ANTI-ULCER ACTIVITY OF APIGENIN -7- *O* - (6′′- *O* - CAFFEOYL) - β - D - GLUCOPYRANOSIDE ISOLATED FROM *COCCINIA GRANDIS* FLOWERS AGAINST PYLORIC LIGATION INDUCED ULCER IN ALBINO RATS

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Abstract : The isolation of Apigenin -7- *O* - (6′′- *O* - caffeoyl) - β - D - glucopyranoside was obtained from *Coccinia grandis* and the structure was established based on analysis of UV, ^{1}H NMR and ^{13}C NMR spectroscopy methods. Anti-ulcer activity of Apigenin -7- *O* - (6′′- *O* - caffeoyl) - β - D - glucopyranoside of *Coccinia grandis* was examined on pyloric ligated induced ulcer model in rats. The anti-ulcer activity of isolated compound from *Coccinia grandis* was estimated with the help of pH, gastric volume, free acidity, total acidity and ulcer index. The isolated compound showed substantial reduction in pH, gastric volume and ulcer index in dose dependent manner as compared to control and did not produce any toxic effects even at high doses.

***IndexTerms-Coccinia grandis,* Apigenin -7- *O* - (6′′- *O* - caffeoyl) - β - D - glucopyranoside, pyloric ligation.**

I. INTRODUCTION

Herbal medicine is a major constituent in all ethnic medicines and a common element in Ayurvedic, Homeopathic, Naturopathic, Traditional and Native Indian medicine. Demand for medicinal plants is increasing both in developing and developed countries. But 90% of them are collected from wild sources without applying scientific management hence many species is under risk to become extinct.[1] Phytochemistry developed from natural products chemistry is reserved to the study of products expanded by plants and it has developed as a distinct discipline between natural product organic chemistry and plant biochemistry in recent years. It compacts with the study of chemical structures of plant constituents, their biosynthesis, metabolism, natural distribution and biological functions.[2]

Coccinia grandis is a type of plant belonging to the family Cucurbitaceae.[3] It plays a major part in the medicinal properties. *Coccinia grandis* contain significant raw material for drug production like bioactive compounds such as secondary metabolite like alkaloids, glycoside and saponin, bamyrine, lupeol, cucubbitacin, cephalandrol, cephalandrine and flavonoids.[4] The plant parts of *Coccinia grandis* such as roots, leaves and fruits are used for numerous medicinal purposes like wound healing, ulcers, jaundice, diabetes and antipyretic.[5] The leaf retains hypoglycemic, anti-hyperglycemic, anti-oxidant properties and is also used to treat infective hepatitis.[6]

Ulcer is defined as the erosion in the lining of the stomach and is caused by the distractions of the gastric mucosal defense and repair organisms.[7] In recent years, there is a dynamic search to discover novel and alternative agents useful to contest gastric dyspepsia and peptic ulcer disease. Pyloric ligation induced ulcer signifies a unique ulcer model in examining the cause, course, consequence and treatment of peptic ulcer. Pylorus ligation induced ulcer is results of auto digestion of the gastric mucosal barrier possibly due to excess production and accumulation of HCl in the stomach.[8] By considering these above and other aspects the present study was assumed to evaluate the gastro protective effect of Apigenin -7- *O* - (6′′- *O* - caffeoyl) - β - D - glucopyranoside isolated from the flower extracts of *Coccinia grandis* against pyloric ligation induced gastric ulcer in rats.

II. MATERIALS AND METHODS

2.1. Extraction and fractionation

The fresh flowers (2 kg) of *Coccinia grandis* (Cucurbitaceae) collected from Thanjavur were extracted with 85% MeOH (6 X 500 mL) under reflux. The alcoholic extract was concentrated *in vacuo* and the aqueous concentrate successively fractionated

with peroxide - free ether (5 X 250 mL) and ethyl acetate (6 X 500 mL). The ether fraction did not yield any isolable solid and could not be studied further. The EtOAc fraction alone was taken up for the study.

***2.2. Ethyl acetate fraction* - Apigenin -7- *O* - (6′′- *O* - caffeoyl) - β - D - glucopyranoside**

The ethyl acetate fraction was concentrated ***in vacuo*** and left in an ice-chest for few days. A yellow solid (m.p. 240-242˚C) that separated was filtered and studied. It developed a green colour with alc. Fe^{3+} and orange red colour with Mg-HCl, yellow colour with NaOH and appeared as a purple spot under UV, turning yellow on exposure to NH_3. It responded to Wilson's boric acid, Molisch and Gibb's tests, but did not answer the Horhammer-Hansal test showing that it could be a flavone glycoside.[9] It had λ_{max}^{MeOH} nm 271, 283sh, 319; +NaOMe 279, 316sh, 364; +$AlCl_3$ 290, 313sh, 343sh, 357; + ($AlCl_3$ / HCl) 290, 312sh, 342sh, 357; +NaOAc 275, 381sh, 375; and + (NaOAc/ H_3BO_3) 275, 342. ^{1}H (500 MHz, $CDCl_3$) δ ppm: 7.614 (H-6), 7.611 (H-8), 7.622 (H-3), 7.811 (H-5′), 7.794 (H- 6′), 5.855 (H-1′′), 8.016 (H-7′′′), 8.015 (H-8′′′), 11.7 (5-OH), 11.5 (4′-OH), 3.00-3.8 (Rest of sugar protons); ^{13}C-NMR (125 MHz, $CDCl_3$) δ ppm: 164.47 (C-2); 103.0 (C-3); 181.9 (C-4); 161.54 (C-5); 98.51 (C-6); 162.23 (C-7); 94.01 (C-8); 157.10 (C-9); 104.60 (C-10); 121.0 (C-1′); 128.5 (C-2′); 116.0 (C-3′); 161.10 (C-4′); 116.24 (C-5′); 128.5 (C-6′); 101.78 (C-1′′); 73.23 (C-2′′); 76.79 (C-3′′); 70.14 (C-4′′); 77.3 (C-5′′); 62.31 (C-6′′); 124.8 (C-1′′′); 121.2 (C-2′′′); 115.31 (C-3′′′); 147.56 (C-4′′′); 115.20 (C-5′′′); 120.8 (C-6′′′); 114.87 (C-7′′′); 113.8 (C-8′′′); 167.51 (C-9′′′).

2.3. Hydrolysis of the glycoside

The glycoside (50 mg) was dissolved in hot aqueous methanol (5 mL; 50%) and an equal volume of H_2SO_4 (7%) was added to it. The reaction mixture was then refluxed at 100˚C for about 2 hrs. The excess of alcohol was distilled off *in vacuo* and the resulting aqueous solution was extracted with ether. The residue from ether fraction was studied as described below.

2.4. Identification of aglycone (flavone- Apigenin)

The Et_2O fraction was concentrated *in vacuo* and left in an ice chest for about a week. A yellow solid that separated was filtered and studied. It came out as pale yellow needles m.p. 238-240˚C on crystallization from MeOH. It was soluble in organic solvents and sparingly in hot water. It gave an orange-red colour with Mg-HCl, green colour with alc.Fe^{3+}, turning very bright yellow colour with NH_3. It answered Wilson's boric acid but did not respond to Horhammer-Hansal and Molisch's test. It developed a bluish green colour in the Gibb's test. It had λ_{max}^{MeOH} nm 270, 282sh, 317; +NaOMe 282, 343sh, 372; +$AlCl_3$ 290, 312sh, 342sh, 353; + ($AlCl_3$ / HCl) 290, 311sh, 340sh, 353; +NaOAc 274, 381sh, 374; and + (NaOAc/ H_3BO_3) 275, 340.

2.5. Animals

Male albino rats (200 - 250 g) of Wistar strain were procured from the animal house, Department of Animal Science, Bharathidasan University, Thiruchirappalli, Tamilnadu, India. Animals were fasted overnight and were divided into control, standard and different test groups each consisting of six animals. They housed in cages and maintained under standard conditions at 26 ± 2˚C and relative humidity 60 - 65 % and 12 h light and 14 h dark cycles each day for one week before and during the experiments. All animals were fed with the standard rodent pellet diet, and water adlibitum. Before starting the experiment on animals, the experimental protocol was subjected to the scrutiny of the Institutional Animal Ethics Committee (IAEC), Bharathidasan University, Trichirappalli, Tamilnadu, India (Approval No. BDU/IAEC/2011/31/29.03.2011).

2.6. Chemicals

Diethyl ether, Sodium hydroxide and omeperazole were purchased from Sigma chemical company, Mumbai, India. All other chemicals and reagents used in this study were of analytical grade with purity and were obtained from Glaxo laboratories and Sisco Research laboratories, Mumbai, India.

2.7. Pyloric ligation induced ulcer in rats

Animals were divided into three groups, each consisting of six rats. Group-1 treated as control group, received saline orally for 7 days. Group-2 treated as standard group was received Omeprazole 30 mg/kg for 7 days. Group 3 treated as treatment groups was received isolated compound (100 mg/kg) for 7 days respectively. Animals in all groups were fasted for 18 hours after the respective assigned treatment and were anaesthetized with diethyl ether at the dose of 35 mg/kg. Ligation was done without causing any wound to the blood supply of the stomach. Animals were allowed to recover and steady in individual cages and were deprived of water during postoperative period. After 4 hours of surgery, rats were surrendered and gastric contents were collected into the centrifuge tubes and centrifuged at 1000 rpm for 10 min and the pH, free acidity, total acidity of the gastric juice was determined.[10] In addition the ulcer index was determined by opening the stomach on greater curvature and scores were given 0 to 3 depending upon the austerity of ulcers.

2.8. Acute toxicity studies

Acute toxicity studies were carried out according to the literature.[11] Animals of either sex were fasted for eighteen hours and used. A dose of 100 mg / kg of isolated compounds from those our selected plants were administrated orally to 12 rats, additionally three rats were kept as control. The control group received distilled water. Then they were observed for 72 hours. Since no mortality was observed and the behavioral pattern was unaffected. No depth was observed at the end of the study.

2.9. Statistical analysis

All experiments were reported as means ± SD. Significant differences for multiple comparisons were determined by one-way analysis of variance (ANOVA) followed by Duncan test with p value less than 0.05 which was considered as statistically significant.

III. RESULTS AND DISCUSSION

Apigenin -7- *O* - (6′′- *O* - caffeoyl) - β - D - glucopyranoside has been isolated from the fresh flowers of *Coccinia grandis*. The UV spectrum of the glycoside exhibited two absorption maxima at 319 nm (band I) and 271 nm (band II) indicating to contain a flavone skeleton. A bathochromic shift of 45 nm in band I detected in its NaOMe spectrum indicated the presence of free OH

group at C-4′. The $AlCl_3$-HCl spectra of the glycoside as well as the aglycone consists of four major peaks which specify the presence of free -OH group at C-5 in both. It was also established by a bathochromic shift of 38 nm and 36 nm respectively in the glycoside and in the aglycone on the addition of $AlCl_3$-HCl. No change was detected in absorption characteristic in band II of the glycoside on the addition of NaOAc. The $AlCl_3$-HCl spectrum was exactly the identical as that of ($AlCl_3$-HCl) revealing the absence of catechol type of substitution in B-ring.

The ^{1}H NMR spectrum (500 MHz, $CDCl_3$) indicated the presence of two meta coupled aromatic doublets at δ 7.614 and 7.611 ppm corresponds to H-6 and H-8 protons. There are four –OH groups in the molecule among this –OH groups present in ring B and C appeared at δ 11.5 and 11.7 ppm respectively. The remaining two –OH groups present in ring D appeared at δ 10.4 ppm. Additionally one singlet signal exhibited at δ 7.622 ppm which has assigned to H-3 of flavone. However, additional resonances ascending from a D-glucose unit with typical signals at δ 5.85 ppm for anomeric proton and the rest of the sugar protons seem between δ 3.00-3.80 ppm (m, unresolved, pyranose protons).[12] ^{13}C-NMR signal (125 MHz, $CDCl_3$) seem at δ 181.25 ppm shows that >C =O at C-4. The sugar carbon signals showing at δ 101.78, 77.3, 76.79, 73.23, 70.14 and 62.31 ppm are similar with those reported for *O*- glucoside.[13] The ^{1}H NMR spectrum shown the presence of anomeric proton signal at δ 5.85 ppm designated the presence of *O*- linked sugar. It has predictable that the sugar moiety bonded to hydroxyl group at C-7 of the aglycone as expected from the correlation between the anomeric proton at δ 5.85 ppm and the C-7 at δ 162.23 ppm.[14] These aromatic protons of D - ring appeared in between δ 7.822 - 7.999 ppm and further these are confirmed by the appearance of respective carbon signals between δ 115.20 - 147.56 ppm. The ^{1}H-NMR spectrum also revealed two doublets, each for 1H, at δ 8.016 (H-7′′′) and 8.015 (H-8′′′). The large value of coupling constant showed the presence of trans-disubstituted ethylene moiety in the molecule. The ^{1}H and ^{13}C chemical shifts of olefinic protons and carbons [δ 114.87 (C-7′′′) and 113.80 (C-8′′′)] were equivalent to those of trans-cinnamic acid.[15] The ^{13}C chemical shifts of a carbon at δ 167.51 displayed the presence of carboxylic functional group in the molecule. The ^{13}C- chemical shifts of carbon atoms at δ 115.31 (C-3′′′), 147.56 (C-4′′′), designated that the hydroxyl group are attached at C-3′′′ and C-4′′′ positions. The position of ethylene function was determined by chemical shift of C-1′′′ carbon at δ 124.80 ppm and the downfield chemical shifts of C-7′′′ carbon and H-7′′′ proton of ethylene moiety. Based on their UV, ^{1}H-NMR and ^{13}C-NMR data's, the flavone glycoside obtained from ethyl acetate fraction of the flower from *Coccinia grandis* could be confirmed as Apigenin - 7- O - (6" - O - caffeoyl) - β -D- glucopyranoside (Fig. 1).

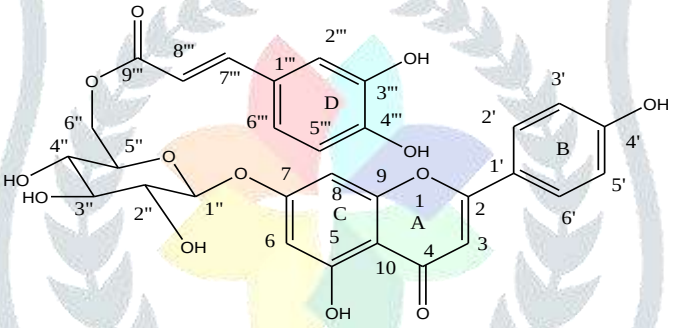
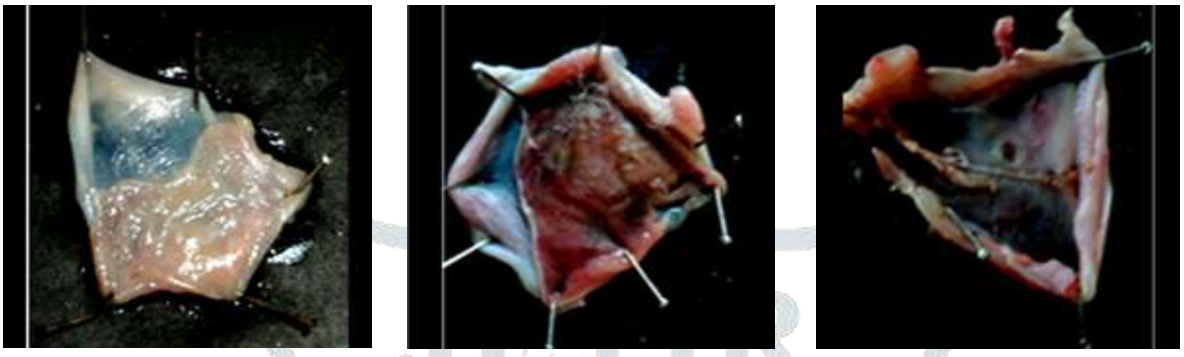


Fig. 1. Apigenin - 7- O - (6" - O - caffeoyl) - β -D- glucopyranoside

The ulcer formation in each of these models occurs by different mechanisms pylorus ligation-induced ulcers are caused by enhanced acid pepsin secretion leading to auto-digestion of the gastric mucosa and break down of the gastric mucosal barrier, and the digestive effect of accumulated gastric juice and interference of gastric blood circulation are responsible for the induction of ulceration.[16] The effect of orally administered four groups on gastric damage induced by pyloric ligation is shown in Table 1. It was detected that increase the ulcer lesion in ulcer control rats. Significant reduction in ulcer lesion was observed in treatment with four groups. It is significant to note that increased the volume, total acidity and free acidity and decreased pH of gastric juice were observed in ulcer control rats as compared to normal rats. Administration of isolated compounds decreased the volume, total acidity and free acidity and increased pH of gastric juice were observed as compared to control rats. Animal groups treated with the isolated compounds exhibited a reduction of gastric damage against pyloric ligation induced gastric ulceration. The percentage of ulcer protection was 64.87% for Group 3 was recorded. Omeperazole, the positive control included for the study also offered significant protection (95.80%) against pyloric ligation induced gastric ulcer. Figure 2 shows the photographic representation of control and experimentally induced ulcer model. The increase in volume in the ulcer control rats is undoubtedly due to increase protection of hydrochloric acid as evident from the total acidity and decrease pH value of gastric juice. In the present study, the decrease in volume of the gastric juice and concomitant decrease in the acidity and increase in pH proving the anti-ulcer activity of isolated compounds. Further evidenced by the reduced edema formation and epithelial lifting were observed in morphometric study. Therefore, it can be believed that the flavonoid glycoside may stimulate the secretion of prostaglandin or possess prostaglandin like substances (Fig.2). Gastro protective role for the flavonoid glycoside Apigenin - 7- O - (6" - O - caffeoyl) - β - D- glucopyranoside against gastric mucosal damage induced by pyloric ligation were investigated in the present study. Pyloric ligation induced gastric ulcer rats show increased gastric volume, acidity and depleted pH. The observed gastro protection is possible mediated to a major extent by a gastric mucosal secretion mechanism as the isolated compounds were able to restore the increased volume, acidity and depleted pH by pyloric ligation almost towards normal levels seen in control. This is further evidenced by morphometric study.

Table 1. Effect of Apigenin - 7- O - (6" - O - caffeoyl) - β -D- glucopyranoside on pH, volume, acidity, ulcer lesion in control and experimental rats

Groups	pH	Volume	Free acidity	Total Acidity	Gastric Ulcer lesion (No.)	% of Ulcer protection
Group I (Control)	2.9 ± 0.34	1.1 ± 0.13	226 ± 14.4	253 ±20.2	1.05± 0.03	-
Group II (Standard)	2.4± 0.14 [b]	1.2 ± 0.06 [b]	228 ± 8.4 [b]	257 ± 11.2 [b]	22± 0.28 [b]	95.80
Group III	2.2 ± 0.16 [b]	1.9± 0.06 [b]	227 ± 13.9 [b]	259 ± 16.8 [b]	3.07 ± 0.18 [b]	64.87



(a) **(b)** **(c)**

(a) Control; (b) Standard (Omeprazole): (c) Apigenin -7- *O* - (6′′- *O* - caffeoyl) - β - D - glucopyranoside

Fig. 2. Photographic representations of control and experimentally induced ulcer model

IV. CONCLUSION

Gastro protective role for Apigenin -7- *O* - (6′′- *O* - caffeoyl) - β - D - glucopyranoside from *Coccinia grandis* against gastric mucosal damage induced by pyloric ligation was investigated in the present study. Pyloric ligation induced gastric ulcer rats show increased gastric volume, acidity and depleted pH. The observed gastro protection is possible mediated to a major extent by a gastric mucosal secretion mechanism as the isolated compound Apigenin -7- *O* - (6′′- *O* - caffeoyl) - β - D - glucopyranoside was able to restore the increased volume, acidity and depleted pH by pyloric ligation almost towards normal levels seen in control.

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REFERENCES

[1] V.P. Kamboj, *Curr. Sci*., 78, 35, (2000).
[2] L.P. Miller, *Phytochemistry*, 1, (1973).
[3] G. Yadav, A. Mishra and A. Tiwari, *Int. Journal of Pharma. Research & Development*, 2, 974, (2010).
[4] M. Umamaheswari and T.K. Chatterjee, *Pcog. Res*., 1, 25, (2009).
[5] M. Gunjan, G.K. Jana, A.K. Jha and U. Mishra, *Int. J. Phytomed*., 2, 36, (2010).
[6] S. Rajalakshmi, N. Kalavathi, G. Sivanadam, R. Thyagarajan, M. Sundaram and G. Veluchamy, *J. Res. Ayurv. Siddha*, 1, 18, (1984).
[7] A. Anoop and M. Jegadeesan, *J. Ethnopharmacol*., 84, 149, (2003).
[8] K. Sairam, S. Priyambda, N.C. Aryya and R.K. Goel, *Journal of Ethanopharmacolgy*, 86, 1, (2003).
[9] M. Kaneta, *Fukushima Tech. Jr. Col. Bull*., 7, 40, (1971).
[10] T.B. Nguelefack, P. Watcho, S.L. Wansi, M.M. Nguelta, D. Ngamaga, P. Tane and A. Kamanyi, *African J. of Trad. Compl. and Alt. Medicine*, 2, 233, (2005).
[11] M.N. Ghosh, Fundamentals of Experimental Pharmacology, 3rd edition, Hilton and Company, Kolkatta, 190, (2005).
[12] R.M. Silverstein, F.X. Webster, Spectrometric identification of organic compounds, 6th edn., John Wiley, New York, (1998).
[13] M. Fairouz, Z. Amar, S. Narimane, T. Ahmed and R. Salah, *Rec. Nat. Prod*., 4, 91, (2010).
[14] R.W. Li, S.P. Myers, D.N. Leach, G.D. Lin and G. Leach, *J. Ethnopharmacol*., 85, 25, (2003).
[15] M. Kumar Roy, K. Nakahara, V. Nathalang, G. Trakoontivakorn, M. Takenaka, S. Isobe and Tsushidat T, *Pharmazie*, 62, 149, (2007).
[16] M. Aditi Chaturvedi, G. Mohan kumar, Bhavani, Harish Chaturvedi and R.K. Mohan Kumar Goel, *Indian J. Physiol. Pharmacol*., 51, 131, (2007).