New Apigenin Glycoside, Polyphenolic Constituents, Anti-inflammatory and Hepatoprotective Activities of Gaillardia grandiflora and Gaillardia pulchella Aerial Parts

Fatma A. Moharram¹, Rabab Abd El Moneim El Dib¹,², Mohamed S. Marzouk³,⁴, Siham M. El-Shenawy⁵, Haitham A. Ibrahim¹

¹Department of Pharmacognosy, Faculty of Pharmacy, Helwan University, Cairo, Egypt; ²Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Kingdom of Saudi Arabia; ³Department of Chemistry, College of Science and Humanities, Prince Sattam bin Abdulaziz University, B3 Ikha, Saudi Arabia; ⁴Chemistry of Natural Products Group, Center for Excellence for Advanced Sciences, National Research Center; ⁵Department of Pharmacology, National Research Center, Egypt

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ABSTRACT

Background: Gaillardia grandiflora Hort. ex Van Houte and Gaillardia pulchella Fouq are flowering plants widely cultivated in Egypt for their ornamental value. Previous reports demonstrated that sesquiterpene derivatives represent the major compounds in both species. Moreover, only few flavones were identified from genus Gaillarda and few studies on the cytotoxicity of G. pulchella were found. Aim of the Study: Investigation of the phenolic constituents of the aerial parts of both species and evaluation of their anti-inflammatory and hepatoprotective activities. Materials and Methods: The 80% aqueous methanol extracts (AME) were prepared for both plants and evaluated for their biological activities. Phytochemical investigation of both extracts resulted in isolation of twelve compounds, which have been identified on the basis of ultraviolet, 1D and 2D nuclear magnetic resonance spectroscopy and negative ESI-MS. Results: The new 8-hydroxyapigenin 6-O-β-D-apiofuranosyl-(1''→4)-Cβ-D-β-D glucopyranoside was isolated from G. grandiflora for the first time in nature, along with schaftoside, luteolin 6-Cβ-D-β-D-glucopyranoside 8-methyl ether, apigenin 6-Cβ-D-β-D-glucopyranoside 8-methyl ether, isoorientin, isovitexin, 6-methoxyapigenin and hispidulin, as well as vicenin-2, vitexin, luteolin and apigenin, which were isolated from G. pulchella together with 6-methoxyluteolin. Furthermore, the AME of both species were found to be nontoxic to mice and exhibited significant anti-inflammatory and hepatoprotective activities in dose dependent manner. Conclusion: Current results shed light on the phenolic constituents of G. grandiflora and G. pulchella aerial parts and the safety of the AME of both species, in addition to their significant anti-inflammatory and hepatoprotective activities. Both plant species may be promising candidates for natural anti-inflammatory and hepatoprotective drugs.

Key words: Anti-inflammatory, Asteraceae, flavone glycosides, Gaillardia grandiflora, Gaillardia pulchella, hepatoprotective

SUMMARY

• Phytochemical investigation of Gaillardia grandiflora and Gaillardia pulchella 80% aqueous methanol extracts of the aerial parts led to the isolation of twelve compounds
• The new compound 8-hydroxyapigenin 6-O-β-D-apiofuranosyl-(1''→4)-Cβ-D-β-D glucopyranoside was isolated from G. grandiflora for the first time in nature
• Schaftoside, luteolin 6-Cβ-D-β-D-glucopyranoside 8-methyl ether, apigenin 6-Cβ-D-β-D-glucopyranoside 8-methyl ether, isoorientin, isovitexin, 6-methoxyapigenin and hispidulin were isolated from G. grandiflora
• Vicenin-2, vitexin, luteolin and apigenin 6-methoxyluteolin were isolated from G. pulchella
• The extracts of both species were nontoxic to mice up to 5 g/kg body weight
• Both extracts exhibited significant anti-inflammatory and hepatoprotective activities in dose dependent manner.

Abbreviations used: ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AME: The 80% aqueous methanol extract of G. grandiflora or G. pulchella aerial parts; AST: Aspartate aminotransferase; br: Broad doublet; Comp-PC: Comparative paper chromatography; d: Doublet; D-2PC: Two-dimensional paper chromatography; DMSO-d6: Deuterated dimethyl sulfoxide; G.: Gaillardia; GPx: Glutathione peroxidase; GRd: Glutathione reductase; GSH: glutathione; GST: Glutathione-S-transferase; J: Nuclear spin-spin coupling constant; m: Multiplet; [M-H]-: Molecular ion peak; MDA: Malondialdehyde; m/z: Mass/charge ratio; NO: Nitric oxide; p: Probability; PC: Paper chromatography; Rf: Retention flow; rpm: Rotation per minute; s: Singlet; SDE: The ethanol extract of Scoparia dulcis; SE: Standard error; SOD: Superoxide dismutase; TMS: Tetramethyloxysilane; λmax: Maximum fluorescence emission wavelength.

Correspondence:
Dr. Rabab Abd El Moneim El Dib, Department of Pharmacognosy, Faculty of Pharmacy, Helwan University, 11795 Cairo, Egypt. E-mail: relsib@yahoo.com DOI: 10.4103/pm.pm_344_16

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INTRODUCTION

Gaillardia grandiflora Hort.ex Van Houte and Gaillardia pulchella Foug. (Asteraceae) are commonly known as firewheel, Indian blanket, Indian blanket flower, or sundance. They are short-lived flowering plants native to the Central United States[1] and are widely cultivated in Egypt for their ornamental value. Genus Gaillardia includes about 23 species, but only three of which are commonly known, these include G. pulchella, which is an annual plant, while G. grandiflora and G. arista are perennial plants.[2] Previous reports demonstrated that sesquiterpene derivatives represent the major compounds isolated from G. pulchella,[3,4] as well as few of which are identified from G. grandiflora.[5,6] Moreover, only few flavones were identified from genus Gaillardia[7,8] and there are few reports about the cytotoxicity of G. pulchella.[9,10] This was found to be encouraging to investigate the phenolic constituents of both species and to evaluate the anti-inflammatory and hepatoprotective activities of the aqueous methanol extract (AME) of G. grandiflora and G. pulchella aerial parts. The study resulted in isolation and structure characterization of several flavones from both extracts for the first time, supported by ultraviolet (UV), 1H and 13C nuclear magnetic resonance (NMR) and ESI-MS spectrometry.

MATERIALS AND METHODS

Plant material

G. grandiflora Hort.ex Van Houte and G. pulchella Foug aerial parts were collected during the flowering stage from El Azhar Park, Cairo, Egypt (March 2006 and April 2007). Authentication of the plant was performed by Terashe Labib, Senior Specialist of Plant Taxonomy and former Head of El Orman Botanical Garden, Giza, Egypt. Voucher specimens (No. G.G.1, G.P.1) have been deposited at the Pharmacognosy Department, Faculty of Pharmacy, Helwan University, Cairo, Egypt.

General experimental procedures

The NMR spectra were run at 300 and 500 (1H), 75 and 125 (13C) MHz, on Varian Mercury 300 and JEOL GX-500 NMR spectrometers, respectively. The chemical shifts (δ) are reported in ppm downfield to TMS in the appropriate deuterated solvent. For ESI-MS analyses, LCQ (Finnigan MAT 95, Bremen, Germany) and LTQ-FIT-MS spectrometers were used, while HR-ESI-MS (Thermo Electron, Finnigan, Germany) was used for HR-ESI-MS analyses. UV spectrophotometer (JASCO UV-630) was used for analysis of pure samples in MeOH and in different UV shift reagents.

Material for column chromatography (CC), including microcrystalline cellulose, polyamide S 6 and Sephadex LH-20, as well as Whatman No. 1 sheets used for paper chromatography were purchased from sources described in our previous literature.[13] Isolated compounds were detected using Naturstoff[14] and/or FeCl3 (1% in ethanol) spray reagents. Solvent systems S1 (n-BuOH/HOAc/H2O: 4:1:5 v/v/v top layer), S2 (HOAc/H2O: 15:85 v/v) and S3 (n-BuOH/iso-propanol/H2O: 4:1:5 v/v/v top layer) were used. Indomethacin was obtained from Epico, Egypt; paracetamol and silymarin from Sedico, Giza, Egypt. Voucher specimens (No. G.G.1, G.P.1) have been deposited at the Pharmacognosy Department, Faculty of Pharmacy, Helwan University, Cairo, Egypt.

Compound 1 (8-hydroxyapigenin 6-O-β-D-apiofuranosyl-(1''→6'')-C-β-D-glucopyranoside)

Yellow amorphous powder; Rf-values: 0.15 (S.), 0.45 (S.) on PC; it gave a dark purple spot under UV-light, which turned to yellowish green fluorescence on exposure to ammonia vapor and Naturstoff spray reagents, as well as green color with FeCl3. UV λmax nm: (MeOH): 272, 340; (+NaOAc): 282, 341, 401; (+NaOAc/H2O/BO): 272, 328, 352; (+AlCl3): 283, 304, 352, 394; (+AlCl3/HCl): 281, 304, 350, 395. ESI/MS m/z 579.1 [M-H]-. 1H NMR (DMSO-d6, 300 MHz): δ ppm 8.13 (2H, d, J= 8.4 Hz, H-2/6'); 6.92 (2H, d, J = 8.4 Hz, H-3/5'); 6.79 (1H, s, H-3), 5.19 (1H, d, J = 1.2 Hz, H-1'), 4.66 (2H, d, J = 9.0 Hz, H-1'), 3.88 (1H, br d, J = 10.5 Hz, H-6a'), 3.74 (1H, t-like, J = 6.9 Hz, H-2'), 3.49 (1H, br d, J = 12.5 Hz, H-6b'), 3.17 (2H, m, H-3''/4''). 13C NMR (DMSO-d6, 125 MHz): δ ppm 182.27, 132.79, 139.17, 133.44, 131.25, 129.39, 118.04, 115.46, 115.10, 114.76, 114.33, 114.09. Extraction and isolation

The powders of G. grandiflora (1150 g) and G. pulchella (950 g) air-dried aerial parts were individually exhaustively extracted with 80% aqueous methanol under reflux (5 L × 5, 60°C, 4 h). The solvent was evaporated under reduced pressure and the residue (180 and 150 g in case of G. grandiflora and G. pulchella, respectively) was refluxed with CHCl3 (1.5 L × 3, 60°C, 1 h). The dried CHCl3 extract and residue were 32 and 140 g in case of G. grandiflora, and 23 and 115 g from G. pulchella. The residue in each case was precipitated from H2O using excess methanol (1:10). This was followed by evaporation of the filtrate under vacuum to afford 105 and 90 g of residue in case of G. grandiflora and G. pulchella, respectively. The dried extract in each case was subjected to CC using polyamide (300 g, 1.1 m x 50 mm) and H2O-MeOH mixtures (100:0–100%) to afford 35 fractions (1 L each), which were collected into six collective fractions (A-F) in case of G. grandiflora. Similar chromatographic procedure afforded 30 fractions, each of 1 L which were collected into five collective fractions (A-E) in case of G. pulchella. The collection of the fractions was done by the aid of comparative paper chromatography (comp-PC), UV-light and spray reagents. In case of G. grandiflora, fraction A (3.15 g) was devoid of polyphenolic compounds, as investigated in our previous study.[11] Fraction B (550 mg) was subjected to CC using Sephadex LH-20 and H2O/MeOH mixtures as eluents (10–90%). This yielded two sub-fractions (i and ii), each containing one major compound. Final purification of both sub-fractions was done on cellulose column (50% MeOH/H2O) to give pure samples of 1 (20 mg) and 2 (17 mg). Fraction C (410 mg) was subjected to successive cellulose columns using 10-90 % aqueous MeOH, to afford pure 3 (16 mg) and 4 (21 mg). The pure compound 5 (17 mg) was obtained by applying fraction D (210 mg) to a Sephadex LH-20 column, using MeOH/H2O mixtures (50–100%). Fraction E (120 mg) was purified by successive cellulose columns using aqueous MeOH mixtures (10–60%) for elution, to afford pure 6 (23 mg). Fraction F (950 mg) was subjected to cellulose CC using 10–90% aqueous MeOH as eluent, which afforded two main sub-fractions. Each of them was individually purified on Sephadex LH-20 column using 50% aqueous MeOH and 100% methanol as eluents, to give pure 7 (19 mg) and 8 (21 mg). Moreover, in case of G. pulchella fraction A (4.50 g) was devoid of polyphenolic compounds, as investigated in our previous study.[11] Fraction B (550 mg) was subjected to Sephadex LH-20 CC using H2O/MeOH mixtures (10–90%) as eluent, to afford pure 9 (25 mg). Fraction C (350 mg) was applied on a cellulose column using δ, for elution and finally purified on Sephadex LH-20 column using MeOH, to give pure sample of 10 (20 mg). Fraction D (150 mg) was purified by successive cellulose columns using aqueous MeOH mixtures (10–90%) for elution, to afford pure 7 (17 mg). Two main sub-fractions (i and ii) were obtained upon purification of fraction E (300 mg) using cellulose column and MeOH/H2O (50%) for elution. Both sub-fractions were individually applied to a Sephadex LH-20 column using δ, for elution, to give pure samples of 11 (21 mg) and 12 (19 mg). The homogeneity of all fractions was tested using 2D- and comp-PC, and solvent systems δ and δ.
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76.49 (C-2”), 74.17 (C-4”), 73.86 (C-1”), 70.44 (C-2”), 70.35 (C-4”), 69.25 (C-6”), 64.71 (C-5”).

Pharmacological studies

Animals
Sprague-Dawley albino rats of both sexes (125–150 g) and Swiss mice (20–30 g) were obtained from the National Research Centre (Animal House), Dokki, Giza, Egypt. The animals were accommodated in standard metal cages at 22°C ± 3°C, 55% ± 5% humidity and were supplied with standard laboratory diet and water ad libitum. All animal procedures were carried out in accordance with the Ethics Committee of the National Research Centre (Egypt, April 2009). In addition, they followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Acute toxicity study
The AME extracts of G. grandiflora and G. pulchella were individually dissolved in distilled water and given orally to mice in graded doses up to 5 g/kg b. wt., while the control group obtained the same volume of distilled water. The percentage mortality was registered 24 h later, and none was observed for both extracts. According to Semler[12] the determination of LD₅₀ in this case is no longer required. The doses of the extract used in this study were 500 and 1000 mg/kg (1/10 and 1/5 of the highest nontoxic tested dose, respectively).

Anti-inflammatory activity study
Paw swelling was induced by sub-plantar injection of carrageenan suspension into the right hind paw (100 μl of 1% in saline).[13] The edema was quantified by measuring hind footpad with a micrometer caliber, before and 1-4 h after carrageenan injection. The results were expressed as percentage change from control values. Rats were divided into six groups, each of six. The control group received orally 0.02 ml saline, the 2nd-5th groups were orally given 500 and 1000 mg/kg of AME of G. grandiflora and G. pulchella. The 6th group was orally given indomethacin (25 mg/kg b.wt., in Na₂CO₃, 5%). The extracts and indomethacin were given 60 min before the injection of the carrageenan suspension.

Hepatoprotective activity study
Hepatotoxicity was induced using paracetamol orally at a dose of 1000 mg/kg in H₂O.[14] Rats were divided into seven groups, each of six. The control group received orally 1 ml saline/day, while the 2nd group received paracetamol. The 3rd-6th groups received orally 500 and 1000 mg/kg of AME of G. pulchella or G. grandiflora, while the last group received silymarin as reference drug (orally, 25 mg/kg). Both AME extracts and silymarin were given for 7 days before paracetamol injection. After 24 h of paracetamol injection, the blood samples were obtained by puncturing rato-orbital plexus. After centrifugation at 2500 rpm, the serum was used for determination of ALT,[15] AST[16] and serum ALP.[17]

Statistical analysis
The results are presented as mean ± standard error. All the data were statistically evaluated using Student’s t-test and one-way ANOVA. The *P <0.05 were considered significant.

RESULTS AND DISCUSSION

Chemistry
The 2D-PC screening of the G. grandiflora and G. pulchella aerial parts disclosed the presence of a mixture of flavone aglycones and glycosides (color properties under UV-light and responses to ammonia, FeCl₃, and Naturstoff spray reagents). Both mixtures were individually fractionated on a polyamide column, followed by successive cellulose and/or Sephadex LH-20 columns to afford the pure compounds 1-12. Compound 1 was isolated from G. grandiflora and nature for the first time, together with compounds 2-8, while compounds 9-12 and 7 were isolated from G. pulchella. All compounds, except 10 were isolated from genus Gaillardia for the first time. The structures of 1-12 [Figure 1] were fully elucidated on the basis of their UV, 1D and 2D NMR, ESI-MS data, and by comparison with previously published data.[18-20] Compound 1 was expected to be an apigenin 6-C-diglycosyl derivative based on its chromatographic properties.[14] UV spectrum in methanol exhibited two characteristic absorption bands at λₘₚₓ = 340 (I) and = 272 nm (II) for an apigenin nucleus. Free 7-OH was concluded from the bathochromic shift in band II, upon addition of NaOAc. In addition, the diagnostic batho- and hypos-chromic shifts observed upon addition of the other shift reagents, indicated the presence of free 3, 7 and 4’-OH groups.[14] 1H NMR and COSY spectra showed the resonances for B-ring protons of apigenin. This was explained from the presence of an A X β spin coupling system consisting of the two ortho doublets, each of 2 protons, at 8.13 and 6.92 for 2’/6’ and 3’/5’, respectively giving evidence to the 4’-hydroxyl B-ring, together with a singlet at δ 6.79 assigned to H-3.[19] Absence of H-6 and H-8 signals gave an evidence for their substitution. The presence of two anomic proton signals at δ 4.66 (d, J = 9 Hz, H-1”) and 5.19 (d, J = 1.2, H-1”) gave an evidence for one C-glucose and O-apiose moieties. The anomeric configurations of D-glucose and D-apiose were identified to be β-configurations, based on the magnitudes of the J₂, coupling constants obtained from the 1H NMR spectrum. Moreover, depending on their δ- and J-values and splitting pattern of all H and 13C-singals, the stereo-structures of glucose and apiose were identified as C-β-C₂- pyranose and O-β-furanose, respectively.[20] The downfield shift of H-6” (3.88) of the glucose moiety affirmed the position of the inter-glycosidic linkage. The 13C NMR spectrum showed the presence of thirteen carbon resonances, including an α/β-unsaturated ketone carbonyl signal at δ 182.27, characteristic for an apigenin nucleus. Downfield shift of C-6 at 108.81 (= +10 ppm) gave an evidence for its substitution by a C-glycosyl

Figure 1: Structures of flavonoids 1-12 isolated from Gaillardia grandiflora and/or Gaillardia pulchella aerial parts
moiety, while the downfield shift of C-8 at about 128.58 (≈ +33 ppm) revealed its hydroxylation.\cite{19,20} In addition, the intrinsic downfield shift of C-6 of the glucose moiety at δ 69.25 (Δ ≈ +9 ppm) in the 13C NMR spectrum was a diagnostic evidence for the inter-glycosidic linkage as 1''→6''.\cite{20} Assignment of the anomeric carbon of apiosyl moiety at 109.03 ppm was a key signal among other C-resonances for the O-β-D-Furanose structure. Moreover, the HMBC spectrum showed a long range correlation between glucose H-1 and C-6 (108.81) of the apigenin aglycone, confirming the site of glycosidation [Figure 2]. Similarly, the inter-glycosidic linkage of the disaccharides were characterized by long range correlations observed between CH-6 of glucose and C-1 (109.03) of O-apiose and the reverse cross peak of H-1 for O-apiose with C-6 (69.25) of glucose. Therefore, compound 1 was identified as 8-hydroxyapigenin 6-O-β-D-apiofuranosyl-(1''→6'')-C-β-D-glucopyranoside.

### Biological activities

Both AME extracts of *Gaillardia* species were nontoxic up to the maximum soluble dose (5 g/kg b.wt.). They exhibited a significant inhibition of edema only at 1000 mg/kg b.wt. by 17.85, 18.88, 18.30, 25.55% (G. grandiflora) and 17.69, 21.21, 20.95, 27.96% (G. pulchella) after 1, 2, 3 and 4 h post carrageenan injection, in comparison to control group [Table 1]. The two extracts exhibited a significant hepatoprotective effect in a dose-dependent manner [Table 2]. Paracetamol group showed significant elevation in ALT, AST and ALP levels by 64.03, 67.91 and 86.12%, respectively compared with control group. The AME of *G. grandiflora* exhibited a significant reduction in ALT (42.04, 41.53%) and AST (22.81, 30.99%) levels by 500 and 1000 mg/kg, respectively. Moreover, *G. pulchella* AME significantly decreased the ALT (25.33, 36.79 %) and AST (24.58, 36.71 %) levels at 500 and 1000 mg/kg, respectively. The AME of *G. grandiflora* and *G. pulchella* significantly decreased ALP level by 15.92% and 14.12%, respectively only at a dose of 1000 mg/kg, as compared to the paracetamol group. Silymarin group exhibited a significant reduction in ALT, AST and ALP serum levels by 35.53, 37.79 and 41.37%, respectively as compared to paracetamol treated group.

Both *Gaillardia* species are characterized by their sesquiterpenes derivatives,\cite{3-6} but only few phenolic compounds were reported from genus *Gaillardia*.\cite{19,20} In this study, we evidence for the first time by chemical and spectrospecic data that AME of *G. grandiflora* and *G. pulchella* contain a large number of phenolic constituents, especially of the flavone type viz. eight glycosylflavones (1-6, 9-10) together with four flavone aglycones (7, 8, 11, 12).

### Table 1: Time course of the effect of oral administration of aqueous methanol extract of *Gaillardia grandiflora, Gaillardia pulchella* and indomethacin on rat paw oedema formation induced by sub-plantar injection of carrageenan

| Group/doses                  | Oedema (cm) |          |          |          |          |          |          |          |
|-----------------------------|-------------|----------|----------|----------|----------|----------|----------|----------|
|                            | 1 h         | 2 h      | 3 h      | 4 h      |          |          |          |          |
|                            | Percentage increase | Potency | Percentage increase | Potency | Percentage increase | Potency | Percentage increase | Potency |
| Control (2 saline)          | 97.95±5.4   | -        | 109.88±6.3 | -        | 111.29±6.3 | -        | 122.1±9.0  | -        |
| *Gaillardia grandiflora* (mg/kg) |            |          |          |          |          |          |          |          |
| 500                         | 90.37±8.3   (7.74) | 0.1      | 103.94±6.2 (5.41) | 0.1      | 106.98±7.5 (3.87) | 0.1      | 107.95±4.8 (11.60) | 0.2      |
| 1000                        | 80.46±4.9*  (17.85) | 0.3      | 89.13±4.5* (18.88) | 0.3      | 90.92±4.8* (18.30) | 0.4      | 90.92±4.8* (25.55) | 0.5      |
| *Gaillardia pulchella* (mg/kg) |            |          |          |          |          |          |          |          |
| 500                         | 94.36±7.4   (3.67) | 0.1      | 100.47±6.2 (8.56) | 0.2      | 102.47±4.3 (7.93) | 0.2      | 102.47±4.7 (16.10) | 0.3      |
| 1000                        | 80.62±4.2*  (17.69) | 0.3      | 86.57±5.8* (21.21) | 0.4      | 87.97±6.6* (20.95) | 0.5      | 87.97±6.6* (27.96) | 0.5      |
| Indomethacin (25 mg/kg)     | 42.01±2.3** (57.10) | 1        | 48.16±2.2** (56.17) | 1        | 52.49±2.6** (52.83) | 1        | 57.32±1.6** (53.06) | 1        |

Data represent the mean±SE of six rats per group of percent changes value versus basal (zero min) values and 1, 2, 3 and 4 h post carrageenan injection; Data were analyzed using Student's t-test, *P<0.01, **P<0.001 as compared with saline treated group at the same time post carrageenan injection; Percent oedema inhibition (the value in between parenthesis) was calculated as regard to saline control group; Potency was calculated as regard to the percentage change of the indomethacin treated group. SE: Standard error.

### Table 2: Effect of oral administration of *Gaillardia grandiflora* and *Gaillardia pulchella* aqueous methanol extract on alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase serum activity in paracetamol induced hepatotoxicity in rats

| Groups                | Dose (mg/kg) | ALT (U/L) | Mean±SE | Percent of change | AST (U/L) | Mean±SE | Percent of change | ALP (IU/L) | Mean±SE | Percent of change |
|-----------------------|--------------|-----------|---------|-------------------|-----------|---------|-------------------|-----------|---------|-------------------|
| Control               | 1 ml saline  | 66.64±2.1 | 39.04   | 60.21±3.5         | 40.45     | 54.74±1.5| 46.28             |           |         |                   |
| Paracetamol           | 1000         | 109.31±6.5* | -       | 101.1±7.6         | -         | 101.9±3.1*| -                 |           |         |                   |
| *Gaillardia grandiflora* | 500         | 63.36±2.3* | 42.04   | 78.04±4.2*        | 22.81     | 96.59±3.9| 5.21              |           |         |                   |
|                       | 1000         | 63.91±1.5* | 41.53   | 69.76±3.9*        | 30.99     | 85.68±2.6*| 15.92             |           |         |                   |
| *Gaillardia pulchella* | 500         | 81.62±3.1* | 25.33   | 76.25±2.8*        | 24.58     | 95.13±4.4| 6.64              |           |         |                   |
|                       | 1000         | 69.10±1.4* | 36.79   | 63.99±3.5*        | 36.71     | 87.51±3.6*| 14.12             |           |         |                   |
| Silymarin             | 25           | 70.47±4.4* | 35.53   | 62.89±5.2*        | 37.79     | 59.74±2.3*| 41.37             |           |         |                   |

Values represent the mean±SE (n=6). *P<0.05: Statistically significant from saline control group; *P<0.01: Statistically significant from paracetamol group by using (Student's t-test). Percent of change was calculated as regard to paracetamol treated group. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; SE: Standard error.
Flavonoids, the most common plant polyphenols, are widely distributed in every plant species and located in different plant parts, including flowers, fruits, bark, stems, and leaves, as well as other parts. They are reported to have a wide variety of biological activities, as some of them act as enzyme inhibitors and antioxidants, and others are reported to have anti-inflammatory and hepatoprotective activities. Inflammation is a normal biological process and is the first response of the body to infections, irritations, or other injuries. It is initiated by migration of immune cells from blood vessels, in addition to the release of mediators at the site of damage. This is followed by recruitment of inflammatory cells, release of reactive oxygen and nitrogen species, and pro-inflammatory cytokines, aiming to neutralize the attacking agents, to repair damaged tissues, and thus to assure the survival of the organism. The usual symptoms of inflammation include warmth, redness, and swelling, in addition to pain and loss of function. In general, normal inflammation is a rapid and self-limiting process, but prolonged inflammation may cause various chronic disorders like rheumatoid arthritis. The response of the immune system may be modified by diet, pollutants present in the environment and chemicals naturally occurring in food. Certain flavonoids can significantly affect the function of the immune system and inflammatory cells. In addition, many flavonoids are reported to possess anti-inflammatory effect and several mechanisms may give a proper explanation. The ability of flavonoids to inhibit the biosynthesis of eicosanoid like prostaglandins, which is involved in various immunologic responses, and to inhibit the activity of cyclooxygenase and lipoxygenase are important inflammatory mediators involved in the generation of inflammatory processes, especially tyrosine and serine-threonine protein kinases. These are enzymes engaged in signal transduction and cell activation processes involving cells of the immune system. These enzymes are inhibited as a result of competitive binding of flavonoids with ATP at catalytic sites on the enzymes. Another anti-inflammatory feature of flavonoids, is their proposed ability to inhibit neutrophil degranulation, which is a direct method to reduce the release of arachidonic acid by neutrophils and other immune cells. Many studies explored the anti-inflammatory activity of apigenin and luteolin, their possible mechanism(s) of action. One study confirmed that the administration of the two flavones markedly inhibited acute carrageenan-induced paw edema in mice, while the flavonol fisetin failed to have an effect. A second study showed that the aqueous extract of Barleria crisata leaves exhibited significant anti-inflammatory activity due to the presence of apigenin, quercetin, naringenin, and luteolin. A third study showed that flavonoid glycosides such as vitexin, regardless of chemical structures of the aglycones, did not significantly inhibit nitric oxide (NO) production. Generally, flavones showed stronger inhibition of NO production than flavonols, as apigenin, naringenin, and luteolin were the most active inhibitors among natural flavonoids tested. In addition, results strongly suggested that the presence of a double bond between C-2 and C-3 is critical for inhibiting NO production and that hydroxyl substitutions on A- and B-rings influence the inhibitory activity. The A-ring 5-/7- and B-ring 3-/4- hydroxylation(s) gave favorable results, while C-3 hydroxylation as in case of flavonol, did not. The results of another study suggested that apigenin has significant anti-inflammatory activity that comprises the blocking of NO mediated cyclooxygenase 2 expression and monocyte adherence.
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