzyme activity of histidine decarboxylase and lessen the formation of histamine in the gastric mucosa has reported that kaempferol significantly inhibited histamine release.

Flavonoids rich extracts have been screened for free radical scavenging and H'-K'-ATPase inhibitory activity in different in vitro models. The inhibitory potency of the flavonoids on the H'-K'-ATPase is attributed due to the presence and position of hydroxyl groups in flavonoids. H'-K'-ATPase inhibitory activity of flavonoids is due to action on the ATPase by competing with ATP binding.

The stomach has mucosa to line and defend the gastric wall from the acid. Without this gastric wall mucus, the stomach wall would be likely to such things as ulcers. Flavonoids show cytoprotective effects by stimulating the mucosal content of prostaglandins and mucus in gastric mucosa. It also treats gastric mucosal lesions produced by various models of experimental ulcer, and protects the gastric mucosa against different necrotic agents.

Lipid peroxidation is a natural process in small amount in the body system, primarily by the cause of numerous reactive oxygen species (hydroxyl radical, hydrogen peroxide, etc.). These ROS readily attack the polyunsaturated fatty acids of the fatty acid membrane, starting a self-propagating chain reaction and change in membrane lipid composition further aggravates gastric damage. The destruction of membrane lipids and the end-products of such lipid peroxidation reactions are especially dangerous for the viability of cells, even tissues. Enzymatic (catalase, superoxide dismutase) and nonenzymatic (vitamins A and E) natural antioxidant defence mechanisms do exist. Dismutation of superoxide anions by superoxide dismutase (SOD) interrupts the free radical chain reaction at the very beginning of the reaction and prevents reflux in oesophagus of rats.

In a recent study, we evaluated the implication of oxygen-derived free radicals in reflux esophagitis of human. Catalase is active in the cells and tissues throughout the body, where it breaks down hydrogen peroxide (H_{2}O_{2}) molecules into oxygen (O_{2}) and water (H_{2}O). At low level, H_{2}O_{2} is involved in chemical signalling pathways, but at high level, it produces toxicity in body cells. Catalase breaks down H_{2}O_{2} and stops production of ROS that can damage DNA, proteins, and cell membranes. Glutathione, a tripeptide...
(glutamyl-cysteinyl-glycine), is an extremely important cell protectant against damage by ROS. The cysteine provides an exposed free and very reactive sulphhydril group (SH), an abundant target for radical attack. This radical attack oxidizes glutathione; however its reduced form is regenerated in a redox cycle involving glutathione reductase and the electron acceptor NADPH. The role of oxygen derived free radicals in GERD has been reported in the induction of GERD in earlier animal studies.

The present study demonstrates that ethyl acetate fraction of Glycine max seeds extract (GMEF) have suppressive effect on gastric acid secretion by stimulation of gastric mucus secretion, blocking of H+-K+-ATPase and opposition to the action of histamine due presence of kaempferol, a flavonoids. Our observations indicate that the ethyl acetate fraction of G. max seeds have beneficial effect in GERD treatment.

Kaempferol [3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1- chromen-4-one], is a natural flavonol, found in a variety of plants and plant derived foods. The molecules with this type of structure have been reported for radical scavenging activity and the antioxidant activity. Therefore, kaempferol standardized extract may be used for treatment of gastroesophageal reflux disease.

Conclusion

It is concluded that molecular structure of kaempferol have been reported for radical scavenging activity, antioxidant activity, stimulation of gastric mucus secretion, blocking of H+-K+-ATPase and opposition to the action of histamine. Kaempferol standardized Glycine max seeds extract play a crucial role in gastric mucus secretion and suppression of gastric acid secretion. The results of the study prove that G. max seeds extract (GMEF) is effective against gastroesophageal reflux disease (GERD) and it protects esophageal in rats. The positive effect of the G. max seeds may be attributed to its anti-secretory and antioxidant potential, and it justifies the use of this seeds to treat gastroesophageal reflux disease.

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