Original Article

Effects of extracts from Gynura bicolor (Roxb. & Willd.) DC. on iron bioavailability in rats

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Article info

Article history:
Received 31 March 2014
Received in revised form 19 August 2014
Accepted 24 November 2014
Available online 24 February 2015

Keywords:
divalent metal transporter
ferroportin
Gynura bicolor
iron bioavailability

Abstract

Gynura bicolor (Roxb. & Willd.) DC. is widely distributed in certain areas of Asia and is very popular in vegetarian cuisine in Taiwan. This study investigates the effects of G. bicolor extracts with different polarities of 80 mg/kg body weight (BW) G. bicolor alcohol extract, 80 mg/kg BW G. bicolor water extract, and 80 mg/kg BW G. bicolor ether extract on Fe bioavailability using the hemoglobin repletion efficiency assay. Wistar rats were assigned to five groups: a group receiving an iron-deficient (ID) diet; a group receiving an ID diet supplemented with ferrous sulfate (20 mg Fe/kg BW); and three groups receiving ID diets supplemented with ferrous sulfate and one of G. bicolor alcohol extract, G. bicolor water extract, or G. bicolor water extract. The results indicated that the levels of hemoglobin, serum iron, serum ferritin, liver ferritin, hemoglobin regeneration efficiency, relative biological value, and hepcidin all were significantly higher than those of the ID diet group. Besides, the iron transporter divalent metal transporter-1 was significantly reduced, but iron release protein expression of ferroportin was significantly increased. It was concluded that G. bicolor extracts may promote iron bioavailability and regulate the expressions of divalent metal transporter-1 and ferroportin.

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1. Introduction

According to the World Health Organization, anemia is a global public health problem that primarily affects the health and social development of populations in developing and developed countries. Iron deficiency is considered to be the most important cause of anemia [1].

Duodenum contains regulatory proteins that, via their role in iron metabolism, facilitate the balance between iron absorption and release. Dietary nonheme iron enters the intestine as Fe^{3+}. It is converted to Fe^{2+} by duodenal apical...
cytochrome b, which exhibits ferrireductase activity and enters the intestinal cells through apical divalent metal transport protein-1 (DMT-1). The iron entering intestinal cells is oxidized to Fe³⁺ by ferritin, and is stored as ferritin and hemosiderin [2]. When tissues need iron, Fe³⁺ is reduced to Fe²⁺ by ferritin and released into the blood circulation through the ferroportin (FPN) located on the basolateral side of intestinal cells. In addition, one of the functions of hepcidin, a heptadecapeptide hormone, is to facilitate iron regulation and metabolism in animals. A previous study showed that hepcidin can bind to FPNs located on the basolateral side of intestinal cells and induce FPN phosphorylation and degradation, thereby increasing iron storage by intestinal epithelial cells, macrophages, and hepatocytes [3].

The main factor affecting nutrition with regard to iron is its low dietary availability. In recent years, there have been many studies regarding food iron fortification and phytomaterials. For example, a study using the Caco-2 cell model showed that, when consumed with iron, garlic can improve iron absorption through increased FPN expression [4]. A different study showed that, when fed to rats, yacon powder, which contains inulin-type fructans, acidified the intestinal mucosa by increasing cecal fermentation to produce short-chain fatty acids, thereby improving the solubility and bioavailability of iron pyrophosphate [5]. Another study showed that, when consumed by mice, Telfairia occidentalis affected iron absorption by decreasing the expression of duodenal DMT-1 [6]. The results of cell and animal models both evidenced that fortification of foods with iron is an effective way to improve iron nutritional status, suggesting that this approach is worth further investigation.

Gynura bicolor (Roxb. & Willd.) DC., a perennial plant belonging to the Asteraceae family, originates from tropical areas in Asia. Leaves of G. bicolor distinctively show a reddish purple color on their abaxial sides, contrasting with the typical green color seen on the adaxial side [7]. Previous studies have shown that G. bicolor possesses high antioxidant activity and performs many biological functions with no toxic effects [8]. The pigment sources and physiological effects of G. bicolor are thought to be related to its rich flavonoid content, including anthocyanins, quercetin, kaempferol, quercitrin, isoquercitrin, and rutin [9,10]. In addition, according to the Nutritional Composition Database of Taiwan, G. bicolor is rich in iron, having iron levels as high as 41 μg/g, and 100 g of G. bicolor contains 15 mg of vitamin C (ascorbic acid), which can promote iron absorption. G. bicolor is consumed as a cooked vegetable and is believed to have a wide range of benefits that include antioxidant, anti-inflammatory, and possibly antihypertensive effects [11,12]. However, promotion of iron absorption by G. bicolor has not been reported.

The purpose of this study was to determine whether the administration of G. bicolor extracts in Wistar rats could promote iron absorption and bioavailability. The blood biochemical indicators, including hemoglobin (Hb), serum iron, serum ferritin, liver ferritin, serum iron, total iron-binding capacity (TIBC), transferrin saturation (TS), Hb regeneration efficiency (HRE), relative biological value (RBV), hepcidin, and duodenal mucosa expressions of iron metabolism proteins (DMT-1 and FPN), were examined using an Hb repletion efficiency assay.

2. Methods

2.1. Preparation of the G. bicolor alcohol extract, G. bicolor water extract, and G. bicolor ether extract

G. bicolor (Roxb. & Willd.) DC. was purchased from Yuanshan Village (Ilan, Taiwan). Leaves of G. bicolor were removed, cleaned, and blended in 95% alcohol or cold water (4°C, w/w: 1/1). After standing for 1 hour, the suspension was centrifuged at 5000g for 10 minutes at 4°C, after which the filtrate was concentrated under reduced pressure (30–40°C). Finally, the extracts were dried in a rotary vacuum dryer. The percent yields of the alcohol and water extracts were 0.9% and 4.2% (w/w), respectively. In addition, the leaves of G. bicolor homogenates were extracted with ether (v/v: 1/1) for 6 hours at 4°C, after which the extracts were stirred on a stirring plate for 4 hours and then dried in a rotary vacuum dryer. The percent yield of the ether extract was 0.3% (w/w).

2.2. G. bicolor composition analysis

The total flavonoid and phenolic content of G. bicolor extracts was determined using the colorimetric method of Jia et al [13]. The rutin content was determined using high-performance liquid chromatography, as described by Krizman et al [14]. The β-carotene and chlorophyll contents were analyzed in accordance with Xu et al [15] and Witham et al [16], respectively. The total phenolic content was determined using the Folin–Ciocalteu procedure described by Singleton and Rossi [17] with gallic acid as the standard and was expressed (μg) as gallic-acid equivalent (GAE)/g of extract. The anthocyanin content was determined according to the method of Padmavati et al [18] with quercetin as the standard and was expressed (μg) as quercetin equivalent/g of extract.

2.3. Experimental design

Weaning male Wistar rats (Lasco, Taipei, Nangang, Taiwan), weighing 50 ± 5 g, were housed individually in stainless-steel cages. The experiments were carried out in accordance to internationally accepted guidelines on laboratory animal use and the protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of National Kaohsiung Marine University of Taiwan.

Weaning male Wistar rats (Lasco, Taipei, Nangang, Taiwan), weighing 50 ± 5 g, were housed individually in stainless-steel cages. The temperature was maintained at 25°C with 12-hour light/dark periods. Food and deionized water were freely available [19]. Each rat was fed a Fe-deficient powder diet (9G0T, test diet, 5 mg Fe/kg) [20] for 14 days (depletion period), and the Hb concentration was monitored via blood drawn from the tail. When the Hb concentration was sufficiently low (6 g/dL), the rats were organized into five groups, based on the average body weight (g).

In the regeneration period, rats were fed a Fe-powder diet containing 20 mg Fe/kg body weight (BW) as ferrous sulfate (FeSO₄ 7H₂O) (Panreac, Barcelona, Spain) and were supplemented with different polarities of the G. bicolor extracts.
[80 mg/kg BW G. bicolor alcohol extract (GBAE), G. bicolor water extract (GBWE) or G. bicolor water extract (GBEE)] for 14 days.

2.4. Iron bioavailability and hematological parameters

Following overnight fasting, Wistar rats were sacrificed by carbon dioxide euthanasia. Blood was withdrawn using the intracardiac method, and serum was prepared for the analysis of hematological parameters by the automated hematology analyzer (Sysmex, Kobe, Japan). The Hb concentrations and Fe consumption results were used to estimate the following parameters [21]:

(1) Hb Fe gain (Fe mg/rat), assuming that the total blood volume was 6.7% of body weight and Fe content in Hb was 0.335%:

\[
\text{Hb Fe gain} = \frac{\text{body weight (g) } \times \text{ Hb (g/dL) } \times 6.7 \times 0.335}{1000}
\]

(2) HRE:

\[
\%\text{HRE} = \frac{\text{Hb Fe (final) } - \text{Hb Fe (initial)} \times 100}{\text{Fe intake (mg/rat)}}
\]

(3) RBV:

\[
\%\text{RBV} = 100 \times \left( \frac{\%\text{HRE test group}}{\%\text{HRE ferrous sulfate group}} \right)
\]

Serum iron and TIBC (Randox Laboratories, Crumlin, Co., Antrim, UK) concentrations were determined using commercial kits for colorimetric iron determination at 590 nm. The protocol described by the manufacturer was used.

2.5. Ferritin contents and TS

Blood samples were centrifuged at 3000g for 10 minutes. The liver samples were scraped (in a buffer containing 8mM KH₂PO₄, 12mM K₂HPO₄, and 1.5% KCl; pH 7.4) with a homogenizer. The homogenate was centrifuged at 3000g for 10 minutes at 4°C. Ferritin was assayed using an enzyme-linked immunosorbent assay kit (R&D System, Minneapolis, MN, USA). Serum and liver ferritin concentrations were extrapolated from standard curves, as described by the manufacturer’s protocol. TS was calculated from serum iron and TIBC using the following equation:

\[
\text{TS (¥)} = \frac{\text{serum iron/TIBC}}{100}.
\]

2.6. Serum hepcidin contents

Serum hepcidin concentrations were determined using commercial enzyme-linked immunosorbent assay kits (Sunred, Shanghai, Baoshan, China). Serum hepcidin concentrations were extrapolated from standard curves, as described in the manufacturer’s protocol.

2.7. Protein expression

Each duodenal mucosa sample was scraped with a glass slide and homogenized in RIPA (radio-immunoprecipitation assay) buffer containing 150mM NaCl, 50mM Tris-HCl (pH 8.0), 0.1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS (sodium dodecyl sulfate), 1mM PMSF (phenylmethylsulfonyl fluoride), 2 µg/mL aprotinin, 1 µg/mL leupeptin, and 1mM sodium orthovanadate] with a homogenizer on ice [6]. The homogenate was centrifuged at 12,000g for 20 minutes at 4°C. Protein concentrations were determined using the Lowry et al’s [22] method. Samples in sample buffer (v/v = 1/1) were heated at 95°C for 5 minutes and then loaded on a 10% gel. The proteins separated were then transferred to polyvinylidene fluoride membranes using a semidry transfer apparatus (Bio-Rad, Philadelphia, PA, USA). Each membrane was blocked with 5% nonfat milk for 2 hours and probed with DMT-1, FPN, or GAPDH (glyceraldehyde-3-phosphate dehydrogenase) (Santa Cruz Bioscience, CA, USA) antibodies diluted in Tris-buffered nonfat milk for 2 hours and probed with DMT-1, FPN, or GAPDH (glyceraldehyde-3-phosphate dehydrogenase) (Santa Cruz Bioscience, CA, USA) antibodies diluted in Tris-buffered saline. Cross-reactivity was observed with peroxidase-linked anti-IgG. Colorimetric protein detection used 3,3’-0-dianinobenzidine (Sigma Chemical, St. Louis, MO, USA). Band intensities were measured with an AlphaImager 2000 (Alpha Innotech, San Leandro, CA, USA).

2.8. Statistical analysis

Data are expressed as the means ± standard deviation from at least three independent experiments. Differences among treatments were analyzed by one-way analysis of variance and Duncan’s test using the SPSS version 12.0 statistical software (SPSS Inc., Chicago, IL, USA). Statistically significant differences were defined as p < 0.05.

3. Results

3.1. G. bicolor extract composition

The composition analysis result of G. bicolor extracts was shown in Table 1. The main phytoactive ingredients contained in GBAE were 3.08 µg/g of quercetin, 506 µg/g of rutin, 29.8 µg/g of gallic acid, 102 µg/g of chlorophyll, 2.39 µg/g of β-carotene, 22.5 µg of GAE/g of total phenolics, 51.4 µg of cyanidin chloride equivalents (CyE)/g of anthocyanin, and 3.26 µg/g of iron; in GBWE were 2.84 µg/g of gallic acid, 22.5 µg/g of chlorophyll, 1.63 µg/g of β-carotene, 2.11 mg of GAE/g of total phenolics, 47.6 mg of CyE/g of anthocyanin, and 10.1 µg/g of iron; and in GBEE were 25.3 µg/g of quercetin, 11500 µg/g of chlorophyll, 7460 µg/g of β-carotene, 2.39 µg/g of total phenolics, 1.44 µg/g of iron.

3.2. Effect of G. bicolor extracts on iron bioavailability and hematological parameters in rats

Changes in Hb content in experimental rats prior to and after iron repletion are shown in Table 2. The statistical results show that, after iron repletion, the ferrous sulfate group and GBAE + ferrous sulfate, GBWE + ferrous sulfate, and
GBEE + ferrous sulfate groups showed significant differences compared to the ID group (p < 0.05). Moreover, GBAE + ferrous sulfate and GBE + ferrous sulfate groups were significantly higher than the other groups.

Table 3 shows the changes in serum iron, TIBC, and TS of the GBAE + ferrous sulfate, GBWE + ferrous sulfate, and GBE + ferrous sulfate groups after iron repletion. The serum iron concentrations of the ferrous sulfate, GBAE + ferrous sulfate, GBWE + ferrous sulfate, and GBE + ferrous sulfate groups were 148 ± 11 μg/dL, 254 ± 30 μg/dL, 178 ± 23 μg/dL, and 225 ± 14 μg/dL, respectively, wherein the GBAE + ferrous sulfate group had the most significant change (p < 0.05). The TS value of the ferrous sulfate group after iron repletion was 35.0 ± 5.3%, and those of the groups that were given GBAE or GBE were significantly increased to 47.4 ± 1.6% and 47.0 ± 5.5%, respectively. Changes in serum ferritin, liver ferritin, and hepcidin are shown in Table 3. The results show that after supplementing iron, the liver ferritin concentrations of the ferrous sulfate, GBAE + ferrous sulfate, GBWE + ferrous sulfate, and GBE + ferrous sulfate groups were 161 ± 5 μg/dL, 220 ± 5 μg/dL, 198 ± 7 μg/dL, and 184 ± 1 μg/dL, respectively, wherein the liver ferritin concentration of the GBAE + ferrous sulfate group was significantly higher than that of the ferrous sulfate, GBWE + ferrous sulfate, and GBE + ferrous sulfate groups (p < 0.05).

The effects of iron repletion on HREs and RBVs of the GBAE + ferrous sulfate, GBWE + ferrous sulfate, and GBE + ferrous sulfate groups are shown in Table 4. The results show that after iron repletion, RBVs of the ferrous sulfate, GBAE + ferrous sulfate, GBWE + ferrous sulfate, and GBE + ferrous sulfate groups were 100 ± 0, 150 ± 8, 135 ± 7, and 132 ± 4, respectively, wherein the GBAE + ferrous sulfate group showed the most significant change in RBV (p < 0.05).

3.3. Effect of G. bicolor extracts on DMT-1 and FPN protein expressions in rat duodenum

The effects of G. bicolor extracts of different polarities on the expressions of DMT-1 and FPN, which regulate iron metabolism of the rat duodenum, are shown in Fig. 1. The results show that, compared with the ID group, DMT-1 expression levels significantly decreased in various groups that were given G. bicolor extracts of different polarities after iron repletion. Likewise, DMT-1 expression levels of the groups that were given GBAE or GBWE were significantly lower compared to those of the ferrous sulfate group (p < 0.05). In addition, the results showed that after iron repletion, FPN expression significantly increased in the groups that were given G. bicolor extracts of different polarities compared with the ID group, and the increasing trend in the GBAE group was significant (p < 0.05).

4. Discussion

Previous studies have shown that phytoactive ingredients can considerably affect iron absorption [23]. Organic acids present in vegetables, such as citric acid, malic acid, and lactic acid, and trace nutrients, such as vitamin A and β-carotene, increase iron absorption [24,25]. The β-carotene in foods may form a complex with iron, maintaining the solubility of iron in the intestine and, at the same time, reducing the inhibition of iron absorption caused by phytic acid, tannic acid, and polyphenols [26]. In addition, some studies have demonstrated that vitamin A and β-carotene also increase the absorption of nonheme iron, thereby increasing Hb content [25], and that β-carotene-rich vegetables (carrots or amaranth) can increase iron availability in grains. Supplementation with β-carotene products can increase the iron bioavailability of grains by 19.6–102%, indicating that β-carotene can promote iron absorption [27].

### Table 1 – Chemical composition analysis of Gynura bicolor and its extracts.

| Ingredients                | G. bicolor | GBAE | GBWE | GBE |
|----------------------------|------------|------|------|-----|
| Quercetin (μg/g)           | 0.11       | 3.08 | ND   | 25.3|
| Rutin (μg/g)               | 6.12       | 506  | ND   | ND  |
| Gallic acid (μg/g)         | 7.50       | 29.8 | 2.84 | ND  |
| Chlorophyll (μg/g)         | 629        | 102  | 22.5 | 11500|
| β-Carotene (μg/g)          | 27.3       | 2.39 | 1.63 | 74600|
| Total phenolic (mg of GAE/g)| 0.13       | 22.5 | 2.11 | 2.40 |
| Anthocyanin (mg of CyE/g)  | 4.65       | 51.4 | 47.6 | 49.1 |
| Iron (mg)                  | 3.94       | 3.26 | 10.1 | 1.44 |

CyE = cyanidin chloride equivalent; GAE = gallic acid equivalent; GBAE = G. bicolor alcohol extract; GBE = G. bicolor ether extract; GBWE = G. bicolor water extract; ND = not detected.

### Table 2 – Effects of Gynura bicolor extracts with different polarities on the 14-day repletion hemoglobin in rats.a,b

| Groups                         | Hemoglobin (g/dl) |
|--------------------------------|-------------------|
|                               | Initial           | 14 d repletion   | 14 d gain       |
| ID                             | 5.05 ± 0.56*a     | 5.34 ± 0.18*b    | 0.29 ± 0.12*c   |
| ID + ferrous sulfate           | 5.11 ± 0.78*b     | 9.93 ± 0.58*c    | 4.82 ± 0.46*d   |
| ID + GBAE + ferrous sulfate    | 4.47 ± 1.08*b     | 9.80 ± 0.53*c    | 5.33 ± 1.11*    |
| ID + GBWE + ferrous sulfate    | 4.90 ± 1.04*b     | 9.63 ± 0.15*b    | 4.73 ± 1.16*    |
| ID + GBE + ferrous sulfate     | 4.70 ± 1.84*b     | 10.20 ± 0.20*b   | 5.50 ± 1.65*    |

Data are presented as mean ± SD. Significance of different groups was evaluated by Duncan’s test. Mean values followed by the same superscript letter are not significantly different (p < 0.05).

GBAE = G. bicolor alcohol extract; GBE = G. bicolor ether extract GBWE = G. bicolor water extract; ID = iron-deficient diet; SD = standard deviation.

*a Iron concentration was 20 mg/kg BW rat.

b GBAE, GBWE, and GBE concentration was 80 mg/kg BW rat.
Quercetin and rutin are phytocompounds with great potential for promoting iron absorption. Previous studies have shown that rutin and quercetin can reduce the oxidation–reduction activity, labile plasma iron, and serum iron overload, and can effectively facilitate the loading of metal ions onto irontransport proteins [23,28]. Quercetin can promote iron absorption and bioavailability by facilitating the reduction of Fe^{3+} to Fe^{2+} in foods [23]. Animal model studies have shown that an increase in the prenatal quercetin intake of adult mice increases the expression of hepcidin in rats. The present study results indicate that G. bicolor extracts are rich in active ingredients that promote iron absorption, such as β-carotene, quercetin, and rutin. The main phytoactive ingredients contained in GBAE were 25.3 μg/g of quercetin and 7460 μg/g of β-carotene.

When given synthetic hepcidin-25 (10μM) and cultured for 24 hours, Caco-2 cells exhibited decreased apical iron absorption via the reduction of mRNA and protein expression of DMT-1 in iron-responsive elements (DMT1 + iron-responsive element). However, the expression of iron-regulated gene-1 (the gene encoding FPN) was not affected by hepcidin, indicating that hepcidin affects mainly the mechanism of apical iron absorption and, thus, reduces absorption of dietary iron [30]. For Caco-2 and J774 cells (rat macrophage cells) that were cultured in synthesized hepcidin-25 for 24 hours, hepcidin-25 can inhibit iron release from macrophages by inducing FPN degradation and can inhibit iron absorption of intestinal cells by inhibiting DMT-1 transcription [31]. When Caco-2 cells were cultured for 24 hours with the active ingredient of garlic (diallyl disulfide) and 55FeCl3, the results showed that the mRNA and protein expression of FPN increased [4], indicating that garlic can promote iron absorption by increasing FPN expression. Mice that received synthesized hepcidin (hepcidin-25) by intraperitoneal injection showed that the injection of hepcidin-25 did not affect liver iron storage or Hb levels, but inhibited the iron absorption of duodenal apical membranes.

| Groups | Serum iron (μg/dL) | TIBC (μg/dL) | TS (%) | Serum ferritin (μg/dL) | Liver ferritin (μg/dL) | Hepcidin (ng/L) |
|--------|-------------------|--------------|--------|------------------------|------------------------|-----------------|
| ID     | 52 ± 4a           | 596 ± 12a    | 11.7 ± 3.1c | 100 ± 6c               | 155 ± 2d               | 98 ± 5b         |
| ID + ferrous sulfate | 148 ± 11d   | 485 ± 20b    | 35.0 ± 5.3b | 133 ± 3b               | 161 ± 5d               | 133 ± 1a        |
| ID + GBAE + ferrous sulfate | 254 ± 30a  | 506 ± 19b    | 47.4 ± 1.6   | 136 ± 1bc              | 220 ± 5a               | 129 ± 3c        |
| ID + GBWE + ferrous sulfate | 178 ± 25c  | 469 ± 25b    | 38.2 ± 7.44  | 147 ± 7b               | 198 ± 7b               | 124 ± 3ac       |
| ID + GBE + ferrous sulfate | 225 ± 14b  | 480 ± 28b    | 47.0 ± 5.55  | 143 ± 7bc              | 184 ± 1c               | 117 ± 3c        |

Data are presented as mean ± SD. Significance of different groups was evaluated by Duncan’s test. Mean values followed by the same superscript letter are not significantly different (p < 0.05).

| Groups | Hemoglobin Fe gain (mg Fe/rat) | HRE (%) | RBV (%) |
|--------|--------------------------------|---------|---------|
| ID     | 3.44 ± 0.23                    | 5.6 ± 0.3b | 100 ± 0c |
| ID + GBAE + ferrous sulfate | 4.03 ± 0.23  | 8.5 ± 0.4a  | 150 ± 8a  |
| ID + GBWE + ferrous sulfate | 3.52 ± 0.10  | 7.6 ± 0.3a  | 135 ± 7b  |
| ID + GBE + ferrous sulfate | 3.27 ± 0.12  | 7.5 ± 0.5a  | 132 ± 4d  |

Data are presented as mean ± SD. Significance of different groups was evaluated by Duncan’s test. Mean values followed by the same superscript letter are not significantly different (p < 0.05).

GBAE = G. bicolor alcohol extract; GBE = G. bicolor ether extract; GBWE = G. bicolor water extract; ID = iron-deficient diet; SD = standard deviation; TIBC = total iron-binding capacity; TS = transferrin saturation.

For Caco-2 and J774 cells (rat macrophage cells) that were cultured in synthesized hepcidin-25 for 24 hours, hepcidin-25 can inhibit iron release from macrophages by inducing FPN degradation and can inhibit iron absorption of intestinal cells by inhibiting DMT-1 transcription [31]. When Caco-2 cells were cultured for 24 hours with the active ingredient of garlic (diallyl disulfide) and 55FeCl3, the results showed that the mRNA and protein expression of FPN increased [4], indicating that garlic can promote iron absorption by increasing FPN expression. Mice that received synthesized hepcidin (hepcidin-25) by intraperitoneal injection showed that the injection of hepcidin-25 did not affect liver iron storage or Hb levels, but inhibited the iron absorption of duodenal apical membranes.
without affecting the proportion of iron transferred into blood circulation [32].

Compared with the ID + ferrous sulfate group, the iron parameters for the groups given *G. bicolor* extracts combined with ferrous sulfate showed that GBAE and GBEE significantly improved Hb levels, TS, serum iron levels, liver ferritin levels, HRE, and RBVs; and GBWE significantly improved serum iron levels, serum ferritin levels, liver ferritin levels, HRE, and RBVs.

Fig. 1 — Effects of *Gynura bicolor* extracts with different polarities on (A) DMT-1 and (B) FPN protein expression in rat duodenum. Results expressed as mean ± SD. Significance of different groups was evaluated by Duncan’s test. Different alphabet letters indicate that the DMT-1 and FPN protein expressions are statistically different from each other (*p* < 0.05). Iron concentration was 20 mg/kg BW rat. GBAE, GBWE, and GBEE concentration was 80 mg/kg BW rat. DMT-1 = divalent metal transporter-1; FPN = ferroportin; GBAE = *G. bicolor* alcohol extract; GBEE = *G. bicolor* ether extract; GBWE = *G. bicolor* water extract; ID = iron-deficient diet (5 mg/kg).
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